Structural elucidation of two unique antimicrobial Cassane – type tricyclic diterpenes from the root of *CALLIANDRA PORTORICENSIS (JACQ)-BENTH (FABACEAE).*

Oguegbulu N. E, Abo A.K. and Afieroho O.E

Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.

Abstract: The burden of infectious diseases by bacteria and fungi had constituted a great concern to the entire human population. *Calliandra portoricensis (Fabaceae)* had been widely used over the years in ethnomedicine for the; treatment of various ailments such as swollen gum, tooth and throat inflammation often associated with microbial infections. At present, no active antimicrobial compound has been reported from this specie. The aim of this research was to identify, isolate and characterize the antimicrobial compounds from the root of *C. portoricensis.* The pulverized root sample (0.8 Kg) was extracted by successive cold maceration respectively for 72 hr. The most bioactive ethyl acetate extract (4.61 gm) was subjected to chromatographic column fractionation (Silica Gel G, 200-400 mesh-stationary phase). Gradient mixtures 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 determinations. Clinically viable human pathogens for the tests were; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Streptococus fecalis, Candida albicans* and *Aspergillus niger*. Two major fractions (F_A and F_B) active against the test organisms were pooled the most active fraction F_B on further purification by preparative TLC (Silica Gel G, 0.5 mm thickness), yielded bioactive pure commands C_1 (9 mg) and C_2 (8 mg). Both compounds exhibited MIC values of 125.00±0.70 µg per milliliter against *Candida albicans* and *Aspergillus niger* These activities were found to be quite significant with respect to the reference controls (Ciprofloxacin and fluconazole) at $P \leq 0.05$. Characterization of C_1 and C_2 by spectroscopic analysis (UV, MS, FT – IR and NMR), identified two novel compounds as Cassane - type tricyclic diterpenoids. C_1 (Molecular Mass: 340, C_{20} H_{36} O_4) is (5,10- 8,9- 12,13)-seco- 4,4,10 – trimethyl, 7 – hydroxy, 14 – hydroxymethyl, 16 – keto, 13(15) – ene – cassane furanoditerpene and compound C_2 (Molecular Mass: 418, C_{24} H_{34} O_6) is (12,13)- seco- 12, 14 – epoxy, 12(16) -Oxo – 16(17), 13(15) – diene, 4, 10, 17 – trimethyl, 4, 7 – di – aceto cassanoate The Cassane - type diterpenoids have been reported for promising antimicrobial properties.

Key words: *Calliandra portoricensis*, antibacterial, antifungal, cassane-type diterpenoid derivatives.

Corresponding Author: eddyoguegbulu@yahoo.com or edwin.oguegbulu@uniport.edu.ng

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 a huge burden of 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 of the globe [4].

Currently, plants are still rated as the most economical and effective alternative source of medicines and 'lead' for novel drug discovery [5; 6]. Studies are therefore needed to validate scientifically, the safety, efficacy, quality and dosage of medicinal plants used [7].

The plant *Calliandra portoricensis*is is a shrub distributed in tropical regions of America, India, West Indies and West African Nigeria [8]. The phytochemical constituents include; saponin, flavonoids, cardiac glycosides, steroids, triterpenoids, reducing compounds and alkaloids [9].

