

Full Length Research Paper

Isolation, characterization and antibacterial activity screening of methoxyamine tetrahydroxyanthocyanidines from *Detarium senegalense* gmelin stem bark

Donatus Ebere Okwu* and Rosemary Uchegbu

Department of Chemistry, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State Nigeria.

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***Detarium senegalense* F. Gmelin (Leguminosae) is well-known Nigerian food and medicinal plant, commonly used in phytomedicine to cure diseases and heal injuries. The ethanol stem barks extract of *D. senegalense* affords an anthocyanidin alkaloid (2-methoxyamine 3,4,5,7 – tetrahydroxy anthocyanidines). The structure was elucidated using NMR spectroscopy in combination with IR and MS spectral data. Antibacterial studies showed that the isolated compound successfully inhibited *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia*. This result authenticates the use of the plant in phytomedicine for the treatment of infections, disease prevention and as a preservative of local palm wine in South Eastern Nigeria.**

Keywords: *Detarium senegalense*, anthocyanidin, alkaloid, antibacterial, preservative, phytomedicine.

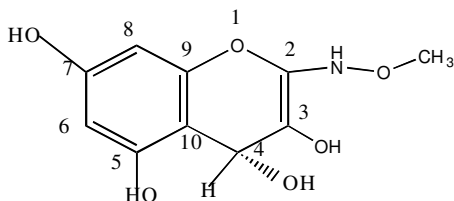
INTRODUCTION

As part of the project on Bioactive Agents from Dry land Biodiversity of Nigeria, *Detarium senegalense* F. Gmelin caesalpiniaceae (leguminosae) was selected for studies because of its use in food and medicine. The tree grows up to 38 m high with a large very leafy crown. It produces its fruits from November to March. The fruits are 4 - 6 cm diameter, fibrous, sweet and one seeded (Keay et al., 1989). The seed flour is used in food as a stabilizer, thickening and flavouring agent (Enwere, 1998). Recent studies showed that *D. senegalense* seed contains a large amount of water soluble, non-starch polysaccharide, zyloglucan. This suggests that it has considerable commercial potential in food, drugs and pharmaceutical industries (Wang et al., 1996, 1997). The stem barks, seeds, leaves and roots extracts of *D. senegalense* are widely used in herbal medicine in Nigeria. They are prepared as infusions or decoctions to treat venereal diseases, urogenital infections, hemorrhoids, rheumatism, stomach-ache, intestinal worms and diarrhea (Keay et al., 1989; Abreu and Relva, 2002). They are also used against malaria and leprosy. A decoction of the powder-

ed bark is widely taken to alleviate pains such as headache, back pain, sore throat and painful menstruation (Keay et al., 1989; Abreu et al., 1998, 1999). The stem bark is macerated in palm wine for bronchitis, pneumonia and for all internal complaints and for leprosy treatment (Burkil, 1995). The Igbo tribe of Nigeria uses this plant as a post partum and for haemostatic medication. The stem bark is macerated for preservation of palm wine in Igbo land. The pulp from the bark is eaten as a remedy for tuberculosis. A bark decoction is given to women at childbirth to expel the placenta. The liquid from the boiled bark is taken for indigestion. A decoction of the leaves is taken to treat convulsions (Kaey et al., 1989). The roots are part of a medicomegal treatment for mental conditions and for protection against evil spirits (Dalziel, 1995).

In veterinary ethno medicine, the leaves and roots are used to treat diarrhea in cattle. The stem bark is also used to treat measles, hypertension, itch and tiredness while a decoction of the leaves or roots is taken against paralysis, meningitis and difficult delivery (Kaey et al., 1989). Herein we report the isolation, characterization and structural elucidation of the anthocyanidine alkaloid; 2 – methoxyamine 3,4,5,7 – tetrahydroxyanthocyanidines from *D. senegalense* stem bark. In addition, we investi-

*Corresponding author. E-mail: okwudonatus@yahoo.com.



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Compound 1. 2-methoxyamine 3,4,5,7-Tetrahydroxy anthocyanidines

Table 1. ^1H (400 MHz) and ^{13}C NMR MHz (75 MHz) Data of compound 1.

Position	δC		δH	
	Chemical shift	Chemical shift	Chemical shift	Multiplicity position
1.				
2.	48.361			
3.	48.57	4.85277		OH (1Hbs)
4.	48.787	1.2005		1Hs CH
5.	110.304			
6.	138.380	7.06512		1Hs
7.	110.304	4.85277		OH (1Hbs)
8.	146.248	6.98759		1Hs
9.	49.000			
10.	49.219			3Hs OCH ₃
OCH ₃	31.909	3.3109		

gate the antibacterial activity of methoxyamine anthocyanidines isolated from *D. senegalense* stem bark.

