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Full Length Research Paper

Isolation, characterization and antibacterial activity of alkaloid from *Datura metel* Linn leaves

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A new antibacterial agent 5^{1}, 7^{1} dimethyl 6^{1}– hydroxy 3^{1}, phenyl 3 α - amine β - yne sitosterol 1 has been isolated from *Datura metel* leaves. The structure of 1 was established using ^{13}C, ^{1}H NMR, IR and MS spectroscopic data. Compound 1 displayed antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabis, Solmonella typhi, Bacillus subtilis* and *Klebsiella pneumonia* but could not inhibit *Escherichia coli*. This result supported the use of *Datura metel* in phytomedicine for the treatment of asthma, cough, burns and healing of wounds in Nigeria.

Key words: *Datura metel*, sterol alkaloid, antibacterial agent, phytomedicine.

INTRODUCTION

Datura metel Linn *(*Thorn-apple, Devil trumpet, Solana-ceae) is a Nigerian medicinal plant widely used in phyto-medicine to cure diseases such as asthma, cough, convulsion and insanity (Duke and Ayensu, 1985; Dabur et al., 2004). The leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, anti-tussive, bronchodilator and as hallucinogenic (Duke and Ayensu, 1985). The whole plant particularly the leaves and seeds are used as anesthetic, anodyne, anti-asthma-tic, antispasmodic, anti-tussive, bronchodilator, and hallu-cinogenic (Duke and Ayensu 1985; Ali and Shuab, 1996; Dabur et al., 2004). The plant finds application in the treatment of catarrh, diarrhea and skin diseases (Chopra et al., 1968, 1986). It is used in the treatment of catarrh, diarrhea, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and treachitis (Dabur et al., 2004).

A variety of phytochemicals have been found to occur in *D. metel*. These phytoconstituents comprises alkaloids, flavonoids, phenols, tannins, saponins and sterols. The solanaceous alkaloids hyoscyamine and scopolamines have been isolated from *D. metel* (Chopra et al., 1986, Oliver-Bever, 1986). Hyoscyamine is the most commonly occurring alkaloid in the solanaceae family and has been associated with varying quantities of hyoscine and in rare cases with traces of atrophine. Ali and Shuaib (1996) isolated a steroidal constituent daturasterol from the leaves of the plant. *D. metel* is an active ingredient in the decoction used presently by herbalists in Eastern Nigeria for the treatment of gonorrhea, asthma, cough, skin ulcers, burns and wounds (Dabur et al., 2004).

Several studies (Okwu and Morah, 2006; 2007a; 2007b; Okoli et al., 2007) have documented the scientific basis for the efficacy of plants in phyto- medicine. This study seeks to ascertain the usefulness of *D. metel* in the treatment of infectious conditions caused by common pathogens. The study involves the isolation, structural elucidation and characterization of the bioactive consti-tuents in the plant and consequently evaluates the anti-bacterial activity against some pathogenic bacteria for possible development of new drugs for the prevention and treatment of infections.

MATERIALS AND METHODS

General experimental procedure

The IR spectra were determined on a Thermo Nicolet 470 FT – IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 FT spectrometer for ^{1}H NMR and 75 FT spectrometer for ^{13} C NMR, using TMS as internal standard. Chemical shifts are expressed in parts per million (ppm) . LC – ESIMS spectra were determined in the positive ion mode on PE Bio-system API 165 single quadruple instruments ; HRESIMS (positive ion mode) spectra were recorded on a Thermofiniga MAT

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Table 1. ^{1}H and ^{13}C NMR Data of 5^{1}, 7^{1}, dimethyl 6^{1} hydroxyl 3^{1} phenyl 3 amine -yne sitosterol.

Position	δ H	δ C
1	1.2083 2Ht	42.512
2	1.2254 2Hm	36.598
3	1.40566 1Hm	59.674
4	1.40566 1Hd	125.582
5		128.241
6	1.58980 1Hd	129.037
7	1.45310 1Ht	130.442
8	1.40566 1Hs	48.575
9	1.43569 1Hs	48.783
10		49.004
11	1.22540 2Hm	36.598
12	1.2083 2Ht	34.364
13		48.575
14	1.43569 1Hs	48.575
15	1.22540 2Hs	48.362
16	1.2083 2Ht	48.362
17	1.43569 1Hs	48.578
18	0.87425 3Hs	16.225
19	1.14465 3Hs	16.228
20	1.40566 1Hs	49.001
21	1.43569 1Hd	125.582
22	1.58980 1Hd	125.582
23	1.40566 1Hm	48.362
24	0.89052 3Hd	25.644
25	0.89784 3Hd	25.644
26	1.19096 3Hd	25.042
NH	4.82631 1Hd	
1^{1}		75.210
2^{1}		75.210
3^{1}		128.241
4^{1}	6.66482 1Hs	143.81
5^{1}		129.037
6^{1} OH	3.3805 1H(bs)	130.44
7^{1}		128.24
8^{1}	6.66482 1Hs	148.00
9^{1}	1.17521 3Hs	27.386
10^{1}	1.17808 3Hs	26.723

