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GC-MS characterization, antimicrobial and antioxidant effects of the leaf essential oil of *Calliandra portoricensis* (Jacq.) Benth

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Abstract
Calliandra portoricensis (*C. portoricensis*) is a reputable traditional herbal medicine in Nigeria with long history of use. The parts often use in traditional medicine are leaves and roots. The aim of the present study was to investigate *C. portoricensis* leaf essential oil (EO) as well as the antioxidant and antimicrobial effects. The EO was obtained by using hydrodistillation technique. Chemical constituents of EO were determined by gas chromatography coupled to mass spectrometry (GC-MS). The antioxidant activity of the EO was evaluated by measuring its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical. The antimicrobial activity of *Calliandra portoricensis* essential oil was assessed employing microdilution method. A total of 39 chemical constituents were identified. The main components identified were monoterpenes (48.05 %) and sesquiterpenes (46.88%). The essential oil showed significant antimicrobial and antioxidant effects.

Keywords: *Calliandra portoricensis*, essential oil, antimicrobial, antioxidant

1. Introduction
Calliandra is a genus of perennial flowering plants in the family Fabaceae and sub family, Mimosoideae. A total of 142 species of the genus has been reported (Renvoize, 1981; Souza *et al*., 2012) [36, 2]. *Calliandra portoricensis* (Jacq.) Benth is native to the tropic and subtropical regions of America such as Mexico and Panama (National Research Council, 1983) [25], many species can also be found in Indonesia (National Research Council, 1983) [25]; India, Madagascar (Renvoize, 1981) [36]; Australia (Palmer *et al*., 1994) [33]; and other tropical areas in Africa such as Nigeria, where they are also cultivated. Nigeria is home to *Calliandra portoricensis* and *Calliandra haematocephala* (Orishadipe *et al*., 2010) [32]. The common name of *Calliandra portoricensis* is corpse awakener. It is called Tude in Yoruba language. The parts often use in traditional medicine are leaves and roots. The plant is used in Nigeria folk medicine as a laxative, worm expeller and as an abortifacient. *Calliandra portoricensis* was reported to have anticonvulsant property (Akah and Nwaiwu, 1988) [5].

This genus is made up of species which range in height, from small shrubs to trees. The flower colours vary between species, but are well known for being small and overshadowed by the numerous and conspicuous stamens (Renvoize, 1981) [36]. The fruits are all straight or slightly curved and compressed pods with thickened margins. The leaves are bi-pinnate with pairs of opposite and sessile leaflets. Many species of this genus are used for firewood, pulp and paper making, and can help in promoting reforestation of exploited forests and farmlands due to their nitrogen fixing ability. Some species also support bee and honey farming, since they can flower all year round (Macqueen, 1996; National Research Council, 1983) [25, 22].

Calliandra portoricensis is a woody shrub that grows to about 6 m in height. The leaves are small, bi-pinnate in structure, while the flowers are pinkish. The fruits are in the form of pods of about 10 cm in length (Renvoize, 1981) [36]. In Nigerian folk medicine, it has been used as anticonvulsant, antipyretic, analgesic, anthelmintic, and treatment for snake bites (Onyeama *et al*., 2012) [29-31]. For example, it was soaked in water for three days with *Olax subscorpioidea*, *Chasmanthera dependens*, *Mimosa pigra*, *Securidaca longipedunculata*, *Crinum jagus*, *Allium ascalonicum* and *Tetrapleura tetraptera* for the treatment of asthma. Numerous pharmacological activities have been reported, including anti asthmatic (Orishadipe *et al*., 2010; Sonibare and Gbile, 2008) [32, 40], antisickling (Amujoyegbe *et al*., 2014) [32], anticonvulsant (Akah and Nwaiwu, 1988; Akah and Nwambie, 1993; El-ghani, 2016) [5, 6, 17], antimicrobial (Orishadipe *et al*., 2010) [32], antiangiogenic (Adaramoye *et al*., 2015; Ogugu *et al*., 2012) [2, 26], antiproliferative (Adaramoye *et al*., 2015; Ogugu *et al*., 2012) [2, 26]; anthelmintic (Aguwa and Lawal, 1988) [4]; hypolipidaemic (Onyeama *et al*., 2012) [29-31]; erythropoietic (Onyeama *et al*., 2012) [29-31] and analgesic (Agunu *et al*., 2005) [3].

