

# Biological Activity of 6,7-Dehydroxyroyleanone and Derivatives Obtained from *Plectranthus aliciae* (Codd) A.J.Paton

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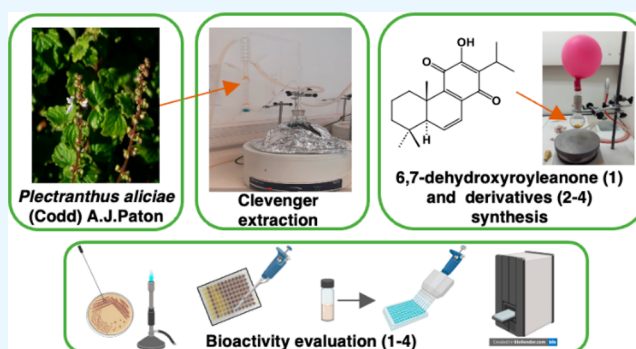


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Supporting Information

**ABSTRACT:** The *Plectranthus* genus (Lamiaceae) is known to be rich in abietane diterpenes. The bioactive 6,7-dehydroxyroyleanone (DHR, **1**) was previously isolated from *Plectranthus madagascariensis* var. *madagascariensis* and var. *aliciae*. This study aimed to explore the occurrence of DHR, **1**, in *P. aliciae* and the potential bioactivities of new semisynthetic derivatives from DHR, **1**. Several extraction methods were evaluated, and the hydro-distillation, using a Clevenger apparatus, afforded the highest yield (77.8 mg/g of **1** in the essential oil). Three new acyl derivatives (**2–4**) were successfully prepared from **1** (yields of 86–95%). Compounds **1–4** showed antioxidant activity, antibacterial effects, potent cytotoxic activity against several cell lines, and enhanced anti-inflammatory activity that surpassed dexamethasone (positive control). These findings encourage further exploration of derivatives **2–4** for potential mechanisms of antitumoral, antioxidant, and anti-inflammatory capabilities, studying both safety and efficacy.

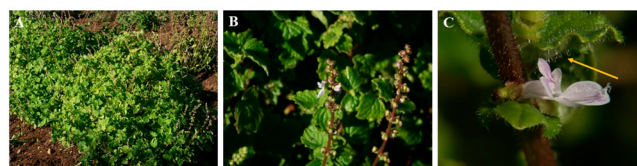


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## INTRODUCTION

The Lamiaceae family represents commercially significant plants, comprising approximately 250 genera and more than 7000 species, many of which are utilized in both folk medicine and modern industries.<sup>1</sup> *Plectranthus* L'Hér. genus, also known as spurflowers, corresponds to approximately 40% of the Lamiaceae family, which consists of over 300 species, distributed in tropical and subtropical areas of the globe.<sup>2,3</sup> *Plectranthus* plants have diverse horticulture and traditional medicine applications with approximately 85% of documented uses being attributed to medicinal purposes.<sup>4</sup> Moreover, *Plectranthus* species are known for their aromatic properties, attributed to their essential oil (EO) production.<sup>3</sup> Phytochemical studies have revealed that the *Plectranthus* genus serves as an abundant reservoir of phenolic compounds and terpenes, particularly diterpenes, which have drawn attention due to their structural diversity and high potential as promising drug candidates.<sup>3,5</sup> Furthermore, the presence of diterpenes serves as a chemotaxonomic marker of these plants.<sup>6</sup>

*Plectranthus aliciae* (Codd) van Jaarsv. & T.J.Edwards (Figure 1) was initially described as a subspecies of *Plectranthus madagascariensis* (var. *aliciae* Codd)<sup>7,8</sup> and later reclassified as *Coleus aliciae* (Codd) A.J.Paton.<sup>9</sup> Distinguishing *Plectranthus* spp. from other closely related species poses a challenge due to the similarities in their morphological characteristics, and consequently, synonyms are highly



**Figure 1.** *P. aliciae* plant: (A) whole plant; (B) corolla and teathed leaves; (C) hairy ovate leaves with red glands. Photograph courtesy of Patrícia Rijo. Copyright 2024.