S = singlet, bs = broad singlet, t = triplet, m = multiplet, d = doublet.

95 XL mass spectrometer. Column chromatography was carried out with silica gel (200 - 300 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25 mm thick silica gel 60 F_{254} aluminum plates 20 x 20 cm Merck, Darmstadt, Germany. Reagents and solvents like ethanol, chloroform, diethyl-lether and hexane were all of analytical grades and procured from Merck. TLC aluminum sheets, silica gel $60F_{254}$ where also purchased from Merck. The nutrient agar was purchased from Scharian Chemie APHA Spain.

Plant materials

Fresh leaves and mature fruits of *D. metel* were harvested from Botanical Garden, Michael Okpara University of Agriculture, Umudike, Nigeria, on 6th February, 2007. Plant samples (fruits, seeds and leaves) were identified by Dr. A. Nmeregini of Taxonomy Section, Forestry Department of the University. A voucher specimen No. DM/122 was deposited at the Forestry Department Herbarium of the University.

Extraction and isolation of plant materials

Plant materials were treated and analyzed at the Chemistry laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves (2 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (1.3 kg) 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 24 h. The ethanolic extract was concentrated using a rotary evaporator at 40°C and then left on the bench to get reddish crude extract (48.5 g). The crude extract was partitioned between chloroform and water. A chloroform soluble fraction 26.8 g was obtained. 15 g of the chloroform fraction were then partitioned between petroleum ether (60-80°C) and aqueous methanol.4 g of the chloroform fractions 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 brown solid 0.48 g, brown oil 0.20 g and green solid 0.52 g. The yield of brown solid (0.48 g) was re-crystallized from hexane afforded compound 1 brown solid (0.21 g). Thin layer chromatography (Chloroform: methanol 7:3) iodine vapour shows the presence of one band Rf (0.72) IR Vamx 3420 cm^{-1} (OH) 2920 cm^{-1} (CH_2), 2853 cm^{-1}(CH_2); 1623 cm^{-1} (C=C – aromatic), 1456 cm^{-1} (NH); 1059 cm^{-1} (CO), HEREIMS m/z 523.4727 (M^{+}) calculated for $C_{36}H_{46}O_2N$ (m/z 524) and m/z 95.0510 base peak calculated for C_7H_{11} (m/z 95). ^{1}HNMR and ^{13}C NMR are shown in Table 1.

Bioassay procedures

The *in vitro* antibacterial activity of Compound 1 was carried out for 24 h culture of seven selected bacteria. The bacteria used were five Gram-negative organisms: *Proteus mirabis*, *Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, and Escherichia coli* and two Gram-positive strains comprising *Staphylococcus aureus* and *Bacillus subtilis*. 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 (Scharlan chemie) and thereafter nutrient agar (peptone 5 g/l and meat extract 3 g/ml) and incubated at 37°C for 24 h.

The antibacterial activity was performed by a filter paper disc diffusion technique. The medium (7 g nutrient agar in 250 ml distilled water, autoclaved at 115°C for 15 min.) was cooled to 50°C. The medium (20 ml) was poured into a sterile Petri dish and allowed to solidify, set for 8 h then observed for contamination. The sterility of the medium was tested using autoclave 121°C 15 psi for 15 min. Compound 1 (1 g) was dissolved in 1 ml of absolute ethanol and made up to 10 ml with distilled water to give a concentration of 100mg/ml (10% dilution). A colony of each test organism was sub-cultured on nutrient broth which contains peptone (5 g/l and meat extract 3 g/l) and incubated aerobically at 37°C for 8 h. 30 ml of the nutrient broth was used to flood the agar plates. A sterilized Whatman No. 1 filter paper disc soaked in compound 1 (0.02 ml) was used to test for the sensitivity or antimicrobial effect

