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Antimicrobial Activity and Chemical Constituents of *Culcasia scandens* P. Beauv

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Abstract: The plant, *Culcasia scandens* P. Beauv. is popularly used in the treatment of tonsillitis, toothache and other inflammatory conditions in the southern part of Nigeria. The hexane, ethyl acetate and methanol extracts of this plant was evaluated for antimicrobial activity against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, Trichophyton mentagrophytes and Cladosporium resinae*. All the extracts examined showed no activity. Phytochemical analysis of the extracts however, revealed the presence of homologous very-long-chain 1,3-alkanediols, homologous series of C31, C33 and C35 alkanols, protoquercitol, methyl β-D-fructopyranoside, palmitic acid, stearic acid, linoleic acid, linolenic acid and their methyl esters.

Key words: *Culcasia scandens* P. Beauv, protoquercitol, alkane 1,3 diol, antimicrobial activity

INTRODUCTION

Culcasia scandens P. Beauv. is a climber growing wild in the southern part of Nigeria. It belongs to the order *Arales*, family *Araceae* and subfamily *Philodendroideae* Engl. of the genus *Culcasia* schott. *Culcasia* species are native to Africa and there has not been any major phytochemical investigation of this genus. The leaves of *C. scandens* are popularly used in the treatment of tonsillitis, toothache and other inflammatory conditions. The ethnomedicinal uses as anti-inflammatory, anti-rheumatic agents (Okoli and Akah, 2000) and as possessing analgesic properties (Okoli *et al*., 2006) have been documented. The present study was undertaken to evaluate the hexane, ethyl acetate and methanol extracts of this plant for antimicrobial activity and to identify the chemical constituents of the various extracts.

MATERIALS AND METHODS

Plant material: Foliage of *Culcasia scandens* were collected in Ibadan, Oyo-state in the south-western part of Nigeria and the collection was identified by Mr. Felix Ussang of the Forestry Research Institute of Nigeria, FRIN, Ibadan; a voucher specimen has been deposited in the herbarium at FRIN (Voucher No. FHI 107234).

Extraction and isolation: *Culcasia scandens* foliage (600 g) was air-dried and crushed. The crushed material was successively extracted with hot hexane, ethylacetate and methanol. The hexanic extract (2 g) was submitted to Column Chromatography on silica gel and eluted with a gradient mixture of hexane/diethyl ether. The eluants were pooled into three fractions (F1-F3) based on the similarities of the components on TLC. The ethylacetate extract was heavily coloured with chlorophyll. The colouring matters were removed (Anna *et al*., 1997) and the combined decolorized fractions (240 mg) was submitted to Column Chromatography on silica gel and eluted with a gradient mixture of hexane/diethyl ether. Fractions were again combined based on the similarities of the components on TLC. Vacuum Short Column Chromatography was run for the methanol extract (4 g) employing butanol/ethanol/water (1:1:1) as eluting solvents. The eluants were pooled into two fractions, F6 and F7 based on the similarities of the fractions on thin layer chromatography plate. F7 was acetylated and submitted to column chromatography (flash) on silica gel using chloroform/acetone (4:1) as eluting solvent. Fraction 2 (500 mg) was further purified by prep-TLC employing 1:1 EtOAc/Hexane mixture as developing solvent: Three major bands were observed (F8, F9 and F10).

Antimicrobial assay: Twenty mililitre of Mueller-Hinton agar were dispersed into sterile universal bottles. These were then inoculated with 0.2 mL of standardized cultures, mixed gently and poured into sterile petri dishes. After setting, a number 4 cup-borer (8 mm diameter) was properly sterilized by flaming and used to make 4 uniform

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cups in each petri dish. A drop of molten agar was used to seal the base of each cup. The cups were thereafter each filled with the different extracts each at a concentration of 350 ug mL^{-1} and allowed to diffuse for 45 min. The solvents used and standard antibiotic/antifungal were similarly treated as negative and positive control. The plates were incubated at 37°C for 24 h. The experiments were carried out in triplicate. The six organisms tested against were: *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, Trichophyton mentagrophytes and Cladosporium resinae.* Chloramphenicol (120 µg mL^{-1}) and Tioconazole (1% w v^{-1}) as antibacterial and antifungal respectively, were the chemotherapeutic drugs used.

Equipment and spectroscopic analysis: NMR spectra (F1, F2, F3, F8, F9 and F10) were acquired in $CDCl_3$ on a Brüker AM-400 spectrometer using the residual solvent signal as internal standard (δ_H7.27, δ_C 77.0; and δ_H 7.15, δ_C 128.0 ppm respectively); Standard Brüker pulse programs were used for DEPT, 2D NMR COSY, HMBC, HMQC spectra; The silica gel used for TLC was precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck); GC-MS analysis (F1, F3, F4, F5, F6) was on Hewlett Packard 5890 series II; 24×0.2 mm i.d. column coated with DB5 bonded phase (0.33 µm film); temp. prog., 10° min^{-1}, then hold at 280°; injector temperature, 250°; detector temperature, 280°; injector volume, typically 1 µL at 70:1 split ratio; flowrate, 0.43 mL min^{-1}.