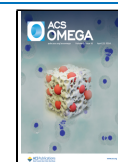
prevalent in this genus.<sup>9</sup> *P. aliciae* is a semisucculent herb commonly found along the northern part of the Eastern Cape Coast of South Africa, from East London to southern KwaZulu-Natal.<sup>10,11</sup> *P. aliciae* has a tradition of being used by local communities for<sup>3</sup> addressing respiratory conditions, such as coughs and asthma, as well as managing flu and colds and treating skin-related illnesses, such as wounds and scabies.<sup>12</sup>

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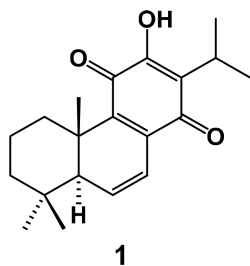
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Recent studies on *P. aliciae* acetic extract showed antimicrobial activity. Toxic effects were observed at a 50% lethal concentration (LC<sub>50</sub>) at 53.48 μg/mL, and the extracts demonstrated cytotoxicity against human colon carcinoma, breast adenocarcinoma, and nonsmall lung cell carcinoma cell lines.<sup>13</sup> In addition, the EO of *P. aliciae* revealed antimicrobial activity and general toxicity accounting for a 59% death rate in the *Artemia salina* model.<sup>13</sup> Although there is scarce information on *P. aliciae* phytochemistry studies, as a variant of *P. madagascariensis* and sharing similar bioactivities, it has been alleged that similar compounds could be present in both varieties.<sup>14</sup> The abietane royleanone-type diterpene 6,7-dehydroxyroyleanone (DHR, **1**) (Figure 2) is the major



**Figure 2.** 6,7-Dehydroxyroyleanone (DHR, **1**) chemical structure, the major compound present in the *P. madagascariensis* EO and acetic extract.

component of *P. madagascariensis* EO.<sup>2</sup> DHR has exhibited moderate to significant cytotoxic activity against various cancer cell lines.<sup>13–15</sup> Thus, exploring *P. aliciae* to obtain the lead natural compound **1** could open opportunities for the synthesis of novel compounds or formulations with enhanced bioactivity.

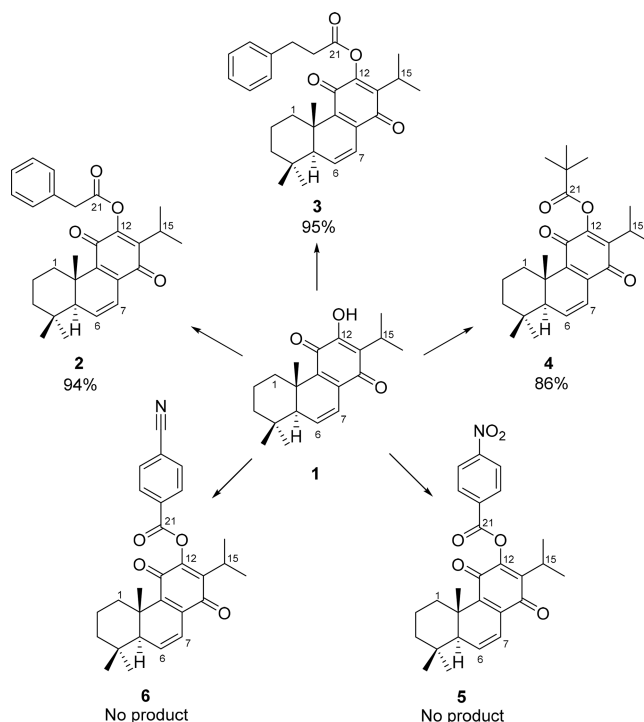
In order to obtain DHR, **1**, from *P. aliciae*, three extraction methods were studied, namely, Clevenger-assisted extraction (CAE), ultrasound-assisted extraction (UAE), and maceration-assisted extraction (MAE). Each method was carefully studied to determine the most effective and efficient way to obtain the desired outcome. EO extraction with CAE afforded a dark orange oil with an agreeable odor, similar to that mentioned in the literature for other *Plectranthus* spp.<sup>2,16</sup> and also characteristic of the presence of compound **1**. The presence of DHR, **1**, in *P. aliciae* EO was confirmed by GC-FID and GC-MS analysis (Table S1, Supporting Information). Fenchone and β-caryophyllene emerged as the major compounds identified in the *P. aliciae* EO. Additionally, the EO was found to comprise of 4.3% of DHR, **1**.

