

Phenolic compounds from the leaves of *Ricinus communis* Linn.

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ABSTRACT

Introduction: *Ricinus communis* Linn. (Castor oil plant) is a monotypic species of *Ricinus* genus (Euphorbiaceae) and widely distributed in all tropical countries. Phytochemical data of this plant are scarce. As part of ongoing research on a survey of Vietnamese medicinal plants, the investigation of this plant was performed. The isolation and structural determination of five phenolic compounds isolated from the leaves of *R. communis* Linn. growing in Binh Phuoc province were addressed. **Method:** The dried power of *R. communis* Linn. leaves was macerated in ethanol to afford the crude extract, which was then separated by liquid-liquid extraction with *n*-hexane, chloroform, and ethyl acetate, respectively to obtain the corresponding extracts. These extracts were applied to multiple silica gel column chromatography and thin-layer chromatography to yield five compounds. Their chemical structures were determined by spectroscopic methods and by comparison of NMR data with literature values. Antioxidant evaluation of **1** was carried out using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) free radical scavenging assay. **Results:** Five phenolic compounds, including one coumarinonignan cleomiscosin A (**1**), two flavonol glycosides kaempferol-3-*O*- β -*D*-glucopyranoside (**2**) and kaempferol-3-*O*- β -*D*-xylopyranoside (**3**), and two aromatic acids gallic acid (**4**) and vanillic acid (**5**) were identified. **Conclusion:** Compound **1** was determined for the first time in *Ricinus* genus and exhibited weak DPPH radical scavenging activity with an SC₅₀ value of 403.23 μ g/mL.

Key words: Euphorbiaceae, *Ricinus communis* Linn., phenolic compound, cleomiscosin A, antioxidant activity.

INTRODUCTION

Ricinus communis Linn. is a single species belonging to the spurge family (Euphorbiaceae) and widespread throughout tropical countries, including South Africa, India, Brazil, and Russia^{1,2}. This castor oil plant has been used for the treatment of inflammation and liver disorders in India, reported having hepatoprotective, laxative, antidiabetic, and antifertility activities in Tunisia³. Its leaves have traditional applications for headache, inflammatories, and antibacterials against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*^{1,4}. Previous studies on the leaves of *R. communis* determined the presence of alkaloids, flavonoids, phenolic compounds, triterpenoids, and steroids⁵⁻⁷. Herein, the isolation and structural elucidation of five phenolic compounds, including one coumarinonignan cleomiscosin A (**1**), two flavonol glycosides kaempferol-3-*O*- β -*D*-glucopyranoside (**2**) and kaempferol-3-*O*- β -*D*-xylopyranoside (**3**), and two aromatic acids gallic acid (**4**) and vanillic acid (**5**) from the leaves of *R. communis* Linn. collected in Bu Dang district, Binh Phuoc province, Vietnam, were reported.

MATERIALS AND METHODS

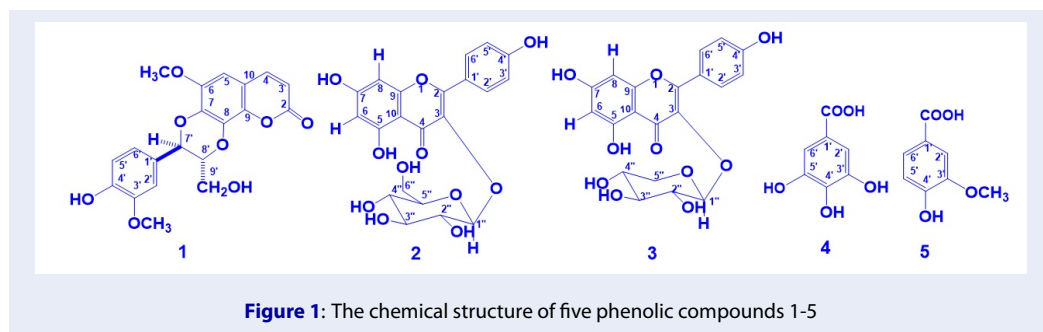
General experimental procedures

The HR-ESI-MS and APCI-MS spectra were carried on a Bruker micrOTOF Q-II and LC-MSD-Trap-SL. The NMR spectra were recorded on a Bruker Avance 500 (500 MHz for ¹H-NMR and 125 MHz for ¹³C-NMR) spectrometer. Column chromatography was applied on silica gel 60 (Merck, 40-63 μ m). TLC was conducted on precoated silica gel 60 F₂₅₄ (Merck Millipore, Billerica, Massachusetts, USA), and spots were visualized by spraying with 10% H₂SO₄ solution followed by heating.

