**Isolation and Identification of Nicotiflorin from the Leaves of *Costus spectabilis* (Fenzl) K. Schum**

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**ABSTRACT**

*Costus spectabilis* is a rhizomatous geophyte used by traditional medicine to treat internal and external wounds, coughs, inflammation, arthritis, rheumatism, fever, maternal and neonatal infections. It is also recommended for its laxative, purgative, diuretic and ichthyotoxic properties. A phytochemical study of the leaves of *Costus spectabilis* revealed the presence of flavonoids, alkaloids, sterols and triterpenes, coumarins, reducing compounds, oses and holosides. The combination of chromatographic (CC and TLC), 1D NMR spectral (1H, 13C), 2D (COZY and HMQC) and spectrometric (ESI-MS positive mode) techniques allowed the isolation and identification of kaempferol 3-O-(6-O-(α-L-rhamnosyl) β-D-glucoside or nicotiflorin of the methanol extract of the leaves of *Costus spectabilis*.

**Keywords:** *Costus spectabilis*, phytochemistry, nicotiflorin, flavonoid, NMR, MS.

**INTRODUCTION**

*Costus spectabilis* is a rhizomatous geophyte native to much of tropical Africa [1]. In Mali, traditional medicine uses its leaves to treat internal and external wounds. Its leaves are also recommended against inflammation, arthritis, rheumatism, maternal and neonatal infections [2,3]. They are also used to prepare the laxative, the purgative, the diuretic [2] and are chewed and swallowed to give up fevers [4]. Its tuberous roots are used for their ichthyotoxic properties [5]. These pharmacological properties, whose interest is obvious, the new perspectives for the scientific valorization of *Costus spectabilis*. A study conducted in the United States at the University of Illinois (Chicago) in 2001 shows that the substances were medicated on the market, 122 transferred from plants. Of these naturally occurring molecules, 80% were used at the same time [6]. This work concerns phytochemical screening, isolation and identification of a bioactive compound, a nicotiflorin methanol extract from the leaves of *Costus spectabilis*. As such, it is a promising field of research, a title of great champion of open applications, as well as the valorization of medicinal plants, the obtaining of new bioactive molecules [7].

**MATERIALS AND METHODS**

**Plant material**

The plant material consists of the leaves of *Costus spectabilis*, harvested in Siby (Bamako) in October 2015. After drying in the shade, the samples were ground to obtain 800 g of fine powder. Botanical identification was carried out at DMT-MALI and confirmed by the nomenclature of the Angiosperms Phylogeny Group classification (APG III, 2009).

**Apparatus**

columns; silica (230-400 Mesh); analytical and preparative TLC plates (GF 254); Koffler's bench; Bruker Avance III 500 MHz spectrometer for 1H and 125 MHz for 13C; Bruker quadrupole spectrometer under electrospray and in positive mode.
PHYTOCHEMICAL SCREENING

We characterized the different chemical groups (sterols and polyterpenes, flavonoids, catechin and gallic tannins, alkaloids, coumarins, saponosides, cardiotonic glycosides, quinone compounds, cyanogenic compounds, reducing compounds, ose and holosides) by referring to the techniques described in works of N’Guessan et al. [8], Muanda [9] and Yahya [10].

EXTRACTION, ISOLATION AND IDENTIFICATION

Extraction and isolation

The powder (500 g) of the leaves of *Costus spectabilis* is extracted with cyclohexane to remove the pigments, then using methanol until exhaustion at room temperature. The resulting extract was evaporated to dryness and weighed. The separation of the methanol extract (JNKE5A2, 5 g) carried out by column chromatography on silica gel (230-400 Mesh), eluting with DCM / MeOH (8: 1) gave three fractions JNKE5A2 (1), 2 and 3. The column chromatographic fraction (JNKE5A2-2) (1.8 g) eluted with AcOEt / MeOH / Water (10: 1: 1) gave in turn three fractions JNKE5A2-3 (1, 2, 3 and 4). The compound JNKE5A2-3-1-2 (42 mg) is finally obtained from the JNKE5A2-3-1 pool (556 mg) selected for purification by preparative MMC in the AcOEt / MeOH / Water system (10: 1: 1).

