ANTIMICROBIAL ACTIVITIES OF COMPOUNDS ISOLATED FROM PYCNANTHUS ANGOLENSIS (WELW.) WARB AND BRYOPHYLLUM PINNATUM (LAM) OKEN

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In the present study a semi-pure residue each from Pycnanthus angolensis (Welw.) Warb and Bryophyllum pinnatum (Lam) for the presence of biologically active compound(s) were investigated. This exercise led to two compounds whose identities have been established to be 1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-pyridine carboxylic acid ethyl ester (1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-nicotinic acid ethyl ester) (5-ethoxycarbonyl, NG-5a) and 1-ethoxy-2-hydroxy-4-proplylengluacetol (vanitrole, KF-1a), respectively using MS and IR spectral techniques. NG-5a was proved to be bacteriostatic against Escherichia coli but recorded no activity against Staphylococcus aureus and Candida albicans. KF-1a recorded only minimal activity against S. aureus but demonstrated no activities against E. coli or C. albicans.

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Introduction

The seeds of P. angolensis are rich in palmitic, linoleic and linolenic acids as useful precursors in phytochemical biogenetics. Furthermore, myristoleic acid (potent anti-arthritis agent) and tocotrenols (antioxidant and anti-inflammatory agents) have been obtained from the plant. Also, four compounds namely, 3-ethoxy-3,7-dimethyl-1,6-octadiene (ethyl linoleol), 1,2-benzenedicarboxylic acid diethyl ester (diethyl phthalate), ethyl cineamate and 9-oximino-2,7-dioxyfluorene (2,7-dioxyfluorene-9-one oxime) have also been isolated from the ethyl acetate fraction of its leaves by column chromatography (CC) and preparative thin-layer chromatography (p-TLC) respectively.

The presence of cardiac glycosides, alkaloids, terpenes and tannins has also been indicated in B. pinnatum. In addition, a steroid, 3-hydroxy-(3β,17β)-Spiroandrost-5-ene-17,1'-cyclobutan-2'-one has been isolated from the butanol fraction of this plant by preparative thin-layer chromatography (p-TLC). In continuation of work on these plants, residues coded NG-5 and KF-1 obtained from previous studies were subjected to preparative thin-layer chromatography (p-TLC) with the aim of isolating more compound(s) from the plants and as well as evaluating the antimicrobial activities of compound(s) so obtained.

Experimental

Preparation of plates

Similar 20 x 20 cm glass plates were washed in detergent solution, rinsed with water and air-dried. Silica gel (Sigma-Aldrich, USA) was treated with CaSO₄ (Bond Chemicals, Nigeria) which served as a binding agent. The slurry obtained there from was vigorously shaken, thereby making it homogenous and free flowing. A thickness of 0.5 mm of the slurry was uniformly applied across the glass plates and allowed to set for 24 h. The coated plates were then activated in a laboratory oven (Gallenkamp, England) at 60°C for at least 10 h prior to use.

Isolation of NG-5a

In order to isolate NG-5, the residue (deep brown, 65 mg) was painstakingly dissolved in some methanol and applied across the coated silica plate using a micro Pasteur pipette (Simax, India) 1 cm above the bottom edge of the plate and then allowed to dry. Afterward, the plate was developed in toluene:(CH₃)₂CO:CH₃OH (40:80:4) inside a large chromatographic glass tank (Pyrex, USA). The obtained chromatogram showed two excellently resolved layers which were carefully scrapped, separately filtered with methanol and concentrated in vacuo on a rotary evaporator (R205D, shensung BS & T, China). The pure sub-fractions were monitored on commercial silica plates in toluene:(CH₃)₂CO:CH₃OH (10:20:1) and (CH₃)₂CO:EtOAc (35:65) using FeCl₃/CH₃OH, Dragendorff’s and vanillin-H₃SO₄ as spray reagents. Further TLC evaluations indicated a spot in NG-5a (yellow compound, R₁ (0.22), 21 mg), C₆H₁₂NO₂, MS (ES): m/z 197 (M⁺, 43.17 %), 179 (M–H₂O⁺, 4.39 %), 151 (M–OC₃H₇–H⁺, 78.71 %), 139 (M–OC₃H₇–N⁺, 9.21 %), 123 (M–OC₃H₇–CO–H⁺, 88.49 %), 110 (M–OC₃H₇–NO⁻, 8.69 %), 95 (M–OC₃H₇–CO–CH₃–OH⁻, 51.26 %), 83 (M–OC₃H₇–CO–CH₃–OH⁻, 45.78 %) 69 (M–OC₃H₇–CO–CH₃–N–OH⁻, 62.02 %) and 42 (M–OC₃H₇–CO–CH₃–N–OH⁻, 100.00 %). FTIR: 717, 863 (alkyl substitution), 1076 (C–O–C), 1621 (C=C), 1715 (C=O), 1732 (C=O), 3456 (NH) and 3567(Ar–OH) cm⁻¹.

