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Journal of Science and Geosciences

Vol. 5 (3), pp. 22 - 26, May, 2017

Segregation, portrayal and antibacterial action screening of methoxyamine tetrahydroxyanthocyanidines from *Detarium senegalense* gmelin stem bark

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Abstract

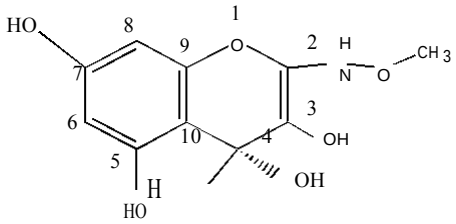
***Detarium senegalense* F. Gmelin (Leguminasae) is well-known Nigerian food and medicinal plant,commonly used in phytomedicine to cure diseases and heal injuries. The ethanol stem barks extract of *D. senegalense* affords an anthocyanidin alkaloid (2-methoxyamine 3,4,5,7 – tetrahydroxyanthocynadines). 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 ofinfections, 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 polysaccha-ride, 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 pre-pared as infusions or decoctions to treat veneral dis-eases, 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 powdered bark is widely taken to alleviate pains such as head-ache, 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 tuber-culosis. 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 medicomegical treatment for mental condi-tions 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-



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

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

δC		δH	
Position	**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^{o}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^{o}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 Rf (0.81) . IR Vmax 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 ($C_{10}H_6O_6N$) and m/z 154.0247 base peak calculated for $C_7H_6O_4$ (m/z 154). ^{1}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^{o}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^{o}C for 15 min) was cooled to 50^{o}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^{o}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 orga-nisms. The plates were incubated at 37^{o}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 $C_{10}H_{11}O_6N$ (m/z 241) with base peak at m/z 154.0247 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 $C_{10}H_{11}O_6N$ (m/z 241) with base peak at m/z 154.0247

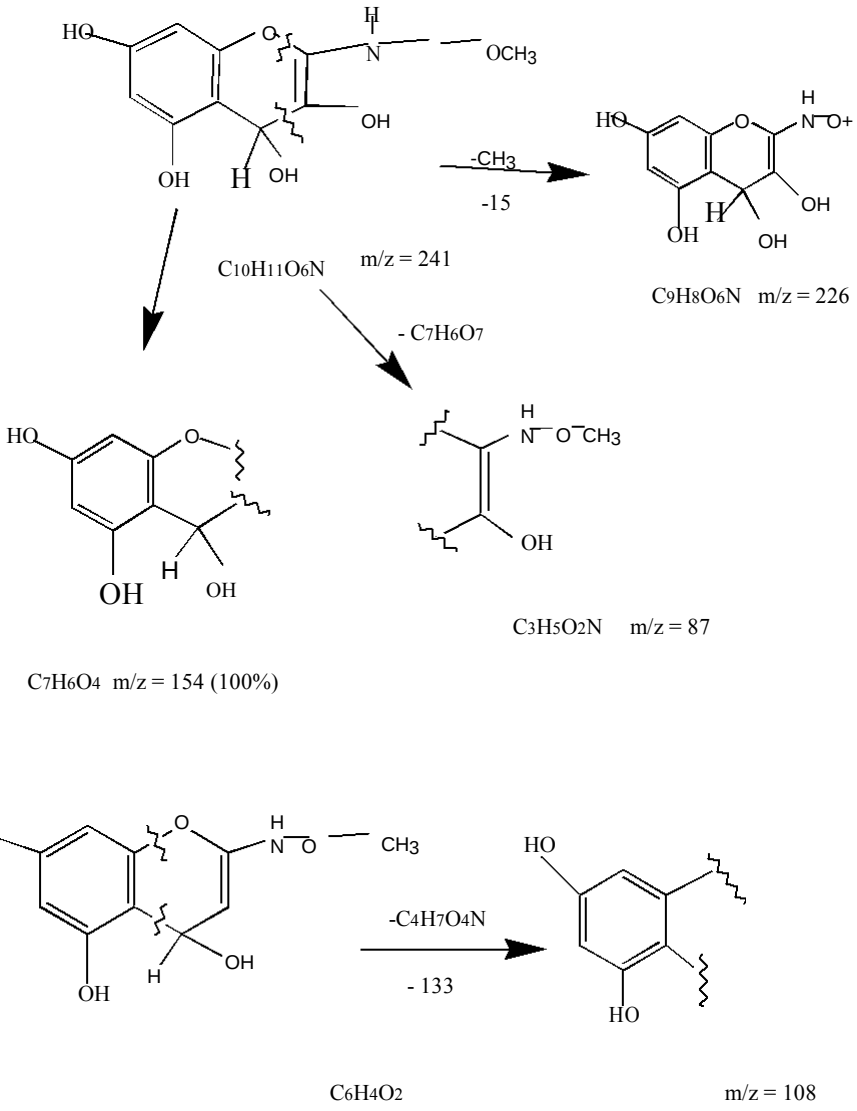

Figure 1. Fragmentation pattern of compound 1.

