Antimicrobial activity of 1,2-benzenedicarboxylic acid, butyldecyl ester isolated from the seeds and pods of Acacia nilotica Linn

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Accepted 13 March, 2016

ABSTRACT

The antimicrobial activity of 1,2-benzenedicarboxylic acid, butyldecyl ester isolated from Acacia nilotica was determined using standard methods. The compound was isolated by directing the fractionation of ethyl acetate extract of the air dried seeds and pod with microbial sensitivity test. The results of the antibacterial screening showed that the ethyl acetate extract of Acacia nilotica Linn exhibited the highest activities against the test microbes with zones of inhibition diameter ranging from 27-32mm against Salmonella typhi, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Candida krusei and Shigella dysentriae. The structure of the compound was identified from ¹³C NMR, ¹H NMR, IR and GC-MS spectral data. The isolation, structural elucidation, NMR spectral assignment and bioactivities are reported.

Keywords; Acacia nilotica, structural elucidation, antimicrobial activity

INTRODUCTION

The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world (Kathe, 2005). They observed that about 74% of 119 plant-derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care. Over hundreds of years, herbal medicines derived from medicinal plants minerals and organic matter is still the mainstay of about 75–80% of the world’s population for health care marketed and gaining popularity in developed and developing countries. Herbs have medicinal property due to presence of different active principles like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols (Anees, 2010).

It is against this background that Acacia nilotica extensively used as herbal preparation in some parts of Nigeria were investigated. It is commonly called Bagarwa in Hausa and Booni by the Yorubas in Nigeria and is used in the treatments of intestinal pains, diarrhea, nerve stimulant, cold, congestion, coughs, dysentery, fever, haemorrhages, leucorrhea, ophthalmia and sclerosis. The plants were found to be of medicinal importance among traditional medicine practitioners in the tropics, including West Africa. On a wider dimension, the disease causing organism commonly found in the affected sites of the patients is the target of this research. In this circumstance they are Salmonella typhi, Escherichia coli, Streptococcus faecalis, Staphylococcus.
Table 1. Results of the zone of inhibition diameter (mm) of fraction 33 against the test microbes

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>1000(µg/cm³)</th>
<th>500(µg/cm³)</th>
<th>250(µg/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella dysentriae</em></td>
<td>22</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>16</td>
<td>9</td>
<td>NI</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>24</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>20</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>28</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>29</td>
<td>27</td>
<td>20</td>
</tr>
</tbody>
</table>

Key; NI means No Inhibition.

*aureus, Candida krusei* and *Shigella dysentriae*. The aim of this research is to determine the antimicrobial potentialities of *Acacia nilotica* and isolate the active compound responsible for the activity.

**MATERIALS AND METHODS**

**Sample collection**

The seeds and pods of *Acacia nilotica* were collected in Nasarawa state. It was identified by Dr Ajibade, at the Herbarium of Biological Sciences, Faculty of Science, Nigerian Defence Academy and assigned a voucher number of 403.

**Extraction**

A portion (100 g) of the ground plant parts was separately percolated in 300 cm³ each of methanol, ethyl acetate, chloroform and petroleum ether each for two weeks. The extracts were separately filtered and evaporated on rotary evaporator at 40°C. The marc was repercolated with the recovered solvents for an additional one week. The extracts were drained, filtered and combined with the previous residue and evaporated on rotary evaporator. Each extract was cooled, weighed and stored in the refrigerator until needed (Garba and Okeniyi, 2012).

**Column chromatography of ethyl acetate fraction**

Ethyl acetate fraction that showed higher activity in most of the tested microbes was subjected to column chromatography. A portion (20g) of the fraction was dissolved in 80mls of ethylacetate and mixed with 15g of silica gel. It was evaporated to dryness in a water bath. The dried extract and silica gel were loaded on the column together with 10g of Celite. The column was first eluded with 6:4 Ethyl acetate: Petroleum ether. This was followed by 8:2 Ethyl acetate: Petroleum ether, 100% Ethyl acetate, 1:1 Ethyl acetate: Methanol and finally 100% Methanol. Each portion collected were evaporated using rotary evaporator (Cannell, 1998). A total of 88 fractions were collected. These fractions were subjected to TLC and similar fractions were pooled together.

