STUDY ON ISOLATION AND STRUCTURAL DETERMINATION OF STEROIDAL SAPONIN FROM THE LEAVES OF *Dracaena fragrans*

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ABSTRACT

Saponins is a chemical compound which could be found in almost every part of plant. Some saponins such as protodioscin and protoneodioscin have been chosen for the National Cancer Institute's anticancer drug screen program. Currently, pharmaceutical companies in the world are focusing on searching for new drugs derived from plants, and also finding how to isolate single compounds in order to remove toxic compounds from initial material and collect compounds possessing biological activity including saponins. In this study, a steroidal saponin with a spirostanol aglycone has been isolated from the leaves of *Dracaena fragrans*. Chemical structure of this compound has been characterized as spirost-5,25(27)-dien-1β,3β-diol-1-O-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, which previously isolated from the stems of *Nolina recurvata* belonging to the *Nolina* genus, thus the Asparagaceae family distributed in Japan. A short review on saponins isolated from the *Dracaena* genus as well as their biological activities has been discussed which further could be used to carry out more experiments on biological activities of spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, in order to complete database on chemical composition and biological activity of *D. fragrans* and the *Dracaena* genus.

NGHIÊN CỨU PHÂN LẬP VÀ XÁC ĐỊNH CẤU TRÚC HÓA HỌC CỦA HỢP CHẤT SAPONIN STEROID TỪ LÁ CỦA LOÀI *Dracaena fragrans*

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TỪ KHÓA

Cấu trúc hóa học
Dracaena fragrans
NMR
Saponin
Spirostane-type

TÓM TẮT

Saponin là một hợp chất hóa học có thể tìm thấy ở hầu hết các bộ phận của cây. Một số saponin như protodioscin và protodioscin đã được lựa chọn cho chương trình sàng lọc thuốc chống ung thư của Viện Ung thư Quốc gia. Hiện nay, các công ty dược phẩm trên thế giới đang tập trung tìm kiếm các loại thuốc mới có nguồn gốc từ thực vật, đồng thời tìm cách phân lập các hợp chất đơn lẻ nhằm loại bỏ các hợp chất độc hại ra khỏi nguyên liệu ban đầu và thu thập các hợp chất có hoạt tính sinh học trong đó có saponin. Trong nghiên cứu này, saponin steroid với aglycone spirostanol đã được phân lập từ lá *Dracaena fragrans*. Cấu trúc hóa học của hợp chất này được đặc trưng là spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, trước đây được phân lập từ thân cây *Nolina recurvata* thuộc chi *Nolina*, họ Asparagaceae phân bố ở Nhật Bản. Tổng quan về hợp chất saponin phân lập từ chi *Dracaena* cũng như hoạt tính sinh học của chúng đã được thảo luận và có thể được sử dụng để thực hiện thêm các thí nghiệm về hoạt tính sinh học của spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, nhằm hoàn thiện cơ sở dữ liệu về thành phần hóa học và hoạt tính sinh học của loài *D. fragrans* và chi *Dracaena*.

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1. Introduction

Saponins is a chemical compound that could be found in almost every part of plant. Some saponins such as protodioscin and protoneodioscin have been chosen for the National Cancer Institute's anticancer drug screen program [1]. Currently, pharmaceutical companies in the world are focusing on searching for new drugs derived from plants, and also finding how to isolate single compounds in order to remove toxic compounds from initial material and collect compounds possessing biological activity including saponins. The *Dracaena* genus contains about 100 species which are widely distributed in the tropical regions of Canary Islands, Madeira, and South East Asia and also from East and West Africa [2]. In Vietnam, 11 species of the *Dracaena* genus are found in natural places for medicinal and ornamental purposes [3]. *D. fragrans* is distributed in western Africa and southeast Asia in which could be found in Vietnam, and this plant has been used for medicinal purposes such as treating malnutrition, inducing labor, treating HIV/AIDS by increasing CD4, and possessing antimicrobial activity [4]-[7]. In our interest of searching for natural compounds in plants, we have isolated a new steroidal saponin from the leaves of *D. fragrans*, along with two known saponins from two species of the *Dracaena* genus, *D. fragrans* and *D. braunii* [8]-[10]. As a continuing study on *Dracaena* plants, we have now investigated on the leaves of *D. fragrans*. A description of the structure of a steroidal saponin has been carried out based on spectroscopic analysis and in combination with mass spectrometry.

