Research Article

Activity of Aristolochia bracteolata against Moraxella catarrhalis

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A bioassay-guided fractionation of methanol extract of Aristolochia bracteolata whole plant was carried out in order to evaluate its antimicrobial activity and to identify the active compounds in this extract. Antibacterial and antifungal activities of methanol extract against gram-positive, gram-negative, and fungal strains were investigated by the agar disk diffusion method. Among the strains tested, Moraxella catarrhalis and sea urchin-derived Bacillus sp. showed the highest sensitivity towards the methanol extract and hence they are used as test organisms for the bioassay-guided fractionation. From this extract, aristolochic acid 1 (AA-1) has been isolated and has showed the greatest antibacterial activity against both standard strain and clinical isolates of Moraxella catarrhalis with equal minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 25 and 50 μg/mL. Modification of the AA-1 to AA-1 methyl ester completely abolished the antibacterial activity of the compound and the piperonylic acid moiety of AA-1 which suggested that the coexistence of phenanthrene ring and free carboxylic acid is essential for AA-1 antibacterial activity.

1. Introduction

Moraxella catarrhalis is a gram-negative, aerobic diplococcus human mucosal pathogen which causes middle ear infections in infants and children [1–3], and it is one of the three major causes of otitis media along with Streptococcus pneumonia and Haemophilus influenzae [4]. Although Moraxella catarrhalis is frequently found as a commensal of the upper respiratory tract, recently it has emerged as a genuine pathogen and is now considered an important cause of upper respiratory tract infections in healthy children and elderly people, lower respiratory tract infections in adults with chronic obstructive pulmonary disease [1, 5], and hospital-acquired pneumonia [6]. Amikacin, cefixime, fosfomycin, cefuroxime, cotrimoxazole, doxycycline, and erythromycin resistant strains of Moraxella catarrhalis were isolated and the widespread production of a β-lactamase enzyme renders the bacterium resistant to the penicillin [7–9].

This has led to the search for new and effective therapeutic alternatives among natural compounds. Plants remain an important source of diverse chemical entities which have been used as drugs or provide scaffolds from which new drugs have been derived [10]. The selection of a suitable candidate species for investigations can be done on the basis of long-term use by humans. This approach is based on
an assumption that the active compounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use [11]. *Aristolochia* is an important genus in the family of *Aristolochiaceae* and is widespread across tropical Asia, Africa, and South America. *Aristolochia bracteolata* is commonly called “worm killer” in English due to supposed anthelmintic activity and trypanocidal effect [12]. It is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery, and snakebites [13]. *Aristolochiaceae* has been used by Sudanese people as analgesic, antiscorpion, and antisnake. It is also used in the treatment of tumors and malaria and for fevers [14], but its usage as an antimalarial is not recommended in its crude form. *Aristolochia bracteolata* showed a definite positive effect on wound healing, with significant increase in the level of powerful antioxidant enzymes. Its root and leaves were bitter and anthelmintic and are medicinally important. Almost every part of the plant has medicinal usage [15]. The whole plant was used as a purgative, antipyretic, and anti-inflammatory. It also possesses a potent antiallergic activity [16]. Organic solvent extracts of the plant showed antibacterial activities while the water extract showed antifungal activity [17]. The plant also showed promising antiarthritic activity [18]. Although *Aristolochia* has been used for thousands of years in many cultures for many indications due to its various pharmacological activities, it was later discovered that consuming these plants can certainly be dangerous. The genus of *Aristolochia* contains a naturally carcinogenic compound AA which has been shown to be the cause of so-called Chinese herb nephropathy or AA nephropathy [19, 20], and mutations in the cells of people who consume it, causes more mutations than two of the best-known environmental carcinogens: tobacco smoke and UV light [21, 22]. There are many cases of nephropathy reported in the literature caused by the systemic and long term application of Chinese snakeroot (*Aristolochia fangchi*); this highlighted the risk of using preparations which contain aristolochic acids [23].