**Thin Layer Chromatography (TLC)**

Fraction 33 was one of the samples that gave a single spot on the TLC plates with an Rf value of 0.65cm using 8:2 Petroleum ether: Ethylacetate, Rf of 0.75cm using 7:3 Petroleum ether: Ethylacetate and 0.5cm using 9:1 Petroleum ether: Ethylacetate.

**Antimicrobial activity**

The isolates of microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria from which the zone of inhibition, Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of fraction 33 were determined against the collected isolates of *Salmonela typhi, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Candida krusei* and *Shigella dysentriae*.

**Procedure**

The antimicrobial activities of the pure compound were determined using agar well diffusion methods as described by Navorro et al. (1996); Okeke et al. (2001).

**RESULTS AND DISCUSSION**

The results of sensitivity test showed that fraction 33 possess different activities against all the test microbes with zone of inhibition diameter of 20, 16, 28, 24, 29 and 22 mm against *Salmonela typhi, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Candida krusei* and *Shigella dysentriae* respectively at a concentration of 1000 µg/ml (Table 1). This showed that the fraction could be used for the treatment of various
Table 2. The Minimum Inhibition Concentration (MIC) of fraction 33 against the test microbes

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>µg/cm² (10⁻³)</th>
<th>µg/cm² (10⁻³)</th>
<th>µg/cm² (10⁻³)</th>
<th>µg/cm² (10⁻³)</th>
<th>Control</th>
<th>µg/cm² (10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sd</td>
<td>10,8,6,4,2,1.05</td>
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<td>Sd</td>
<td>10,8,6,4,2,1.05</td>
</tr>
<tr>
<td>Ec</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>Ec</td>
<td><em>,-,</em>,+,#,^</td>
</tr>
<tr>
<td>Sa</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>Sa</td>
<td><em>,-,</em>,+,#,^</td>
</tr>
<tr>
<td>St</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>St</td>
<td><em>,-,</em>,+,#,^</td>
</tr>
<tr>
<td>Sf</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>Sf</td>
<td><em>,-,</em>,+,#,^</td>
</tr>
<tr>
<td>Ck</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>Ck</td>
<td><em>,-,</em>,+,#,^</td>
</tr>
</tbody>
</table>

**Key:** - means no colour change
* means MIC
+ means colon growth (light pink)
^ means moderate colon growth
# means high colon growth
Control is Ampicloxacinil, St; Means Salmonela typhi, Ec means Escherichia coli, Sf means Streptococcus feacalis, Sa means Staphylococcus aureus, Ck means Candida krusei and Sd means Shigella dysentiae

Table 3. The Minimum Bactericidal Concentration (MBC) of fraction 33 against the test microbes

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<th>Test organisms</th>
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<th>Control</th>
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<td>Sd</td>
<td>10,8,6,4,2,1.05</td>
</tr>
<tr>
<td>Ec</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>Ec</td>
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</tr>
<tr>
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<td><em>,-,</em>,+,#,^</td>
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<td><em>,-,</em>,+,#,^</td>
<td><em>,-,</em>,+,#,^</td>
<td>Sa</td>
<td><em>,-,</em>,+,#,^</td>
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<tr>
<td>St</td>
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<tr>
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<td>Ck</td>
<td><em>,-,</em>,+,#,^</td>
</tr>
</tbody>
</table>

**KEY:** - means no colony growth, * means MBC/MFC, + means scanty colony growth, ^ means heavy colony growth. Control is Ampicloxacinil, St; Means Salmonela typhi, Ec means Escherichia coli, Sf means Streptococcus feacalis, Sa means Staphylococcus aureus, Ck means Candida krusei and Sd means Shigella dysentiae

diseases caused by the tested microbes. The results of the MIC for this fraction showed no colour change at 1000µg/cm³ for all the microbes (Table 2). The lowest concentration where there is no colour change (MIC value), ranges from 4×10⁻² - 8×10⁻⁵ µg/cm³. The results of the MBC showed the MBC value ranges from 10×10⁻² - 8×10⁻⁵ µg/cm³ (Table 3). However, Fraction 33 recorded a broad spectrum of antimicrobial activities against the test microbes. Fraction 33 had a weight of fraction of 0.44g with an Rf value of 0.75cm leading to the isolation of a pure compound whose spectral identity showed that it is 1,2-benzenedicarboxylic acid, butyldecyl ester. The compound boils at 887K.