The extraction yield was highest for UAE using water (16.3%), followed by water:acetone UAE (10.2%) and MAE (0.9%). However, CAE had the lowest extraction yield (0.04%) (Table S2, Supporting Information). Despite this, CAE had a higher recovery of DHR **1**, resulting in 77.8 mg/g of compound **1** in the EO. The amount of DHR, **1**, in each extract was assessed by HPLC-DAD. Compound **1** was identified from the chromatogram based on its retention time (37.79 min) and comparison of UV spectra (Figure S1, Supporting Information) present in the literature.<sup>15,17</sup> Despite detection of DHR, **1**, in the UAE and MAE extracts, it was not possible to quantify the amount since in both instances **1** was lower than the limit of quantification (LOQ). These results are in agreement with those published for *P. madagascariensis* var. *madagascariensis*,<sup>15</sup> which describes the greatest recovery of

DHR, **1**, by CAE. One hypothesis for the low quantity of compound **1** in the MAE and UAE methods, compared to the high yield from CAE, can be attributed to the fact that DHR, **1**, is an artifact in this kind of extraction method as the high temperature causes oxidation of a precursor abietane into DHR **1**. Extractions can induce compound transformation; terpenes are often extracted with a Clevenger distillation device; as such, the highest yield of this bioactive compound obtained could be caused by the conditions linked to the technique itself.<sup>15</sup> In fact, there are some examples compiled in the literature of artifacts derived from abietane-type compound extraction.<sup>18</sup> In terms of biosynthesis, royleanones, such as DHR, **1**, taxoquinone, 7α-acetoxyroyleanone, horminone, 7-oxoroyleanone, and inuroyleanol are considered oxidative derivatives of the diterpene ferruginol.<sup>5,19,20</sup>

DHR, **1**, was isolated following the procedure described in ref 21, characterized by spectroscopic and spectrometric methods (<sup>1</sup>H NMR, <sup>13</sup>C NMR, UV-vis, GC-MS, and LC-HRMS-ESI-MS), and confirmed using the literature.<sup>15</sup> In this study, the preparation of new derivatives was also explored with the aim of improving the bioactivities of the lead compound **1**. The synthesis of derivatives from royleanones has been documented, and it has been demonstrated that ester derivatives are not only stable but also bioactive molecules.<sup>21</sup> Based on this, several esterification reactions were performed (Figure 3) using DHR, **1**, as the starting material.

Five esterification reactions were performed to obtain new DHR, **1**, ester derivatives (Figure 3); however, only three products (**2–4**) were successfully achieved with overall good yields of 94%, 95%, and 86%, respectively. Derivatives **2–4** were obtained in mild conditions, ranging from a few hours to 3 days. For the preparation of derivatives **5** and **6**, several



**Figure 3.** Scheme of DHR, **1**, derivatives **2–6** preparation. Derivatives **2–4** were successfully prepared using a small excess of pyridine and the corresponding acyl chloride. Derivatives **5** and **6** were not obtained.