2. Methodology

2.1. General procedures

^{1}H and ^{13}C NMR spectra (δ in ppm, J in Hz) were obtained on an NMR Inova spectrometer (Agilent Technologies®, USA). Pyridine-d_5 was used as a reference standard for ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz). HR-ESI-MS was determined on a micrOTOF II mass spectrometer (Bruker®, Germany). Extraction of materials was carried out on an Elmasonic S10H ultrasound cleaner (Elma, Switzerland). Crude extract was separated using vacuum liquid chromatography (VLC) on Silicycle silica gel RP-18 (Canada) of particle size 75-200 µm as the stationary phase, and a solvent system of H_2O-EtOH (1-0, 1-1, 0-1, v-v) as the mobile phase. Column chromatography (CC) was used to remove impurities in fractions using Merck silica gel 60 (Germany) of particle size 15–40 µm as the stationary phase, and a solvent system of $CHCl_3$-MeOH-H_2O at different ratios. Medium pressure liquid chromatography (MPLC) was carried out on Merck silica gel 60 (Germany) of particle size 15-40 µm as the stationary phase and the same solvent system used for CC. All the fractions were monitored by thin-layer chromatography (TLC) on Merck silica gel $60F_{254}$ (Germany). Compounds were visualized as colored spots by spraying with vanilin-H_2SO_4 and then by heating on a hot plate.

2.2. Plant materials

Figure 1. *Morphological characteristic of D. fragrans*

Leaves of *D. fragrans*. were collected in Thai Nguyen, Vietnam, in September 2021 (Figure 1). A voucher specimen (NDH202103TNDDF) is maintained at Faculty of Biology, Thai Nguyen University of Education, Vietnam.

2.3. Extraction and isolation

The dried leaves of *D. fragrans* (389.5 g) were successively extracted with a solvent system of EtOH-H_2O (75:35, 700 mL each, 30 W, 50 °C, 30 min). The resulting extract was concentrated to almost dryness under reduced pressure to give a residue (6.8 g) which was submitted to a VLC on RP-18 (H_2O-EtOH, 1-0, 1-1, 0-1; 500 mL each) resulting three fractions (D1–D3). Fraction D2 (327.5 mg) was submitted on a CC over silica gel and eluted successively with $CHCl_3$-MeOH-H_2O 70-30-5 (v-v-v) to afford five crude saponin subfractions: D2.1 (23.8 mg), D2.2 (39.2 mg), D2.3 (53.1 mg), D2.4 (44.3 mg) and D2.5 (67.1 mg). Subfraction D2.3 (53.1 mg) was chromatographed on a MPLC silica gel 60 with $CHCl_3$-MeOH-H_2O 75-25-3, 70-30-5 (v-v-v) to give four subfractions including saponin **1** (3.2 mg).

*Spirost-5,25(27)-dien-1β,3β-diol-1-O-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (**1**):* white amorphous powder; ^{1}H NMR (600 MHz in pyridine-d_5) and ^{13}C NMR (150 MHz in pyridine-d_5) spectral data are given in Table 1; HR-ESI-MS (positive mode): *m/z* 861.4238 $[M+Na]^+$ (calculated for $C_{43}H_{66}NaO_{16}^+$, 861.4243).