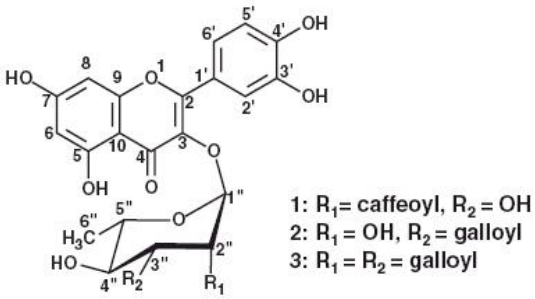
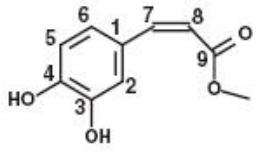
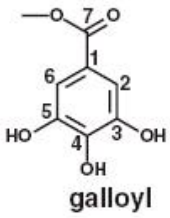
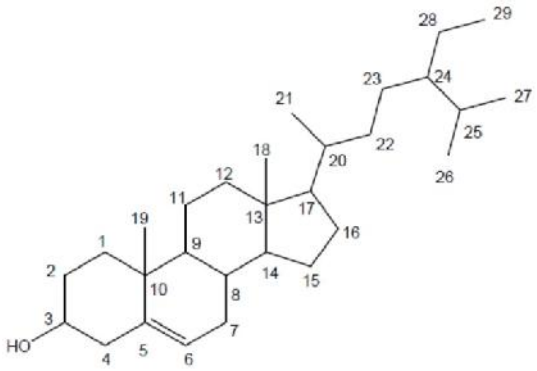
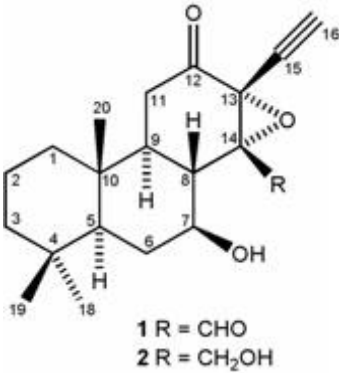
Figure 1: Photograph of *Calliandra portoricensis* showing, twigs, leaves and flowers.

Ethno botanically, the common names of *Calliandra portoricensis* include, “Sleeping plants” and “Corpse awakener,” Tude (Yoruba of South Western Nigeria); [10]; “Ekweanahi” and “Avuvuagu” or “Eriagbo” among the Igbos of South Eastern Nigeria. In these regions, the plant has been used extensively in traditional medicine for the treatment of various ailments such as; throat and tooth inflammations, swollen tonsils, mouth ulcers. These medical conditions are usually caused by bacteria and fungi. [11].

There were some previous scientific reports in the following domains; worm expeller, laxative, abortificient, antidote to viparean venon [12; 13] Antidiarrhoea, anticonvulsant and antipyretic properties. [14; 15; 16]. Also crude extracts of *C. portoricensis* exhibited antimicrobial activity. [17]. Antioxidant, antiangiogenic, and antiproliferative activities in human prostate cancer cells [18]. Antisickling properties [19]. Antioxidant and antihepatotoxic *[20]*

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

Structural formulary	Name of Isolated compound	Morphological part	References

	1.) Quercitrin 2′′-*O*-caffeate 2.) Quercitrin 3′′-*O*-gallate 3.) Quercitrin 2′′,3′′-di-*O* galate	Leaves and stem of *Calliandra haematocephala*	[21]; *)*
			
			
	**β-sitosterol**	Leaves of *C.haematocephal a.*	[22]
	**Cassane derivatives: Escobarine A (1) Escobarine B (2)**	*Root of Calliandra californica*	([23]

Aims and objective of the study:

To identify, isolate and characterize the antimicrobial compounds from the root of *C. portoricensis.*

Materials and Methods:

Plant Material:

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

The methodology adopted the techniques as described earlier [24], for; extraction by successive cold maceration using n-hexane, ethyl acetate and 70 % aq. Methanol for 72 hr respectively, Antimicrobial *in vitro* susceptibility evaluation and Minimum Inhibitory Concentration (MIC) determinations by agar well diffusion method, preparation of test micro organisms which were; *Staphylococcus aureus* (Gram +ve cocci*), Streptococcus fecalis* (Gram +ve cocci), *Escherichia coli* (Gram –ve rod), *Bacillus subtitlis* (Gram +ve rod), *Klebsiella pneumoniae* (Gram -ve rod*), Candida albican* (fungi), Aspergillus niger (fungi), **Also conducted was** column Fractionation of bioactive ethyl acetate extracts (Silica Gel G, 200-400 mesh-stationary phase) with gradient mixtures 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) used for the elution , The bioactive fraction (B) was subjected to further purification by preparative TLC (Silica Gel G, 0.5 mm thickness), The resultant bioactive pure compounds C_1 and C_2 were subjected to spectroscopic analysis (UV, MS, FT – IR and NMR).