**Table 1. Antioxidant Capacity of DHR 1 and Its Derivatives 2–4 Using Different Methodologies<sup>a</sup>**

samples	ABTS IC <sub>50</sub> (mM)	DPPH IC <sub>50</sub> (mM)	ORAC (TE)	HORAC (TE)	NO IC <sub>50</sub> (mM)	TAOC EC <sub>50</sub> (mM)
1	0.582	>1.5	0.291	0.294	0.833	>1.5
2	0.296	0.685	0.550	0.608	>1.5	0.412
3	0.362	0.706	0.757	0.630	1.050	0.457
4	0.302	0.542	0.826	0.642	0.544	0.480
*trolox	0.160	0.221	1	1	>1.5	0.143
*ascorbic acid	0.350	0.350				0.244

<sup>a</sup>ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate); DPPH, 2,2-diphenyl-1-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; HORAC, hydroxyl radical absorbance capacity; NO, nitric oxide; TAOC, total antioxidant capacity; TE, trolox equivalent. \*Trolox and \*ascorbic acid, positive controls.

reaction conditions were explored, namely, increasing the amount of reagent, temperature, under reflux temperature, and reaction time up to 5 days. However, despite our efforts, no product was obtained.

With the aim of evaluating the bioactive capacity of the lead molecule as well as the new compounds, several assays were tested. The antioxidant activity was evaluated through ABTS, DPPH, ORAC, HORAC, NO, and TAOC assays. The results (Table 1) of the natural compound 1 and derivatives 2–4 are expressed in IC<sub>50</sub> (mM) and trolox equivalent (TE). The results showed that all of the derivatives increased the antioxidant activity when compared with the starting material DHR, 1, yet had lower antioxidant activity when compared with the positive controls used for the DPPH, ORAC, HORAC and NO assays. In the ABTS assay, all of the compounds surpass the lead compound 1 and exceed or equaled the positive control. On the other hand, in the NO assay, compound 4 showed antioxidant activity and none the other compounds showed promising results. These results imply that the ester derivatives improved the antioxidant activity.

The antibacterial activity of compound 1 and derivatives 2–4 was evaluated through the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) with vancomycin as a positive control. The results are presented in Table 2. All compounds (1–4) showed

**Table 2. MIC and MBC Values of the Compounds Obtained by the Microdilution Method against Gram-Positive Strains (in  $\mu\text{M}$ )<sup>a</sup>**

samples	microorganisms			
	<i>S. aureus</i> (MSSA)		methicillin-resistant <i>S. aureus</i> (MRSA)	
	MIC	MBC	MIC	MBC
1	12.44	99.39	12.44	99.39
2	9.04	<72.24	9.04	<72.24
3	8.76	<69.97	8.76	<69.97
4	10.04	<80.22	10.04	<80.22
vancomycin	1.35	1.35	0.67	0.67

<sup>a</sup>Vancomycin, positive control; *S. aureus*, *Staphylococcus aureus*. The results represent a median of at least three independent experiments.

moderate antibacterial activity against both the *Staphylococcus aureus* (MSSA) and the resistant *S. aureus* (MRSA) strains with MIC values ranging from 8.76 to 12.44  $\mu\text{M}$  and MBC values from 69.97 to 99.39  $\mu\text{M}$  for both Gram-positive bacteria. It is worth noting that all of the derivatives (2–4) slightly increased the antibacterial activity against both strains when compared with the parent compound 1 (MIC of 12.44  $\mu\text{M}$  for compound

1 vs 9.04, 8.76, and 10.04  $\mu\text{M}$  for derivatives 2, 3 and 4, respectively). Compound 3 exhibited the most promising results with a MIC of 8.76  $\mu\text{M}$  and an MBC < 69.97  $\mu\text{M}$  against both strains. For compound 2, a MIC of 9.04  $\mu\text{M}$  and an MBC < 72.24  $\mu\text{M}$  was observed, suggesting that an extra aromatic moiety in the royleanone core could be an advantage to the antibacterial activity.