Calliandra portoricensis leaf extracts are said to possess anti-diarrhoeal, antispasmodic, antipyretic, anti-rheumatic and analgesic properties (Aguwa and Lawal, 1988) [4]. *Calliandra portoricensis* roots and leaves have high analgesic activity (Agunu *et al*., 2005) [3]. *Calliandra portoricensis* has been

reported to possess antioxidant and antibacterial properties (Moharram *et al*., 2006) [24].

Previous phytochemical studies revealed the presence of phenols and polyphenols like quercetin, tannins, saponins, glycosides, cardiac glycosides, steroids, alkaloids, fatty acids, esters and methyl esters, gallic acid, caffeic acid and betulinic acid. A GC-MS analysis of the hexane extract revealed methyl 14-methylpentadecanoate as a major component. Other fatty acids and fatty acid methyl esters detected were hexadecanoic acid, methylhexadecanoate and 9-oxomethyl nonanoate. The extract was found to be active against *S. aureus, E. coli* and *S. gallinallum* (Onyeama *et al*., 2012; Orishadipe *et al*., 2010) [29, 30, 31, 32].

Essential oils are made up of numerous volatile compounds and can be isolated by distillation techniques, from the leaves, flowers, roots, stems or whole plant. Essential oils have numerous applications especially in the food, cosmetic and fragrance industries due to the possession of characteristic and unique aroma, antimicrobial activities and moisturising properties (Bakkali *et al*., 2008; Buchbauer, 2010; Carrasco *et al*., 2016; Heinrich *et al*., 2012; National Research Council, 1983; Onyeama *et al*., 2012) [9, 10, 12, 18, 25, 29, 30, 31].

To our knowledge, no chemical investigation of the leaf essential oil of *C. portoricensis* has been reported. This study aimed at determining the chemical constituents and evaluating the antioxidant and antimicrobial activity of the leaf essential oil of *Calliandra portoricensis* grown in Northern Nigeria.

2. Materials and Methods

2.1 Plant materials

Leaves of *Calliandra portoricensis* were collected in August, at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The plant was identified by a taxonomist at the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

2.2 Essential oil isolation

The air dried leaves of *Calliandra portoricensis* (500 g) were chopped into small pieces and hydrodistilled using a Clavenger type apparatus for 4 h. *Calliandra portoricensis* leaf yielded 1.2 ml (0.24% v/w) light yellow essential oil. The light yellow essential oil obtained was dried over anhydrous sodium sulphate. The oil was filtered through 0.22 microns filter paper and stored at 4 ^{o}C in sealed vials until analysis.

2.3 Gas Chromatography-Mass Spectral Analysis

The oils were analysed by GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and Shimadzu GCMSsolution data system. The GC column was HP-5MS fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, length 30 m, internal diameter 0.25 mm and film thickness 0.25 µm. The carrier gas was helium with flow rate of 1.61 ml/min. The program used for GC oven temperature was isothermal at 60 ^{o}C, followed by 60-180 ^{o}C at a rate of 10 ^{o}C/min, then held at 180 ^{o}C for 2 minutes; followed by 180-280 ^{o}C at a rate of 15 ^{o}C/min, then again held at 280 ^{o}C for 4 minutes. The injection port temperature was 250 ^{o}C. The ionization of sample components was performed in the E.I. mode (70eV). Injector temperature was 250 ^{o}C while detector temperature was 280 ^{o}C. Helium was used as carrier gas at a flow rate of 1.61 ml/min. 1.0 µl of diluted sample (1/100 in hexane, v/v) was injected using autosampler and in the split mode. Split ratio was 10:90 (Okhale *et al*., 2016) [28].

2.4 Antioxidant activity

The antioxidant potential activity of essential oil of *Calliandra portoricensis*was evaluated by spectrophotometric method using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging activity assay. Fifty microlitres of various concentrations of the samples (1, 5, 10, 15, 20, 25, 30, 40, 50, 80 and 100 µg/mL) were added to 5 mL of a 0.004% ethanol solution of DPPH. Tests were carried out with essential oil and reference antioxidant, the synthetic butylated hydroxytoluene (BHT) in concentrations ranging from 1 to 100 µg/mL. The DPPH test is based on the ability of the extracts to donate radical hydrogen to neutralize the stable DPPH radical. When this radical reacts with the antioxidant compound, it is reduced with colour of solution changing from deep violet to light-yellow. The absorbance was measured at 517 nm on a visible light spectrophotometer. The percentage of DPPH radical scavenging capacity was calculated as follows:

DPPH radical scavenging capacity (%) = $[(Abs_{blank} - Abs_{essential\ oil})/Abs_{blank}]x100$.