3. Results and discussion

Compound **1** was obtained as a white amorphous powder with a molecular formula of $C_{43}H_{66}O_{16}$, proved by HR-ESI-MS at *m/z* 861.4238 $[M+Na]^+$. Analysis of the 2D NMR resulted three sugar units as one arabinose (Ara), one xylose (Xyl) and one rhamnose (Rha). The structure of the aglycone of **1** was also confirmed by observation the signals exhibited in the ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of **1** exhibited signal attributed to anomeric protons at δ_H = 4.68 (d, J = 7.6 Hz), δ_H = 4.95 (d, J = 7.6 Hz), δ_H = 6.32 (br s), an olefinic proton at δ_H = 5.57 (d, J = 5.3 Hz, aglycone H-6), two angular methyl protons at δ_H = 1.44 (s, aglycone H-18), δ_H = 0.86 (s, aglycone H-19), one secondary methyl proton at δ_H = 1.05 (d, J = 7.0 Hz, aglycone H-21) (Figure 2).

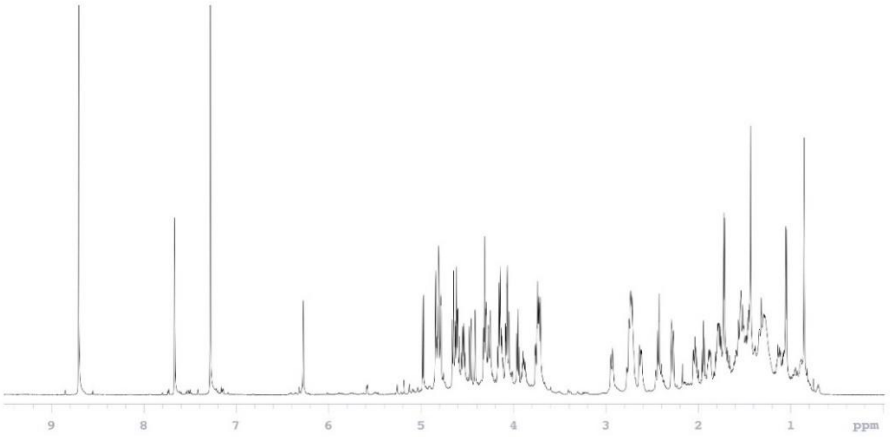

Figure 2. *The ^{1}H NMR spectrum of compound **1***

The ^{13}C NMR spectrum showed a total of 27 carbons arising from the aglycone moiety (Figure 3). The fundamental steroid structure of **1**, based upon spirostanol-type, was suggested by a quaternary carbon signal at δ_C = 109.4 assignable to C-22 of the spirostanol skeleton in the ^{13}C NMR spectrum, and the oxymethylene proton signals at δ_H = 4.04 (br d, J = 11.7 Hz) and δ_H = 4.45 (br d, J = 11.7 Hz) attributable to 26-H_2, as well as the above ^{1}H NMR data (Table 1). Thus, the structure of the aglycone of **1** was assigned as spirost-5,25(27)-dien-1β,3β-diol.