RESULTS AND DISCUSSION:

Table 2: Result of antimicrobial susceptibility tests of TLC bands (C_1 and C_2) on selected human pathogens at concentration of 1 mg / ml:

	TLC Band [C_1]	**TLC Band [C_2]**

Microorganism	**C-$_1$**	**CTR**	**C-$_2$**	**CTR**
Staphylococcus aureus	*25.00±0.40	23.00±0.70	*31.00±0.12	24.00±0.70
Escherichia. Coli	*35.00±0.60	38.00±0.90	*20.00±0.60	25.00±0.70
Bacillus subtitis	*45.00±0.50	40.00±0.80	*22.00±0.50	35.00±0.40
Klebsiella pneumonae	*35.00±0.70	30.00±0.60	*18.00±0.40	30.00±0.80
Streptococcus. fecalis	*30.00±0.20	35.00±0.10	*20.00±0.80	25.00±0.40
Candida albicans	*33.00±0.90	35.00±.00	*20.00±0.90	25.00±0.70
Aspergillus n.	*25.00±0.50	14.00±0.80	*30.00±0.50	45.00±0.60

Values are Diameter Zone of Inhibition (mm) and expressed as mean ±SEM; n = 3; CTR. = Control - Ciproflaxacin (20 µg per milliliter for bacteria) and Fluconazole (1000 µg per milliliter for fungi); (-) = no inhibition; 10 % aqueous DMSO (negative control, no inhibition).

* Represent significant values with respect to the control of P ≦ 0.05

Table 3: Minimum Inhibitory Concentrations (MIC) values of TLC bands (C_1 and C_2) in µg per ml against the selected human pathogens:

S/N	**Micro organisms**	**TLC Bands.**	
		C_1	**C_2**
1.	*Staphylococcus. Aureus*	125.00±0.80	125.00±0.10
2.	*Escherichia. Coli*	25000±0.30	125.00±0.70
3.	*Bacillus. Subtitis*	125.00±0.20	250.00±0.30
4.	*Klebsiella pneumonae*	250.00±0.60	125.00±0.80
5.	*Streptococcus fecalis*	12500.±0.50	125.00±0.10

6.	*Candida albicans*	125.00±0.70	125.00±0.40
7.	*Aspergillus niger*	125.00±0.20.5	125.00±0.60

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

Table 4: Interpretation of ^{1}H and ^{13}C NMR Spectral data (Deuterated Chloroform _$CDCl_3$ as solvent) for compounds C_1 and C_2.

Assigned position/ identity of atoms	(δ) -^{13}C (ppm)		δ_H (ppm)		^{1}H-H COSY		HMBC ^{2,3,4} JAC		DEPT-135	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
C-1	22.0	29.85	-0.88	1.2-1.3					CH_2	CH_2
C-2	18.2	29.51	0.9,1.2	1.2-1.3					CH_2	CH_2
C-3	22.8	33.97	1.32	1.2-1.3					CH_2	CH_2
C-4	45.8	54.66		-						-
C-5	29.8	22.85	1.33	0.9					CH_2	CH_2
C-6	21.1	33.39	1.3	1.2-1.3					CH_2	CH_2
C-7	21.7	44.49	0.9	3.7		H_8			CH_2	CH
C-8	25.0	41.85	1.68	3.3		H_7			CH	CH
C-9	23.0	22.24	1.45	0.8					CH	CH_2
C-10	38.0	52.96	2.4	-					CH	-
C-11	30.2	32.08	1.4,1.75	1.2	H12				CH_2	CH_2
C-12	65.7	95.21	4.3	5.98	H11				CH_2OR	CH
C-13	127.9	111	7.6	5.40	H15				=CH	CH
C-14	28.7	82	2.04	3.60				C_{21}	CH_2	CH
C-15	129.9	109	7.4	6.05	H13				=CH	CH
C-16	170 ca	140	-	-					RO-C=O	-
C-17	13.1	95.09	0.99	6.04					CH_3	CH
C-18	11	14.23	0.95	0.95					CH_3	CH_3
C-19	14	14.53	0.9	0.75			C2,		CH_3	CH_3

						C4		
C-20	67.2	170	4.23	-			CH_2OH	R-O-C= O
C-21		164		-				R-O-C= O
C-22		18.81		1.0				CH_2
C-23		56.71		3.80		C_{20}		O-CH_3
C-24		55.89		3.70		C_{21}		O-CH_3

Both compounds exhibited DZI of 33.00±0.90 and 25.00±0.50 respectively against Candida *albicans* and *Aspergillus niger* as well as MIC values of 125.00±0.70 µg per ml for each. These activities were found to be quite significant with respect to the reference controls (Ciprofloxacin and fluconazole) at $P \leq 0.05$.

