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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, Solanaceae) is a Nigerian medicinal plant widely used in phytomedicine 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, antitussive, bronchodilator and as hallucinogenic (Duke and Ayensu, 1985). The whole plant particularly the leaves and seeds are used as anesthetic, anodyne, anti-asthmatic, antispasmodic, anti-tussive, bronchodilator, and hallucinogenic (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 constituents in the plant and consequently evaluates the antibacterial 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 $5^{/}$, $7^{/}$, dimethyl $6^{/}$ hydroxyl $3^{/}$ phenyl 3α amine β-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

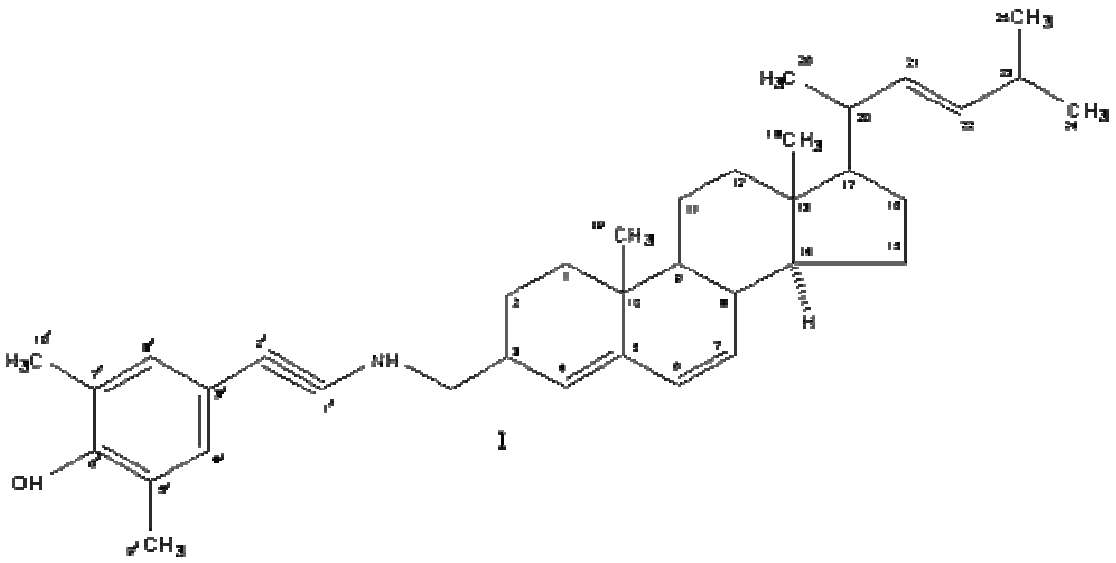


**Figure 1.** Compound 1 [$C_{36}H_{46}O_2N$].

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 ciprofloxacin. 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 resonates as singlet at δH 4.82 and the OH proton appeared 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 Figure 2. The IR spectrum showed peaks at Vmax 3420 $cm^{-1}$ (OH), 2926 $cm^{-1}$ (CH), 2853 $cm^{-1}$ (aliphatic C-H stretching) and 1059 $cm^{-1}$ (C-O) stretching. The paper reported the isolation and characterization of a new steroidal 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$^{l}$, 7$^{l}$, dimethyl 6$^{l}$ hydroxyl 3$^{l}$ phenyl 3α amine β-yne sitosterol and ciproflaxacin®.

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

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.

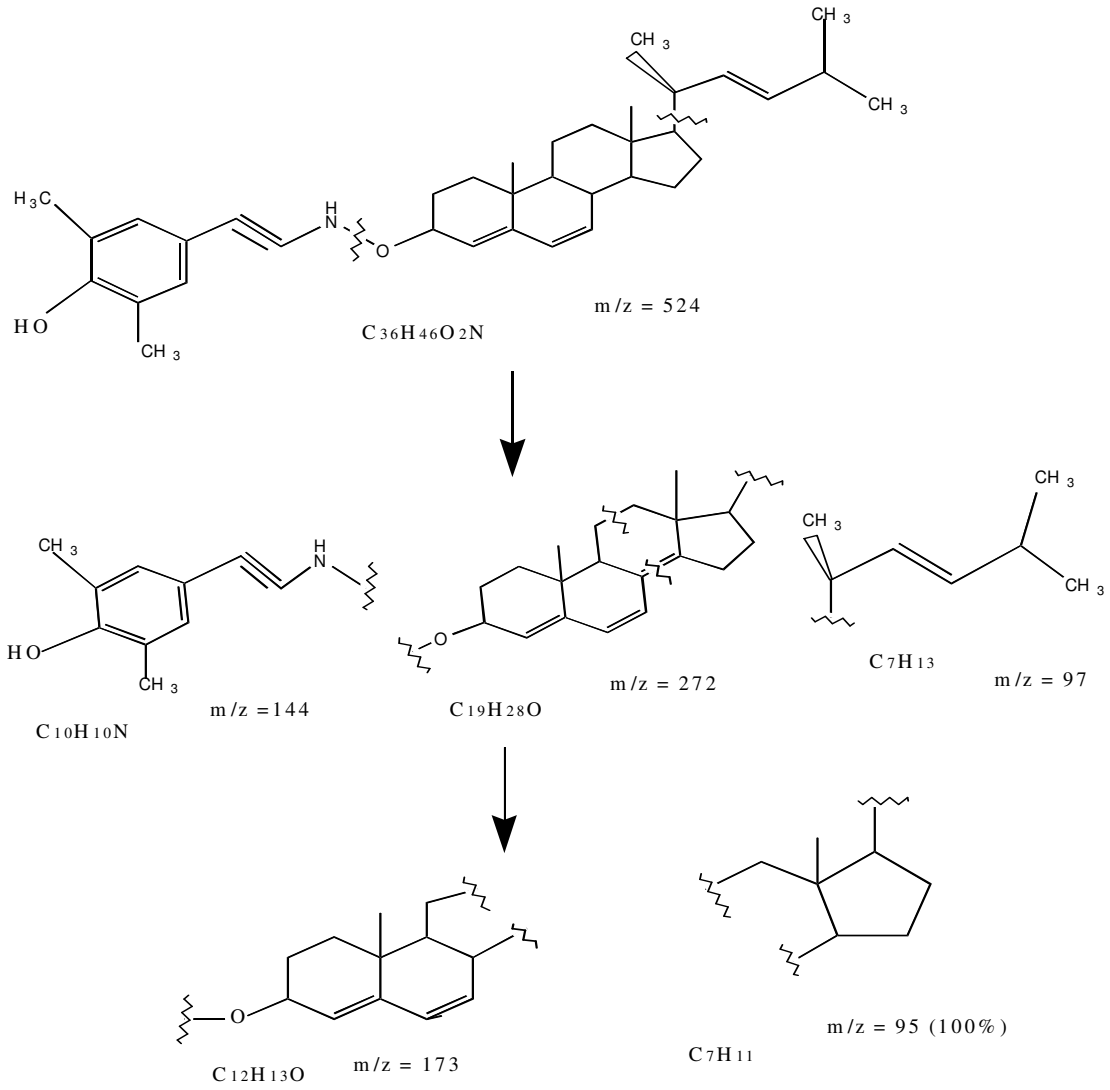


**Figure 2.** Fragmentation pattern of compound 1.

**Table 3.** Minimum inhibitory concentration of 5′, 7′, dimethyl 6′ hydroxyl 3α amine β-yne sitosterol on the pathogens mg/ml.

| Pathogens | Concentration of 5′, 7′, dimethyl 6′ hydroxyl 3α amine β-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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