

Full Length Research Paper

Gas chromatography – mass spectrometry analysis of the hexane extract of *Calliandra portoricensis* and its antimicrobial activity

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Calliandra portoricensis is widely used in traditional medicine preparation in South western part of Nigeria for the management of sickle cell crisis and other health challenges. Analysis of the hexane extract of *C. portoricensis* by GC-MS revealed 14 – methyl methylpentadecanoate as the major component. Other fatty acids/fatty acid methyl esters detected were hexadecanoic acid, methylhexadecanoate, 9 – oxo – methyl nonanoate and some other minor components. The extract was also investigated for preliminary antimicrobial activity using the following pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella gallinallum*, *Klebsiela pneumonia*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The extract was found to be active against *S. aureus*, *E. coli* and *S. gallinallum*, on the other hand it was not active against *K. pneumonia*, *B. subtilis* and *P. aeruginosa*. The results of the GC-MS analysis and the biological assay are discussed.

Key words: *Calliandra portoricensis*, gas chromatography, mass spectrometry, antimicrobial, fatty acid methyl esters.

INTRODUCTION

Calliandra portoricensis (Jacq.) Benth. is a straggling perennial shrub and belongs to the family mimosaceae (Hutchinson and Dalziel, 1937). It is used in Nigeria folklore medicine as a laxative/worm expeller (Adesida, 1976) and an abortifacient in human beings (Ayensu, 1978). The plant has also been reported to have anticonvulsant (Akah and Nwaiwu, 1988; Adesina, 1982), antidiarrheal, antispasmodic, antipyretic, antirheumatic and analgesic (Aguwa et al., 1988) activities in human beings. In addition *C. portoricensis* has also been reported to exhibit anticholinergic, antacid, antiulcer, molluscidal and ovucidal activities in laboratory animals (Aguwa et al., 1988). The plant extracts have been reported to have antimicrobial activities against the following organisms: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecium* and *Candida albicans* (Adesina, 1982).

Though the genus *Calliandra* consists of many species

distributed Worldwide the only other specie growing in Nigeria is *Calliandra haematocephala*. This has been extensively evaluated for its chemical constituents. Three acylated quercetin rhamnosides were recently reported from the leaves and stem of *C. haematocephala* and their structures were established as quercitrin 2"-O-caffeate, quercitrin 3"-O-gallate and quercitrin 2",3"-di-O-gallate (Mohoarran et al., 2006). Also, 17 known compounds were reported for the first time from the genus *Calliandra* they are gallic acid, methyl gallate, myricitrin, quercitrin, myricetin 3-O- β -D-⁴C₁-glucopyranoside, afzelin, isoquercitrin, myricetin 3-O-(6"-O-galloyl)- β -D-glucopyranoside, myricitrin 2"-O-gallate, quercitrin 2"-O-gallate, afzelin 2"-O-gallate, myricitrin 3"-O-gallate, afzelin 3"-O-gallate (17), 1,2,3,4,6-penta-O-galloyl- β -D-⁴C₁-glucopyranose, myricitrin 2",3"-di-O-gallate, quercetin 3-O-methyl ether (Mohoarran et al 2006). Moreover caffeic acid, betulinic acid were previously reported (Nia et al., 1999). Compounds myricitrin, quercitrin, myricitrin 2"-O-gallate, quercitrin 2"-O-gallate, myricitrin 3"-O-gallate, and myricitrin 2",3"-di-O-gallate, exhibited moderate to strong radical scavenging properties on lipid peroxidation, hydroxyl radical, superoxide anion

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Table 1. Results of phytochemical screening on different parts of *C. portoricensis*.

Groups	Leaves	Stem	Root
Saponins	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Tannins	-ve	-ve	-ve
Glycosides	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve
Anthraquinones	-ve	-ve	-ve
Digitalis glycosides	+ve	+ve	+ve
Fatty acids	+ve	+ve	+ve

Key: +ve = present, -ve = absent.

generation and DPPH radical in comparison with that of quercetin as a positive control *in vitro* (Mohoarran et al., 2006). Despite these reported biological activities of *C. portoricensis*, no detailed investigation of the chemical constituents has been reported. The dried root of *C. portoricensis* was pulverized and extracted with dichloromethane, the dichloromethane extract was fractionated by flash column chromatography from which fraction 1 eluted with n-hexane was obtained and subjected to Gas-Chromatography/Mass-Spectrometry (GC-MS) analysis. The results of the Gas-Chromatography/Mass-Spectrometry (GC-MS) analysis, phytochemical screening and preliminary antimicrobial activity of the hexane fraction of the root extract of *C. portoricensis* were reported.