The susceptibility tests result shown in (Table 1) was found to be consistent with the report which suggested that Diameter Zone of Inhibition 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 potent [25].. Further, the result shown on (Table 2), on MIC values was in line with the 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, whereas natural products with MIC values below 1 mg /ml should be considered as noteworthy [26].

.Compound C_1 had a R_f value of 0.91 (Silica Gel, 0.25 mm, n-hexane: ethyl acetate: methanol – 12: 4: 1) and fluoresced light green under UV lamp at 365. The C_1 was a semi-solid oily and dark brown compound. Ultraviolet (UV) spectrum exhibited absorption maximum at 280 nm. This was consistent with values reported on cassane - type tricyclic diterpenoids [27], and supported by the presence of conjugated chromospheric group on ring C of compound C_1. The structure of this compound was elucidated by using FT - IR, NMR (1- D and 2 – D experiments) and MS spectroscopy. The IR bands were in the region; 3402.54 cm^{-1} and 2959.47 cm^{-1}, representing the -0H stretching and –CH vibrations respectively. Also evident was the carbonyl stretch at 1727.30

cm^{-1} , α and β, unsaturation at 1415.52 cm^{-1} and the structure of the pure compound was established by ^{1}H – NMR, ^{13}C – NMR, ^{1}H – ^{1}H COSY, DEPT – 135, HSQC, and HMBC.(Table 4).

The proton (^{1}H) – NMR contained five peaks of deshielded protons at δ_H (ppm); 7.6, 7.4, 4.26, 4.25 and 2.04

The cosy spectrum revealed the correlation of the proton peaks and exhibited cross – peak correlations as in; proton H -12 with H – 11 and H – 13 with H – 15.

Three methyl protons singlets were evident at δ_H: (ppm); H – 17 – Me (0.99); H – 18 – Me (0.95) and H – 19 – Me (0.90). Evident too were ten methylene (CH_2) protons corresponding to H – 1, H – 2, H – 3, H – 5, H – 6, H – 8, H – 9, H – 11, H – 12 and H – 20 respectively. Also present were five methine (CH) protons at; H – 7, H – 10, H – 13, H – 14 and H – 15 respective. The olefinic (Sp^2) = CH protons were evident at H – 13 and H – 15. The methoxy (–OCH_2 – protons at H – 12 were evident too. The clear designations and identity of atoms were achieved by the use of 2 – Dimensional proton to carbon correlation (HMBC and HSQC). The other proton chemical shift peaks were equally rationalized on the same Table 4.

A total of twenty spectral peaks were identified in ^{13}C – NMR experiments of compound C_1 These were rationalized by the aid of DEPT -135. Three methyl groups at δc (ppm); 13.10, 11.00 and 14.00 corresponding to C17 – Me, C18 – Me and C19 – Me respectively. Olefinic group (C = C) at δc (ppm): 127.90 (C – 13 and 129.90 (C – 15) respectively. Ten methylene (-CH_2) groups at δc (ppm); 22.00 (C-1), 18.20 (C – 2) 22.80 (C – 3), 29.80 (C -5), 21.10 (C -6), 25.00 (C -8), 23.00 (C-9) 30.20 (C-11) 65.70 (C-12) and 67.20 (C-20$^{'}$). Two oxymethylene groups (-$CH_2$0) at; 65.70 (C-12) and 170.00 (C – 16). Present also were five methine groups (-CH-) at; 21.70 (C – 7), 38.00 (C – 10), 127.90 (C – 13), 28.70 (C – 14) and 129.90 (C – 15.). Evident also were two quaternary groups at; 45.80 (C – 4) and 170.00 (C – 16) respectively.

