DOI: 10.31276/VJSTE.66(2).27-32

Chemical structure of steroidal saponin isolated from the leaves of *Dracaena draco* L.

Hung Duc Nguyen*, Tan Quang Tu, Mau Hoang Chu

Faculty of Biology, Thai Nguyen University of Education,
20 Luong Ngoc Quyen Street, Quang Trung Ward, Thai Nguyen City, Thai Nguyen Province, Vietnam

Received 27 July 2023; revised 6 September 2023; accepted 19 January 2024

Abstract:

Saponins are naturally occurring compounds ubiquitously present in various organisms, predominantly in plants. They are noted for forming colloidal solutions in water, which foam on shaking and precipitate cholesterol. Several *in vitro* and *in vivo* tests on mixtures and individual saponins have revealed a broad spectrum of biological and pharmacological activities, including cancer-related activity, antiphlogistic and antiallergic, immunomodulating, antihepatotoxic, antiviral, hypoglycemic, antifungal and molluscicidal activities. The *Dracaena* genus is renowned for an abundance of steroidal saponins utilized in treating digestive and hematological disorders. Previous studies on this genus revealed the presence of steroidal saponins, some of which showed cytotoxic activities against cultured tumor cells and interesting biological activities. This study procured steroidal saponin from the foliage of *D. draco* L. using various chromatographic techniques and 1D and 2D nuclear magnetic resonance (NMR) spectroscopic analysis. The structure was deduced as 26-*O*-β-D-glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1β,3β,22α,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl}; a compound previously isolated from the underground parts of *Ruscus aculeatus* in Japan. Future evaluation of the saponin's biological activity is imperative to enhance chemotaxonomic data concerning the chemical composition and biological functionality of *D. draco* L. and, by extension, the *Dracaena* genus.

Keywords: **Asparagaceae, *Dracaena draco* L., nuclear magnetic resonance, ornamental plant, steroidal saponin.**

Classification numbers: **2.2, 3.3**

1. Introduction

The *Dracaena* genus, part of the Asparagaceae family, comprises approximately 110 species predominantly native to tropical and subtropical zones [1]. Species from this genus are employed in treating digestive ailments. Traditional Chinese medicine has documented the substitution of the red resin from *D. cochinchinensis* since the 1970s to augment blood circulation and treat inflammation, diarrhea, diabetes, and haemorrhage [2]. Chemical studies on *Dracaena* species have disclosed the presence of saponins with antituberculosis, anti-inflammatory, antimicrobial, and cytotoxic properties [3-6].

D. draco L. is indigenous to the Canary Islands but is also observed in Vietnam. Its trunk and leaves exhibit a dark red hue, known as 'dragon's blood', and are employed in traditional medicine. Previous studies have examined the isolation and structural elucidation of steroidal saponins with biological efficacy from *D. draco* L. [7]. To facilitate comparison between specimens found in Japan and Vietnam, an exhaustive phytochemical analysis of *D. draco* L.'s leaves was undertaken, culminating in the isolation of a steroidal saponin via chromatographic and spectroscopic techniques. The paper delineates the isolation and structural elucidation of this saponin.

2. Materials and methods

2.1. Plant material

The foliage of *D. draco* L. was gathered in Thai Nguyen Province, Vietnam, during July 2019 and was authenticated by Dr. Hung Duc Nguyen, a co-author. The voucher specimen is conserved at the Faculty of Biology, Thai Nguyen University of Education (Fig. 1).

*Corresponding author: Email: hungnd@tnue.edu.vn

cc BY NC ND
Crossref Similarity Check Powered by iThenticate

Fig. 1. The species *D. draco* L.