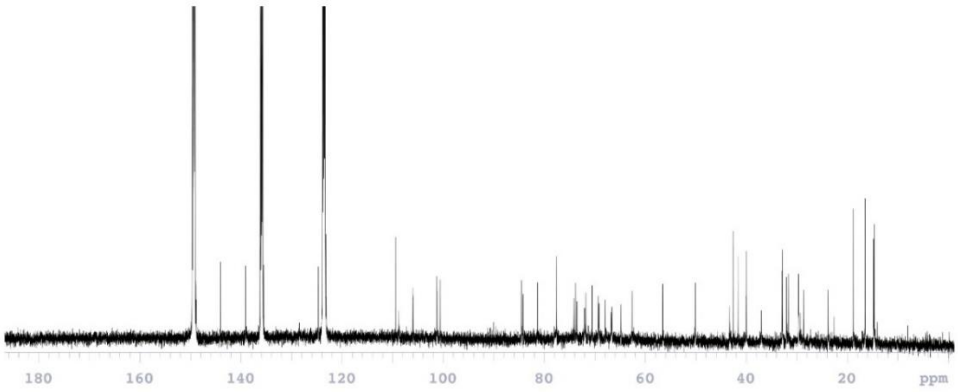

Figure 3. *The ^{13}C NMR spectrum of compound* **1**

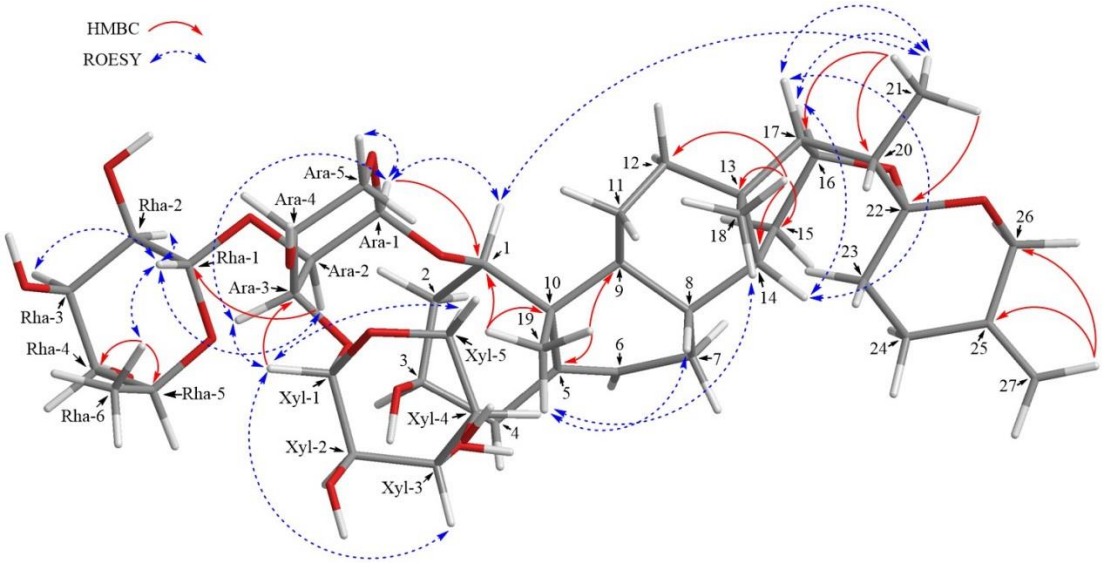

Figure 4. *The 3D structure of compound* ***1*** *with the keys of HMBC and ROESY*

The study on monosaccharide region started from the anomeric proton signals at δ_H = 4.68 (d, J = 7.6 Hz), δ_H = 4.95 (d, J = 7.6 Hz) and δ_H = 6.32 (br s). The signals in the HSQC spectrum exhibited the presence of an α-L-Ara unit at δ_H = 4.68 (d, J = 7.6 Hz), a β-D-Xyl unit at δ_H = 4.95 (d, J = 7.6 Hz), and an α-L-Rha unit at δ_H = 6.32 (br s). The signals of a α-L-Ara unit was indicated by characteristic five signals at δ_C = 100.5 (CH), δ_C = 74.4 (CH), δ_C =84.3 (CH), δ_C = 69.2 (CH) and δ_C = 66.8 (CH_2) in the ^{13}C NMR spectrum, which accomplished with signals in the ^1H NMR spectrum at δ_H = 4.68 (d, J = 7.6 Hz, CH), δ_H = 4.53 (CH), δ_H = 4.10 (CH), δ_H = 4.35 (CH) and δ_H = 3.74, 4.29 (CH_2). The remaining five signals due to another monosaccharide were assigned to a β-D-Xyl at δ_C = 106.2 (CH), δ_C = 74.4 (CH), δ_C =77.8 (CH), δ_C = 70.5 (CH) and δ_C = 66.9 (CH_2) in the ^{13}C NMR spectrum, and at δ_H = 4.95 (d, J = 7.6 Hz, CH), δ_H = 3.94 (CH), δ_H = 4.19 (CH), δ_H = 4.12 (CH) and δ_H = 3.68, 4.35 (CH_2) in the ^1H NMR spectrum. The signals of an α-L-Rha unit were identified by an observation of six signals in the ^{13}C NMR spectrum at δ_C = 101.5 (CH), δ_C = 72.2 (CH), δ_C =72.1 (CH), δ_C = 73.9 (CH), δ_C = 69.5 (CH) and δ_C = 18.6 (CH_3), which was good in agreement with the signals in the ^1H NMR spectrum at δ_H = 6.32 (br s, CH), δ_H = 4.77 (CH), δ_H = 4.65 (d, J = 9.9, 2.3 Hz, CH), δ_H = 4.32 (d, J = 9.7, 9.3 Hz, CH), δ_H = 4.77 (d, J = 9.7, 5.8 Hz, CH) and δ_H = 1.74 (d, J = 5.8 Hz, CH_3) (Table 1). The monosaccharide sequence of **1** was assigned by correlations observed in the HMBC and ROESY spectra. The HMBC spectrum showed the ^1H-^{13}C long-range correlations between δ_H = 4.68 (d, J = 7.6 Hz,

