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ISOLATION OF AN ANTIOXIDANT FLAVONONE DIGLYCOSIDE FROM THE NIGERIAN MEDICINAL PLANT *CLERODENDRON SPLENDENS* A. CHEVAL

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ABSTRACT

The ethanolic extract of *Clerodendron splenden*, A. Cheval (Lamiaceae) that had previously been shown to have biological activity were studied. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, steroids, glycoside and phenolic compounds. The ethanolic extract of *Clerodendron splenden* resulted in the isolation and characterization of flavonone diglycoside, 3',5', 5 – trihydroxy 4'- methoxy flavonone 7-O-β-D gluconopyranosyl methyl glucopyranose from its spectral data. EI-MS and ^{1}H NMR spin system analysis were performed to characterize the higher order of the glucoside linked to carbon 7 of the flavonone ring system of the isolate. It is concluded that 3',5',5-trihydroxy-4'-methoxy flavonone 7-O-β-D gluconopyranosyl methyl glucopyranose may be a contributor to the antioxidants, anti-inflammatory, antimalarial and antimicrobial properties exhibited by *Clerodendon splenden* .

Key words: *Clerodendron splendens*, Verbenaceae, Flavonone, Glucoside, Phenolic compounds, Antioxidant, Phytomedicine.

INTRODUCTION

The genus *Clerodendrum* L. (Family Lamiaceae: Verbenaceae) is very widely distributed in regions of the world. More than five hundred species of the genus are identified comprising small trees, shrubs and herbs[1].

Species belonging to the genus *Clerodendrum* have been used as a traditional medicine against syphilis, typhoid, cancer, jaundice and hypertension[1]. Few species of the genus like *Clerodendrum inerme*, *C. thomosonae, C. indicum and C. speciosum* are ornamental and are being cultivated for aesthetic purposes[1].

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Roots and leaf extracts of *C. indicum, C. phlomidis, C. serratum, C. trochotomum, C. chinense and C. petasites* have been used for the treatment of rheumatism, asthma and other inflammatory diseases [2-6]. Plant species such as *C. indicum* and *C. inerme* were used to treat coughs, scrofulous infection, buboes problems, venereal infections, skin diseases and as a vermifuge, febrifuge and also to treat Beriberi disease [7].

Tribal use of *C. inerme*, as antidote of poisoning from fish, crabs and feeds have been reported [8]. *C. phlomidis, C. colebrookianum, C. clabitosium and citrichofomum* have been reported to have antidiabetic, antihypertensive and sedative properties [8-12].

C. cyrtophy llum and C. chinense were used for the treatment of fever, jaundice, typhoid and syphilis [6].

Roots, leaves and fresh juice of leaves of *C. infortunasum* were used in eliminating ascarids and tumors and also as a laxative[1]. *C. phlomidis* has been used as an astringent and also in the treatment of gonorrhea [12,13]. The roots of *C. serratum* have been claimed to be used in dyspepsia, seeds in dropsy and leaves as a febrifuge and in cephalagia and ophthalmia [1].

C. calamitosum was used as a medicine for the treatment of kidney, gall and bladder stones. This plant is also reported to have diuretic and antibacterial properties [12]. Flavonoids are one of the major phytoconstituents present in *Clerodendron* species and they are also responsible for the few biological activities[1]. The main flavonoid contents isolated from *Clerodendrom* species are cynaroside, 5 – hydroxy – 4$^{'}$ – 7 – dimethoxy methyl flavine, kaemferol, salvingin, 4 – methyl, scutetellarein 5, 7, 4-0- trihydroxyflavone and naringin 4$^{'}$ – 0 - α - glucopyranoside[1]. This research describes the isolation and identification of new phytoconstituents from the leaves of *Clerodendron splendens* commonly used in herbal medicine in Nigeria.

EXPERIMENTAL

Materials and methods

Sample collection: The leaves of *Clerodendron* spendens were collected from Umudike Nigeria on 20th June 2004. The plant materials (leaves, flowers and seeds) were identified and authenticated by Dr. A. Nmeregini of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria. The voucher specimens were deposited in Forestry Department Herbarium of Michael Okpara University of Agriculture, Umudike, Nigeria.