Although *Aristolochia* is being known in many countries that is containing a toxic compound AA, but this has not stopped it from being a popular herbal remedy for thousands of years. It is still extensively used in India and in traditional Chinese medicine for slimming, menstrual symptoms, and rheumatism. It is also widespread used in Sudan and other African countries as one of the most effective herbal remedies for infectious diseases. Therefore, it was our objective to assess the potential antimicrobial activity of *Aristolochia bracteolata* using a bioassay-guided fractionation, in order to produce pure compound that can act as the lead compound in developing new, safe, and effective drug to replace the use of the harmful crude plant material.

2. Materials and Methods

2.1. Materials

2.1.1. General. Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F254s plates (Merck) were used for thin-layer chromatography (TLC) analysis. High resolution FAB-MS and ESI-MS were recorded on JEOL JMS700N and JMS-100TD, respectively. H- and C-NMR, H COSY, NOESY, HSQC, and HMBC spectra were recorded with a Unity plus 500 spectrometer (Varian Inc., U.S.A.) operating at 500 MHz for H and 125 MHz for C, respectively. H-NMR chemical shifts are expressed in values referring to the solvent peak H, 2.49 for DMSO and coupling constants are expressed in Hz. C-NMR chemical shifts are expressed in values referring to the solvent peak C, 39.5 for DMSO. Piperonylic acid was purchased from commercial sources (TCI) and used without further purification.

2.1.2. Plant Material. The plant material (whole) was collected in the period from (October to December 2012) from Khartoum state in Sudan. The plant was kindly identified and authenticated by the Taxonomist Dr. Haider Abdelgadir and Mr. Yahia Mohammed, Medicinal and Aromatic Plants Research Institute (MAPRI).

2.1.3. Test Microorganisms


(b) Clinical strains: *Moraxella catarrhalis*, *Bacillus cereus*, *Aeromonas hydrophila*, *Salmonella typhi*, *Vibrio cholerae*, and *Yersinia enterocolitica*.

2.2. Extraction of Plant Material. The air-dried powdered whole plant (200 g) was exhaustively extracted with cold maceration method with sufficient quantity of 70% methanol for 7 days at room temperature. The methanolic extract was passed through Whatman number 1 filter paper (Whatman England) and the concentrated extract (40 g) was digested with 100 mL distilled water and successively partitioned with n-hexane (4 × 400 mL), chloroform (3 × 400 mL), ethyl acetate (5 × 400 mL), and n-butanol (2 × 400 mL). Each fraction was concentrated under reduced pressure to a constant weight to give the corresponding n-hexane fraction (0.4 g), chloroform...
The inoculated plates. These plates were incubated for 24–
were impregnated with individual extract were placed on
inoculated on nutrient agar plates supplemented with 2%
mined by disk diffusion method [25, 26]. Fungi strains were
measured by millimeter scale. The experiment was replicated
clearly active and pure compound AA-1 (150 mg).

2.4.2. Antifungal Assay. The antifungal activity was
determined by disk diffusion method [25, 26]. Fungi strains were
impregnated with the plant extract (1–4 mg) and
pure compound (10–100 µg) were placed aseptically over the
bacterial culture on nutrient agar plates. After incubation at
37°C for 24 hours, the zone of inhibition around the discs was
measured by millimeter scale. The experiment was replicated
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2.4.1. Antibacterial Assay. The antibacterial activity was
tested by agar disc diffusion assay [24]. Suspension of the
tested bacteria (100 µL of 10^8 cfu/mL) was spread onto solid
media plates. The sterile paper discs (6 mm in diameter)
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3. Results and Discussion

3.1. Antimicrobial Activity. Initial steps in new drug discovery
involve identification of new chemical entities, which can be
either sourced through chemical synthesis or can be isolated
from natural products through biological activity guided
fractionation. Bioassay-guided fractionation of the identified
plant may lead to standardized extract or isolated bioactive
lead compounds as the new drug [11].