Ara H-1) and δ_C = 84.1 (aglycone C-1), δ_H = 6.32 (br s, Rha H-1) and δ_C = 74.4 (Ara C-2), δ_H = 4.95 (d, *J* = 7.6 Hz, Xyl H-1) and δ_C = 84.3 (Ara C-3). The ^{1}H-^{1}H long-range correlations were exhibited in the ROESY spectrum at δ_H = 4.68 (d, *J* = 7.6 Hz, Ara H-1) and δ_H = 3.73 (d, *J* = 11.1, 2.3 Hz, aglycone H-1), δ_H = 6.32 (br s, Rha H-1) and δ_H = 4.53 (Ara H-2), δ_H = 4.95 (d, *J* = 7.6 Hz, Xyl H-1) and δ_H = 4.10 (Ara H-3) (Figure 4). Accordingly, the structure of **1** was determined to be spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, which was previously isolated from the stems of *Nolina recurvata* (Figure 5) [11].

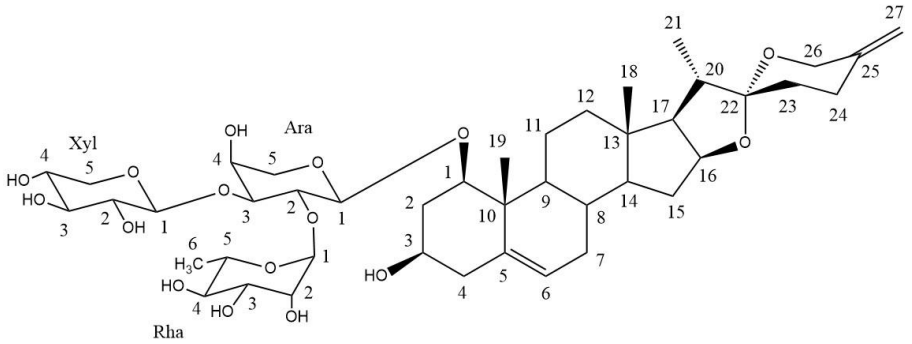

Figure 5. *The structure of compound **1***

Table 1. *^{13}C and ^{1}H NMR spectra data of compound **1** (C_5D_5N, δ in ppm, J in Hz)*

Carbon	δ_C (ppm)	δ_H (Hz)	Carbon	δ_C (ppm)	δ_H (Hz)
1	84.1	3.73 dd (11.1, 2.3)	23	32.6	1.77 m. 1.79 m
2	37.2	2.42 q (11.1),2.71	24	28.5	2.25, 2.71
3	68.2	3.93 m	25	144.3	-
4	43.2	2.61, 2.77	26	64.6	4.04 br d (11.7), 4.45 br d (11.7)
5	139.0	-	27	108.8	4.83 br s, 4.87 br s
6	124.3	5.57 d (5.3)	Ara-1	100.5	4.68 d (7.6)
7	32.1	1.85, 2.02	2	74.4	4.53
8	33.3	1.51 m	3	84.3	4.10
9	50.3	1.53 m	4	69.2	4.35
10	42.7	-	5	66.8	3.74, 4.29
11	23.5	1.57, 2.95 dd (11.3, 3.1)	Rha-1	101.5	6.32 br s
12	40.2	1.29, 1.52	2	72.2	4.77 br s
13	40.4	-	3	72.1	4.65 dd (9.9, 2.3)
14	56.7	1.16	4	73.9	4.32 dd (9.7, 9.3)
15	31.9	1.44 m, 2.06 m	5	69.5	4.77 dq (9.7, 5.8)
16	81.1	4.56 q (6.4)	6	18.6	1.74 d (5.8)
17	62.3	1.74	Xyl-1	106.2	4.95 d (7.6)
18	14.9	1.44 s	2	74.4	3.94
19	16.5	0.86 s	3	77.8	4.19
20	41.6	1.95 dd (7.0, 6.4)	4	70.5	4.12
21	14.7	1.05 d (7.0)	5	66.9	3.68, 4.35
22	109.4	-			