The general toxicity of the compounds was assayed using the *Artemia salina* model. The results showed that the semi-synthetic derivative 4 demonstrated an increased general toxicity, with a 52.16% mortality rate, when compared with the starting material 1 (36.60% mortality rate). However, derivatives 2 and 3 are responsible for 32.43% and 13.60% mortality rates, respectively, suggesting that the *tert*-butyl moiety could be responsible for the increase in the general toxicity, while aromatic groups could lead to a decrease in toxicity. Next, the cytotoxicity activity of compounds 1–4 was evaluated against gastric carcinoma (AGS), colorectal adenocarcinoma (CaCo-2), breast carcinoma (MCF-7), and lung adenocarcinoma (NCI-H460) cell lines. Additionally, the hepatotoxicity was evaluated in a primary culture obtained from pig liver (PLP2). Derivative 2 showed the most promising results against all cancer cell lines tested (Table 3), with increased cytotoxic activity when compared to DHR, 1. Moreover, compound 2 showed slight selectivity toward the NCI-H460 cancer cell line. Derivative 4 showed selectivity against AGS cells, since a slight increase in cytotoxic activity in this cell line was observed when compared to DHR, 1. Derivatives 2–4 exhibited GI<sub>50</sub> (concentration of drug to cause a 50% reduction in proliferation of cancer cells) values with less than one-half the concentration of DHR, 1, against MCF-7 and NCI-H460 cell lines, and compound 3 slightly increased the cytotoxic activity against the CaCo-2 cell line.

In addition, the cytotoxic activity of DHR 1 and derivatives 2–4 was also evaluated in the aggressive MDA-MB-231S triple-negative breast cancer cell line. Antiproliferative results (Table 4) evidenced that the starting material possessed a greater cytotoxic activity (GI<sub>50</sub> = 4.3  $\mu\text{M}$ ) in this cell line than its counterpart derivatives (2–4, GI<sub>50</sub> values > 10  $\mu\text{M}$ ).

The final biological test conducted involved assessing the anti-inflammatory activity using RAW 264.7 macrophages, and the outcomes are outlined in Table 5. The lead molecule 1 did not show significant anti-inflammatory capacity. However, derivatives 2–4 exhibited a promising anti-inflammatory activity in a range of 16–53 times higher than that of the starting material 1. Notably, the activity observed for derivatives 2–4 exhibited a 2–4-fold increase compared to the positive control.

Table 3. Cytotoxicity Results ( $GI_{50}$ ,  $\mu M$ ) of Compounds 1–4 against Different Cell Lines<sup>a</sup>

samples	AGS	CaCo-2	MCF-7	NCI-H460	PLP2
1	24.31 ± 1.41	31.62 ± 2.74	60.44 ± 3.69	82.98 ± 2.98	13.39 ± 0.61
2	20.74 ± 1.71	28.61 ± 0.14	27.45 ± 0.33	16.04 ± 1.68	27.54 ± 0.26
3	66.16 ± 4.49	20.38 ± 1.91	17.77 ± 0.75	12.99 ± 0.31	18.02 ± 0.19
4	18.53 ± 1.75	51.36 ± 1.56	38.4 5 ± 1.14	38.53 ± 3.03	36.07 ± 0.90
ellipcin (control)	4.99 ± 0.12	4.91 ± 0.08	4.14 ± 0.08	4.10 ± 0.08	5.68 ± 0.41

<sup>a</sup>AGS, gastric carcinoma; CaCo-2, colorectal adenocarcinoma; MCF-7, breast carcinoma; NCI-H460, lung cancer; PLP2, nontumor cell line.

Table 4. Antiproliferative Effect in MDA-MB-231S Cancer Cell Line<sup>a</sup>

samples	IC <sub>50</sub> ( $\mu M$ )
1	4.3
2	>10
3	>10
4	>10
doxorubicin	0.07 ± 0.01

<sup>a</sup>Dimethyl sulfoxide (DMSO), negative control. Doxorubicin, positive control.

Table 5. Anti-inflammatory Activity Using RAW 264.7 Macrophages<sup>s</sup>

samples	NO Production Inhibition (IC <sub>50</sub> $\mu M$ )
1	>159.00
2	9.94 ± 0.70
3	3.48 ± 0.18
4	4.19 ± 0.13
dexametasona	16.05 ± 1.02

<sup>s</sup>Dexametasona, positive control.