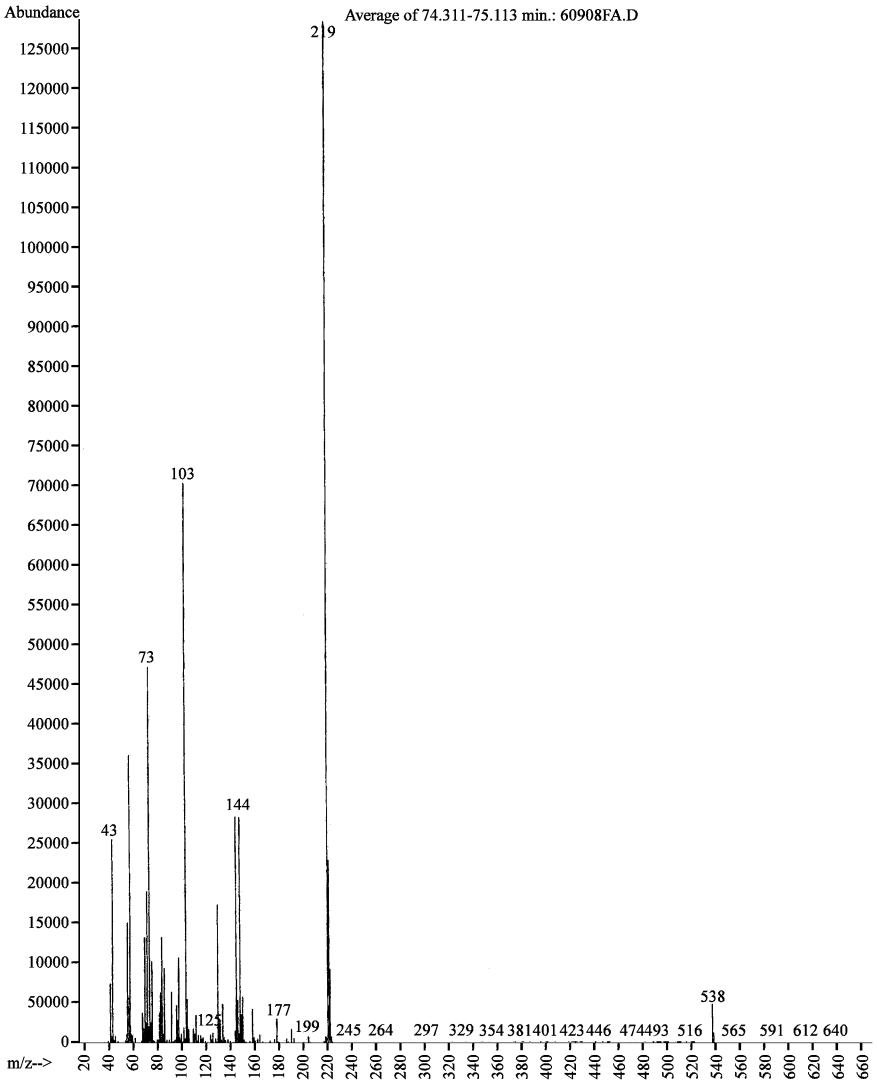

Fig. 1: a 1,3 relationship was established evident from the base peak of 219, corresponding to $[C_3H_5(TMSiO)_2]^+$

RESULTS AND DISCUSSION

F1 (15 mg) yielded white crystalline precipitates upon evaporation *in vacuo*. ^{1}H-NMR, ^{13}C-NMR, DEPT 135 and low-resolution mass spectrum showed F1 to be a homologous series of odd carbon-chain alkanols. This was confirmed by silylating a portion of the fraction and from the low-resolution mass spectrum and GC-MS data, F1 was found to be a homologous series of C31, C33 and C35 alkanols. F2 (65 mg) was identified as a mixture of stigmasterol and β-sitosterol in the ratio 1.2:1.0. ^{1}H-NMR spectrum of Fractions F3 (20 mg) was similar to Fractions F1 except that the absorption at δ_H 3.65 ppm due to a hydroxylated methylene carbon was shifted downfield to 3.88 ppm and instead of the triplet, a multiplet was observed. ^{13}C-NMR indicated the presence of two oxygen-bearing carbon atoms at δ_C 72 and 62 ppm and with DEPT experiment, one of the oxygen-bearing carbon atoms was found to be primary and the other, secondary. To establish the relationship between these oxygenated carbon atoms, F3 was silylated; the GC-MS was run for the silylated product and a 1,3 relationship was established evident from the base peak of 219, corresponding to $[C_3H_5(TMSiO)_2]^+$ (Fig. 1). From the GC-MS and low-resolution mass spectra of F3 (Fig. 2), homologous series of C32, C34 and C36 1,3 alkanediol with C34 being the most abundant, was deduced. To the best of our knowledge, this is the longest homologous series of 1,3 alkanediol isolated so far (Cornelia *et al*., 2003; Huang *et al*., 1994; Reinhard *et al*., 1996).