Isolation of KF-1a

The KF-1 residue (yellow, 47 mg) was dissolved in some methanol and applied across the coated silica plate using a micro Pasteur pipette (Simax, India) 1 cm above the bottom edge of the plate and then allowed to dry. Afterward, the plate was developed in toluene: (CH₃)₂CO:CH₃OH (40:80:4)
inside a large chromatographic glass tank (Pyrex, USA).
The obtained chromatogram showed three layers which were
carefully scrapped, separately filtered with methanol
and concentrated in vacuo on a rotary evaporator (R205D,
Shensung BS & T, China).

The pure sub-fractions were monitored on commercial
silica plates in toluene:(CH$_3$)$_2$CO:H$_2$O (10:20:1) and
(CH$_3$)$_2$CO:EtOAc (35:65) using FeCl$_3$:CH$_3$OH and vanillin-
H$_2$SO$_4$ as spray reagents. Further TLC evaluations indicated
a spot in KF-1a (amorphous pale yellow solid, R$_f$(0.61),
0.18 mg).

**KF-1a:** C$_{14}$H$_{10}$O$_4$, MS (ES) m/z 178 (M$^+$ (100.00 %), 161
(M-OH)$^+$ (5.16 %), 149 (M-C$_3$H$_3$)$^+$ (54.91 %), 131 (M-
OC$_3$H$_7$-2H)$^+$ (38.87 %), 121 (M-OC$_2$H$_5$:OH+5)$^+$ (7.33 %),
103 (M-C$_6$H$_4$:OH)$^+$ (30.29 %), 91 (M-OC$_3$H$_7$-CH$_2$-OH-10)$^+$
(20.81 %), 77 (M-C$_6$H$_5$:OH -7)$^+$ (27.89 %), 66 (M-C$_6$H$_7$-
OH-CH$_3$-3)$^+$ (12.82 %) and 55 (M-C$_6$H$_5$:OH-CH$_2$:C$_2$H$_3$)$^+$
(21.80 %). FTIR: 767, 823 (alkyl substitution), 1056 (-C-O-
C), 1618 (Ar-C=C), 1642 (exocyclic -C=C) and 3312 (Ar-
OH) cm$^{-1}$.

**Structural elucidation**

The mass spectra of the compounds were obtained on
Kratos MS 80 (Germany) while the infra-red analyses were
done on Shimadzu FTIR 8400S (Japan).

**Antimicrobial screening**

The microorganisms used in this study were limited to
each microorganism was introduced into each petri-
plate agar diffusion method (100 %) shows the removal of ethox-
and identified as a

It is pertinent to note that this could be due to partial
esterification of 3-pyridine carboxylic acid in ethanol during
extration. In addition, **NG-5a** tested positive for the ferric
chloride and Dragendorff's reagents indicating the presence
of a hydroxyl group and an alkaloidal nucleus respectively.

Due to the nature of its matrix, many fragmented ions
could be seen in the mass spectrum of the compound. Those
that are easily identifiable include (M$^+$ at m/z 197 (43.17 %)
while the peak at 179 (4.39 %) indicates the loss of water
from the matrix. However, the fragments at 151(79.71 %)
and 139 (9.21 %) represent the removal of ethoxy and ethoxy
and nitrogen units respectively from the molecule.
Furthermore, ions at 123 (88.49 %), 110 (28.69 %),
95(51.26 %) and 83 (45.78 %) correspond to the excisions of
ethoxy and carboxyl, ethoxy, carboxyl and nitrogen and
ethoxy, carboxyl, methyl and hydroxy groups respectively
from **NG-5a**. The most abundant ion (base peak) at 42
(100 %) shows the removal of ethoxy, carboxylate, methyl,
nitrogen and hydroxyl units from the molecular matrix.

The IR spectrum of the compound shows characteristic
stretching bands at 717, 863, 1070, 1621, 1715, 1732, 3450
and 3567 cm$^{-1}$ indicating alkyl substituions, ether linkage,
diendocyclic -C=C, carboxyl, -NH and aromatic hydroxyl
absorptions respectively.

