African Journal of Pharmaceutical Research & Development

Vol. 5 No.1 pp.25-29 (2013)

Characterization and Antimicrobial Evaluation of Epiafzelechin from the Stem Bark of *Calliandra surinamensis* Benth

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ABSTRACT

The stem bark of *Calliandra surinamensis* Benth was investigated for chemical constituents. The isolated compounds were characterized by NMR, IR and Mass spectral studies, and the extract fractions and pure compounds were subjected to antimicrobial screening against different organisms. *p*-hydroxybenzoic acid (**1**) and (-)-epiafzelechin (**2**) were isolated from the chloroform soluble fraction.

KEYWORDS: *Calliandra surinamensis*, Epiafzelechin, Antimicrobial activity

INTRODUCTION

The genus Calliandra belongs to the family Leguminosae and it consists of about 200 species distributed in tropical and subtropical America, East Pakistan (Bangladesh) and China [1, 2]. Many Calliandra species are eye-catching with flowers clustered into showy heads [2]. Calliandra is the genus of woody shrubs and small trees [3]. The word 'Calliandra' comes from the Greek; kalli, meaning beautiful and andros meaning stamens, for its prominent and attractive stamens. The species of the genus Calliandra are jointly called powder puff trees, or shrubs and all species are attractive [4]. Various Calliandra species have been reported for the treatment of eye diseases, diarrhea, indigestion [4], diabetes, obesity [5], Calliandra species exhibit a broad spectrum of biological activities including antioxidant [6], anti-asthmatic [7], insecticidal [8], cytotoxic [9], antimicrobial [10], hemolytic [11], analgesic [12] anticonvulsant [13], trypsin inhibitory activity [14], *in vivo* toxicity [15], antibacterial [16] and molluscicidal activities [17]. Different classes of compounds have been reported in literature e.g. diterpenes [16], imino acids [18], flavones and flavonoids [16, 19], acylated quercetin rhamnosides [20], pipecolic acid derivatives [21], saponins [22], volatile constituents [23], polyphenols [24], tannins [25] and oleanene triterpenoids [26] etc.

The alcoholic extract of the root bark of *Calliandra surinamensis* is used in Nigerian ethnomedicine for the treatment of bacterial infections such as cough, bronchitis and pneumonia. The study was undertaken to validate the ethno medicinal claim of the medicinal plant.

MATERIALS AND METHODS

General Experimental Procedures: Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. The IR spectrum was recorded on JASCO FT/IR-5300 spectrophotometer. 1 H (500 MHz), ^{13}C (125 MHz), and 2D (HMBC and HMQC or HSQC, COSY, NOESY) NMR spectra were recorded using JEOL JNM-A500 and/or Varian INOVA-500 spectrometers with tetramethylsilane (TMS) as an internal standard in $CDCl_3$. A JEOL

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JMS-700 instrument was used to obtain the FABMS and the HRFABMS. Silica gel 60 (Merck, 40-63 ím) column chromatography was used for the separations, and pre-coated silica gel plates (Merck, silica gel 60 F_{254}, 0.20 mm) were used for analytical TLC. The spots were visualized by spraying with 10% H_2SO_4 and then heating.

Plant Material

Fresh stem bark of *Calliandra surinamensis* was collected from the Ugbowo Campus of the University of Benin, Benin City, Nigeria in April, 2009. Botanical identification and authentification of the sample was done by a plant Taxonomist Prof. M. Idu, Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Extraction and Isolation of Chemical Constituents