EXPERIMENTAL

Plant materials

The leaves, stem and root of *C. portoricensis* were collected from the medicinal plant garden of the National Institute for Pharmaceutical Research and Development, Idu Abuja FCT Nigeria. Large quantities of the root were collected from Owo, Ondo State Nigeria. These plants parts were sun-dried and pulverized using a hammer mill (Trapp TRF 80, Trapp Metallurgica, Brazil).

Extraction and phytochemical screening

The leaves and stem extracts were dissolved with appropriate solvent for phytochemical screening. All solvents used were analytical grade from sigma. The root was extracted in a giant soxhlet extractor using n-hexane, dichloromethane and methanol as solvents. The extracts were subjected to phytochemical screening in accordance with the J. B. Harbone methods (Harbone, 1973). The hexane extract of the root was chromatographed on a silica gel (60–120 mesh) column by gradient elution employing n-hexane (100%), n-hexane/ethyl acetate in varying proportions, ethyl acetate/ 5% methanol. Fractions (100 mL each) were collected, concentrated and monitored by thin layer chromatography (TLC). The fraction eluted by hexane (F1) from the column was analyzed by GC-MS.

Gas chromatography - mass spectrometry analysis.

The GC-MS analysis was carried out on a Shimadzu QP 500 using a programmed temperature vaporizer (PTV) sampling technique in the split mode. The GC was equipped with a DB-1 column (30 m, 0.32b mm I. d, film thickness 0.25 micron) manufactured by J and W Scientific, (Folsom, CA, U.S.A). The column oven temperature was programmed from 80 to 250 °C at 10 °C/min, employing helium as carrier gas (1.6 ml/min). Mass spectra were acquired with an ionization voltage of 70eV.

Identification of components was based on a comparison of mass spectra of the individual component against the Shimadzu NIST62 library and by injection of standard FAMES obtained from Sigma (St Louis, MO, USA).

ANTIMICROBIAL ACTIVITY DETERMINATION MATERIALS AND METHODS

Materials used include stock culture of standard American typed culture collection, molten agar, Petri dishes and MacFarland standard. The test organisms were standardized, after inoculation with freshly prepared nutrient broth from an overnight culture for 2 - 5 h before use. The standardized bacteria suspension was compared to the turbidity of half (0.5) McFarland standard (105 cfu). Employing agar dilution method.

The test extract (48 mg) was dissolved in sterile water (3 ml) to give a concentration of 16 mg/ml of the extract solution. Thereafter 1 ml of this solution was taken and added into 15 ml of water with sterile nutrient agar. This was mixed well and poured into plates and allowed to solidify. The final concentration of the media infused with the extract was 1 mg/ml. The plates were inoculated with standardized pure culture of each test organism (0.1 ml). The cultures were incubated at 37 °C for 18 - 24 h, and the degree of inhibition was measured as a zone of inhibition (area of no growth) diameter (mm).

RESULTS AND DISCUSSION

The phytochemical screening of the leaf, stem and root extracts of *C. portoricensis* reveals the presence of saponins, glycosides, steroids, fatty acids and digitalis glycosides in the plant. The summary of the results is shown in Table 1. The gas chromatogram (Figure 1) shows 23 distinct peaks some of which could be identified by GC-MS. The mass spectrum of each compound was compared to that in the NIST 62 Library. The compounds identified are listed in Table 2 along with their GC and MS data that were employed for identification. Some of the compounds were not identified and all of these were present in concentration of less than 2% as auto quantified by the GC-MS machine.

Table 3 shows the retention times and m/z values of the major ions of two standard mixtures that was analyzed on the same machine. The preliminary antimicrobial activity investigation was carried out in triplicate. The n-hexane extract was found to be active against *S. aureus*, *E. coli*, and *S. gallinallum*, on the other hand it was not active against *K. pneumonia*, *B. subtilis* and *P. aeruginosa* as shown in Table 4. This result is in agreement with a previous antimicrobial test done on the plant extract (Akah and Nwaiwu, 1988).