Treatment of plant materials

Plant materials were treated and analysed at the Chemistry Laboratory, Michael Okpara University of Agriculture Umudike. The plant leaves (1 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (560 g) using Thomas Wiley Machine (Model – 5 USA). The powdered plant materials is dried and stored in airtight bottles for chemical analysis. The powdered plant sample (500 g) was packed into a Soxhlet apparatus (2 L) and extracted exhaustively with 1000 mL – ethanol for 24 hrs. The ethanolic extract was concentrated using a rotary evaporator at 45ºC and in a hot air circulating oven to get brown residue. Thin layer chromatogram (chloroform : methanol 7 : 3) with iodine vapour showed the presence of two bands R_f 0.88 (yellow) and R_f 0.75 (purple). The eluted sample was re-chromatographed through the column with 50% chloroform in diethyl ether, which afforded yellow crystals (8.2 mg). This was recrystallized from hexane to afford compound

(1) Yellow solid (6.2 mg) UV(MeOH) max 290 nm, IR ν_{max} 1641(C = C) aromatic, 1726 (C = O) ketones, 2859 (OH) phenol, 1125 (-CO-) ether, EI – MS m/z 657.6 $[M^+]$ calculated for $C_{29}H_{37}O_{17}$. 657. ^{1}H NMR $CDCl_3$ TMS; δ-Scale (ppm). 7.7 ^{1}H ($H_{2'}$ and $H_{6'}$), 7.46 I H (H_6 and H_8), 4.6 bs (OH) 4.2 I H m, 3.0 – 4.2 unresolved (sugar OH, and CH_2 protons) 3.0 3H (H4'), 3.72 3H (H_6) 4.80 (^{1}H d H – Glc – 1); 4.36 (I H m)

Acid hydrolysis of (1)

Compound **(1)** (5 mg) was refluxed with 1M HCl/dioxane 1 : 1 v/2 mL) on a water bath for 6 h. The reaction mixture was partitioned four times between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated and subjected to silica gel column chromatography $CHCl_3$/EtOAc to provide compound **(2)** (3.2 mg), white amorphous powder IR (KBr) ν_{max} 3416, 2933, 1456, 1068, 1050 cm^{-1}; ^{1}H NMR (pyridine - d5). 4, 36 (1H d H –Glc–1) 4.60 (1H d – H Glc – 1 4.80 2H (m– H_6).

Acid hydrolysis of (2)

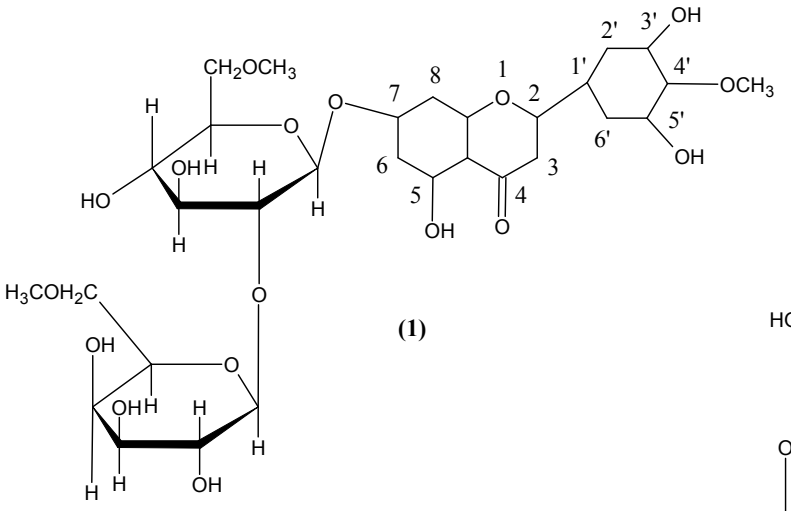
Compound **(2)** was subjected to acid hydrolysis as described for **(1)** to give D-glucose and 6-methyl D-glucose as sugar moieties shown by sugar analysis with Benedict's reagent [15].