The chemical structure of **KF-1a** was established by a
combination of spectroscopic techniques as highlighted
above. These data were matched with those in the library
data of organic compounds and were found to be consistent
with those in literature. Consequently, **KF-1a** (Figure 2) has
been identified to be 1-ethoxy-2-hydroxy-4-prophenyl
guaethol (vanitrope).

**Results and discussion**

3-Pyridine carboxylic acid (3-nicotinic acid) and its
derivatives are well-known compounds and can easily be
identified by their MS and IR spectra. **NG-5a** was isolated
and identified as an ethyl ester derivative of 3-nicotinic acid.

**Figure 1.** 1,6-Dihydro-2-methyl-4-hydroxy-6-oxo-3-pyridine

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Compound Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KF-1a</strong></td>
<td>Amorphous pale yellow solid, R$_f$(0.61), 0.18 mg.</td>
</tr>
<tr>
<td><strong>NG-5a</strong></td>
<td>Ethyl ester derivative of 3-nicotinic acid.</td>
</tr>
</tbody>
</table>

The experiments were carried out in triplicates. The plates
were labeled on the underside and left at room temperature
for 2 h to allow for diffusion. The plates were then
incubated at 37± 2 °C for 24 to 48 h. Zones of inhibition
were measured in mm with the aid of a ruler.
Table 1. Antimicrobial screening of crude extract, ethyl acetate fraction, NG-5a and KF-1a at different concentrations on test microbes in water.

<table>
<thead>
<tr>
<th>Test microbe</th>
<th>CE/CE 20 mg mL⁻¹</th>
<th>ET/BT 10 mg mL⁻¹</th>
<th>NG-5a 2 mg mL⁻¹</th>
<th>KF-1a 2 mg mL⁻¹</th>
<th>deionized water</th>
<th>SP 10 μg mL⁻¹</th>
<th>NY 1 μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>6</td>
<td>6</td>
<td>6.5</td>
<td>7.5</td>
<td>6</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>E. coli</td>
<td>6</td>
<td>6</td>
<td>11.5</td>
<td>6</td>
<td>6</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>C. albicans</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. 1-Ethoxy-2-hydroxy-4-prophenyl guaethol (KF-1a).

Due to the nature of the matrix, many fragmented peaks appeared in the MS of the compound. Those that are easily identifiable include (M)⁺ which shows as the most abundant ion (base peak) at m/z 178 (100.00 %) while fragments at 161 (51.16 %) and 149 (54.41 %) represent the loss of hydroxy and ethyl groups from the molecule, respectively. Furthermore, the ions at 103 (30.29 %), 77 (27.89 %) 66 (12.82 %) and 55 (21.80 %) indicate the disintegration of the molecular matrix by the excisions of phenyl and some smaller units such as methyl and hydroxy from (M)⁺. However, peaks at 131 (38.87 %) and 121 (7.33 %) reveal the removal of ethoxy groups from the compound.

The IR spectrum of KF-1a shows absorptions at 767, 823, 1056, 1618, 1642 and 3312 cm⁻¹ indicating diagnostic alkyl substitutions, an ether linkage (-C-O-C), aromatic C=C, exocyclic -C=C and aromatic -OH functional groups respectively. It should be noted that KF-1a was isolated with a sweet fragrance characteristic of oils, spices, perfumes and food additives.

Antimicrobial tests

The spectrum of microbes employed in these tests was narrow, encompassing one each of gram-positive (S. aureus) and gram negative (E. coli) bacterial strains and a fungus (C. albicans). The results presented in Table 1 show that the crude extracts and fractions were inactive against S. aureus, E. coli and C. albicans. However, NG-5a was appreciably bacteriostatic against E. coli. It but recorded no activities against S. aureus and C. albicans. KF-1a was minimally active against S. aureus but recorded no activities against E. coli or C. albicans. The activity given by NG-5a against E. coli has importance because of the resistance of this microorganism against known antimicrobial agents. This resistance is believed due to the presence of a three-layered envelope which does not allow permeation of external agents. The compounds demonstrated no antifungal activity against C. albicans.

Conclusion

The isolation of these compounds is being reported for the first time from the plants. Hence, NG-5a and KF-1a are expected to serve as chemotaxonomic markers for both plants. Furthermore, the results of the antimicrobial screening lend some justification to the uses of these plants especially in the treatment and management of some bacterial infections. However, the compounds will be screened against other bacterial and fungal strains in the future with the aim of obtaining better activities.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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