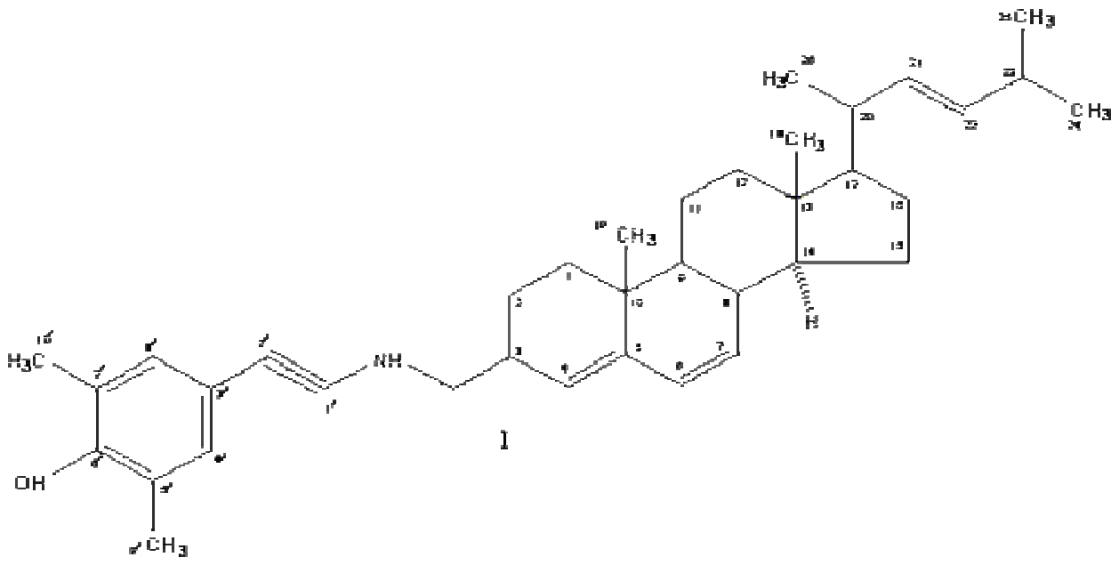

Figure 1. Compound 1 [$C_{36}H_{46}O_2N$].

of compound 1 isolated from *D. metel.* 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. The sensitivity susceptibility of the test bacteria to standard drug was tested using incubated agar plate and ciproflo-xacin. The zones of inhibition of ciprofloxacin on the test organisms were measured and compared with those of compound 1 of the same concentration.

Statistical analysis

All measurements were replicated three times and standard deviations determined. The student t-test at $P < 0.05$ was applied to assess the difference between the means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Compound 1 has a molecular formula of $C_{36}H_{46}O_2N$ as established by HREIMS (Figure 1). The ^1H NMR spectrum revealed the presence of four tertiary methyls (δH 0.8742, 1.1446, 1.1752 and 1.1780), three secondary methyls (δH 1.4056, 1.4356, 1.4531 and 1.5898). The ^1H NMR spec-trum of the two angular tertiary methyl groups (C_{18}, C_{19}) resonate as singlet at δH 0.8905 and 1.1446 respectively. The C_{18} methyl protons resonate up field compared to the C_{19} methyl groups, which resonate downfield. The C_{24} and C_{25} secondary methyl protons attached to C - 23 methine group give rise to a doublet at δ 0.8905 and 0.8978. The other aromatic tertiary methyl group protons at C_9^1 and C_{10}^1 appeared as a singlet at δ 1.175 and 1.178.

The presence of the aromatic ring is easily established and identified by the IR characteristics signal Vmax 1623 Cm^{-1} and ^1H and ^{13}C spectra. The ^1H spectrum give the aromatic proton at δH 6.66482 and the ^{13}C spectrum give the resonance at δC 125.58 (C_3^1), 143.81 (C_4^1), 129.03 (C_5^1), 130.44 (6^1), 128.24 (C_7^1) and 148. (C_8^1).