The whole plant of Aristolochia bracteolata was extracted
successively with MeOH and subjected to liquid-liquid frac-
tionation with n-hexane, chloroform, ethyl acetate, and n-
butanol. The resulting fractions were tested for antibacterial
and antifungal activities. The crude extract and chloroform
fraction were significantly active against sea urchin-derived
Bacillus sp. and both standard strain and clinical isolates of
Moraxella catarrhalis and were moderately active against S.
aureus, B. subtilis, and P. aeruginosa. The n-hexane fraction
had moderate activity against S. aureus and B. subtilis while
ethyl acetate fraction showed moderate activity against P.
aeruginosa and B. subtilis. All fractions were active against
sea urchin-derived Bacillus sp. (Table 1). The crude extract
failed to inhibit the growth of all test fungi in addition to the
following bacterial strains: Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Streptococcus pyo-
genes, Streptococcus agalactiae, Staphylococcus epidermidis,
Neisseria lactamica, Enterobacter cloacae, Bacillus cereus,
Aeromonas hydrophila, Salmonella typhi, Vibrio cholerae, and
Yersinia enterocolitica.

The chloroform soluble fraction was therefore selected for
further chromatographic separations and resulted in the
isolation of known compound AA-1 (Figure 1). AA-1
showed strong activity against Moraxella catarrhalis (stand-
ard strain and clinical isolates) and sea urchin-derived
Bacillus sp. (Table 2), with equal MIC and MBC values of
25 and 50 µg/mL. Both the piperonyl alcohol moiety of AA-1
(Figure 1) and AA-1-methyl ester showed no activity against
cellulase (Table 2), which suggests that the coexistence of
phenanthrene ring and free carboxylic acid is essential for
AA-1 antibacterial activity.

3.2. Structure Elucidation of AA-1. Bioassisted fractionation of
methanolic extract of Aristolochia bracteolata led to isolation
of AA-1 and its structure was elucidated by interpretation of
Figure 1: Structure of AA-1, AA-1 methyl ester, and piperonylic acid.

Table 1: Antibacterial activity of crude plant extract and fractions.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Crude extract</th>
<th>n-Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>n-Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>15</td>
<td>12</td>
<td>16</td>
<td>12</td>
<td>—</td>
</tr>
<tr>
<td>Marine Bacillus sp.</td>
<td>25</td>
<td>18</td>
<td>25</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>S. aureus</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>10</td>
<td>—</td>
<td>11</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>12</td>
<td>—</td>
<td>14</td>
<td>9</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2: Antibacterial activity of AA-1 and its derivatives.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>AA-1</th>
<th>AA-1 methyl ester</th>
<th>Piperonylic acid</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine Bacillus sp.</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>M. catarrhalis T</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>M. catarrhalis 1 CI</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>M. catarrhalis 2 CI</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>M. catarrhalis 3 CI</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>M. catarrhalis 4 CI</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>M. catarrhalis 5 CI</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>19</td>
</tr>
</tbody>
</table>

T: type strain; CI: clinical isolate.

Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies and an examination of their effects on beneficial normal microbiota [10].

In spite of the fact that herbal remedy is a mixture of many chemicals in unknown doses and might result in unpleasant side effects, many people believe that treatments that are natural are somehow magically safe and effective. Aristolochia is used in traditional medicine for the treatment of various diseases [13, 15], including those associated with bacteria. This study showed clearly that the excellent effect of Aristolochia in treating such diseases is due to the toxic compound AA-1. Although AA-1 is highly effective in killing M. catarrhalis, it is ineffective against the other microorganisms tested. This highlights the importance of M. catarrhalis in discovering the cellular target of AA-1 and the mechanism of AA-1 toxicity. The widespread use of Aristolochia is not sufficient to ensure that it is effective or even that it is safe. Therefore, hit-to-lead exploration...
is necessary to identify related compounds with low toxicity, low cost, and improved potency that can replace the use of the harmful crude plant material.

It is impossible to ban the use of these remedies, especially in the rural areas in Sudan and other African countries; therefore, we strongly recommend educating the public of the risks versus the benefits of Aristolochia and gradually replacing them with either economical new drugs or standardized extracts and homogenous batches of other plant material with known levels of safe active constituents.

4. Conclusion

Using bioassay-guided fractionation technique, the present study directly linked the antibacterial activity of Aristolochia bracteolata to the AA-1. Although AA-1 had strong activity against M. catarrhalis, it had a narrow spectrum of activity than expected based on the activity of the crude extract from which it was isolated or from its traditional usage. This may be the result of synergism between different compounds in the complex extracts or may be due to placebo effect.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References