F4 (100 mg), the major component from the ethylacetate extracts was found to be a mixture of fatty acids and were therefore methylated with CH_2N_2. GC-MS confirmed the presence of palmitic, stearic, oleic, linoleic acid and linolenic. F5 was identified by GC-MS as methyl esters of the acids in F4. The identities of these compounds were confirmed by comparison of retention time with retention time of authentic materials purchased from Aldrich chemicals. Systematic ^{1}H-NMR/GC-MS check on F6 only showed overwhelming presence of previously identified long-chain fatty acids. ^{1}H-NMR of F7 indicated signals attributable to sugar molecules; it was therefore acetylated. F8 (15 mg) was identified by ^{1}H-NMR and ^{13}C-NMR as protoquercitol pentacetate. This is the first mention of the presence of protoquercitol in any *Araceae*. F9 (7 mg) was identified as methyl β-D-fructopyranoside by comparing its NMR data with data documented for synthesized methyl β-D-fructopyranoside per-acetylated by Grice *et al.* (2005). This appears to be the first mention of the occurrence of methyl β-D-fructopyranoside in nature. However, the suspicion is that this compound might be an artifact formed by methanolysis during the hot methanol extraction process of the plant sample evidently full of free carboxylic acids. Library search showed F10 to be a mixture of β-D glucose- and β-D glucose pentaacetate; all signals in the broadband ^{13}C-NMR could be correlated.

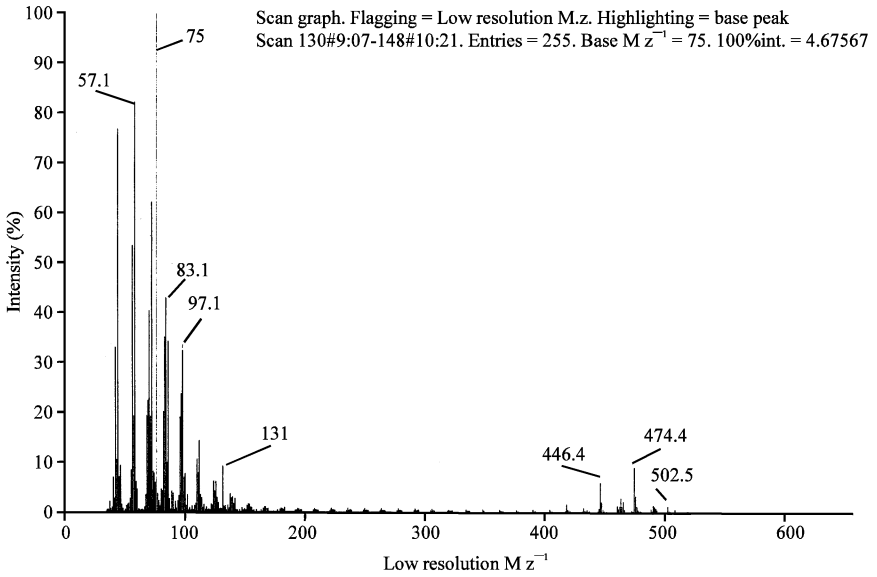

Fig. 2: The GC-MS and low-resolution mass spectra of F3

CONCLUSION

Araceae appear to be a very rich source of long chain saturated, unsaturated and aromatic phenyl ring fatty acids (Chee *et al*., 2001; Torsten *et al*., 1982; Saglik *et al*., 2002; Serap *et al*., 2002). This probably explains why some of them find use as traditional anti-inflammatory and anti-rheumatic agents (Okoli and Akah, 2000; Saglik *et al*., 2002, Serap *et al*., 2002) since many polyunsaturated fatty acids, especially linoleic acid and linolenic acid have been variously implicated in literature as possessing anti-inflammatory effects (Guixiang *et al*., 2005; Zhao *et al*., 2004). Though the ethnomedicinal uses of *C. scandens* as anti-inflammatory, anti-rheumatic agents (Okoli and Akah, 2000) and as possessing analgesic properties (Okoli *et al*., 2006) have been documented, our finding showed that the hexane, ethylacetate and the methanol extracts of *C. scandens* showed no activity against any of the tested organisms.

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