Other correlations were evident in HMBC as rationalized in Table 4.

The number assignment of hydrogen, carbon and oxygen was further supported by the MS The mass and NMR (1D AND 2D) spectral data suggested the presence of cassane – type tricyclic diterpenoid. This skeleton is usually linked to certain sub group in *Fabaceae* family [28]. Again, a peak was shown at m/z 341 and corresponded to [M + 1] equivalent to molecular mass of 340 ($C_{20}H_{36}0_4$).

Compound C_1 is therefore a Cassane – type tricyclic diterpenoid derivative with (IUPAC) name as; (5, 10- 8, 9- 12, 13)-seco- 4,4,10 – trimethyl, 7 – hydroxy, 14 – hydroxymethyl, 16 – keto, 13(15) – ene – cassane furanoditerpene. (Figure 2).

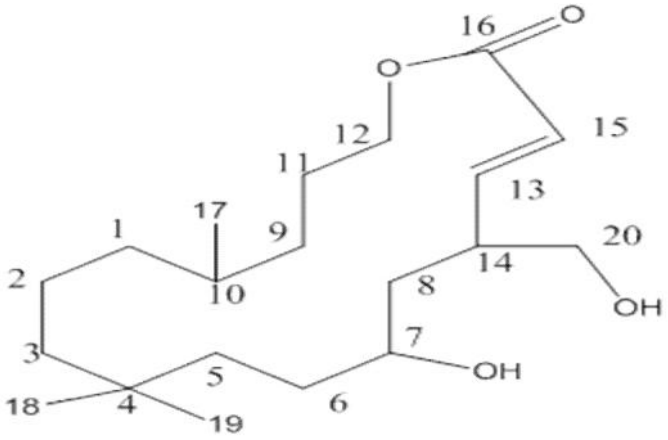


Figure 2: The structure of compound C_1. (5, 10- 8, 9- 12, 13)-seco- 4,4,10 – trimethyl, 7 – hydroxy, 14 – hydroxymethyl, 16 – keto, 13(15) – ene – cassane furanoditerpene.

Compound C_2 had a R_f value of 0.46 on analytical TLC plate (Silica Gel, 0.25 mm, n-hexane: ethyl acetate: methanol – 12: 4: 1) and fluoresced deep purple under a UV lamp at 365 nm it was also a semi-solid and oily compound with molecular formula of $C_{24}H_{34}O_6$ as could be deduced from the MS data by a peak showing at m/z 419 corresponding to [M+1] equivalent to its molecular mass of 418.

FT–IR of C_2 showed a broad stretching at 3375.40 cm^{-1} of -0H groups and C= 0 stretching in the band region of 1710.33 $cm^{-1.}$ Presence of –CH, CH_2, CH_3 vibrational frequencies were also evident.

The ^{1}H-NMR for compound C_2 exhibited signals of deshielded protons with chemical shifts at δ_H (ppm) 6.05, 6.04, 5.98, 5.40, 3.80, 3,70, 3.60 and 3.30, corresponding to; H-15, H-17, H-12 , H-13, H-23, H-7/24, H-14 and H-8 respectively. Five quaternary carbons with no protons at; C-4, C-10, C- 16, C – 20, and C – 21, were evident. Also evident were the three methyl protons singlets at chemical shifts δ_H (ppm); 0.95, 0.75 and 1.0, corresponding to; C-10-Me, C-4-Me, and C-17-Me respectively. Olefinic (Sp^2) protons at δ_H (ppm); H-15 (6.05) and H-17 (6.04) (ppm); Present too were the oxymethine protons at δ_H (ppm); H-12 (5.98) and H-14 (3.60) respectively.

The structural configuration of this compound C_2 was further supported by a total of twenty-four carbon signals in ^{13}C - NMR spectroscopy these resonances were rationalized on the basis of DEPT- 135. (Table 4). Five quaternary carbons at δc (ppm); C-4 (54.66), C-10 (52.96), and C – 16 (140.0), C-20 (170.0) and C – 21 (164.0).Three methyl group carbons were evident at δc (ppm): Me - C-18 (14.23), Me - C-19 (14.53), and Me - C-22 (18.81). Five methylene groups (-CH_2 -) at δc (ppm) C-1 (29.85), C-2 (29.51), C-3 (33.97), C-6 (33.39) and C-11 (32.08). Present too were the two oxymethine groups (>CHO) at δc (ppm): C-12 (95.21) and C-14 (82.00). Two olefinic methine groups were evident at δc (ppm) C-15 (109.00) and C-17 (95.09).