Plant material

R. communis Linn. leaves were collected in Thong Nhat commune, Bu Dang district, Binh Phuoc province, Viet Nam in February 2017. The scientific name was identified by botanist Dr. Dang Van Son, Institute of Tropical Biology, Viet Nam. A voucher specimen (N^o SGU-MT004) was deposited in the laboratory of Faculty of Environmental Science, Sai Gon University, Ho Chi Minh City, Viet Nam.

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Extraction and isolation

The *R. communis* leaves were washed, dried, and ground into powder (15.0 kg), which was then extracted with ethanol (10 x 5 L) by the maceration method at room temperature. The filtrated solution was evaporated under reduced pressure to yield the crude ethanol extract (1.15 kg). This crude extract dissolved in solvent systems of methanol: water (1:9, v/v) was partitioned against *n*-hexane, chloroform, and ethyl acetate, respectively. The obtained solutions were evaporated to afford the corresponding residues: *n*-hexane (300.0 g), chloroform (220.0 g), and ethyl acetate (210.0 g) extracts.

The chloroform extract (220.0 g) was dissolved in chloroform again to get the precipitation (22.0 g) and the filtrated solution. The latter was evaporated under *vacuum* to obtain the corresponding extract (154.2 g). This extract was chromatographed on silica gel column eluting with a solvent system of *n*-hexane: ethyl acetate (stepwise, 8:2, 6:4, 4:6, 2:8, 0:10) and then methanol to afford five fractions (C.A–E). Fraction C.C (16.2 g) was subjected to silica gel column chromatography and eluted by *n*-hexane: chloroform (50:50, 25:75, 0:100), then chloroform: methanol (98:2, 95:5, 90:10, 0:100) to give eight subfractions (C.C1–8). Subfraction C.C3 (570.0 mg) was rechromatographed on the silica gel column eluting with *n*-hexane: chloroform (1:9) to yield 1 (72.0 mg). The same procedure for subfraction C.C4 (1.13 g) was conducted, eluting with chloroform: methanol (97:3, 95:5, 90:10) to obtain 5 (34.3 mg).

The ethyl acetate extract (210.0 g) was fractionated by silica gel column chromatography, eluting with *n*-hexane: ethyl acetate (stepwise, 6:4, 4:6, 2:8, 0:10) and then methanol to get five fractions (EA.A–E). Fraction EA.B (43.0 g) was separated by silica gel column chromatography and eluted with *n*-hexane: ethyl acetate (3:7, 2:8, 1:9, 0:10) to give five subfractions (EA.B1–5). Subfraction EA.B3 (2.8 g) was rechromatographed on silica gel eluting with chloroform:methanol

(10:0, 9:1, 8:2) to obtain 4 (78.2 mg). Fraction EA.C (47.8 g) was applied to silica gel column chromatography and eluted with *n*-hexane: ethyl acetate (9:1, 8:2, 7:3, 0:10) to give four subfractions (EA.C1–EA.C4). Subfraction EA.C1 (10.5 g) was rechromatographed on silica gel, eluting with chloroform: methanol (9:1) to obtain 2 (34.8 mg). The same procedure for fraction EA.D (55.3 g) was carried out, eluted by chloroform:methanol (9:1, 8:2) to obtain three subfractions (EA.D1–3). Subfraction EA.D3 (29.6 g) was rechromatographed on silica gel, eluting with chloroform: methanol (90:10, 85:15, 80:20) to obtain 3 (15.4 mg).