## RESULTS AND DISCUSSION

In summary, DHR, **1**, the major constituent of the *P. aliciae* EO, was obtained through the CAE method. This compound was quantified by HPLC, corresponding to 77.8 mg/g (mg DeRoy/g EO) in the EO. The obtained DHR, **1**, was further derivatized with the aim of developing a small library of semisynthetic compounds. Successful esterification reactions at the C-12 moiety led to the synthesis of three semisynthetic compounds (**2–4**) with overall good yields (86–95%). Several biological activities were evaluated, and the anti-inflammatory activity displayed the most promising results. Esterification at the C-12 position of DHR, **1**, resulted in a significant increase in the anti-inflammatory activity observed in derivatives **2–4**. Additional studies are under consideration with the intent of evaluating the antitumoral and anti-inflammatory mechanisms of action, efficacy, and security in vivo of DHR, **1**, and its derivatives. The work presented herein enlightens the multifaceted pharmacological potential of *Plectranthus* derivatives.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c10071>.

Experimental description; data for plant and reagents; HPLC, GC-FID, and GC-MS analyses; synthesis; evaluation of bioactivities assays (PDF)

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have read and agreed to the published version of the manuscript.

### Notes

The authors declare no competing financial interest.

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### ABBREVIATIONS

DHR, 1	6,7-dehydroxyroyleanone
<i>P. aliciae</i>	<i>Plectranthus madagascariensis</i> var. <i>aliciae</i>
EO	essential oil
LC <sub>50</sub>	50% lethal concentration
CAE	Clevenger-assisted extraction
UAE	ultrasound-assisted extraction
MAE	maceration-assisted extraction

GC-MS	gas chromatography-mass spectrometry
R <sup>2</sup>	correlation coefficient
LOD	limit of detection
LOQ	limit of quantification
UV	ultraviolet
HPLC-DAD	high-performance liquid chromatography with diode-array detection.