The ^1H-H COSY indicated that proton H – 8 (δ_H = 3.30 ppm) exhibited cross – peak correlation with H -7(δ_H = 3.70 ppm). At the same time, the following correlations were observed with HMBC spectrum proton H -14 (δ_H = 3.60 ppm) with C - 21(δ_C = 164.00 ppm), H – 23 (δ_H = 3.80 ppm) with C – 20 (δ_C = 170.00 ppm) and H – 24 (δ_H = 3.70 ppm) with C – 21 (δ_C = 164.00 ppm).

The absorption maximum of UV- VIS experiment was at 270 nm this is consistent with earlier reports on Cassane skeleton.

This novel compound C_2 isolated from the root of *Calliandra portoricensis* was identified as (12,13) – seco - 12, 14 – epoxy, 12(16) -Oxo – 16(17), 13(15) – diene, 4, 10, 17 – trimethyl, 4, 7 – di – aceto cassanoate. (Figure 3).

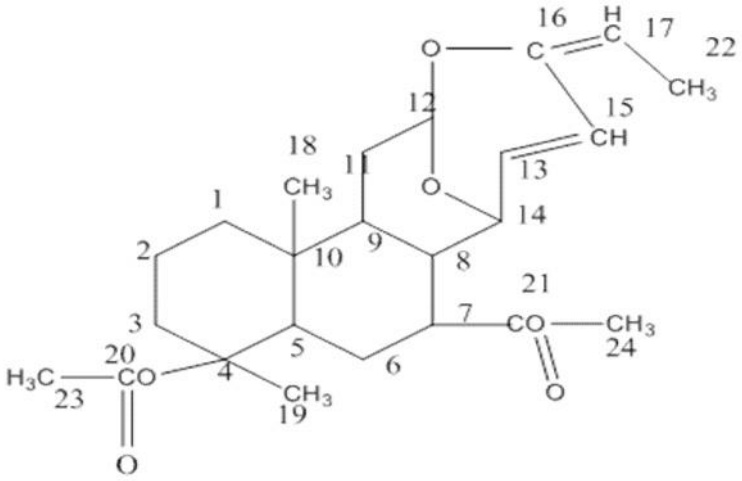


Figure 3: The structure of compound C_2: (12,13)- seco- 12, 14 – epoxy, 12(16) -Oxo – 16(17), 13(15) – diene, 4, 10, 17 – trimethyl, 4, 7 – di – aceto cassanoate.

Both compounds C_1 and C_2 being Cassane – type diterpenoids derivatives could be linked to earlier reported activities exhibited by similar moieties against test bacteria, viruses and *candida albican* – fung*us [*23; 29; 30]

Conclusion:

This study had successfully isolated, identified and characterized two novels Cassane - type tricyclic diterpenoid derivatives. Compound C_1 as; (5, 10- 8, 9- 12, 13)-seco- 4,4,10 – trimethyl, 7 – hydroxy, 14 – hydroxymethyl, 16 – keto, 13(15) – ene – cassane furanoditerpene. (Molecular mass: 340 and molecular formularies: C_{20} H_{36} O_4). and Compound C_2 as; (12, 13)- seco- 12, 14 – epoxy, 12(16) -Oxo – 16(17), 13(15) – diene, 4, 10, 17 – trimethyl, 4, 7 – di – aceto cassanoate (Molecular mass: 418 and molecular formula: C_{24} H_{34} O_6). These Cassane - type diterpenoids have shown promising activities against bacteria and fungi (*Candida albican*).

Recommendations:

The two compounds C_1 and C_2 should be further investigated for toxicity and Structure Activity Relationships (SAR) for drug development, possibly formulated into lozenges for treatment of the throat, oral thrush and tooth infections. Their tinctures could be prepared for nail-fungal and other mixed dermatological infections, douches and pessaries for vaginal candidiasis.