Identification of the compound (JNKE5A2-3-1-2)

Yellow crystals soluble in MeOH, Rf = 3.6 (AcOEt / MeOH / Water: 10: 1: 1), mp 200°C. The SM / ESI spectrum shows a quasi-molecular ion [M-H] + at m / z 595 suggesting a molecular weight of 594u indicating the molecular formula C_{27}H_{30}O_{15}. Fragmentation in ESI + of [M + Na] + reveals the ions at m / z 449 and at m / z 287 indicating the successive loss of a deoxyhexose (-146 u) then of a hexose (-146-162 u). In addition, the most abundant fragment at m / z 287 assumes the presence of a kaempferol genome (Figure 1)[11,12].

The 13C-NMR spectrum (Table 1) confirms the characteristic signals of the kaempferol genome: signal at δC 179.37 ppm of the carbonyl group, nine quaternary carbons (eight between 130 and 170 ppm and one at δC 105.56 ppm) and five aromatic CH (Table 1)[11-14]. The GHMBC spectrum indicates that these carbons correlate respectively with the anomic protons at δH 4.53 and 5.00 ppm. These elements confirm the presence of two hexoses [11-14]. The 13 C-NMR shifts in the sugar region are characteristic, on the one hand, of a glucopyranose (C-2 '', C-3 '', C-4 '', C-5 '' and C-6 at δ C 75.76, 78.14, 71.43, 72.08 and 68.55 ppm) in the β-configuration (H-1''J = 7.4 Hz) and on the other hand, rhamnopyranose (C-2'', C-3'', C-4'', C-5'' and C-6 '') respectively at δC 71.43; 72.28; 73.89; 69,72 and 17,91) in α (H-1 '' 'J = 1.3 Hz) configuration (Figure 2)[11-14].

Moreover, the Heteronuclear Single-Quantum Correlation (HSQC) observed between the protons at δH 4.53 ppm (H-1 '' ') and the carbon located at δ 68.55 ppm (C-6 '') made it possible to show that the two sugars are bound in 1-6 and identify the diglycoside as rutinose (rhamnopyranosyl- (α1-6) -glucopyranoside) [11-14]. Rutinosin is attached to the genome Kaempferol at position 3 as evidenced by the HSQC correlation between C-3 carbon and the H-1 'anomeric proton (Figure 3)[11-14].

These data allowed us to identify JNKE5A-2-3-1-2 as kaempferol 3-O- (6-O- (α-L-rhamnosyl) β-D-glucoside known as nicotiflorin.

RESULTS AND DISCUSSION

Phytochemical screening of the leaves of *Costus spectabilis* revealed the presence of flavonoids, coumarins, alkaloids,
Table 1: 1-H (500 MHz), 13-C (125 MHz) chemical shifts in CD3OD and GHSQC and GHMBC.

<table>
<thead>
<tr>
<th>n°</th>
<th>δC</th>
<th>δH (J en Hz)</th>
<th>HSQC</th>
<th>HMBC</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>159,36</td>
<td>-</td>
<td>-</td>
<td>C-2', C-6'</td>
</tr>
<tr>
<td>3</td>
<td>135,50</td>
<td>-</td>
<td>-</td>
<td>C-1''</td>
</tr>
<tr>
<td>4</td>
<td>179,37</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>162,98</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100,06</td>
<td>6,23 d (2,1)</td>
<td>H-6/C-6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>166,35</td>
<td>-</td>
<td>-</td>
<td>C-6</td>
</tr>
<tr>
<td>8</td>
<td>94,98</td>
<td>6,43 d (2,1)</td>
<td>H-8/C-8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>158,57</td>
<td>-</td>
<td>-</td>
<td>C-8</td>
</tr>
<tr>
<td>10</td>
<td>105,56</td>
<td>-</td>
<td>-</td>
<td>C-8, C-6</td>
</tr>
<tr>
<td>1'</td>
<td>122,74</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>2'</td>
<td>132,37</td>
<td>8,10 d (8,9)</td>
<td>H-2'/C-2'</td>
<td>C-6'</td>
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<tr>
<td>3'</td>
<td>116,13</td>
<td>6,93 d (8,9)</td>
<td>H-3'/C-3'</td>
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</tr>
<tr>
<td>4'</td>
<td>161,50</td>
<td>-</td>
<td>-</td>
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<tr>
<td>6'</td>
<td>132,37</td>
<td>8,10 d (8,9)</td>
<td>H-6'/C-6'</td>
<td>C-2'</td>
</tr>
</tbody>
</table>