Where Abs_{blank} is the absorbance of the blank sample (time = 30 min) and $Abs_{essential\ oil}$ is the absorbance of the essential oil sample (time = 30 min).

2.5 Antimicrobial Activity

The minimum inhibitory concentration (MIC) values of the essential oil of *Calliandra portoricensis* leaf were determined by microdilution broth method in 96-well microplates (Klacnik *et al*., 2010). The oil sample was dissolved in dimethyl sulfoxide (DMSO) followed by addition of sterile Mueller-Hinton nutrient broth for bacteria and Sabouraud-Dextrose nutrient broth for fungi, to achieve concentration of 200 µg/ml. The final DMSO concentration was 20% (v/v) and this solution was used as a negative control. The inoculum was adjusted for each organism to yield a cell concentration of 2×10^7 colony forming units (cfu) per ml.

Ciprofloxacin (Fidson, Nigeria) was used as a positive control for bacteria and Fluconazole (Pfizer, UK) was used as the standard drug for fungi at stock concentration of 50 µg/ml. Controls of sterility for the Mueller-Hinton nutrient broth, control culture (inoculum), Ciprofloxacin,

Fluconazole, essential oil and DMSO were performed. The microwell plates were closed and incubated aerobically at 37°C for 24 h. Post incubation, to indicate respiratory activity, to the plates were added 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium (MTT), incubated for another 2 h to indicate colour change in wells where there is no activity. MICs were defined as lowest concentration of essential oil at which the red formazan of MTT was not observed. All assays were carried out in triplicate (Okhale *et al*., 2016) [28]. Results are shown in Table 2.

3. Results

GC–MS chromatogram of *Calliandra portoricensis* is as shown in Figure 1. The compounds that were successfully identified and characterized sequel to the GC-MS analysis are as shown in the table 1. The major constituents found were the mono and sesquiterpenes with percentage composition being 52.69 % and 43.30 % respectively. The other constituents were diterpenes, hydrocarbons and amides.

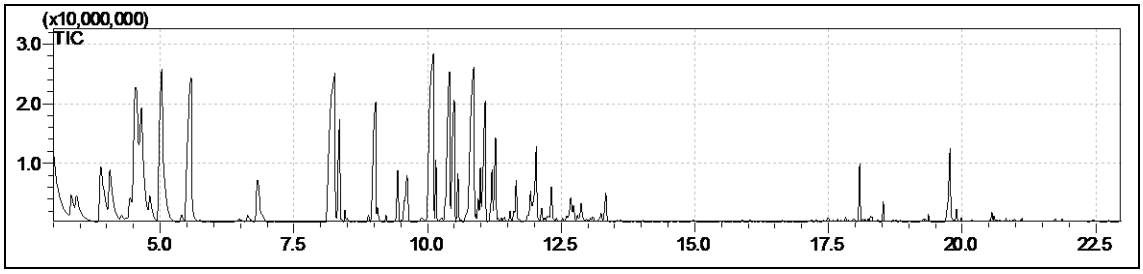

Fig 1: GC–MS chromatogram of *Calliandra portoricensis* leaf essential oil analyzed on GC–MS (Shimadzu, Japan) using a capillary column (HP 5-MS) attached with mass detector. The chromatogram showed the presence of chemical components found in the essential oil.