Acknowledgement

Staff members and laboratory facilities of Pharmacognosy & Phytotherapy and Pharmaceutical & Medicinal Chemistry Departments both of the Faculty of Pharmaceutical Sciences, University of Port Harcourt. Rivers State, Nigeria. Also, the Central Laboratory of Kwame Nkuruma University of Science and Technology, Kumasi Ghana for hosting me and undertaking the laboratory high-Tech spectroscopic analysis of all my samples.

Conflict Of Interest:

There was no conflict of interest involved in this research work.

References

1.Phillipson, J.D., 2001. Phytochemistry and medicinal plants.*Phyto chemistry* 56, 237 – 243.

2.Yoneyama, I.J, Katsumala, R. 2006, Antibiotic Resistance in bacteria and its future for novel antibiotic development. Bio Science, *Biotechnology and Biochemistry. 70(5); 1060–1075.*

3.WHO (1985). Bulletin of the World Health Organization 63(6); 965 – 981.

4.Farnsworth, N.R., 1994. The Role of Medicinal Plants in drug development. In; Krogsgaard-Larsen, S., Brogger – Christensen, S., Kofod, H. (Eds). Natural Products and Drug Development Munksgaard, Copenhagen.

5.Vander Watt, E., Pretorius, J.C; 2001 – Purification and identification of active components of *Carpobiotusedulisl*L. *Journal of Ethno Pharmacology*, 7687-91.

6.Cos, P; Vlietinck, A.J., Berghe, D.V., (2006) Anti-infective potential of Natural Products: How to develop a stronger *in vitro* 'Proof – of concept'. *Journal of Ethnopharmacology 106, 290 – 302.*

7. Maska, P.J., Afolayan, A.J. 2002. Antimicrobial activity of some plants used for the treatment of livestock disease in the *Eastern cape South Africa. Journal of Ethno*pharmacology 83, 129 – 134.

8.Burkill, H.M. (1985). The useful plants of West Tropical Africa. Families, Royal Botanic Gardens. Kew. Great Britain vol. 3. 177 – 266.

9.Oguegbulu1, E.N, Abo, A.K, Afieroho,O.E (2020). Comparative Evaluation of the Antimicrobial Activities of some plants used in Natural Medicine –Spondiasmombin, Calliaidra portoricensiss, Dennettia tripetala, Anthocleista djalonensis and Cronton zambesicus. *Saudi Journal of Pathology and Microbiology*. 5(5): 257-262.

10.Fatokun, O.T, Wojuola, T.E, Esievo, K.B. Kunle, O.F. (2006). Medicinal Plants used in the Management of Asthma; A Review. *European Journal of Pharmaceutical and Medicinal Research* 3(7):82-92.

11.Holist, N.O. (2004).A collection of Traditional Yoruba oral and Dental Medicaments. Book Builders. Ibadan, Nigeria 123 pp.

12.Onyeama, H.P., Ebong, P.E. Eteng, M.U., Igile, G.O., Ibekwe, H.A.; Ofemile, P.Y. (2012). Histopathological responses of the Heart, Lines and kidney to *Calliandria portoricensis* Extracts in Wisbor Rats challenged with venom of Echisocellatus. *Journal of App. Pharma.Sciences* 02 (06): 164 – 171.

13.Ayensu, E.S. (1972). Medicinal plants of West Africa References, publications, Inc. Algonac Michigan USA.

14.Aguwa, C.N., Lawal, A.M. (1988). *Pharmacological studies on the active principle of Calliandra portoricensis* leaf extracts *Journal of Ethnopharmacology*. 22:63 – 71.

15.Akah, A.P., Nwiwu, I.J. (1988). Anti convulsant activity of the root and stem of *Calliandriaportoricesis*. *Journal of Ethnopharmcology*. 22, 205 – 210.

16.Orishadipe, A.T., Okogun, J.I., Mishelia, L. (2010). Gas chromatography – mass spectrometry analysis of the hexane extract of *Calliandria portoricensis* and its antimicrobial activity. *African Journal of Pure and Applied Chemistry*. Vol. 4 (7): 131 – 134.