Compound **(3),** yellow amorphous powder. IR (KBr) νmax 3492, 2938, 2359, 1639, 1594, 1462, 1369, and 1166 cm^{-1} ^{1}H NMR (400 MHz) 6.56 (2H brs $H_{2'}$, $6'$) 5.97 (1H brs H-8) 5.92 (1H brs H – 6δ), 5.26 (1H d) 4.20 (1Hd H – $_3$, 3.80 (3H brs $4'$ OMe. EI –

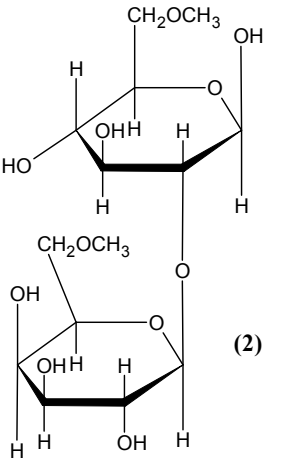
MS (m/z) 333.05 calculated for $C_{16}H_{13}O_8$ (333).

RESULTS AND DISCUSSION

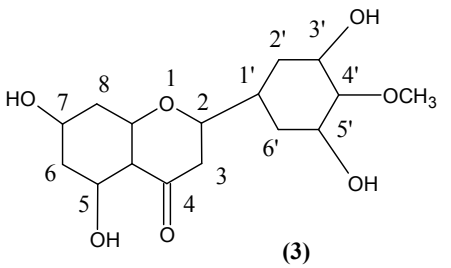
Compound **(1)**, yellow solid was assigned the molecular formula $C_{29}H_{37}O_{17}$ on the basis of EI -MS. The IR spectrum revealed the hydroxyl, carbonyl and aromatic bands at 2859, 1726 and 1641 cm $^{-1}$, respectively. The ^{1}H NMR spectrum showed four aromatic protons (δH 7.42 (1H brs) and 7.46 (1H brs), 7.7 (2Hs), two methine protons (δH 5.26 and 4.20 (each 1H d) and two methoxy groups (δH 3.0 and 3.72) respectively. The compound was identified as flavonone glycoside; Apigenin – 7-O-β-D glucuronopyranoside, that is 3', 5', 5- trihydroxy -4' methoxy flavonone 7-O- β-D- glucucronopyranosyl methyl glucopyranose.



(1)



(2)



(3)

Acid hydrolysis of compound **(1)** produces the glucurono-pyranoside moiety **(2)** comprising two glucose residues linked to carbon 7 of the flavonone ring system and 3',

5', 7 trihydroxy - 4 methoxy flavonone, a non bitter yellow compound. Compound **(3)** was assigned the molecular formula $C_{16}H_{13}O_8$ on the basis of EI-MS. The 1H NMR spectrum showed four aromatic protons (δH 6.56 2H brs), 5.97 and 5.92 (each 1H brs), two methine protons δH 5.26 and 4.20 (each 1Hd), and one methoxy group (δH 3.80).

Clerodendron splenden has been used to treat malaria in Nigeria due to the presence of the bitter principle. However, acid hydrolysis removes the glycoside and produces compound **(3),** a non-bitter yellow compound with antioxidant activity [1,16]. Antioxidant compounds are responsible for scavenging free radicals, which are produced during normal metabolism or during adverse conditions that can be harmful to biological systems and thus leading to the death of an organism. Apigenin 7-O-β-D glucuronopyranoside (AGC) isolated from *C. tricholomum* leaves decreased the volume of gastric juice and increased the gastric pH in a dose-dependent manner, decreasing the number of gastric lesions[1]. Apigenin glucuronopyranoside also decreases mucous glutathione (GSH) levels; thus suggesting that AGC possessed free radical scavenging activity. In present lifestyles, where stress conditions are common leading to excess production of free radicals, these natural products will prove a support to our biological system to sustain and balance metabolism. These findings supported the use of *Clerodendron splenden* in phytomedicine as antioxidant and anti-inflammatory agent.

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