The dried and powdered stem bark of *Calliandra surinamensis* (1 kg) was extracted with MeOH (5 L) at room temperature for 48 hours. The solvent was evaporated under reduced pressure and the crude extract (57 g) was obtained. The combined methanol (200 mL of MeOH and 50 mL of H_2O) extract (57 g) was successively extracted out with n-heptane, chloroform and EtOAc. The $CHCl_3$ soluble fraction (9.9 g) was subjected to column chromatography over silica gel eluting with n-heptane, n-heptane-EtOAc, EtOAc, EtOAc-MeOH and MeOH in increasing order of polarity. The sub fractions were subdivided into three fractions: CSC-1 (70% EtOAc-n-heptane), CSC-2 (10% MeOH-EtOAc) and CSC-3 (100% MeOH). The fraction CSC-1 was subjected again to column chromatography and the elution with EtOAc-n-heptane (60 %) gave *p*-hydroxybenzoic acid (**1**) (36 mg) and with EtOAc-n-heptane (90 %) (-)-epiafzelechin (**2**) (125 mg) was isolated. The structures of the isolated compounds were established using NMR spectral techniques (such as COSY, HMBC, HSQC, NOESY) and direct comparison with the published information.

***p*-Hydroxybenzoic acid:**

^{1}H NMR (250.13 MHz, CD_3OH): δ = 6.71 (d, *J* = 8.83 Hz, 2H, ArH), 7.77 (d, *J* = 8.83 Hz, 2H, ArH). ^{13}C NMR (62.89 MHz, CD_3OH), δ = 116.0 (2CH), 122.9 (C), 133.0 (2CH), 163.3 (C), 170.3 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3439, 2916, 2848, 2544, 1658, 1650, 1589, 1423, 1242, 1167, 910, 848, 767, 618. HRMS (ESI) calcd for $C_7H_5O_3$ [M-H]: 137.02442 found 137.02475.

(-)-Epiafzelechin:

Colourless solid (349 mg); $[\alpha]^{23.9}_{546}$ -61.9 (*c* 0.01 THF); mp 224–226 °C; R_f = 0.68 (EtOAc).

^{1}H NMR (300.13 MHz, Acetone-d_6): δ = 2.84 (m, -CH_2-, envelope, 2H, H-4), 3.67 (d, *J* = 5.85 Hz, 1H, H-2), 4.21-4.26 (m, 1H, H-3), 4.95 (br s, 1H, (CH)OH), 5.95 (d, *J* = 2.27 Hz, 1H, H-8), 6.05 (d, *J* = 2.27 Hz, 1H, H-6), 6.83 (d, *J* = 8.69 Hz, 2H, H-3′, H-5′), 7.37 (d, *J* = 8.31 Hz, 2H, H-2′, H-6′), 7.97 (br s, 1H, ArOH), 8.13 (br s, 1H, ArOH), 8.25 (br s, 1H, ArOH). ^{13}C NMR (75.46 MHz, Acetone-d_6): δ = 29.2 (-CH_2-, C-4), 66.9 (CH, C-3), 79.5 (CH, C-2), 95.8 (CH, C-6), 96.2 (CH, C-8), 99.8 (C, C-4a), 115.5 (2C, C-3′, C-5′), 129.2 (2C, C-2′, C-6′), 131.5 (C, C-1′), 157.2 (C, C-4′), 157.5 (C, C-7), 157.6 (2C, C-5, C-8a). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3416 (w), 3317 (w), 2936 (w), 1614 (s), 1513 (s), 1469 (m), 1355 (w), 1284 (w), 1219 (m), 1167 (w), 1140 (s), 1094 (m), 1013 (s), 916 (w), 864 (w), 842 (w), 816 (m), 792 (s), 715 (w), 722 (s), 543 (w). GC-MS (EI, 70 eV): *m/z* (%): 274 (M^+, 80), 167 (17), 140 (25), 139 (100), 136 (87), 108 (47), 107 (82), 77 (14), 69 (17), 53 (4), 43 (7), 39 (7).

Antimicrobial Activity:

Three extracts (CS-Crude, CSCE, and CSEE) and one pure compound (CS-5) were subjected to antimicrobial evaluation. Two of these extracts (CSCE and CSEE) and one pure compound (CS-5) were solubilized with 1-2 mL of MeOH and the remaining one extract (CS-crude) was solubillized with 1-2 mL (1 mg/ml) DMSO. These four solutions were diluted with approximate volumes of sterile distilled water to yield 1 mg/mL as working stock solution.