17.Oguegbulu1,E.N, Abo, A.K, Afieroho,O.E (2020). Comparative Evaluation of the Antimicrobial Activities of some plants used in Natural Medicine –Spondiasmombin, Calliaidra portoricensiss, Dennettia tripetala, Anthocleista djalonensis and Cronton zambesicus. *Saudi Journal of Pathology and Microbiology*.. 5(5): 257-262.

18Adaramoye, O, Erguen, B, Oyebode, O, Nitzsche, B, Hopfner, M, Juna, K., Rabien, A. (2015). Antioxidant, antiangiogenic and antiproliferation activities of root methanol extract of *Calliandra portoricensis* in human prostrate cancer cells. *Journal of Integrative Medicine*. 18(3): 185-93.

19.Amujoyegbe, O.O, Agbedahunsi, J.M, Akanmu, M.A. (2014). Antisickling properties of two *Calliandria* species *C. portoricensis* and *C. haematocephala* (*Fabaceae). European Journal of Medicinal Plants*, 4(2); 206 – 219.

20.Amr, M., Abo-Elhamd, Ahmed, M. Aboul-Enein, Samy, M, Mohamed Ahmed, S., Shalaby, Usama. Konsowa, U, Hassan E.M., Metwally, N.S.(2016). Chemical Characterization, antioxidant and anti-hepatotoxic activities of *Calliandrahaematocephala* (Hassk), vgrowing in Egypt. *Journal of Chemical and Pharmaceutical Research* 8(4): 828-845.

21.Moharram, FA. Marzouk, MS. Ibrahim, MT., Mabry, TJ. (2006). Antioxidant galloylated flavonol glycosides from *Calliandrahaematocephela. Journal of Natural Product Research*. 20(16):927-34.

22.El-Sayed, ME. (2014). Phytoconstituents from *Calliandrahaematocephala* and their biological activities. *Journal of Pharmaceutical Sciences*. 49: 259-268.

23.Encarnacion-Dimayuga, R, Agundez-Espinoza, J, Gorcia, A, Delgado, G, Molina-Salinas, G. (2006). Two New Cassane-Type Diterpenes from *Calliandra californica. Letter. Planta Medica. 72*: 757 -761.

24.Oguegbulu1,E.N, Abo, A.K.(2020). Chromatographic Isolation of Antimicrobial Compounds ofCalliandraportoricensis(Jacq)-Benth (Fabaceae) Root. *Middle-East Journal of Scientific Research* 28 (3): 225-234.

25.Anyanwu, M.U., Okoye, R.C. (2017). Antimicrobial activity of Nigerian Medicinal Plants. *Journal* of Intercultural Ethnopharmocology. 6(2); 240 – 259.

26.Bios, J.L., Recio, M.C. (2005). Medicinal Plants and Antimicrobial. *Journal of Ethnopharmacology*. 100; 80 – 84.

27.Berania, B.C, Djalma, M.O, Ezequias, P.S, Elaine, M.S, Adriano, M.C, Santos, D.M, Rabelo, A, Zani, C.L. (2011). New cassane diterpenes from Caesalpina echinate. Fitoterapia. 82(7):969 -975.

28.Barba, B, Dias, J.G, Goedken, V.L, Herz, W, Dominguez, X.A, (1992). Unusual Cassanes from Chamaecrista species. Tetrahedron.48; 4725 – 4732.

29.Jiang, R, Ma, S.C, But, P.P.H. (2001). Antiviral cassane furanditerpenes from Caesalpinia minax, Journal of Natural Products. 64(10): 200 – 217

30.Favre – Godal, G, Dorsaz, S, Queiraz, E.F, Marcourt, L, Ebrahim, S.N, Allard, P, Voinesco, F, Hamburger, M, Gupta, M.P, Gindro, K, Sanglad, D, Wolfender, T. (2015). Anti – Candida Cassane – Type Diterpenoids from the Root Bark of Swartzia simplex. Journal of Natural Products. 78(12): 2994 – 3604.

Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.info **ISSN 2458-5920**