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 coumarinolignan cleomiscosin A (1), two flavonol glycosides kaempferol-3-O-β-D-glucopyranoside (2) and kaempferol-3-O-β-D-glucopyranoside (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 μg/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, anti diabetic, and antifertility activities in Tunisia.³ 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 steroids.⁵-⁷ Herein, the isolation and structural elucidation of five phenolic compounds, including one coumarinolignan cleomiscosin A (1), two flavonol glycosides kaempferol-3-O-β-D-glucopyranoside (2) and kaempferol-3-O-β-D-glucopyranoside (3), and two aromatic acids gallic acid (4) and vanillic acid (5) from the leaves of *R. communis* Linn. collected in Bu Dank 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 µm). TLC was conducted on precoated silica gel 60 F₅₄₄ (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 Dank 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° 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 extract was chromatographed on silica gel, eluting with chloroform:methanol (9:1) to obtain 1 (72.0 mg). The ethyl acetate extract (210.0 g) was fractionated by silica gel column chromatography, eluting with n-hexane: ethyl acetate (stepwise, 8:2, 6:4, 4:6, 2:8, 0:10) and then methanol to afford five fractions (EA.A–E). Fraction EA.A (55.3 g) was carried out, eluted by chloroform:methanol (9:1, 8:2) to yield 1 (72.0 mg). The same procedure for 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 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 afford five fractions (EA.A–E). Fraction EA.A (55.3 g) was carried out, eluted by chloroform:methanol (9:1, 8:2) to yield 1 (72.0 mg). The same procedure for 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 ethyl acetate extract (210.0 g) was fractionated by silica gel column chromatography, eluting with n-hexane: ethyl acetate (stepwise, 8:2, 6:4, 4:6, 2:8, 0:10) and then methanol to afford five fractions (EA.A–E). Fraction EA.A (55.3 g) was carried out, eluted by chloroform:methanol (9:1, 8:2) to yield 1 (72.0 mg). The same procedure for 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 ethyl acetate extract (210.0 g) was fractionated by silica gel column chromatography, eluting with n-hexane: ethyl acetate (stepwise, 8:2, 6:4, 4:6, 2:8, 0:10) and then methanol to afford five fractions (EA.A–E). Fraction EA.A (55.3 g) was carried out, eluted by chloroform:methanol (9:1, 8:2) to yield 1 (72.0 mg). The same procedure for 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, 8:2) to yield 1 (72.0 mg). The same procedure for 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, 8:2) to yield 1 (72.0 mg). The same procedure for 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, 8:2) to yield 1 (72.0 mg).
The assay was carried out following the method reported previously \(^{10}\). Trolox was used as a positive control. Compound 1 was analyzed in triplicate, and results are given as averages ± SD.

**RESULTS**

Compound 1 was obtained as a white amorphous powder. HR-ESI-MS spectrum indicated the molecular formula as \(C_{20}H_{13}O_8\) due to the pseudomolecular peak at \(m/z\) 409.0899 for \(C_{20}H_{13}O_8\). The \(1^H\)-NMR spectrum displayed the signals of two olefin protons at \(\delta_H 6.31 (1H, d, 9.5, H-3)\) and 7.88 \((1H, d, 9.5, H-4)\), and one aromatic proton signal at \(\delta_H 6.82 (1H, s, H-5)\), which demonstrated the presence of a coumarin skeleton. Additionally, its \(1^H\)-NMR spectra also identified the two typical proton signals of lignan skeleton at \(\delta_H 5.07 (1H, d, 8.0, H-7')\) and 4.22 \((1H, ddd, 10.0, 7.5, 3.5, H-8')\).

**DPPH scavenging assay**

The assay was carried out following the method reported previously \(^{10}\). Trolox was used as a positive control. Compound 1 was analyzed in triplicate, and results are given as averages ± SD.

**RESULTS**

Compound 1 was obtained as a white amorphous powder. HR-ESI-MS spectrum indicated the molecular formula as \(C_{20}H_{13}O_8\) due to the pseudomolecular peak at \(m/z\) 409.0899 for \(C_{20}H_{13}O_8\). The \(1^H\)-NMR spectrum displayed the signals of two olefin protons at \(\delta_H 6.31 (1H, d, 9.5, H-3)\) and 7.88 \((1H, d, 9.5, H-4)\), and one aromatic proton signal at \(\delta_H 6.82 (1H, s, H-5)\), which demonstrated the presence of a coumarin skeleton. Additionally, its \(1^H\)-NMR spectra also identified the two typical proton signals of lignan skeleton at \(\delta_H 5.07 (1H, d, 8.0, H-7')\) and 4.22 \((1H, ddd, 10.0, 7.5, 3.5, H-8')\).