Table 1: Constituents of the leaf essential oil of *Calliandra portoricensis*

No.	Retention time (min)	Compound	Area (%)
1	3.336	α-Thujene	0.79
2	3.431	α-Pinene	1.09
3	3.889	β-Phellandrene	3.00
4	4.058	β-Myrcene	2.68
5	4.541	o-Cymene	7.51
6	5.031	γ-terpinene	7.90
7	5.576	Linalool	7.07
8	6.821	α-Terpineol	1.88
9	8.263	Thymol	9.64
10	8.354	Carvacrol	2.26
11	9.028	Thymol acetate	4.23
12	9.442	α-Copaene	1.14
13	9.615	α-Cadinene	1.96
14	10.113	β-Caryophyllene	9.15
15	10.154	β-Cubebene	0.89
16	10.413	β-Farnesene	5.34
17	10.498	α-Caryophyllene	3.71
18	10.568	Alloaromadendrene	0.81
19	10.861	β-Cubebene	7.48
20	11.078	β-Bisabolene	4.60
21	11.275	δ-Cadinene	3.07
22	11.275	1,4-Cadinadiene	1.57
23	11.661	Cis-Nerolidol	1.05
24	11.928	Germacrene D-4-ol	1.09
25	12.034	Caryophyllene oxide	2.00
26	12.138	Guaiol	0.31
27	12.684	(+)-Epi-Bicyclosesquiphellandrene	1.24
28	12.876	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalene-2-ol	0.72
29	13.336	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5,3,1]undec-1-ene	0.75
30	18.085	Phytol	0.91
31	18.527	Octadecanamide	0.28
32	19.773	9-Octadecanamide (z)	0.82
33	19.897	Octadecanamide	0.25
34	19.285	Nonacosane	0.24
35	20.559	Tetracosane	0.29
36	21.121	2-Methyihexacosane Total	0.09 97.81

Classification of the essential oil constituents: monoterpenes (48.05 %); sesquiterpenes (46.88 %); diterpene alcohol (0.91 %); amides (1.35 %); aliphatic hydrocarbons (0.62 %). Total: 97.81%

3.1 Antioxidant activity

Table 2: Antioxidant activity of essential oil of *Calliandra portoricensis* and BHT

Concentration (µg/ml)		1	5	10	15	20	25	30	40	50	80	100
% Radical scavenging activity	Essential oil	4	9	15	20	25	28	32	38	43	55	75
	BHT	6	15	25	35	40	45	50	60	75	86	95

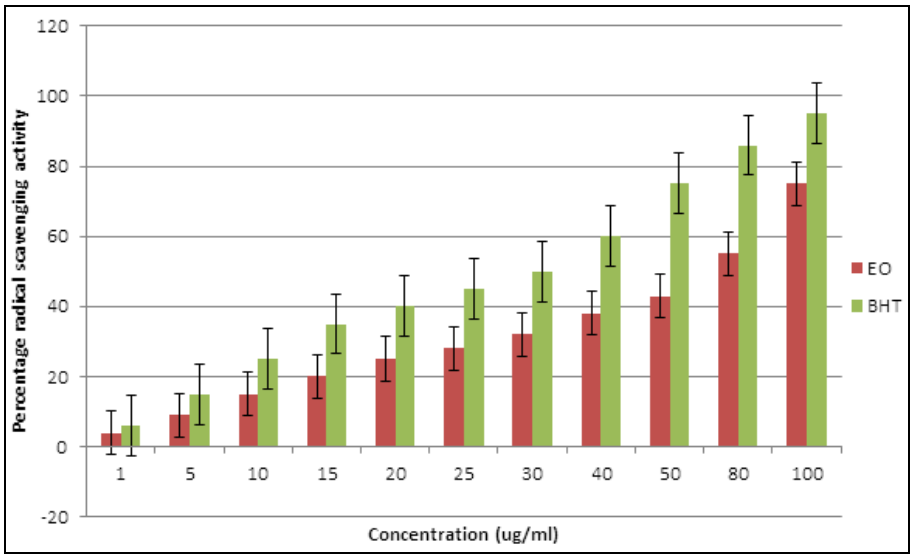

Fig 2: A graphical representation of the percentage radical scavenging activity of the leaf essential oil of *Calliandra portoricensis* (EO) and BHT

Table 3: MIC of leaf essential oil of *Calliandra portoricensis*

S/N	Microorganisms	Minimum inhibitory concentrations(µg/ml)	
		Essential oil	Standard drug
1*	*Pseudomonas aeruginosa*(ATCC 27853)	50	0.049
2*	*Klebsiella pneumonia* (ATCC 13883)	100	0.391
3*	*Escherichia coli* (ATCC 10798)	50	0.391
4*	*Staphylococcus aureus*(ATCC 25923)	100	0.098
5**	*Candida albicans* (ATCC 2876)	50	6.25

*Bacterial strain; **fungal strain; Ciprofloxacin (standard drug for bacterial strains); Fluconazole (standard drug for fungi strain)