The C_1^1 and C_2^1 indicate the triple bond carbon and appeared at δC 75.21 respectively. The amine proton re-sonates as singlet at δH 4.82 and the OH proton ap-peared as broad singlet at δH 3.3805. The high-resolution mass spectrum afforded the molecular mass calculated for C_{36} $H_{46}O_2N$ (m/z 524). The mass spectrum apart from the molecular ion peak at (m/z 523.4727 $[m^+]$) showed fragments at m/z 95.0510 base peak, corresponding to C_7H_{11}. There are also fragments peaks at m/z 272.2115 and 271.2081 respectively corresponding to $C_{19}H_{28}O$ (M-1). In this case proton migration and rearrangement occurs. The fragmentation pattern of compound 1 is shown in Fi-gure 2. The IR spectrum showed peaks at Vmax 3420 cm^{-1} (OH), 2926 cm^{-1} (CH), 2853 cm^{-1} (aliphatic C-H stre-tching) and 1059 cm^{-1} (C-O) stretching. The paper re-ported the isolation and characterization of a new steroi-dal alkaloid 5^1, 7^1 dimethyl 6^1 hydroxy 3^1 phenyl 3 α-amine β-yne sitosterol from the leaves of *D. metel*. The compound exhibited anti-bacterial activity *in vitro* against a wide range of pathogenic microorganisms (Table 2). The compound successfully inhibited *P. aeruginosa, B. subtilis, S. typhi, K. pneumonia, S. aureus* and *P. mirabis* but could not inhibit *E. coli*. *P. aeruginosa and B. subtilis* were found to be more sensitive to compound 1. Many of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man (Esimone et al., 2008).

These micro-organisms are infections of wounds and boils (Duguid et al., 1985; Ijeh and Omodamiro, 2006). Evaluation of the effect of compound 1 on clinically

Table 2. Diameter of zones of inhibition (mm) of 5′, 7′, dimethyl 6′ hydroxyl 3′ phenyl 3 amine -yne sitosterol and ciproflaxacin®.

Test organisms	Concentration of 5′, 7′, dimethyl 6′ hydroxyl 3 amine - yne sitosterol on the pathogens mg/ml 100mg/ml	Ciprofloxacin® 100mg/ml
Proteus mirabis	5.0 ± 0.10^{d}	35.0 ± 0.01^{a}
Klebsiella pneumonia	7.0 ± 0.20^{d}	12.0 ± 0.10^{c}
Pseudomonas aeruginosa	11.0 ± 0.20^{c}	14.0 ± 0.01^{c}
Staphylococcus aureus	6.0 ± 0.02^{d}	25.0 ± 0.11^{b}
Escherichia coli	-	-
Salmonella typhi	7.0 ± 0.11^{d}	23.0 ± 0.10^{b}
Bacillus Subtilis	10.0 ± 0.02^{c}	30.0 ± 0.20^{a}

Data are means ± standard deviation of triplicate determinations. Values with superscript that are the same in each row are not significantly different at ($P < 0.05$).
- No inhibition.

