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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 IC_{50} value in the µg/mL range. This compound gave the highest activity against CLK1 with an IC_{50} 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.

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].

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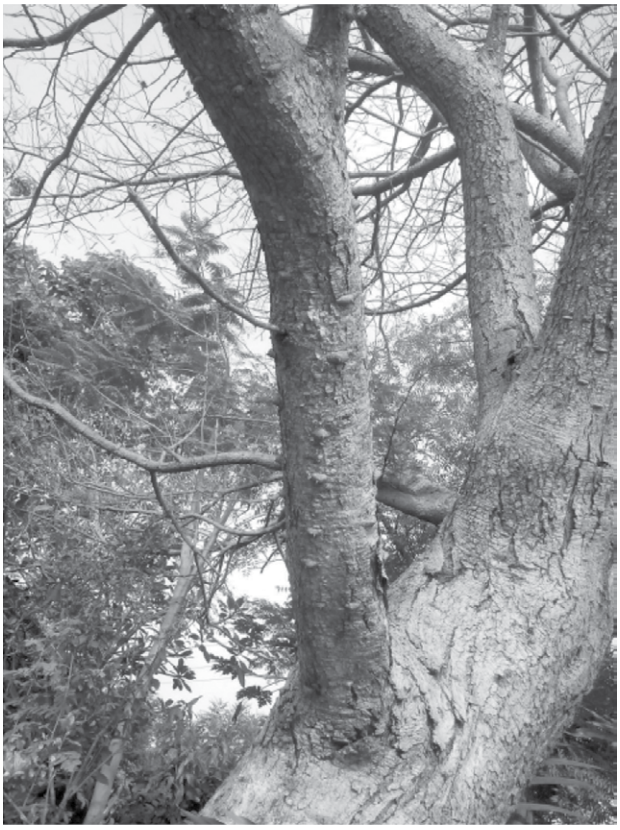
a



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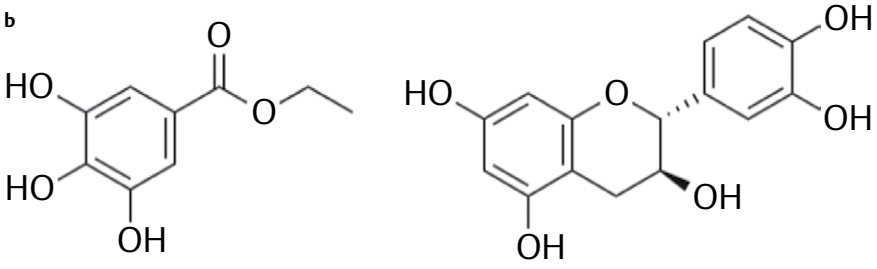


▶ **Fig. 1** **a** Pictures of *A. nilotica* branches (Zaria, Nigeria). **b** Chemical structures of the purified compounds.

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.**

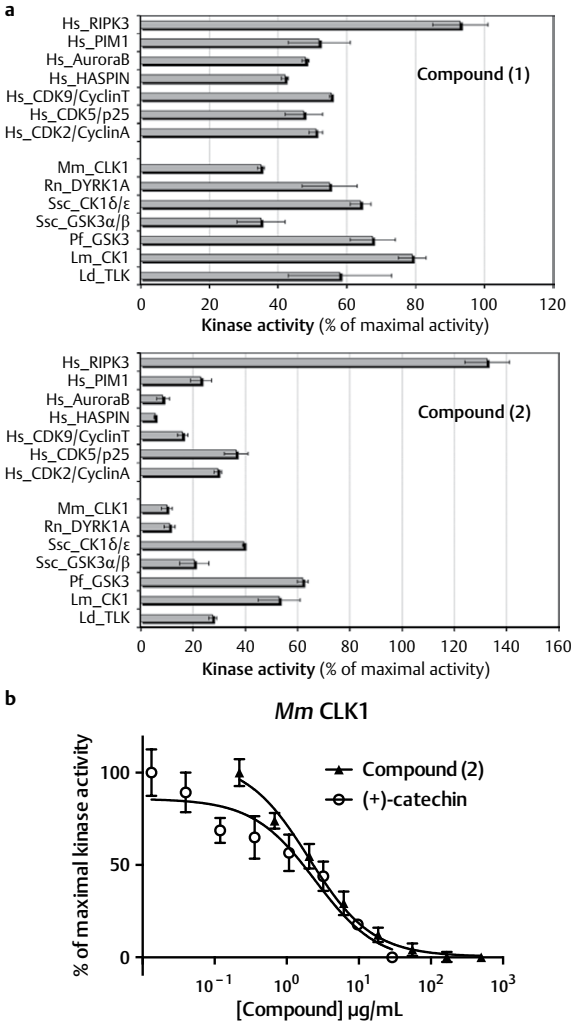
UV, NMR, and MS of these compounds were consistent with that reported in the literature for ethyl gallate [13, 14] and (+)-catechin [15, 16].