3-O-C(6)-glucoside

| 1''  | 104,63 | 5,00 d (7,4) | H-1''/C-1'' | C-2''          |
| 2''  | 75,76  | 3,41-3,49 m  | H-2''/C-2'' | C-3''          |
| 3''  | 78,14  | 3,41-3,49 m  | H-3''/C-3'' | C-4'', C-2''   |
| 4''  | 72,08  | 3,28-3,33 m  | H-4''/C-4'' | C-6'', C-3'', C-5'' |
| 5''  | 77,20  | 3,41-3,49 m  | H-5''/C-5'' | C-4'', C-6''   |
| 6''a | 68,55  | 3,83 d (9,6) | Ha-6''/C-6'' | -              |
| 6''b |       | 3,41-3,49 m  | Hb-6''/C-6'' |                |

6''-O-rhamnosyl

| 1''' | 102,42 | 4,53 d (1,3) | H-1''''/C-1'''' | C-6''          |
| 2''' | 71,43  | 3,67 dd (3,4 ; 1,6) | H-2''''/C-2'''' | C-4'''          |
| 3''' | 72,28  | 3,56 dd (9,5 ; 3,5) | H-3''''/C-3'''' | C-2'''', C-5'''' |
| 4''' | 73,89  | 3,29-3,36 m  | H-4''''/C-4'''' | C-6'''', C-5'', C-3'''' |
| 5''' | 69,72  | 3,46-3,50 m  | H-5''''/C-5'''' | C-4'''', C-4'''', C-6'''' |
| 6''' | 17,91  | 1,16 d (6,2) | H-6''''/C-6'''' | C-4''''        |

Figure 2: GHMBC Correlations for Compound JNKE5A2-3-1-2.
sterols and triterpenes, reducing compounds, ose and holosides. The methanolic extract showed no moderate selectivity between cancer and non-cancer cells [7]. These results suggest toxicity and should caution the traditional use of Costus spectabilis, despite its traditional pharmacological potential [7]. Flavonoids are compounds known for their antioxidant properties, they are therefore at the origin of physiological effects beneficial for the human organism and deserve the growing interest that the research brings to them [15,16]. Nicotiflorin (Figure 4) is a flavonoid di-glycosyl type isolated from many plants including Astragalus verrucosus and Astragalus cruciatus [17], Heteropappus alticus and H. Biennis [18], Solidago canadensis [19], Ficaria verna [20], Clitoria ternatea [21], Staphylea bumalda [22], Trigonotis peduncularis [23], Acalypha indica [24], Carthamus tinctorius [25], Caragana Bungei [26], Solanum campaniform [27], Oxyris wightiana [28], Ampelopsis heterophylla [29], Amaranth [30] and Aspergillus awamori [31]. Although it is not an original structure, we note for the first time, the isolation of this compound from the leaves of Costus spectabilis.

Various studies have shown the pharmacological effects of nicotiflorin, such as inhibitors on hACAT1 (human AcylCoA: cholesterol transferase 1) [23], protective against memory dysfunction and oxidative stress in model rats with multiple infarct dementia [25], decreased blood pressure and heart rate [32], anti-glycation [33], hepatoprotective

Figure 3: GHSQC Correlations for Compound JNKE5A2-3-1-2.

Figure 4: Chemical structure of Nicotiflorin.
effect on CCl₄-induced hepatic injury [34,35], anti-inflammatory, anti-nociceptive [36], inhibitors of α-glucosidase, with an activity more than 8 times higher than that of the reference antidiabetic drug, acarbose [37], antioxidant [37,43], antihypertensive, anti-anaphylactic [38] and neuroprotective [39,40]. Other studies have shown that it inhibits adipogenesis [41] and protects against ischemic brain damage [42,43].

CONCLUSION

We have found the presence of flavonoids, coumarins, alkaloids, sterols and triterpenes, reducing compounds, oses and holosides in the leaves of Costus spectabilis. This work also isolated nicotiflorin for the first time from Costus spectabilis. This molecule could be used to study and explain the medicinal properties of Costus spectabilis. For greater efficiency, we envision many perspectives including expanding the panel of activities by determining the acute oral toxicity, haemolysis, and cytotoxicity of the aqueous extract to assess the safety of use of Costus spectabilis, and continue the chemical investigations of other extracts of Costus spectabilis.

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REFERENCES