### REFERENCES

- (1) Rattray, R. D.; Van Wyk, B. E. The Botanical, Chemical and Ethnobotanical Diversity of Southern African Lamiaceae. *Molecules* **2021**, *26*, 3712.
- (2) Ascensao, L.; Figueiredo, A. C.; Barroso, J. G.; Pedro, L. G.; Schripsema, J.; Deans, S. G. *Plectranthus madagascariensis*: Morphology of the Glandular Trichomes, Essential Oil Composition, and Its Biological Activity. *Int. J. Plant Sci.* **1998**, *159*, 31.
- (3) Rice, L. J.; Brits, G. J.; Potgieter, C. J.; Van Staden, J. *Plectranthus*: A Plant for the Future? *South African Journal of Botany* **2011**, *77*, 947–959.
- (4) Lukhoba, C. W.; Simmonds, M. S. J.; Paton, A. J. *Plectranthus*: A Review of Ethnobotanical Uses. *J. Ethnopharmacol.* **2006**, *103*, 1–24.
- (5) Gáborová, M.; Šmejkal, K.; Kubínová, R. Abietane Diterpenes of the Genus *Plectranthus* Sensu Lato. *Molecules* **2022**, *27*, 166.
- (6) Grayer, R. J.; Paton, A. J.; Simmonds, M. S. J.; Howes, M. J. R. Differences in Diterpenoid Diversity Reveal New Evidence for Separating the Genus: *Coleus* from *Plectranthus*. *Nat. Prod Rep* **2021**, *38*, 1720–1728.
- (7) Van Jaarsveld, E. J.; Edwards, T. J. Notes on *Plectranthus* (Lamiaceae) from Southern Africa. *Bothalia* **1997**, *27*, 1–6.
- (8) Codd, L. E. *Plectranthus* (Labiatae) and Allied Genera in Southern Africa. *Bothalia* **1975**, *11*, 371.
- (9) Paton, A. J.; Mwanyambo, M.; Govaerts, R. H. A.; Smitha, K.; Suddee, S.; Phillipson, P. B.; Wilson, T. C.; Forster, P. I.; Culham, A. Nomenclatural Changes in *Coleus* and *Plectranthus* (Lamiaceae): A Tale of More than Two Genera. *PhytoKeys* **2019**, *129*, 1–158.
- (10) Van Jaarsveld, E.; Thomas, V. *The Southern African Plectranthus: And the Art of Turning Shade to Glade*; Fernwood Press: Cape Town, South Africa, 2006.
- (11) Van Jaarsveld, E. J.; Edwards, T. J. Notes on *Plectranthus* (Lamiaceae) from Southern Africa. *Bothalia* **1997**, *27*, 1.
- (12) Lambrechts, I. A.; Thihe, V. C.; Katti, K. V.; Mandiwana, V.; Kalombo, M. L.; Ray, S. S.; Rikhotso, R.; Janse van Vuuren, A.; Esmear, T.; Lall, N. Targeting Acne Bacteria and Wound Healing In Vitro Using *Plectranthus aliciae*, Rosmarinic Acid, and Tetracycline Gold Nanoparticles. *Pharmaceuticals* **2022**, *15*, 933.
- (13) Garcia, C.; Ntungwe, E.; Rebelo, A.; Bessa, C.; Stankovic, T.; Dinic, J.; Diaz-Lanza, A.; Reis, C. P.; Roberto, A.; Pereira, P. Parvifloron D from *Plectranthus strigosus*: Cytotoxicity Screening of *Plectranthus* Spp. Extracts. *Biomolecules* **2019**, *9*, 616.
- (14) Lambrechts, I. A.; Lall, N. Traditional Usage and Biological Activity of *Plectranthus madagascariensis* and Its Varieties: A Review. *J. Ethnopharmacol.* **2021**, *269*, 113663.
- (15) Garcia, C.; Silva, C. O.; Monteiro, C. M.; Nicolai, M.; Viana, A.; Andrade, J. M.; Barasoain, I.; Stankovic, T.; Quintana, J.; Hernández, I.; et al. Anticancer Properties of the Abietane Diterpene 6, 7-Dehydroroyleanone Obtained by Optimized Extraction. *Future Med. Chem.* **2018**, *10*, 1177–1189.
- (16) Mota, L.; Figueiredo, A. C.; Pedro, L. G.; Barroso, J. G.; Ascensão, L. Glandular Trichomes, Histochemical Localization of Secretion, and Essential Oil Composition in *Plectranthus grandidentatus* Growing in Portugal. *Flavour Fragr J.* **2013**, *28*, 393–401.
- (17) Hensch, M.; Rüedi, P.; Eugster, C. H. Horminon, Taxochinon Und Weitere Royleanone Aus 2 Abessinischen *Plectranthus*-Spezies (Labiatae). *Helv. Chim. Acta* **1975**, *58*, 1921–1934.
- (18) Ladeiras, D.; Monteiro, C. M.; Pereira, F.; Reis, C. P.; Afonso, C. A. M.; Rijo, P. Reactivity of Diterpenoid Quinones: Royleanones. *Curr. Pharm. Des.* **2016**, *22*, 1682–1714.

- (19) Matsumoto, T.; Harada, S. Synthesis of Taxoquinone, 7 $\alpha$ -Acetoxyroleanone, Dehydroroleanone, Horminone, 7-Oxoroleanone, And Inuroyleanol. *Chem. Lett.* **1976**, *5*, 1311–1314.
- (20) Edwards, O. E.; Feniak, G.; Los, M. Diterpenoid Quinones of *Inula Royleana* D. C. *Can. J. Chem.* **1962**, *40*, 1540–1546.
- (21) Garcia, C.; Isca, V. M. S.; Pereira, F.; Monteiro, C. M.; Ntungwe, E.; Sousa, F.; Dinic, J.; Holmstedt, S.; Roberto, A.; Díaz-Lanza, A. Royleanone Derivatives From *Plectranthus* Spp. as a Novel Class of P-Glycoprotein Inhibitors. *Front. Pharmacol.* **2020**, *11*, 557789.