Preparation of Organism:

Six clinical isolates comprising of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogens*, *Klebsiella pneumonia*, *Escherichia coli* and *Alkaligenes sp*. were challenged with the dilutions of 50 µg/mL, 75 µg/mL, 100 µg/mL, and 150 µg/mL of the different samples. The clinical isolates were obtained from the Department of Pharmaceutical Microbiology of the Faculty of Pharmacy, University of Benin, Benin city. Each pure isolate of the bacteria was grown in Nutrient broth (Oxoid) for 18-24 hrs at approximately 10^6 of cfu/mL. 0.2 mL was used to seed the agar towards determination of the Minimum Inhibitory Concentration (MIC). The agar-well diffusion method was used in determining the antimicrobial activity [27]. The equivalent volumes of the extracts

containing the various concentrations were pipetted into sterile petri dishes and made up to 20 mL with nutrient agar at 46 °C. This was mixed thoroughly and allowed to set. The agar plates were dried at 60 °C for ten minutes and spotted with 0.01 mL of the various organisms at the different concentrations including two standard organisms *Staphylococcus aureus* ATTC 25923 and *Esherichia coli* (ATTC 25922). The plates were allowed to stand on the bench for 30 minutes and incubated at 37 °C for 24 hrs.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of compound (**1**) showed two doublets for symmetric methine protons resonating at δ_H = 6.71 (d, *J* = 8.83 Hz, 2H) and δ = 7.77 (d, *J* = 8.83 Hz, 2H) which indicates 1,4-disubstituted benzene ring. The ^{13}C spectrum showed total five signals comprising two signals for symmetric methine carbons (δ_C = 116.0, 133.0) and the signal at δ_C = 170.3 was assigned for the carbon of the carboxylic acid. The IR spectrum showed the presence of hydroxyl group at 3439 cm^{-1} and carbonyl group at 1658 cm^{-1}. The HRMS (ESI) exhibited fragment ion at *m/z* 137 [M$^+$-H]. Thus the isolated compound was determined as *p*-hydroxybenzoic acid with the molecular formula $C_7H_6O_3$ established on the basis of the NMR and MS data. The experimental data for compound (**1**) was found in agreement with the already published data [28].

The ^{1}H NMR spectrum of compound (**2**) showed total 14 protons consisting of 4 hydroxyl goup protons, 8 methine protons and 2 methylene protons. Using HSQC and HMBC spectra, it is confirmed that the methylene protons resonating at δ_H = 2.84 show correlation with C-2, C-3, C-4a and C-8a. The spectroscopic data for the compound (**2**) was found in agreement with the previous published data [29].

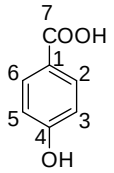

Fig. 1: *p*-Hydroxybenzoic acid

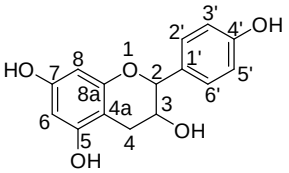

Fig. 2: (-)-Epiafzelechin

The clinical isolates were all resistant to the different concentrations of the extracts. The ATTC 25923 was sensitive to the 75 µg/mL, 100 µg/mL and 150 µg/mL of the methanol extracts and the crude, while the ATTC 25922 *Escherichia coli* were also resistant to all the dilution.

Table 1: Antimicrobial screening of pure isolates of *C. Surinamensis*

	CS-Crude				CSCE				CSEE				CS-5			
	50	75	50	75	100	150	100	150	50	75	100	150	50	75	100	150
P. aeruginosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. aerogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkakaligenes sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key:
+: (growth resistant)

CONCLUSION

The isolation and characterization of Epiafzelechin from the Stem Bark of *Calliandra surinamensis* was achieved through the assay guided technique.

ACKNOWLEDGEMENTS

The authors thank HEC (Pakistan), DFG, REMEDIS (Germany) for the financial support.

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