- Cleomiscosin A (1). White amorphous powder. HR-ESI-MS, positive mode: m/z 409.0831 $[M+Na]^+$ (calcd. for $C_{20}H_{18}O_8+Na$ 409.0899). The 1H -NMR data (Methanol- d_4 , δ ppm, J in Hertz): 6.31 (1H, *d*, 9.5, H-3), 7.88 (1H, *d*, 9.5, H-4), 6.82 (1H, *s*, H-5), 7.08 (1H, *d*, 1.5, H-2'), 6.89 (1H, *d*, 8.5, H-5'), 6.97 (1H, *dd*, 8.5, 1.5, H-6'), 5.07 (1H, *d*, 8.0, H-7'), 4.22 (1H, *ddd*, 10.0, 7.5, 3.5, H-8'), 3.59 (1H, *dd*, 12.5, 4.0, H-9'a), 3.87 (1H, *ddd*, 12.5, 6.5, 2.5, H-9'b), 3.90 (3H, *s*, 6-OCH₃) and 3.89 (3H, *s*, 3'-OCH₃). The ^{13}C -NMR data (Methanol- d_4): 163.1 (C-2), 114.1 (C-3), 146.3 (C-4), 102.6 (C-5), 147.6 (C-6), 139.4 (C-7), 133.5 (C-8), 140.1 (C-9), 113.2 (C-10), 128.6 (C-1'), 112.7 (C-2'), 149.4 (C-3'), 148.8 (C-4'), 116.5 (C-5'), 122.1 (C-6'), 78.2 (C-7'), 80.1 (C-8'), 61.9 (C-9'), 56.7 (6-OCH₃), and 57.1 (3'-OCH₃).
- Kaempferol-3-*O*- β -*D*-glucopyranoside (2). Yellow amorphous powder. HR-ESI-MS, positive mode: m/z 449.1074 $[M+H]^+$ (calcd. for $C_{21}H_{20}O_{11}+H$ 449.1083). The 1H -NMR data (Acetone- d_6 , δ ppm, J in Hertz): 6.28 (1H, *d*, 2.0, H-6), 6.52 (1H, *d*, 2.0, H-8), 8.14 (2H, *d*, 8.0, H-2'; H-6'), 6.97 (1H, *d*, 8.0, H-3'; H-5'), 5.24 (1H, *d*, 7.5, H-1''), 3.22–3.31 (6H, *m*, H-2'', H-3'', H-4'', H-5'', H-6'') and 12.37 (1H, *s*, OH-5). The ^{13}C -NMR data (Acetone- d_6):

157.9 (C-2), 135.4 (C-3), 179.1 (C-4), 162.9 (C-5), 99.7 (C-6), 165.2 (C-7), 94.6 (C-8), 158.6 (C-9), 105.5 (C-10), 122.6 (C-1'), 132.1 (C-2'; C-6'), 115.8 (C-3'; C-5'), 161.0 (C-4'), 104.8 (C-1''), 75.4 (C-2''), 77.8 (C-3''), 71.2 (C-4''), 78.0 (C-5''), and 62.7 (C-6'').

- Kaempferol-3-O- β -D-xylopyranoside (**3**). Yellow amorphous powder. HR-ESI-MS, negative mode: m/z 417.0817 [M-H]⁻ (calcd. for C₂₀H₁₇O₁₀ -H 417.0821). The ¹H-NMR data (DMSO-*d*₆, δ ppm, *J* in Hertz): 6.16 (1H, *d*, 2.0, H-6), 6.39 (1H, *d*, 2.0, H-8), 7.94 (2H, *d*, 8.5, H-2'; H-6'), 6.85 (1H, *d*, 9.0, H-3'; H-5'), 5.20 (1H, *d*, 7.0, H-1''), 3.22 -3.31 (3H, *m*, H-2'', H-3'', H-4''), 3.59 (1H, *dd*, 11.5, 12.0, H-5''a), 2.95 (1H, *dd*, 10.0, 9.0, H-5''b) and 12.41 (1H, *s*, OH-5). The ¹³C-NMR data (DMSO-*d*₆): 157.2 (C-2), 133.9 (C-3), 178.1 (C-4), 161.8 (C-5), 99.6 (C-6), 164.8 (C-7), 94.7 (C-8), 157.4 (C-9), 104.7 (C-10), 121.5 (C-1'), 131.7 (C-2'; C-6'), 116.2 (C-3'; C-5'), 160.6 (C-4'), 102.6 (C-1''), 76.4 (C-2''), 74.4 (C-3''), 70.1 (C-4'') and 66.4 (C-5'').
- Gallic acid (**4**). White amorphous powder. HR-ESI-MS, positive mode: m/z 193.0098 [M+Na]⁺ (calcd. for C₇H₆O₅ +Na 193.0112). ¹H-NMR data (Acetone-*d*₆, δ ppm, *J* in Hertz): 7.16 (2H, *s*, H-2, H-6). ¹³C-NMR data (Acetone-*d*₆): 167.9 (COOH), 111.9 (C-1), 110.1 (C-2, C-6), 145.9 (C-3, C-5) and 138.6 (C-4)⁸.
- Vanillic acid (**5**) white amorphous powder. APCI-MS, positive mode: m/z 207.8 [M+K]⁺ (calcd. for C₈H₈O₄ +K 207.0596). ¹H-NMR (Acetone-*d*₆, δ ppm, *J* in Hertz): 7.56 (1H, *d*, 2.0, H-2), 6.91 (1H, *d*, 8.5, H-5), 7.89 (1H, *dd*, 8.5, 2.0, H-6), and 3.91 (3H, *s*, 3-OCH₃). ¹³C-NMR data (Acetone-*d*₆): 168.5 (COOH), 123.0 (C-1), 113.5 (C-2), 148.1 (C-3), 152.1 (C-4), 115.5 (C-5), 124.9 (C-6) and 56.4 (3-OCH₃)⁹.