4. Discussion

The phytoconstituents of *Calliandra portoricensis* leaf essential oil fall into seven main categories namely: monoterpenes, sesquiterpenes, diterpenes, aliphatic hydrocarbons and amides. The monoterpenes and sesquiterpenes make up a major part of the oil, monoterpenes (48.05 %), sesquiterpenes (46.88 %). These most likely contribute to the ethnomedicinal use of *Calliandra portoricensis* as an antimicrobial (Zeraib *et al*., 2014) [44]. The results obtained from this study corroborate other claims associated with *Calliandra portoricensis* (Adaramoye *et al*., 2015; Agunu *et al*., 2005; Aguwa and Lawal, 1988; Moharram *et al*., 2006; Ogugu *et al*., 2012; Orishadipe *et al*., 2010) [2, 3, 4, 24, 26, 32]. In fact one could posit that due to the superior occurrence of some of the oil's constituents the major ethno-medicinal indications could thus be further corroborated. For instance thymol (9.64%) takes up the greatest percentage composition of the oil and hence is most likely the reason behind its antimicrobial activity (Santurio *et al*., 2014) [39] which as portrayed in table 3 is quite significant although sub-par to the efficacy of the standard drugs. This deduction is based on earlier reports that classify the antimicrobial activities of plant extracts thus: MIC < 100 µg (good); 100 µg > MIC < 500 µg (moderate); 500 µg > MIC < 1000 µg (weak); MIC > 1000 µg (inactive) (Scur *et al*., 2016) [40]. O-cymene most likely confers on the oil its antioxidant, anti-inflammatory, anticancer and some of its antimicrobial properties (Marchese *et al*., 2017) [23]; Gamma terpinene has antioxidant properties, most likely holds the key to some of the antioxidant activity of the oil (Rajeshwari and Andallu, 2011) [35]. The antioxidant properties of this essential oil were quite impressive, given that it rivals that of BHT as shown in figure 1 above.

β-cubebene could contribute to the antimicrobial activity of the oil together with linalool although the latter possessed some anti-inflammatory and antinociceptive activity (Zeraib *et al*., 2014; Chang *et al*., 2015; Peana and Moretti, 2008) [44, 13, 34].

Other constituents such as beta caryophyllene, beta myrcene, beta bisabolene, thymol acetate could contribute to its ethnomedicinal uses as antiathritic, cardio-protective, antiulcerogenic, spasmolytic respectively (Andrade *et al*.,

2011; Burcu *et al*., 2016; Jeena *et al*., 2013; Vijayalaxmi *et al*., 2015) [8, 11, 20, 43].

It is noteworthy that most essential oils from other plants in the same family Fabaceae, are devoid of thymol as one of their constituent (Isiaka *et al*., 2013; Santi *et al*., 2017) [19, 38]. This could place *Calliandra portoricensis* on a different pedestal with respect to ethno-medicine. For instance, compared to the essential oils from *Cassia siamea, Cassia occidentalis* and *Cassia ferruginea*, *Calliandra portoricensis* possessed a higher concentration of β-caryophyllene and linalool (Isiaka *et al*., 2013). [19] However, *Cassia ferruginea* featured a greater concentration of phytol relative to *Calliandra portoricensis* (Isiaka *et al*., 2013) [19]. Likewise, essential oils from *Calliandra portoricensis* contained more β-phellandrene and β-crayophyllene compared to essential oils from *Myrocarpus frondosus* (Santi *et al*., 2017) [38]. *Calliandra portoricensis* had higher monoterpenes and sesquiterpenes than *Alhagi maurorum* (Samejo *et al*., 2012). Similar result was obtained on comparison with essential oils from *Gliricidia sepium*, *Cassia bakeriana* as regard linalool, β-caryophyllene, germacrene D-4-ol (Chaverri and Ciccio, 2015; Cunha *et al*., 2013) [14, 16].

5. Conclusion

A total of 36 compounds were identified as constituents of the leaf essential oil of *Calliandra portoricensis*. The majority of these compounds were monoterpenes and sesquiterpenes. This finding provided insight into the volatile chemical constituents as well as the antimicrobial and antioxidant potential of *Calliandra portoricensis* leaf.

6. Conflict of Interests

The authors declare no conflict of interests

7. Acknowledgements

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