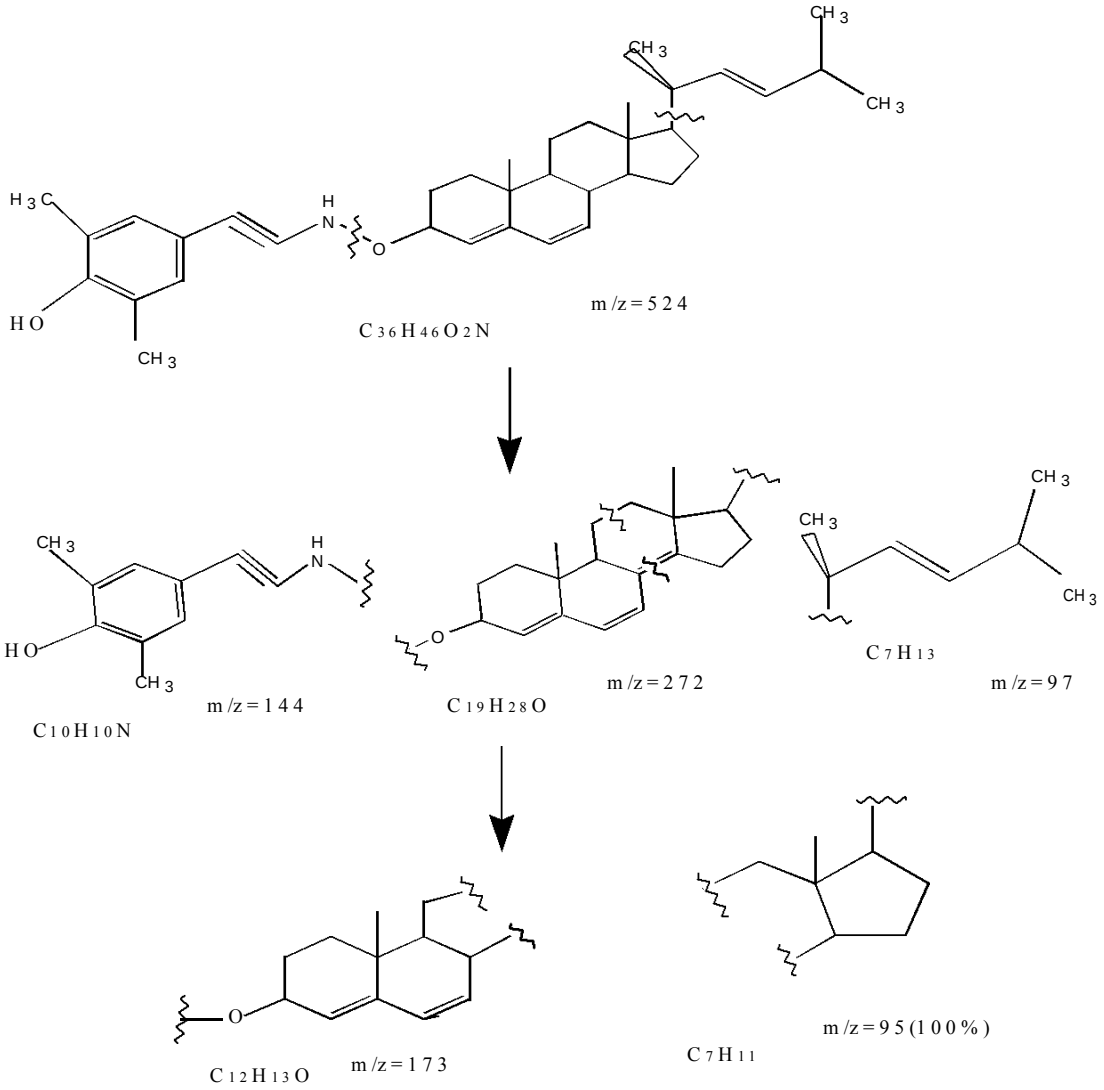

Figure 2. Fragmentation pattern of compound 1.

Table 3. Minimum inhibitory concentration of 5^{I}, 7 ^{I}, dimethyl 6 ^{I} hydroxyl 3 amine -yne sitosterol on the pathogens mg/ml.

Pathogens	Concentration of 5^{I}, 7 ^{I}, dimethyl 6 ^{I} hydroxyl 3 amine -yne sitosterol on the pathogens mg/ml				MIC mg/ml
	50	25	12.5	6.25	
Proteus mirabis	4.0	3.0	1.0	-	12.5
Klebsiella pneumonia	6.0	4.0	1.0	-	12.5
Pseudomonas aeruginosa	8.0	4.0	1.0	-	12.5
Staphylococcus aureus	4.0	2.0	-	-	25.
Salmonella typhi	5.0	2.0	-	-	25.
Bacillus subtilis	7.0	4.0	2	-	12.5

Data are means of triplicate determinations.
- No zone of inhibition.

isolated microbial contaminants of boils, wounds and sores showed varying levels of inhibitory activity on these pa-thogens (Table 2). The inhibition effect of these pathogenic organisms may be the reason behind the use of *D. metel* in herbal medicine for the treatment of asthma, cough, catarrh, diarrhea, gonorrhea and skin diseases (Nadkarni, 1976; Duke and Ayensu, 1985).

The spectrum of activity of inhibition of compound 1 when compared with standard conventional drug (ciprofloxacin®) is relatively narrow (Table 2). However, the level of activity is still good as inhibiting concentration at 100 mg/ml. The minimum inhibitory concentration (mic) of the compound was 12.5-25 mg/ml (Table 3). *P. mirabis* and *S. aureus* are the common cause of urinary track infections and travelers diarrhea (Jawetz et al., 1999; Okigbo and Omodamiro, 2006). Compound 1 cause varying degrees on inhibition of the growth of these pathogens. This finding supported the use of the leaves of *D. metel* in the treatment of diarrhea and urogenital infections in herbal medicine (Duke and Ayensu, 1985; Barefort, 1992). Compound 1 showed inhibition against *K. pneumonia, S. aureus* and *P. aergunosa*. These findings supported the use of *D. metel* leaves for the treatment of wounds for which these pathogens are associated (Okigbo and Omodamiro, 2006). The leaves of *D. metel* possess phyto- constituents capable of inhibiting the growth of microbial wound contaminants; accelerate wound healing and consequently resulting to cell proliferation.

This study demonstrates that *D. metel* possess antibacterial activities. These findings justify the traditional use of *D. metel* in phyto-medicine. The isolated compound from *D. metel* can be used by pharmaceutical firms for drug formulation.

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