2.2. General experimental procedure

Nuclear magnetic resonance (NMR) spectra were acquired utilising an Inova NMR spectrometer (Agilent Technologies®, USA) at frequencies of 600 MHz for ^{1}H and 2D NMR (HSQC, HMBC, ROESY) and 150 MHz for ^{13}C NMR. Chemical shifts (δ) are reported in parts per million (ppm), with samples dissolved in pyridine-d5. Electrospray Ionisation Mass Spectrometry (ESI-MS) readings were conducted on a micrOTOF II mass spectrometer (Bruker®, Germany). Extractions utilised an Elmasonic S10H ultrasound cleaner (Elma, Switzerland). Isolations employed a Medium-Pressure Liquid Chromatography (MPLC) system with a silica gel 60 (40-63 µm, Merck®, Germany) stationary phase. Reversed-phase silica gel RP-18 (75-200 µm, SiliCycle, Canada) and Sephadex LH20 (Sigma Aldrich, France) were utilised for Vacuum Liquid Chromatography (VLC) and Column Chromatography (CC), respectively. Thin-layer chromatography (TLC) was conducted on pre-coated silica gel plates 60F254 (Merck, Germany). The saponins were detected with a spray reagent of vanillin-sulphuric acid, comprising 1% vanillin in an EtOH:H_2SO_4 solution (50:1, v/v).

2.3. Extraction and isolation of saponin

Dried, powdered leaves (130.6 g) underwent successive extractions via an ultrasound-assisted method with an EtOH:H_2O (75:35, 800 ml each) solvent system at 30 W and 50°C for 35 minutes. Post evaporation under reduced pressure at 55°C using a Büchi Rotavapor R-210, 6.8 g of ethanolic-aqueous extract was procured and then dissolved in 30 ml H_2O, subsequently subjected to VLC using silica gel RP-18 and 500 ml of solvents sequentially: 100% H_2O, 50% EtOH-50% H_2O, and 100% EtOH. This process yielded three fractions, including the saponin-rich fraction DR.2 (418.1 mg). Fraction DR.2 was further separated on an MPLC with silica gel 60 using a $CHCl_3$-MeOH-H_2O solvent system at varying ratios (80-20-2, 70-30-7, 60-32-7, v/v/v) to produce five subfractions (DR.2.1-DR.2.5). Subfraction DR.2.2 (35.6 mg) underwent further MPLC to yield three subfractions, DR.2.2.1-DR.2.2.3. The subfraction DR.2.2.2 (6.3 mg) was purified using CC on Sephadex LH20 with MeOH, resulting in compound 1 (DSC02) (2.9 mg) (Fig. 2).

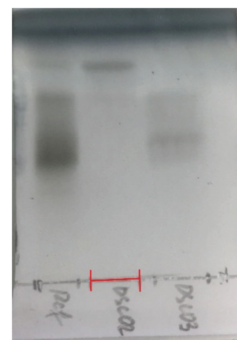

Fig. 2. TLC of compound 1 (in red).

Table 1. Spectroscopic data of compound 1 (C_5D_5N, δ in ppm, *J* in Hz).

Carbon	δ_C	δ_H	Carbon	δ_C	δ_H
1	83.7	3.71 dd (11.3, 2.9)	23	37.3	2.20, 2.25
2	37.3	2.34, 2.68	24	28.3	2.66, 2.70
3	67.7	3.79	25	146.6	-
4	43.1	2.52, 2.72	26	72.1	4.34 br d (12.3), 4.59 br d (12.3)
5	139.0	-	27	110.9	5.03 br s, 5.35 br s
6	124.4	5.54 d (5.1)	OCH_3	47.2	3.30 s
7	31.5	1.81, 1.84	Ara-1	100.7	4.67 d (7.0)
8	33.1	1.55	2	74.7	4.52
9	50.5	1.45	3	75.2	4.17
10	42.6	-	4	69.9	4.26
11	23.5	1.60, 2.94	5	66.9	3.72, 4.25
12	40.2	1.35, 1.66	Rha-1	101.6	6.29 br s
13	40.4	-	2	72.3	4.75 br s
14	56.7	1.15	3	72.2	4.64 dd (9.4, 3.1)
15	32.2	1.42, 2.04	4	73.9	4.38 dd (9.4, 9.3)
16	81.5	4.86 q (7.0)	5	69.4	4.88 dq (9.3, 5.8)
17	63.2	1.93	6	18.9	1.72 d (5.8)
18	16.7	0.94 s	Glc-1	103.6	4.90 d (7.6)
19	14.6	1.41 s	2	74.9	4.08
20	40.2	2.28 t (7.0)	3	78.2	4.29
21	16.5	1.26 d (7.0)	4	71.5	4.20
22	112.1	-	5	78.1	3.97
			6	62.6	4.35, 4.56