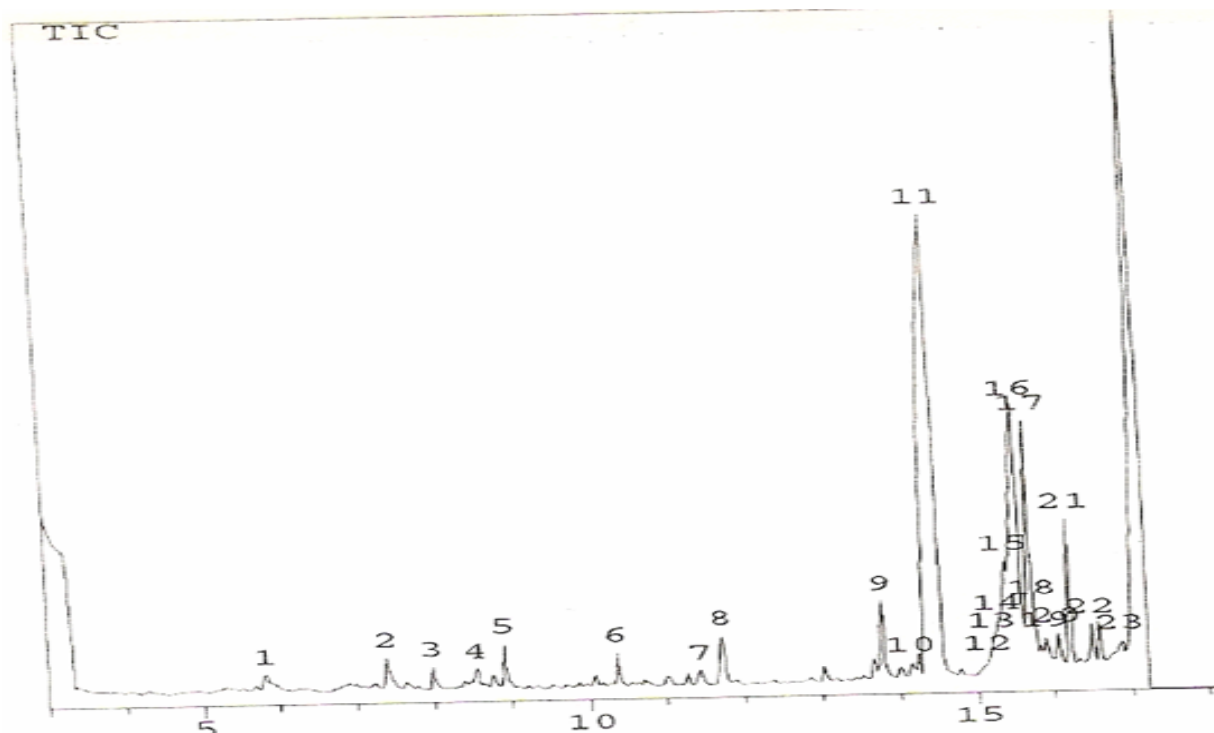


Figure 1. The total ion chromatogram of the hexane fraction.

Table 2. Data of compounds present in the hexane fraction.

GC peak no.	Retention time	Mass spectral data	% total	Identified compound
1	5.821	43 57 71 85 99 156	1.41	Undecane
2	7.399	43 57 71 85 145 170	2.20	Dodecane
3	7.988	43 57 70 83 97 154	1.15	Decenal
4	8.556	43 57 71 85 115 154	1.15	4-Ethoxycyclohexanone
5	8.927	43 57 71 85 99 156	1.95	3,7-Dimethylundecane
6	10.376	43 57 71 100 128 198	1.15	Tetradecane
7	11.435		1.02	2-Methyltetradecane
8	11.733	41 55 74 87 143 156	2.71	Tetradecanoic acid, methyl ester
9	13.786	41 55 74 98 194 256	3.36	9-Hexadecenoic acid, methyl ester
10	14.166		0.93	NI
11	14.533	43 55 74 85 143 270	31.46	14-Methylpentadecanoic acid, methyl ester
12	15.158		0.95	NI
13	15.239		1.61	NI
14	15.297		1.84	NI
15	15.411	43 60 73 129 213 256	6.61	Hexadecanoic acid
16	15.583	43 60 73 129 213 256	20.75	Hexadecanoic acid
17	15.725	43 55 88 101 157 284	6.61	Hexadecanoic acid, ethyl ester
18	15.769		1.53	NI
19	15.913		1.35	NI
20	16.064		1.17	NI
21	16.214	41 55 74 87 143 284	4.21	14-Methylhexadecanoic acid, methyl ester
22	16.504		1.14	NI
23	16.886		1.06	NI

NI = Not identified.

Table 3. Data obtained for reference samples.

GC peak no.	Retention time (min)	Mass spectra data	% of total	Reference compound
1	10.513	41 55 74 115 153 200	42.44	Undecanoic acid, methyl ester.
2	13.200	41 55 74 87 228	57.66	Tridecanoic acid, methyl ester.

Table 4. Results of the preliminary antimicrobial assay.

Concentration of extracts	Organisms					
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. gallinallum</i>	<i>K. pneumonia</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
1000 mg/ml	++	++	++	-	-	-
500 mg/ml	++	-	-	-	-	-
OVC	++	++	++	++	++	++
MSC	-	-	-	-	-	-

Key: ++ = activity observed - = No activity observed.

Conclusion

The GC-MS results of the hexane fraction of the root of *Calliandra portoricensis* indicated the extract is rich in fatty acids/methyl esters which have been implicated in plant's antimicrobial activities. It is therefore suggested that detailed phytochemical evaluation of this plant be carried out to isolate compounds responsible for some of the reported biological activities.

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