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].

* These authors share senior authorship.
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
Ahmadu AA et al. Constituents of Acacia nilotica… Planta Med Int Open 2017; 4: e108–e113

The 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 ethylacetate 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 of the extract. The results of the primary screening performed using 50 µg/mL of the mentioned extract or purified compound are shown in Table 1. 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.

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

<table>
<thead>
<tr>
<th>Extract or compound</th>
<th>CDK2/ cyclinA</th>
<th>CDK5/ p25</th>
<th>CDK9/ cyclinT</th>
<th>Mm CLK1</th>
<th>Ssc GSK-3</th>
<th>Pf GSK-3</th>
<th>Rn Dyrrk1A</th>
<th>Plm1</th>
<th>Haspin</th>
<th>RIPK3</th>
<th>Lm 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>23</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>73</td>
<td>23</td>
<td>10</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](image-url) Effect of purified compounds 1 and 2 on the catalytic activity of a selected set of mammalian and parasitic kinases. Kinase activity in the presence of 50 µg/mL of tested compound is 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 ... Planta Med Int Open 2017; 4: e108–e113

... Planta Med Int Open 2017; 4: e108–e113

Table 2

<table>
<thead>
<tr>
<th>Extract or compound</th>
<th>Cdk2 / CyclinA</th>
<th>Cdk5 / p25</th>
<th>Cdk9 / CyclinT</th>
<th>Mm CK1</th>
<th>Ssc GSK3</th>
<th>Pf GSK3</th>
<th>Rn Dyrk1A</th>
<th>Pim1</th>
<th>AuroraB</th>
<th>Haspin</th>
<th>RIPK3</th>
<th>Ssc CK1</th>
<th>Lm CK1</th>
<th>Ld TLK</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>12.0</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>&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>&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>&gt;50</td>
<td>&gt;50</td>
<td>35.0</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>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Compound 2</td>
<td>14.0</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>11.0</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>
<td>&gt;50</td>
<td>&gt;50</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.
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 suspended 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).

Acknowledgements

The authors wish to acknowledge the assistance of Dr. Zulfiqar Ali of the National Center for Natural Products Research, University of Mississippi, Oxford, MS, USA, for MS of isolated compounds and Dr. Nikolas Fokialakis of the Department of Pharmacognosy and Natural Products Chemistry, University of Athens, Greece, for the LC-MS analysis. The authors also thank Cancéroplège Grand-Ouest (axis: Marine Natural Products in Cancer Treatment), GIS IBiSA (Infrastructures en Biologie Sante et Agronomie, France), and Bioge- noest (Western France Life Science and Environment Core Facility Network) for supporting the KISSf screening facility (Roscoff, France). T.-N.-D. Nguyen is supported by the government of Vietnam (recipient of a “Project 911” PhD fellowship) and the French Embassy in Vietnam (Campus France). B. Serive is supported by a Bach project is supported by the ANR/Investissements d’Avenir programme (grant #ANR-11-BTBR-0008) and INCa (NECROTAIL Program).

Conflict of Interest

The authors declare no conflict of interest.

References


[27] Chen ZP, Schell JB, Ho CT, Chen KY. Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Lett 1998; 129: 173–179