Constituents of Acacia nilotica (L.) Delile with Novel Kinase Inhibitory Activity

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Detailed descriptions of the isolation, structural characterization, and protein kinase assays are available online at http://www.thieme-connect.de/products as Supporting Information.

ABSTRACT
Acacia nilotica (L.) Delile belongs to the genus Acacia, which includes about 1400 species in subtropical and tropical Africa including Nigeria, Senegal, Egypt, and Mozambique as well as Asia from India to Burma. This plant is traditionally used to treat several pathologies such as mouth, ear, and bone cancer. Moreover, it possesses many other biological activities (antidiarrheal, anti-inflammatory, antimicrobial, and antifungal). We report here the extraction, purification, and identification of two known compounds [ethylgallate and (+)-catechin] from the bark of the tree that were further tested for their inhibitory activities against a panel of disease-related protein kinases. Both compounds were active, and (+)-catechin showed the best activity by inhibiting nine out of fourteen protein kinases with an IC50 value in the µg/mL range. This compound gave the highest activity against CLK1 with an IC50 of 2.1 µg/mL. The ethyl acetate extract and its components, such as catechins and other polyphenols, which also had protein kinase inhibitory activity, can be exploited in the research for anticancer agents.

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The genus Acacia includes some 1400 species of trees and shrubs widespread throughout warm and semiarid regions of the world including Nigeria. Acacia nilotica (L.) Delile belongs to the subgenus Acacia [1, 2]. It grows naturally in tropical Africa including Nigeria, Senegal, Egypt, and Mozambique as well as Asia from India to Burma, where it was probably introduced. A. nilotica is a shrubby tree, 5–20 m high and is widely used in traditional medicine in Africa (▶ Fig. 1a). In the traditional Hausa ethnomedicine of Northern Nigeria, the leaves and bark are used to treat diarrhea and inflammation [3]. While naringenin, and several galloyl and catechin derivatives have been isolated from the bark [4, 5], flavonol glycosides from the seeds [6], antimicrobial, antifungal, and anti-inflammatory properties have been reported previously [7–10]. Studies regarding the Fabaceae family (i.e., those of A. nilotica) have shown that 106 of their phytochemicals possess activities related to cancer treatment. Amongst them, A. nilotica compounds were described for anticancer activity and for cancer preventive activity [11].
Previously, we have isolated two new peltogynoids from the stem bark of this plant [12]. In the present study, as part of our continuing chemical exploration from the species *A. nilotica*, we report here the isolation of two phenolic compounds. For the first time, ethyl gallate and (+)-catechin were tested against a panel of 14 protein kinases for their potential inhibitory activity.

Bark samples of *A. nilotica* were collected in Zaria, Nigeria, in July 2015, as described previously [12]. After an ethanolic extraction, chloroform, ethyl acetate, and n-butanol fractions were produced and two major compounds from the ethyl acetate fraction were purified (compounds 1 and 2). Compound 1 is a pale white solid corresponding to a known compound ethyl gallate (MW = 198.17) according to various types of spectroscopy analyses. The chemical structure of the purified compound 1 is depicted in Fig. 1b.

Compound 2 is a pale yellow powder. It was validated and confirmed as the known compound (+)-catechin (MW = 290.26) according to the spectroscopy analysis. The chemical structure of the purified compound 2 is depicted in Fig. 1b.

UV, NMR, and MS of these compounds were consistent with that reported in the literature for ethyl gallate [13, 14] and (+)-catechin [15, 16].

A primary screening of the extracts and isolated compounds against 14 disease-related protein kinases was undertaken. Table 1 and Fig. 2a show the results of the primary screening of the extracts and compounds against a panel of 14 protein kinases. The
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result indicates the activity that remains in the tube after treating the mentioned kinases with 50 µg/mL of the extracts and compounds compared to the control assay treated with DMSO. The results revealed that the chloroform extract showed the worst inhibition against the tested kinases (e.g., 50 µg/mL of the chloroform extract inhibits only 10% of the total CDK5 kinase activity). In contrast, the ethyl acetate extract is highly active against the Haspin kinase: 50 µg/mL of the extract inhibits 99% of the maximal kinase activity. A similar trend was observed for the n-butanol soluble fraction.

Table 1. Primary screening of fractions from A. nilotica and purified compounds against 14 disease-related protein kinases.

<table>
<thead>
<tr>
<th>Extract/compound</th>
<th>CDK2/CLink</th>
<th>CDK5/CLink</th>
<th>CDK9/CLink</th>
<th>Mm CLK1</th>
<th>Ssc GSK-3</th>
<th>Pf Gly</th>
<th>Rn Dyrk1A</th>
<th>Pim1</th>
<th>Haspin</th>
<th>RIPK3</th>
<th>Mm CK1</th>
<th>Ld TUK</th>
<th>Ssc CK1</th>
<th>AuroraB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>87</td>
<td>90</td>
<td>67</td>
<td>44</td>
<td>71</td>
<td>74</td>
<td>81</td>
<td>61</td>
<td>45</td>
<td>114</td>
<td>75</td>
<td>65</td>
<td>73</td>
<td>52</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>37</td>
<td>25</td>
<td>11</td>
<td>10</td>
<td>15</td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td>25</td>
<td>20</td>
<td>50</td>
<td>9</td>
<td>-2</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>59</td>
<td>60</td>
<td>15</td>
<td>18</td>
<td>11</td>
<td>9</td>
<td>17</td>
<td>20</td>
<td>0</td>
<td>43</td>
<td>29</td>
<td>23</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Compound 1</td>
<td>51</td>
<td>47</td>
<td>55</td>
<td>35</td>
<td>35</td>
<td>68</td>
<td>55</td>
<td>52</td>
<td>42</td>
<td>93</td>
<td>79</td>
<td>58</td>
<td>64</td>
<td>48</td>
</tr>
<tr>
<td>Compound 2</td>
<td>29</td>
<td>36</td>
<td>16</td>
<td>10</td>
<td>20</td>
<td>62</td>
<td>11</td>
<td>23</td>
<td>6</td>
<td>132</td>
<td>53</td>
<td>27</td>
<td>40</td>
<td>8</td>
</tr>
</tbody>
</table>