Compound 1: amorphous powder. ^{1}H-NMR (pyridine-d_5, 600 MHz) and ^{13}C-NMR (pyridine-d_5, 150 MHz), see Table 1. ESI-MS (positive mode) *m/z* 923.4602 $[M+Na]^+$ (calcd. for $C_{45}H_{72}NaO_{18}$, 923.4616).

3. Results and discussion

Compound **1** was obtained as a white amorphous powder and formed a soapy lather when shaken with water. The ESI-MS peak of compound **1** at *m/z* 923.4602 gave evidence for the molecular formula of $C_{45}H_{72}O_{18}$. The ^1H NMR spectrum of compound **1** showed distinct signals for one olefinic proton at δ_H 5.54 (d, *J*=5.1 Hz, aglycone H-6), two exomethylene protons at δ_H 5.03 and 5.35 (br s, aglycone H-27), two tertiary methyl groups at δ_H 0.94 (s, aglycone 18-Me) and 1.41 (s, aglycone 19-Me), one secondary methyl group at δ_H 1.26 (d, *J*=7.0 Hz, aglycone 21-Me), and a methoxyl group at δ_H 3.30 (s) (Fig. 3). The ^{13}C NMR spectrum displayed a total of 27 carbons for the aglycone, excluding one for the methoxyl carbon at δ_C 47.2 (Fig. 4). Accordingly, the structural feature of the aglycone moiety was inferred to be a 22-methoxyl furostanol derivative, which is in agreement with literature [8]. The configurations of the H-1, H-3, H-6, H-9, H-16 and H-21 positions in the aglycone were determined to be in the α-axial orientation via observations from the ROESY spectrum at δ_H/δ_H 3.71 (dd, *J*=11.3, 2.9 Hz, aglycone H-1)/3.79 (aglycone H-3), δ_H/δ_H 3.79 (aglycone H-3)/5.54 (d, *J*=5.1 Hz, aglycone H-6), and δ_H/δ_H 1.26 (d, *J*=7.0 Hz, aglycone 21-Me)/ 4.86 (q, *J*=7.0 Hz, aglycone H-16). On the other hand, the configurations of the H-8, H-14, H-18, H-19, H-16 and H-21 positions in the aglycone were inferred to be of β-axial orientation by observations of the ROESY spectrum at δ_H/δ_H 1.55 (aglycone H-8)/0.94 (s, aglycone 18-Me), δ_H/δ_H 1.55 (aglycone H-8)/1.41 (s, aglycone 19-Me), δ_H/δ_H 1.55 (aglycone H-8)/1.15 (aglycone H-14), δ_H/δ_H 1.15 (aglycone H-14)/0.94 (s, aglycone 18-Me), δ_H/δ_H 1.15 (aglycone H-14)/1.41 (s, aglycone 19-Me), and δ_H/δ_H 0.94 (s, aglycone 18-Me)/1.41 (s, aglycone 19-Me) (Fig. 5). Furthermore, the cross peak observed in the ROESY spectrum between δ_H 4.86 (q, *J*=7.0 Hz, aglycone H-16), and δ_H 3.30 (s, aglycone methoxyl proton) confirmed the C-22α configuration. Thus, the structure of the aglycone in compound **1** was confirmed to be 22-*O*-methylfurosta-5,25(27)-diene-1β,3β,22α,26-tetrol.