DPPH scavenging assay

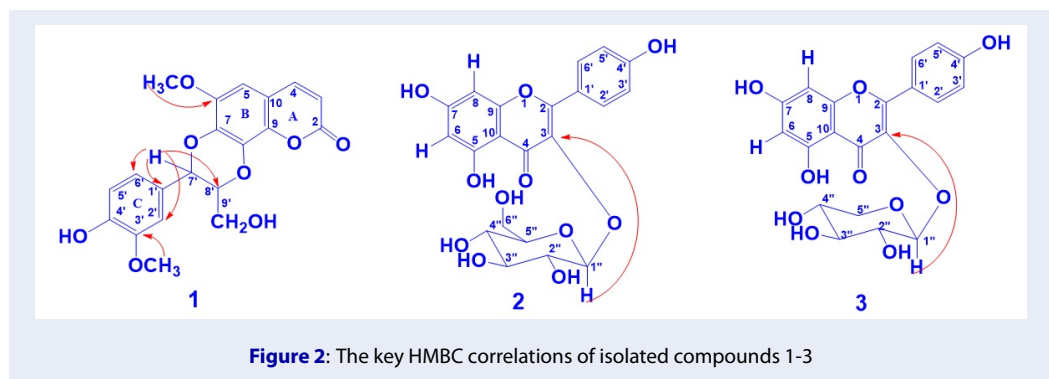
The assay was carried out following the method reported previously¹⁰. Trolox was used as a positive control. Compound **1** was analyzed in triplicate, and results are given as averages \pm SD.

RESULTS

Compound **1** was obtained as a white amorphous powder. HR-ESI-MS spectrum indicated the molecular formula as C₂₀H₁₈O₈ due to the pseudo-molecular peak at m/z 409.0831 [M+Na]⁺ (calcd.