**Infra-red analysis**

The IR spectrum showed a strong absorption at 3,456.55cm⁻¹ which is due to a O-H band, 1,649.19cm⁻¹ which is due to carbonyl stretching (G=0), 1,194.94cm⁻¹ is due to the presence of C-O-C stretch of an ester, 2104.41 cm⁻¹ is due to the presence of C=C of an aromatic ring. 1433.16 is due to the presence of methyl and methylene groups. 532.37 cm⁻¹ indicates the presence of C-H bending of an aromatic ring. 2,930 cm⁻¹ indicates the presence of C-H stretching. The presence of methyl groups were further confirmed by the presence of a peak at 1257.63 cm⁻¹. (Williams and Fleming). Figure 2.

**GC-MS Analysis**

The GC/MS gave the molecular weight of the molecule as 362. The most stable signal occur at m/z 223 which correspond to the loss of C₆H₄⁺ from the molecular ion, the signal at 208 correspond to the loss of CH₃⁺ and the signal at 167 correspond to the loss of C₃H₆⁺. The signal at 149 corresponds to the base peak and is due to the loss of H₂O molecule. The signal at 121 is due to the loss of C₂H₅⁺. The signal at 76 is due to the loss of COOH⁺ radical. Taking all the above information into consideration, compound 33 is interpreted as 1,2-benzenedicarboxylic acid, butyldecyl ester. Figure 2.
The $^{1}H_{\text{NMR}}$ and $^{13}C_{\text{NMR}}$ of compound 33, obtained by analyzing the sample on Agilent –NMR- vnmrs 400. The $^{1}H_{\text{NMR}}$ recorded signals at $\delta$2.421 which are methyl protons and integrates into three (3) methyl protons (C8 and C21), $\delta$3.68 are methylene protons that integrates to 26 methylene protons (C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20) and $\delta$6.826 which integrates to four (4) aromatic protons and one (1) carboxylic proton (C1, C2, C3, C4 and C7). Figure 3.

$^{13}C_{\text{NMR}}$ Analysis

The $^{13}C_{\text{NMR}}$ spectrum had signals recorded at $\delta$168.002 which correspond to a carboxylic carbon and carbonyl carbon (C7 and C7' respectively). The signal at $\delta$145.712 corresponds to the two quartenary carbon (C5 and C6). The signal at $\delta$138.352 is aromatic carbons (C1 and C4),
Figure 3. $^1$H NMR of fraction 33

δ120.834 correspond to the aromatic carbons (C2 and C4) and the signals at 109.051 corresponds to the carbon at position 12 (methine group). The signal at δ41.471 corresponds to the methylene carbons attached (C11 and C13); the signal at δ40.123 corresponds to the methylene carbon (C10 and C14); the signal at δ39.926 corresponds to the methylene carbons attached (C11 and C13); the signal at δ40.123 corresponds to the methylene carbon (C10 and C14); the signal at δ39.926 corresponds...
to the methylene carbons (C9 and C20); the signal at δ39.713 corresponds to the methylene carbons (C19 and 18); the signal at δ39.501 corresponds to the methylene carbons (C17 and C16); the signal at δ39.296 corresponds to the methylene carbon (C15); the signal at δ38.879 corresponds to the methyl carbons (C8 and C21). Figure 4 and 5.

CONCLUSION

The ethyl acetate extracts of the seeds and pods of Acacia nilotica Linn were found to have higher activities against the test microbes. Chromatographic separation and thin layer chromatography carried out on it led to the isolation of a compound with a boiling point of 887K. Structural elucidation using 1H NMR, 13C NMR, IR and GC-MS showed that the compound is 1,2-benzenedicarboxylic acid, butyldecyl ester.

ACKNOWLEDGEMENT

Authors acknowledge the contribution of Alhaji Muh’d Munir, all the laboratory technologist of Chemistry Department laboratory and staffs of Chemistry Department, Nigerian Defence Academy, Kaduna for their contributions towards the success of this research work.

REFERENCES