The table reports the results of the primary screening performed using 50 µg/mL of the mentioned extract or purified compound. Data are expressed in % of maximal activity, i.e., measured in the absence of inhibitor. ATP concentration used in the kinase assays was 15 µM (values are means; n = 2). Kinases are from human (Hs, Homo sapiens) origin unless specified: Ssc, Sus scrofa; Rn, Rattus norvegicus; Mm, Mus musculus; Pf, Plasmodium falciparum; Lm, Leishmania major; Ld, Leishmania donovani.

Fig. 2. Effect of purified compounds 1 and 2 on the catalytic activity of a selected set of mammalian and parasitic kinases.

The % of maximal kinase activity in the presence of 50 µg/mL of purified compounds 1 and 2 was assayed in the presence of increasing concentrations of (+)-catechin. Kinase activities are expressed in % of maximal activity, i.e., measured in the absence of inhibitor (mean ± SD; n = 3).
Ahmadu AA et al. Constituents of Acacia nilotica (IC50).

The ethyl acetate fraction is the most active against the mentioned kinases. As shown here, (+)-catechin is inactive against various cancer cell lines and mycobacteria [19–22]. No strong significant kinase inhibition was reported in this study.

### Table 2

<table>
<thead>
<tr>
<th>Extract or Compound</th>
<th>Cdk2</th>
<th>Cdk5</th>
<th>Cdk9</th>
<th>Mm CLK1</th>
<th>Ssc GSK3</th>
<th>Pf GSK3</th>
<th>Rn Dyrk1A</th>
<th>Pim1</th>
<th>AurorA</th>
<th>Haspin</th>
<th>RIPK3</th>
<th>Ssc CK1</th>
<th>Lm CK1</th>
<th>Ld TKL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>&gt;50</td>
<td>31.0</td>
<td>8.0</td>
<td>6.5</td>
<td>11.0</td>
<td>4.5</td>
<td>10.0</td>
<td>3.9</td>
<td>2.9</td>
<td>1.2</td>
<td>27.0</td>
<td>4.0</td>
<td>12.0</td>
<td>&gt;50</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>15.0</td>
<td>6.0</td>
<td>19.0</td>
<td>3.0</td>
<td>2.2</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Compound 1</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>27.0</td>
<td>30.0</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>45.0</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Compound 2</td>
<td>14.0</td>
<td>&gt;50</td>
<td>11.0</td>
<td>2.1</td>
<td>11.0</td>
<td>&gt;50</td>
<td>11.0</td>
<td>7.0</td>
<td>21.0</td>
<td>18.0</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>32.0</td>
</tr>
</tbody>
</table>

Values are reported in µg/mL for both extracts and purified compounds (n = 3; independent experiments). ATP concentration used in the kinase assays was 15 µM. Kinases are from human origin unless specified: Ssc, Sus scrofa; Rn, Rattus norvegicus; Mm, Mus musculus; Pf, Plasmodium falciparum; Lm, Leishmania major; Ld, Leishmania donovani.

...and inhibiting lipid peroxidation. This molecule has a hepaprotective effect against CCl4-induced acute liver injury as well, and has displayed anti-hyperglycemic activity in STZ-diabetic rats. Furthermore, some flavonoids like Acacia have been used daily since before 3000 BC in China for its medicinal effects. One example is that flavonoids are known to possess suppressive effects in human cancer [27]. Moreover, (+)-catechin has been known as an effective antioxidant by scavenging hydroxyl radicals, inhibiting the consumption of other antioxidants such as tocopherol and β-carotene, and inhibiting lipid peroxidation. This molecule has a hepaprotective effect against CCl4-induced acute liver injury as well, and has displayed anti-hyperglycemic activity in STZ-diabetic rats.
Thus, (+)-catechin might be further explored as a lead in the discovery of anticancer agents.

Materials and Methods

Plant collection and extraction

Dried pulverized bark (400 g), collected in Zaria (Nigeria), was extracted with 70% ethanol to exhaustion at room temperature. Removal of the solvent at a reduced temperature afforded a brownish crude extract (25 g). A portion of this extract (20 g) was resuspended in distilled water (100 mL) and partitioned successively to exhaustion with 2 × 500 mL each of chloroform, ethyl acetate, and n-butanol. Removal of the organic solvents afforded 1.25 g of chloroform, 3.2 g of ethyl acetate, and 5.2 g of n-butanol, respectively. Commercial (+)-catechin was obtained from Sigma-Aldrich (99% purity, reference product #43412).

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Conflict of Interest

The authors declare no conflict of interest.

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