MATERIALS AND METHODS

Plant materials

The stem barks and seeds of *D. senegalense* were collected from the forest in Lokpaukwu, Umunneochi Nigeria on 14th January, 2007. Authentication of plant materials was done by Dr. A. Nmeregini of Taxonomy Section, Forestry Department Michael Okpara University of Agriculture Umudike Nigeria. A voucher specimen No. DS/102 has been deposited at the Forestry Department Herbarium of Michael Okpara University of Agriculture Umudike, Nigeria.

Treatment of plant material

The stem barks (2kg) were air dried on the laboratory bench at Chemistry Department, Michael Okpara University of Agriculture, Umudike for 10 days. The dry sample was milled and grounded into powder (1.2kg) using Thomas Wiley machine (model 5 USA). The powdered plant sample (1 kg) was packed into a soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24h. The ethanolic extract was concentrated using a rotary evaporator at

40°C and then left on the bench to get reddish crude extract (62.10 g). The crude extract was partitioned between chloroform and water. A chloroform soluble fraction (20.8g) was obtained. 15g of the chloroform fraction were then partitioned between petroleum ether (60 - 80°C) and aqueous methanol. 4.0 g of the chloroform fraction was subjected to column chromatography over silica gel and eluted gradually with petroleum ether, petroleum ether-chloroform (90:10; 80:20; 70:30) to get a yellow solid 0.52g, yellow crystal 0.73 g and yellow oil 0.20 g.

The yield of yellow crystal (0.73g) was recrystallised from hexane afforded compound 1 yellow crystal solid (0.15g). Thin layer chromatography (chloroform: methanol 7:3) iodine vapour shows the presence of one band R_f (0.81). IR V_{max} 3350 Cm⁻¹ (N-H); 3122 cm⁻¹ (OH), 1697 cm⁻¹ (C = C aromatic), 1033 cm⁻¹ (C-N). HEREIMS m/z 241.1973 [m⁺] calculated for m/z 241 (C₁₀H₆O₆N) and m/z 154.0247 base peak calculated for C₇H₆O₄ (m/z 154). $^1\text{HNMR}$ and ^{13}C NMR were presented in Table 1.

Bioassay procedures

The *in vitro* antibacterial activity of compound 1 was carried out for 24 h culture of five selected bacteria. The bacteria organisms used were *Escheria coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia*. All the test organisms are clinical isolates of human pathogens obtained from the Federal Medical Centre (FMC) Umuahia, Nigeria.

Cultures were brought to laboratory conditions by resuscitating the organism in buffered peptone broth and thereafter agar medium and incubated at 37°C for 24 h. The antibacterial activity was performed by filter paper disc diffusion technique. The medium (7 g nutrient agar in 250 ml distilled water, auto claved at 115°C for 15 min) was cooled to 50°C. 20 ml of the medium was poured into a sterile Petri dish and allowed to solidify. It was allowed to stay for 8 h and observed for contamination. The sterility of the medium was tested. 1 g of compound 1 was dissolved in 1 ml of absolute ethanol and made up to 10 ml with distilled water to give a concentration of 100 mg/ml (10% dilution) a colony of each test organism was sub-cultured on nutrient broth and incubated at 37°C for 8 h. This was then used to flood the agar plates. Sterilized filter paper disc soaked in compound 1 was placed on the plates with test organisms. The plates were incubated at 37°C for 24 h. After incubation, plates were observed for zones of inhibition (in mm diameter). The minimum inhibitory concentration was determined by comparing the different concentrations of compound 1 having different zones of inhibition and selecting the lowest concentration.

The sensitivity susceptibility of the test bacteria to the standard drug was tested using inoculated agar plate and ciproflaxacin. The zones of inhibition were measured and compared with those of compound 1.

RESULTS AND DISCUSSION

The yellow crystal solid obtained after the column chromatography was re-crystallized from hexane to afford compound 1.