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 ^{1}HNMR 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 ^{1}HNMR 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	*D. senegalese* stem bark constituent (mg/100g)	Ciprofloxacin (mg/100g)
Escherichia coli	5.00 ± 0.01	11.00± 0.20
Pseudomonas aeruginosa	7.00 ± 0.20	26.00± 0.01
Staphylococcus aureus	7.00± 0.03	11.00± 0.10
Klebsiella pneumonia	9.00± 0.0.6	16.00± 0.02
Proteus mirabilis	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)					
Escherichia coli	5.00	3.00	2.00	-	-	25
Pseudomonas aeruginosa	7.00	4.00	3.00	1.00	-	12.5
Staphylococcus aureus	7.00	3.00	2.00	-	-	25
Klebsiella pneumonia	9.00	2.00	2.00	-	-	25
Proteus mirabilis	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. mirabillis, S. aureus, P. aeruginosa* and *E. coli* (Table 2). This compound isolated from the stem barkof *D. senegalese* has exhibited highest antibacterial activity against *K. pneumonia* and *P. mirabillis.* In general, the order of activity of the compound against the bacteria was: *K. pneumonia*>*P. mirabillis*>*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. mirabillis*, *P. aeruginosa, S. aureus* and *E. coli* are human commensals and have been incriminatedin the infection of wounds (Ijeh and Omodamiro, 2006). *P. mirabilis* and *E. coli* are the common cause of urinarytrack 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.senegalense* in the treatment of diarrhea and urogenitalinfections in herbal medicine (Keay et al., 1989; Burkill, 1995). The compound showed inhibition against *K. pneu-monia*, *S. aureus* and *P. aergunesa*. This supported theuse of *D. senegalense* 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. senegalense* 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.

ACKNOWLEDGEMENTS

We are grateful to Barrister Kingsley Edeh of Obafemi Awolowo University, Ile Ife Nigeria, Mrs. Adaku Asuoha of Mobil Eket Nigeria and Miss Nkechi Ibisi for their kind assistance in running the NMR, IR and Mass spectra of the samples.

REFERENCES

Abreu PM, Rosa VS, Araujo EM, Canda AB, Kayser O, Bindsei KG, Siems K, Seemann A (1998). Phytochemical analysis and antimicrobial evaluation of *Detarium Microcarpum* bark extracts. Pharmaceutical and Pharmacological letters 8 (3): 107-109.

Abreu PM, Martins ES, Kayser O, Bindseil KU, Siems K, Seemann A, Frevert J (1999). Anti-microbial, anti-tumor and anti-eishmania screening of medicinal plants from Guinea – Bissau.Phytomedicine 6(3): 187 – 195.

Abreu P, Relva A (2002). Carbohydrates from *Detarium microcarpum* bark extract. Carbohydrate Research. 337: 1663-1666.

Burkill HM (1995). The useful plants of West Tropical Africa Royal Botanic Gardens. London. 3: 101.

Dalziel JM (1995). The useful plants of West Tropical Africa. Crown Agents for overseas Government and Administration. London pp. 53.

Enwere NJ (1998). Foods of Plant origin. Afro-orbis Publications Ltd Nsukka Nigeria. Pp. 64 –65.

Ijeh II, Omodamiro OD (2006). Antimicrobial effects of aqueous and ethanolic fractions of some Local spices *Ocimum gratissimum* and *Xylopia aethiopica*. Recent Progress in Medicinal plants 13: 455

– 460.
Jawetz M, Adelberg EA, Brooks GF, Butel JS, Ornston LN (1989). Medical microbiology. 18th Edu. Prentic – Hall. International UK London. p. 592.
Keay RWJ, Phil D, Biol FT (1989). Trees of Nigeria. Oxford University Press, New York. Pp. 204-207.
Okigbo RN, Ajalie AN (2005). Inhibition of some human pathogens with tropical plants extracts *Chromlanena Odorata* and *Citrus aurantifolia* and some Antibiotics. International Journal of Molecular Medicine and Advance Sciences 1 (1): 34 – 40.
Okigbo RN, Omodamiro OD (2006). Antimicrobial effects of leaf extracts of Pigeon Pea Cajamus Cajan (L) millop on some human pathogens. J. Herbs, Spices, Medicinal Plants 12: 117-127.
Sultana GN, Rose SA, Burandt CL (2006). Two new alkaloids from *Zanthoxylum Syncarpum*. J. med. Aromatic Plant Sci. 28: 580-584.
Vollhardt KC, Schore NE (1994). Organic Chemistry WH Freeman and Company New York Pp. 825-826.
Wang Q, Ellis PR,Ross-Morphy SB, Grant JS (1996). A new polysaccharides from a Traditional Nigerian plant food: *Detariumsenegalense Gmelin.* Carbohydrate Research 284: 229-239.
Wang Q, Ellis PR, Ross-Morphy SB, Burcjard W (1997). Solution characteristics of the xyloglucan extracted from Detarium senegalense Gmelin. Carbohydrate polymers 31: 115 – 124.