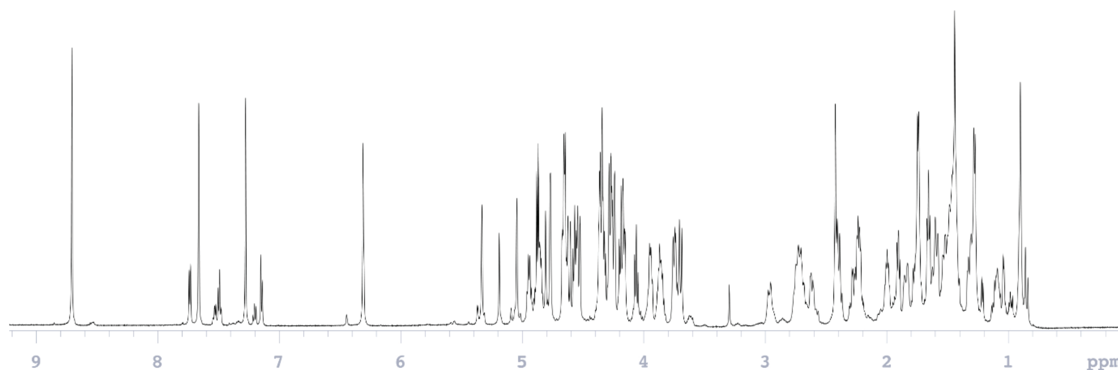

Fig. 3. ^1H NMR spectrum of compound 1.

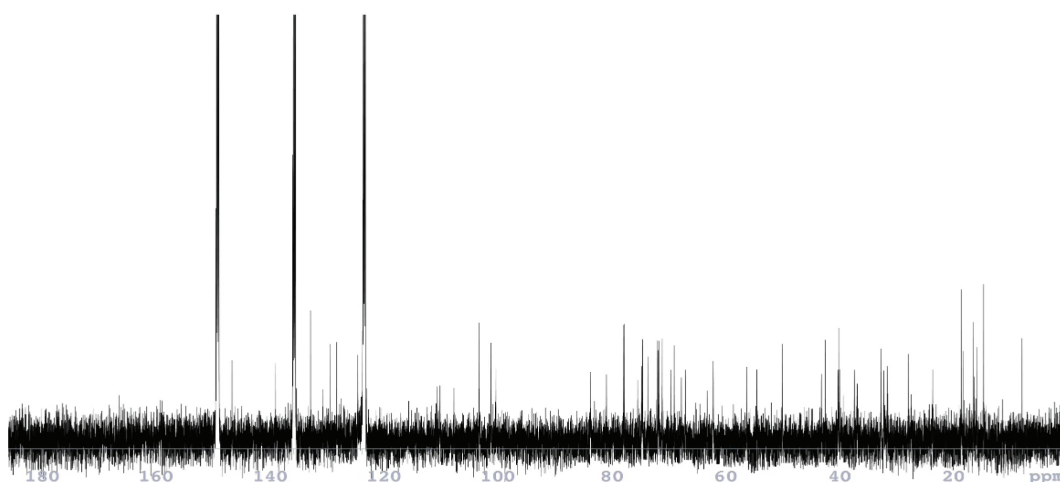

Fig. 4. ^{13}C NMR spectrum of compound 1.