Compound 1 was identified as 2-methoxyamine 3,4,5,7-Tetrahydroxy anthocyanidines was assigned the molecular formula m/z 241.1973, calculated for C₁₀H₁₁O₆N (m/z 241) with base peak at m/z 154.0247 calculated for C₇H₆O₄ (m/z 154) on the basis of HREIMS. The IR spectrum revealed hydroxyl, aromatic and amine bands at (3122; 1697 and 1033 cm⁻¹) respectively. The relative molecular mass of 241.1973 with base peak at C₁₀H₁₁O₆N (m/z 241) with base peak at m/z 154.0247

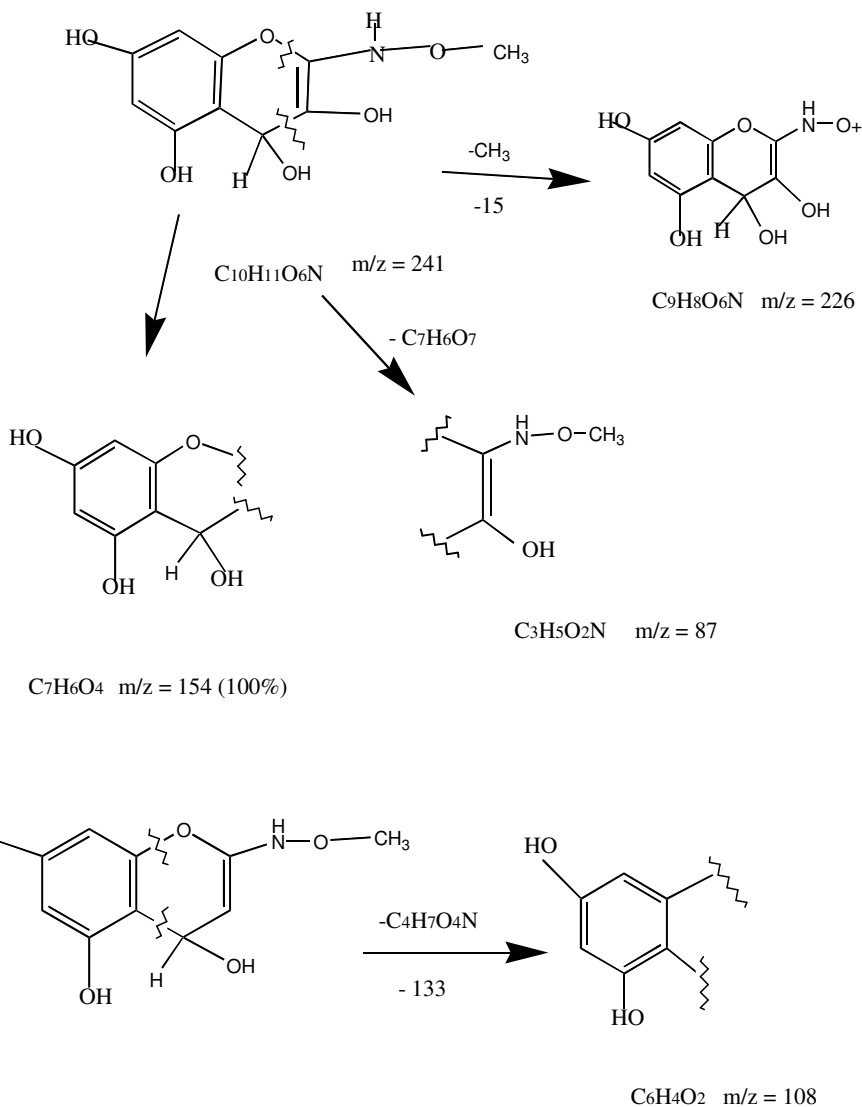


Figure 1. Fragmentation pattern of compound 1.

calculated for $C_7H_6O_4$ (m/z 154) on the basis of HREIMS. The IR spectrum revealed hydroxyl, aromatic and amine bands at (3122; 1697 and 1033 cm^{-1}) respectively. The relative molecular mass of 241.1973 with base peak at 154.0247 ($C_7H_6O_4$) confirm compound 1 as 2-methoxy amine 3,4,5,7 – Tetrahydroxy anthocyanidine. The pattern of fragmentation (Figure 1) showed that compound 1 undergoes cleavage or detachment to afford the base peak of $C_7H_6O_4$ (m/z 154.0247). Also, detachment of $C_{10}H_{11}O_6N$ from the compound affords the peak 109.0276 calculated for m/z 108 and loss of methyl group from the methoxy group produced the peak at 227.0364 calculated for m/z 226.