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

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

Extract or compound	CDK2/ CyclinA	CDK5/ p25	CDK9/ CyclinT	*Mm* CLK1	*Ssc* GSK-3	*Pf* GSK-3	*Rn* Dyrk1A	Pim1	Haspin	RIPK3	*Lm* CK1	*Ld* TLK	*Ssc* CK1	AuroraB
Chloroform	87	90	67	44	71	74	81	61	45	114	75	65	73	52
Ethyl acetate	37	25	11	10	15	11	12	7	1	23	20	50	9	-2
n-Butanol	59	60	15	18	11	9	17	20	0	43	29	73	23	10
Compound **1**	51	47	55	35	35	68	55	52	42	93	79	58	64	48
Compound **2**	29	36	16	10	20	62	11	23	6	132	53	27	40	8

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. **a** Kinase activities in the presence of 50 µg/mL of tested compound are expressed in % of maximal activity, i.e., measured in the absence of inhibitor. ATP concentration used in the kinase assays was 15 µM (mean ± range; n = 2). *Hs, Homo sapiens; Ssc, Sus scrofa; Rn, Rattus norvegicus; Mm, Mus musculus; Pf, Plasmodium falciparum; Lm, Leishmania major; Ld, Leishmania donovani*. **b** Dose-dependent effect of compound **2** and commercial (+)-catechin on mouse CLK1. Recombinant GST-CLK1 was assayed in the presence of increasing concentrations of the two batches of (+)-catechin. Kinase activities are expressed in % of maximal activity, i.e., measured in the absence of inhibitor (mean ± SD; n = 3).

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 frac-

tion and compounds **1** and **2**. The ethyl acetate and n-butanol fractions and the isolated compounds **1** and **2** were tested over a wide range of concentrations (from 0.016 to 50 µg/mL) and the IC_{50} values were determined from the dose-response curves. ▶ **Table 2** reports the inhibitory activity of the fractions and isolated compounds against the mentioned kinases. The result revealed that the ethylacetate fraction is the most active against the Haspin kinase (IC_{50} of 1.2 µg/mL) followed by Aurora B kinase (IC_{50} of 2.9 µg/mL). This extract is more active than the n-butanol. Chloroform extract was not tested since the primary screening did not show any activity against the kinase panel at 50 µg/mL. Inhibitory activity of the two compounds showed that (+)-catechin (**2**) was the most active protein kinase inhibitor as it inhibits the activity of nine out of the fourteen protein kinases. As shown here, (+)-catechin is inactive against *Hs*CDK5/p25, *Pf*GSK3, *Hs*RIPK3, *Ssc*CK1, and *Lm*CK1 (IC_{50} > 172 µM). Note that the IC_{50} value of compound **2** against *Mm*CLK1, the best target, was estimated at 2.1 µg/mL for the natural product purified from Acacia and 2.5 µg/mL for the molecule commercially available (▶ **Fig. 2b**). The kinase panel tested in this study represents only 2 % of the human kinome (total of 518 protein kinases). Since plant flavonoids have been shown to modulate the activities of some enzyme systems, e.g., those involved in cell surface signal transduction, immune function and transformation, tumor growth, and metastasis [17, 18], some other targets might also be affected.

Regarding ethyl gallate, although the literature reported numerous activities as an anti-inflammatory and antioxidant compound against various cancer cell lines and mycobacteria [19–22], no strong significant kinase inhibition was reported in this study. Compound **1** affected only four kinases with IC_{50}s under 50 µg/mL (< 252 µM).

(+)-Catechin (**2**) is naturally present in green tea. Green tea has been used daily since before 3000 BC in China for its medicinal effects. These last decades, it has been demonstrated that catechins represent the molecular family, which hold the activity. Furthermore, the presence of polyphenol derivatives with reported pro-apoptotic activity [23] could prevent cancer [24]. Such molecules are not only present in green tea but also in many other land plants like Acacia. Their role is not fully understood but plants can acquire an ecological/survival benefit (e.g., space colonization, protection against grazing, activity against pathogens) to synthesize such compounds [25]. This phenomenon of so-called allelopathy is a source to identify new inhibitors of human therapeutic targets. As an example, it has been shown that the inhibitory effect of flavonoids in the growth of malignant cells could be a consequence of their interference with the protein kinase activities involved in the regulation of cellular proliferation and apoptosis [26]. Studies have shown that some polyphenols (e.g., catechins that are flavanols) possess suppressive effects in human cancer [27]. In diabetic rats, they may delay the loss of functional beta-cell mass and delay the progression of diabetes by preventing oxidative stress and beta-cell apoptosis [28]. Moreover, (+)-catechin has been known as an effective antioxidant by scavenging hydroxyl radicals, delaying the consumption of other antioxidants such as α-tocopherol and β-carotene, and inhibiting lipid peroxidation. This molecule has a hepatoprotective effect against CCl_4-induced acute liver injury as well, and has displayed anti-hyperglycemic activity in STZ-diabetic

▲ **Table 2** Effect of fractions and purified compounds on kinase inhibitory activity of *A. nilotica* (IC_{50}).

Extract or compound	Cdk2 / CyclinA	Cdk5 / p25	Cdk9 / CyclinT	*Mm* CLK1	*Ssc* GSK3	*Pf* GSK3	*Rn* Dyrk1A	Pim1	AuroraB	Haspin	RIPK3	*Ssc* CK1	*Lm* CK1	*Ld* TLK
Ethyl acetate	> 50	31.0	8.0	6.5	11.0	4.5	10.0	3.9	2.9	1.2	27.0	4.0	12.0	> 50
n-Butanol	> 50	> 50	11.0	15.0	8.1	6.0	19.0	15.0	3.0	2.2	> 50	27.0	8.5	> 50
Compound **1**	> 50	> 50	> 50	27.0	30.0	> 50	> 50	35.0	> 50	45.0	> 50	> 50	> 50	> 50
Compound **2**	14.0	> 50	11.0	2.1	11.0	> 50	11.0	7.0	21.0	18.0	> 50	> 50	> 50	32.0

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 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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