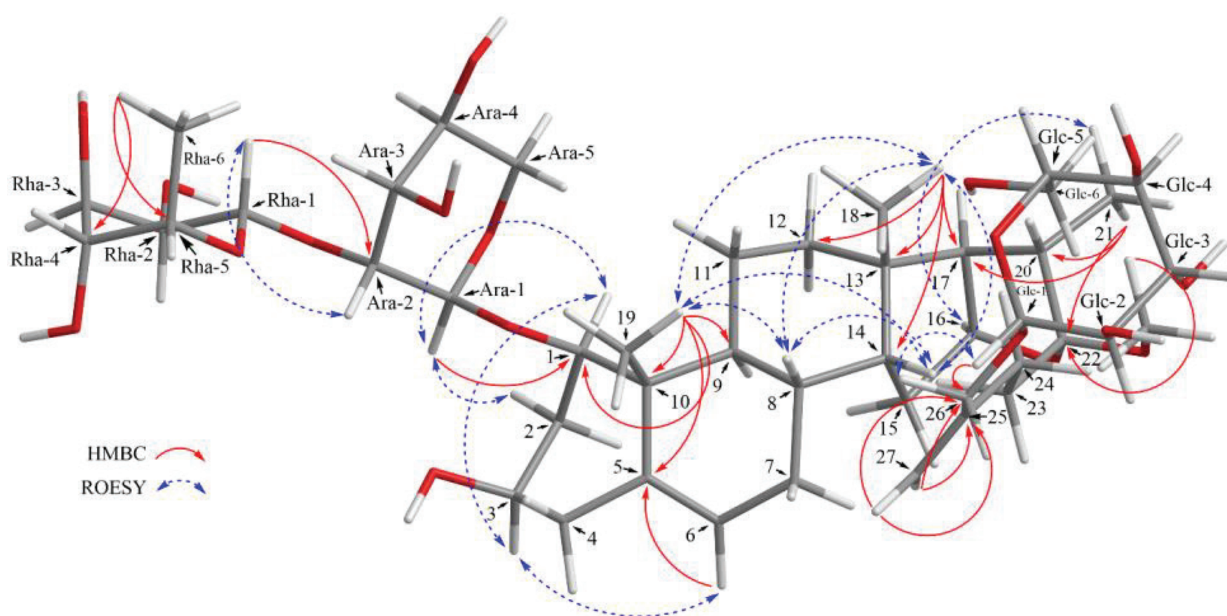

Fig. 5. Three-dimensional structure of compound 1 (HMBC correlations in red, ROESY correlations in blue).

The ^{1}H NMR spectrum in the sugar region of compound **1** exhibited the presence of three anomeric protons at $\boldsymbol{\delta}_H$ 4.67 (d, *J*=7.0 Hz), 4.90 (d, *J*=7.6 Hz), and 6.29 (br s), which were further revealed to be one arabinose (Ara), one glucose (Glc), and one rhamnose (Rha) unit, respectively. These were delineated through systematic ^{1}H-^{13}C HMBC and [1]H-^{1}H ROESY spectra mapping from the anomeric proton signals. Acid hydrolysis and comparison with antecedent research data [9] elucidated their absolute configurations as α-L for Rha and Ara, and β-D for Glc [9]. The Ara moiety's connection to the C-1 position of the aglycone was confirmed through the HMBC correlation between δ_H 4.67 (d, *J*=7.0 Hz, Ara H-1) and δ_C 83.7 (aglycone C-1), and a ROESY correlation with δ_H 3.71 (dd, *J*=11.3, 2.9 Hz, aglycone H-1). The sugar sequence extending from the C-1 position of the aglycone was conclusively identified as α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl. This was validated by the HMBC correlation from δ_H 6.29 (br s, Rha H-1) to δ_C 74.7 (Ara C-2), and the ROESY correlation between δ_H 6.29 (br s, Rha H-1) and δ_H 4.52 (Ara H-2). Furthermore, the linkage of the Glc unit to the C-26 position of the aglycone was established via the HMBC correlation from δ_H 4.90 (d, *J*=7.6 Hz, Glc H-1) to δ_C 72.1 (aglycone C-26), complemented by the ROESY correlation with δ_H 4.59 (br d, *J*=12.3 Hz, aglycone H-26a). Consequently, the complete molecular architecture of compound 1 was resolved as 26-*O*-β-D-glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1β,3β,22α,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} (Fig. 6), analogous to the compound isolated from the subterranean segments of *Ruscus aculeatus*, a member of the Asparagaceae family, sourced from Japan [10].