Analysis of the 1H NMR spectrum is shown in Table 1. Four signal protons appeared at δ 1.2005 (1Hs), 3.3102 (3Hs) 4.85277 (OH bs) and 7.06512 (2Hs). The 1H NMR spectrum showed the presence of methoxy group at δ

3.3109 (3Hs) and two aromatic protons at 7.06512 (2Hs). The N-H appeared at δ 1.26700 (1Hs) ^{13}C -NMR spectra showed that the aromatic at C_5 and C_7 appeared at (δC 110.304) have OH group attached to it. Also C_3 and C_4 appeared at δC 48.574 and 48.787 have OH group attached to them. The methoxy carbon appeared at δC 31.909 (Table 1). The paper reported the isolation and characterization of an anthocyanidine alkaloid 1 from the stem bark of *D. senegalense*. Several simple amine compounds exhibit antiplasmodial and antibacterial activities and are used as drugs (Sultana et al., 2006). Not only are the molecules potent central nervous system stimulants but also increase cardiovascular activity and raise body temperature and cause loss of appetite (Vollhardt and Schore, 1994). This may be the reason behind the use of the stem bark of *D. senegalense* not only as a preservative against bacteria but also a

Table 2. Diameter of zones of inhibition (mm) of Compound 1 isolated from *D. senegalese* stem bark and ciprofloxacin.

Test organisms	<i>D. senegalese</i> stem bark constituent (mg/100g)	Ciprofloxacin (mg/100g)
<i>Escherichia coli</i>	5.00 ± 0.01	11.00± 0.20
<i>Pseudomonas aeruginosa</i>	7.00 ± 0.20	26.00± 0.01
<i>Staphylococcus aureus</i>	7.00± 0.03	11.00± 0.10
<i>Klebsiella pneumonia</i>	9.00± 0.0.6	16.00± 0.02
<i>Proteus mirabilis</i>	8.00± 0.01	19.00± 0.05

Data are means ± standard deviation of triplicate determinations.

Table 3. Minimum inhibitory concentration of Compound 1 isolated from stem bark of *D. senegalese* on the pathogens mg/ml.

Pathogens	Concentration of stem bark isolate (100 mg/ml)					Mic mg/ml
	100	50	25	12.5	6.25	
	Zone of inhibition (mm)					
<i>Escherichia coli</i>	5.00	3.00	2.00	-	-	25
<i>Pseudomonas aeruginosa</i>	7.00	4.00	3.00	1.00	-	12.5
<i>Staphylococcus aureus</i>	7.00	3.00	2.00	-	-	25
<i>Klebsiella pneumonia</i>	9.00	2.00	2.00	-	-	25
<i>Proteus mirabilis</i>	8.00	7.00	3.00	2.00	-	12.5

Data are means of triplicate determinations

- No zone of inhibition.

stimulant.

The antibacterial activity of the compound isolated from the bark showed potent inhibition on some micro-organisms. The anthocyanidine alkaloid isolated from the stem bark of *D. senegalese* successfully inhibited *K. pneumonia*, *P. mirabilis*, *S. aureus*, *P. aeruginosa* and *E. coli* (Table 2). This compound isolated from the stem bark of *D. senegalese* has exhibited highest antibacterial activity against *K. pneumonia* and *P. mirabilis*. In general, the order of activity of the compound against the bacteria was: *K. pneumonia* > *P. mirabilis* > *P. aeruginosa* > *S. aureus* > *E. coli*. The minimum inhibitory concentration (mic) of the compound was 12.5 – 25 mg/ml (Table 3). *K. pneumonia*, *P. mirabilis*, *P. aeruginosa*, *S. aureus* and *E. coli* are human commensals and have been incriminated in the infection of wounds (Ijeh and Omodamiro, 2006). *P. mirabilis* and *E. coli* are the common cause of urinary track infection and travelers diarrhea (Jawetz et al., 1989; Okigbo and Ajalie, 2005; Okigbo and Omodamiro 2006). This finding supported the use of the stem bark of *D. senegalese* in the treatment of diarrhea and urogenital infections in herbal medicine (Keay et al., 1989; Burkill, 1995). The compound showed inhibition against *K. pneumonia*, *S. aureus* and *P. aeruginosa*. This supported the use of *D. senegalese* stem bark for the treatment of wounds for which *S. aureus* is associated (Okigbo and Omodamiro, 2006). The ability of this compound to inhibit this microorganism may be the reason behind the use of stem bark of *D. senegalese* in preserving palm wine by the natives in Nigeria. This result authenticates the use of

the plant in phytomedicine for the treatment of infections, disease preventions and as a preservative of local palm wine in South Eastern Nigeria.

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