409.0899 for C₂₀H₁₈O₈+Na). The ¹H-NMR spectrum displayed the signals of two olefin protons at δ_H 6.31 (1H, *d*, 9.5, H-3) and 7.88 (1H, *d*, 9.5, H-4), and one aromatic proton signal at δ_H 6.82 (1H, *s*, H-5), which demonstrated the presence of a coumarin skeleton. Additionally, its ¹H-NMR spectra also identified the two typical proton signals of lignan skeleton at δ_H 5.07 (1H, *d*, 8.0, H-7') and 4.22 (1H, *ddd*, 10.0, 7.5, 3.5, H-8'). Furthermore, there were signals of other aromatic protons of a 1,3,4-trisubstituted benzene ring at δ_H 7.08 (1H, *d*, 1.5, H-2'), 6.89 (1H, *d*, 8.5, H-5') and 6.97 (1H, *dd*, 8.5, 1.5, H-6') and signals of two methoxy proton groups at δ_H 3.90 (3H, *s*, 6-OCH₃) and 3.89 (3H, *s*, 3'-OCH₃) in ¹H-NMR spectrum. These data suggested that **1** should be a coumarinolignan derivative. The ¹³C-NMR spectrum was consistent with the previous statement, showing the presence of 20 carbons, including signals of one carboxyl carbon at δ_C 163.1 (C-2), two oxymethine carbons at δ_C 78.2 (C-7') and 80.1 (C-8'), one oxymethylene carbon at δ_C 61.9 (C-9'), two methoxy carbon groups at δ_C 56.7 (6-OCH₃) and 57.1 (3'-OCH₃), and the quaternary carbons in the range δ_C 114.1 to 149.4 ppm. The COSY, HSQC and HMBC spectra determined the structure of **1**. Indeed, HMBC cross peaks of the oxymethine proton at δ_H 5.07 (1H, *d*, 8.0, H-7') to carbons at δ_C 128.6 (C-1'), 112.7 (C-2'), 122.1 (C-6'), and 80.1 (C-8') defined the chemical structure of the C-ring. Likewise, HMBC correlations of proton H-7' to C-7 and of H-8' to C-8 indicated the attachment of B and C rings at C-7' and C-8'. The relative configuration of H-7' and H-8' was defined by its large coupling constant of 8.0 Hz. Comparison of NMR data **1** and cleomiscosin A in the literature¹¹ gave the consistency, thus, the structure of **1** was elucidated as cleomiscosin A. The result of DPPH radical scavenging activity assay indicated that **1** showed weak antioxidant potential with C₅₀ value of 403.23 μ g/mL (compared with Trolox, C₅₀ value of 7.53 μ g/mL).

Compound **2** was obtained as a yellow amorphous powder. Its ¹H-NMR spectrum exhibited a down field signal at δ 12.37 (1H, *brs*), indicating the presence of a chelated hydroxy group at C-5 position. The ¹H-NMR spectrum also showed two *meta*-coupled signals at δ_H 6.28 (1H, *d*, 2.0, H-6) and 6.52 (1H, *d*, 2.0, H-8), corresponding the presence of a 5,7-dihydroxy A ring system in flavonol. The 1',4'-disubstituted B ring system in flavonol were determined by displaying two aromatic proton signals on ABX system at δ_H 8.14 (2H, *d*, 8.0, H-2', H-6') and 6.97 (1H, *d*, 8.0, H-3'; H-5'). These spectroscopic



data indicated the presence of a kaempferol skeleton. Moreover, the $^1\text{H-NMR}$ spectrum showed one anomeric proton signal at δ_H 5.24 (1H, *d*, 7.5, H-1'') and other oxygenated protons at δ_H 3.22 -3.31 (6H, *m*, H-2''-6'') of a β -D-glucopyranosyl moiety, indicating that compound **2** was a kaempferol glycoside. The $^{13}\text{C-NMR}$ spectrum displayed 21 carbon signals, including 15 carbons of kaempferol skeleton and six carbons of a β -D-glucopyranosyl moiety, fully supporting the previous finding. The kaempferol skeleton was confirmed by the presence of one carbonyl carbon signal at δ_C 179.1 (C-4), six oxygenated aromatic carbon signals from 135.4 to 165.2 ppm, and eight sp^2 carbon signals in the range 94.6 to 132.1 ppm. The β -D-glucopyranosyl unit was determined by the presence of one anomeric carbon at δ_C 104.8 (C-1''), four oxymethine carbons at δ_C 75.4 (C-2''), 77.8 (C-3''), 71.2 (C-4''), 78.0 (C-5'') and one oxymethylene carbon at δ_C 62.7 (C-6''). The linkage of the β -D-glucopyranosyl unit at C-3 was established by the HMBC correlation of the anomeric proton at δ_H 5.24 (1H, *d*, 7.5, H-1'') to the oxygenated carbon at δ_C 135.4 (C-3). The other correlations on HSQC and HMBC spectra were definitely agreed with the assignment. The molecular formula of **2** was determined as $\text{C}_{20}\text{H}_{18}\text{O}_{11}$ through the protonated molecular ion peak at m/z 449.1074 $[\text{M}+\text{H}]^+$ in HR-ESI-MS spectrum (calcd. 449.1083 for $\text{C}_{21}\text{H}_{20}\text{O}_{11}+\text{H}$). Therefore, **2** was elucidated as kaempferol-3-*O*- β -D-glucopyranoside (Astragalinalin), whose NMR data were identical to those in the literature¹².