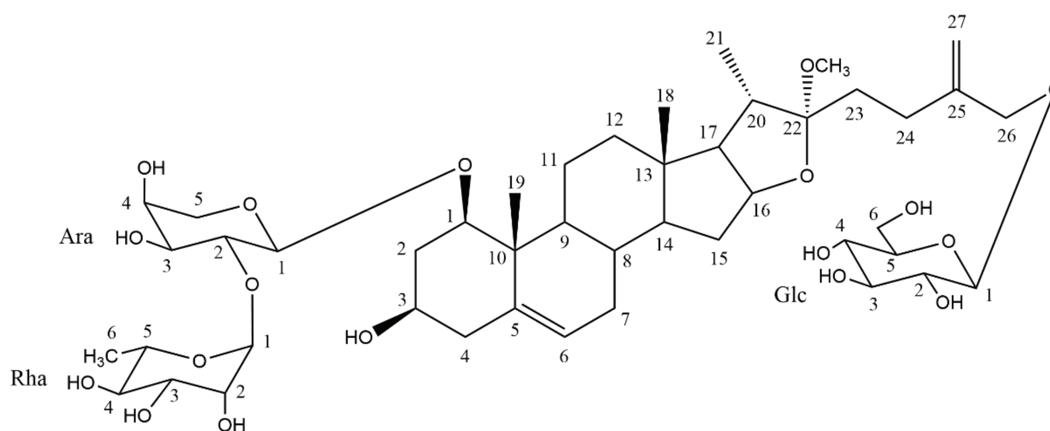

Fig. 6. Structure of compound 1.

Saponins are recognized for their hemolytic, antimicrobial, piscicidal, molluscicidal, insecticidal, antifeedant, antifertility, cytotoxic, and antitumor properties. Notably, three steroidal saponins including namonin A, namonin B, and (23*S*,24*S*)-spirosta-5,25(27)-dien-1β-3β-23,24-tetrol 1-*O*-{[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside isolated from the roots and rhizomes of *D. angustifolia* showed significant antiproliferative activity against HT-1080 fibrosarcoma cells with IC_{50} values of 0.2, 0.3, and 0.6 µM, respectively, compared to doxorubicin with IC_{50} values of 0.2 µM [11]. The biological activity of saponins correlates with their chemical structure. For example, mannioside A and other compounds such as pennogenin, floribundasaponin A, and spiroconazole A, isolated from the stem bark of *D. mannii* collected in Cameroon, were evaluated for anti-inflammatory effects. Results indicated that after one-hour post-carrageenan injection, the maximal inhibitory activity of 80.57, 71.22, 66.19, and 30.21% was achieved for mannioside A, floribundasaponin A, spiroconazole A, and pennogenin, respectively. It was inferred that the attachment of a Glc unit to the aglycone markedly enhances the anti-inflammatory efficacy of these compounds. Additionally, the inclusion of a Rha unit to the C-3 position of the Glc unit in mannioside A resulted in marginally greater activity compared to floribundasaponin A. Interestingly, two Rha units connected at the C-2 and C-3 positions of Glc in spiroconazole A slightly diminished the anti-inflammatory effect [12]. Further studies on the cytotoxic activity of saponins from the *Dracaena* genus were conducted by Y. Luo, et al. (2014) [13] on *D. cambodiana*'s dragon's blood collected in China, which led to the isolation of thirteen steroidal saponins. Among these, diosgenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside, pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside, and spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside exhibited significant inhibitory activities against various cancer cell lines with IC_{50} values indicating potent efficacy, whilst other compounds were inactive (IC_{50}>10 µM), on par with paclitaxel [13]. This overview suggests that the *Dracaena* genus is a promising source of saponins with remarkable biological activities, particularly in cytotoxicity against cancer cell lines. Consequently, future testing of the biological activity of saponin 26-*O*-β-D-glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1β,3β,22α,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} is warranted to augment chemotaxonomy data on the chemical composition and biological activity of the *D. draco* L. species and the *Dracaena* genus at large.

4. Conclusions

In this study, a steroidal saponin was extracted from the foliage of *D. draco* L. using some chromatographic and spectroscopic methods. The structure of this compound was determined to be 26-*O*-β-D-glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1β,3β,22α,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl}, which is similar to the saponin isolated previously from the underground portions of *Ruscus aculeatus* collected in Japan.

CRediT author statement

Hung Duc Nguyen: Investigation, Methodology, Writing original draft, Editing, Data analysis; Tan Quang Tu: Investigation and Writing, Visualisation; Mau Hoang Chu: Writing - Original editing, Conceptualisation, Supervision, Methodology, Idea.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] Z.M. Thu, S.M. Oo, T.M. Nwe, et al. (2021), "Structures and bioactivities of steroidal saponins isolated from the genera *Dracaena* and *Sansevieria*", *Molecules*, **26(7)**, DOI: 10.3390/molecules26071916.