A number of saponins have been described for their cytotoxic activities including antimicrobial, antioxidant, antiallergic, immunomodulatory, immunostimulatory, antiviral, antihepatotoxic, antidiabetic, antifungal, molluscicidal, cardiac and cancer-related activities (cytotoxic activity, antitumor activity, chemo preventive activity). The first study on isolation and evaluation of saponin has been carried out on sea cucumber which further showed an interesting antitumor activity [12]. Since then, a number of publications on isolation and evaluation on cancer-related activities have been carried out on different organisms [13], [14]. A new steroidal saponin named afromontoside along with ten known saponins were isolated from the methanolic

extract of the twigs of *D. afromontana* collected from Kenya. The test for biological activity was further carried out on cytotoxicity against cultured KB cells. The result showed that the compound afromontoside possessed a significant cytotoxic activity on this cell line [15]. Biological activity of saponins has a relationship with their chemical structures. Eighteen saponins including six new saponins and twelve known compounds were isolated from *D. thalioides* collected from Japan. All the isolated compounds were then tested for their cytotoxicity against HL-60 cell lines. Three of those compounds possessed a potential cytotoxic activity with the IC_{50} values ranged from 0.38 to 0.74 µM, while other compounds showed weaker activity with the IC_{50} values ranged from 1.66 to 17.3 µM. The structure-activity relationship was further concluded that the presence of the triacetylated α-L-Rha moiety and the β-D-Fuc group linked to C-24 position of the aglycone was the key to the potent cytotoxic activity of saponins. One of those potential compounds was chosen for test of apoptosis induction activity. The result showed that caspase-3 exhibited an important role in apoptotic signaling pathways and is the enzyme that executes apoptosis [16]. Evaluation on cytotoxic activity of saponins isolated from the *Dracaena* genus has been continued with the study of Teponno et al., (2017) on the *D. viridiflora* collected from Cameroon. This research resulted the isolation of six steroidal saponins and three of those exhibited a significant activity with the IC_{50} values ranged from 0.42 to 16.13 µg/mL while three other compounds displayed weaker cytotoxic activity with the IC_{50} values ranged from 13.72 to 93.46 µg/mL against four cancer cell lines (human ovarian carcinoma Skov-3, lung adenocarcinoma A549, human T-Cell leukemia cells JURKAT, and human epithelial colorectal adenocarcinoma cells Caco-2) [17]. The basis of the above overview suggests that the *Dracaena* genus is a source of saponins possessing remarkable biological activities, especially in cytotoxic activity on cancer cell lines. Therefore, it is proposed to continue to evaluate the biological activities of spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside isolated in this study in order to complete the chemotaxonomy data on chemical composition and biological activity of the *D. fragrans* species and thus the *Dracaena* genus.

4. Conclusion

A steroidal saponin has been successfully isolated from the leaves of *D. fragrans*. This compound has been characterized as spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside which was previously isolated from the stem of *Nolina recurvata*, the *Nolina* genus, thus the Asparagaceae family. An overview of some saponins isolated from the *Dracaena* genus, as well as their biological activities have been discussed. A short review on saponins isolated from the *Dracaena* genus as well as their biological activities has been discussed which further could be used to carry out more experiments on biological activities of spirost-5,25(27)-dien-1β,3β-diol-1-*O*-α-L-rhamnopyranosy-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, in order to complete database on chemical composition and biological activity of *D. fragrans* and the *Dracaena* genus.

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