**DISCUSSION**

The \(1^H\)-NMR spectrum was consistent with the previous statement, showing the presence of 20 carbons, including signals of one carboxyl carbon at \(\delta_C 163.1\) (C-2), two oxymethine carbons at \(\delta_C 78.2\) (C-7') and 80.1 (C-8'), one oxymethylene carbon at \(\delta_C 61.9\) (C-9'), two methoxy carbon groups at \(\delta_C 56.7\) (6-OCH\(_3\)) and 57.1 (3'-OCH\(_3\)), and the quaternary carbons in the range of \(\delta_C 114.1\) to 149.4 ppm. The COSY, HSQC and HMBC spectra determined the structure of 1. Indeed, HMBC cross peaks of the oxymethine proton at \(\delta_H 5.07 (1H, d, 8.0, H-7')\) to carbons at \(\delta_C 128.6\) (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' and H-8' 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 of 1 and cleomiscosin A in the literature \(^{11}\) 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 \(C_{50}\) value of 403.23 \(\mu\)g/mL (compared with Trolox, \(C_{50}\) value of 7.53 \(\mu\)g/mL).

Compound 2 was obtained as a yellow amorphous powder. Its \(1^H\)-NMR spectrum exhibited a down field signal at \(\delta 12.37 (1H, brs)\), indicating the presence of a chelated hydroxy group at C-5 position. The \(1^H\)-NMR spectrum also showed two meta-coupled signals at \(\delta_H 6.28 (1H, d, 2.0, H-6)\) 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 \(\delta_H 8.14 (2H, d, 8.0, H-2'; H-6')\) and 6.97 \((1H, d, 8.0, H-3', H-5')\). These spectroscopic
data indicated the presence of a kaempferol skeleton. Moreover, the 1H-NMR spectrum showed one anomeric proton signal at δH 5.24 (1H, d, 7.5, H-1") and other oxygenated protons at δH 3.22 -3.31 (6H, m, H-2"-6") of a β-D-glucopyranosyl moiety, indicating that compound 2 was a kaempferol glycoside. The 13C-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 δC 179.1 (C-4), six oxygenated aromatic carbon signals from 135.4 to 165.2 ppm, and eight sp2 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 δC 104.8 (C-1"), four oxymethylene carbons at δC 75.4 (C-2"), 77.8 (C-3"), 71.2 (C-4"), 78.0 (C-5") and one oxymethylene carbon at δC 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 δH 5.24 (1H, d, 7.5, H-1") to the oxygenated carbon at δC 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 C20H18O11 through the protonated molecular ion peak at m/z 449.1074 [M+H]+ in HR-ESI-MS spectrum (calcd. 449.1083 for C21H20O11+H). Therefore, 2 was elucidated as kaempferol-3-O-β-D-glucopyranoside (Astragalin), 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 δC 102.6 (C-1") and four oxymethylene carbons at δC 76.4 (C-2"), 74.4 (C-3"), 70.1 (C-4") and 66.4 (C-5") in 13C-NMR spectrum and one anomeric proton at δH 5.20 (1H, d, 7.0, H-1"), three oxymethylene protons at δH 3.22 -3.31 (3H, m, H-2", H-3", H-4") and one oxymethylene group [δH 3.59 (1H, dd, 11.5, 12.0, H-5"a) and 2.95 (1H, dd, 10.0, 9.0, H-5"b)] in 1H-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 C20H18O10 based on a pseudomolecular ion peak at m/z 417.0817 ([M-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 turbinata13 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. Astragalin (2), a potential therapeutic compound, was isolated from many higher plants, Cuscuta chinensis or Cassia alata13. This compound was found in the roots of R. communis which was considered to possess mast cell stabilizing, antinaphylactic 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 steatorrhophilus with the IC50 value of 19.0 μM19.

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-xylpyranoside (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 a C50 value of 403.23 μg/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,

13C NMR: Carbon-13 nuclear magnetic resonance,

CC: column chromatography, TLC: Thin layer chromatography, HSCQC: 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.T, 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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