[2] Q.A. Zheng, H.Z. Li, Y.J. Zhang, et al. (2006), "Dracaenogenins A and B, new spirostanols from the red resin of *Dracaena cochinchinensis*", *Steroids*, **71(2)**, pp.160-164, DOI: 10.1016/j.steroids.2005.09.007.

[3] V.A.N. Ngnitedem, L.K. Omosa, S. Derese, et al. (2022), "Cytotoxic flavonoids from the seeds of *Dracaena steudneri* Engl against leukemia cancer cell lines", *Phytomedicine Plus*, **2(2)**, DOI: 10.1016/j.phy.plu.2022.100234.

[4] N.D. Hung, T.Q. Tan (2022), "Study on phytochemical composition and isolation steroidal saponin from the leaves of *Dracaena fragrans* (L.) Ker Gawl.", *TNU J. Sci. Technol.*, **227(14)**, pp.93-99, DOI: 10.34238/tnu-jst.6241 (in Vietnamese).

[5] L.T. Nga, T.Q. Tan, N.T.H. Yen, et al. (2023), "Study on antibacterial activity and isolation of steroidal saponin from the roots of *Dracaena braunii* Engl.", *TNU J. Sci. Technol.*, **228(05)**, pp.105-112, DOI: 10.34238/tnu-jst.7180 (in Vietnamese).

[6] T.Q. Tan, N.D. Hung (2023a), "Study on isolation and structure elucidation of steroidal saponins from the leaves of *Dracaena fragrans* (L.) Ker Gawl.", *TNU J. Sci. Technol.*, **228(05)**, pp.3-10, DOI: 10.34238/tnu-jst.6834 (in Vietnamese).

[7] Y. Mimaki, M. Kuroda, A. Ide, et al. (1999), "Steroidal saponins from the aerial parts of *Dracaena draco* and their cytostatic activity on HL-60 cells", *Phytochemistry*, **50(5)**, pp.805-813, DOI: 10.1016/s0031-9422(98)00614-1.

[8] T.Q. Tan, N.T.H. Yen, C.H. Mau, et al. (2023b), "One new steroidal saponin from the leaves of *Cordyline fruticosa* (L.) A. Chev.", *Lett. Org. Chem.*, **20(6)**, pp.579-583, DOI: 10.2174/1570178620666230116114218.

[9] D.H. Nguyen, Q.T. Tu, H.M. Chu (2022), "Triterpenoid saponins from the root of *Weigela florida*", *Vietnam Journal of Science, Technology and Engineering*, **64(4)**, pp.79-83, DOI: 10.31276/VJSTE.64(4).79-83.

[10] Y. Mimaki, M. Kuroda, A. Kameyama, et al. (1998), "New steroidal constituents of the underground parts of *Ruscus aculeatus* and their cytostatic activity on HL-60 cells", *Chem. Pharm. Bull.*, **46(2)**, pp.298-303, DOI: 10.1248/cpb.46.298.

[11] Q.L. Tran, Y. Tezuka, A.H. Banskota, et al. (2001), "New spirostanol steroids and steroidal saponins from roots and rhizomes of *Dracaena angustifolia* and their antiproliferative activity", *J. Nat. Prod.*, **64(9)**, pp.1127-1132, DOI: 10.1021/np0100385.

[12] L.A. Tapondjou, K.B. Ponou, R.B. Teponno, et al. (2008), "*In vivo* anti-inflammatory effect of a new steroidal saponin, mannioside A, and its derivatives isolated from *Dracaena mannii*", *Arch. Pharm. Res.*, **31(5)**, pp.653-658, DOI: 10.1007/s12272-001-1208-3.

[13] Y. Luo, H.Y. Shen, W.J. Zuo, et al. (2014), "A new steroidal saponin from dragon's blood of *Dracaena cambodiana*", *J. Asian Nat. Prod. Res.*, **17(4)**, pp.409-414, DOI: 10.1080/10286020.2014.967229.