Compound **3** was also a kaempferol derivative, having similar NMR data with those of **2**, except for the difference in the sugar unit. The β -D-xylopyranosyl moiety was identified by the presence of one anomeric carbon at δ_C 102.6 (C-1'') and four oxymethine carbons at δ_C 76.4 (C-2''), 74.4 (C-3''), 70.1 (C-4'') and 66.4 (C-5'') in $^{13}\text{C-NMR}$ spectrum and one anomeric proton at δ_H 5.20 (1H, *d*, 7.0, H-1''), three oxymethine protons at δ_H 3.22 -3.31 (3H, *m*, H-2'', H-3'',

H-4'') and one oxymethylene group [δ_H 3.59 (1H, *dd*, 11.5, 12.0, H-5''a) and 2.95 (1H, *dd*, 10.0, 9.0, H-5''b)] in $^1\text{H-NMR}$ spectrum. The linkage of the β -D-glucopyranosyl unit at C-3 was established by the HMBC spectrum. The molecular formula of **3** was established as $\text{C}_{20}\text{H}_{18}\text{O}_{10}$ based on a pseudo-molecular ion peak at m/z 417.0817 $[\text{M}-\text{H}]^-$ of HR-ESI-MS spectrum. Based on the good compatibility of the NMR data of **3** and kaempferol-3-*O*- β -D-xylopyranoside¹², **3** was elucidated as kaempferol 3-*O*- β -D-xylopyranoside.

DISCUSSION

Cleomiscosin A (**1**), found for the first time in *Aesculus turbinata*¹³ showed various biological activities, i.e. anti-inflammatory¹⁴, antihepatotoxicity¹⁵, and antitumor activities¹⁶. Derivatives of this compound were prepared to evaluate the structure-activity relationship¹⁴. To the best of our knowledge, this is the first isolation of **1** from *Ricinus* genus. Astragalinalin (**2**), a potential therapeutic compound, was isolated from many higher plants, *Cuscuta chinensis* or *Cassia alata*¹³. This compound was found in the roots of *R.communis* which was considered to possess mast cell stabilizing, antianaphylactic activity and antiasthmatic activity¹⁷. Kaempferol 3-*O*- β -D-xylopyranoside (**3**) was also found in the roots of *R.communis* and the leaves of this plant growing in Sri Lanka¹⁸. This compound showed moderate inhibitory activity against α -glucosidase type IV from *Bacillus stearothermophilus* with the IC_{50} value of $19.0 \mu\text{M}$ ¹⁹.

CONCLUSION

From the leaves of *R.communis* collected in Binh Phuoc province, using various chromatographic methods provided five isolated phenolic compounds. Their structures were determined as cleomiscosin A (**1**), kaempferol-3-*O*- β -D-glucopyranoside (**2**),

kaempferol-3-*O*- β -*D*-xylopyranoside (3), gallic acid (4), and vanillic acid (5). Among them, compound 1 was found for the first time in the genus *Ricinus* and showed weak DPPH radical scavenging activity with C_{50} value of 403.23 μ g/mL.

ABBREVIATIONS

HR-ESI-MS: High resolution electrospray ionization mass spectrometry, APCI-MS: Atmospheric pressure chemical ionization mass spectrometry, ^1H NMR: Proton nuclear magnetic resonance, ^{13}C NMR: Carbon-13 nuclear magnetic resonance, CC: column chromatography, TLC: Thin layer chromatography, HSQC: Heteronuclear single quantum coherence, HMBC: Heteronuclear multiple bond correlation, s: singlet, d: doublet, m: multiplet.

CONFLICTS OF INTEREST

The authors declare no competing financial interest.

AUTHOR CONTRIBUTION

Pham N.K.T has contributed in conducting experiments, acquisition of data, and interpretation of data. Tran T.T.L., Dinh V.S, Nguyen V.T, Dang V.S., Nguyen T.Q.T., Nguyen D.X.K, Nguyen T.P. interpreted NMR and MS data as well as searched the bibliography. Huynh B.L.C and Duong T.H. gave final approval of the manuscript to be submitted.

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