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Diterpenes and Phenolic Compounds from *Salvia brachyodon* Vandas

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Abstract: *Salvia brachyodon*, the short-tooth sage, is one of the rarest plant species and endemic in the Adriatic area of the Balkan Peninsula. As aside from its essential oil, only limited information on its phytochemical composition is known, a more detailed study of the leaves was undertaken. From its leaves two diterpenes, agastanol (**2**), and a new natural compound **1**, i.e., 3-methyl-4-methylen-11,12,14-trihydroxy-8,11,13-abietatrien-7-one, were isolated and identified by NMR spectroscopy and mass spectrometry. In addition, caffeic acid, isoquercitrin, luteolin 7-O-glucoside and rosmarinic acid were identified by comparison with reference compounds. The fraction containing the diterpenes as well as the isolated compound **1** showed significant antimycobacterial activity against *Mycobacterium smegmatis*. The diterpenes of *S. brachyodon* represent promising antimycobacterial substances for further evaluation. Due to the endangered nature of the plant, the wide use of *S. brachyodon* and its bioactive compounds could be achieved by growing the plants in culture.

Keywords: *Salvia brachyodon* Vandas, diterpenes, phenolic compounds, antimycobacterial activity, LC-PDA-ESI-MS, NMR.

INTRODUCTION

TAXA of the genus *Salvia* L. (family Lamiaceae) are widely known for their essential oils, which is why they have long been used primarily for medicinal purposes.^[1] This is especially true for the species *Salvia officinalis* L. (Dalmatian sage), for which numerous bioactive components and their activities are well known.^[2–4] However, it is also known that some of the phytochemicals isolated from *S. officinalis* (for example, thujones) have negative side effects with prolonged usage.^[5] Therefore, detection and investigation of other taxa of the genus *Salvia* whose bioactive compounds would not have a negative effect is of vital importance for both producers and users of products with sage essential oils. In this sense, it is interesting to note that in the area of Dalmatia, sage collectors have long collected also the endemic species *S. brachyodon* Vandas (short-tooth

sage), emphasizing its more useful medicinal properties than those in Dalmatian sage. This knowledge, as well as preliminary phytochemical studies of the species *S. brachyodon*^[2] directed us to this more extensive study of its bioactive compounds, which strives for the elucidation of their phytochemical composition.

Relatively recently, only two of the four previously mentioned sites in the early 20th century, have been confirmed: the Mount Orjen on the border between Bosnia and Herzegovina and Montenegro (which is the *locus classicus* for this species) and on the Pelješac peninsula in Croatia.^[6] Therefore, the short-tooth sage is one of the rarest plant species and endemic in the Adriatic area of the Balkan Peninsula. Its endangerment in nature is confirmed by morphological and molecular analysis.^[6,7]

In Croatia, *S. brachyodon* has the status of an almost endangered species (NT).^[8] It grows on the highest peak of

the Pelješac peninsula (St. Ilija) on dolomitic limestones within open habitat formed after the Dalmatian black pine (*Pinus nigra* Arnold) forest was burned about 20 years ago.^[7]

Short-tooth sage is a small shrub (up to 80 cm), hemicryptophyte which develops dense and relatively large leaves with a long petiole at the base of the stem. Young leaves are densely hairy, while developed leaves are mostly glabrous on the upper, and mainly hairy on the lower side. Pale lilac-blue flowers and their pedicels have glandular hairs and are grouped in loosely racemes. The species is diploid, with the $2n = 14$ chromosomes.^[9] Flowering period is from July to early September.

For *S. brachyodon* leaves phytochemical analysis showed that there was no presence or only the presence of traces of thujones;^[10] and that they contained 1.6 % of essential oil, with sesquiterpenes as the main compounds (67.8 %).^[9] Due to the limited phytochemical data, in the current study a more detailed exploration of the leaves of *S. brachyodon* was undertaken. In recent years, increasing infection rates by non-tuberculous mycobacteria (NTM) have been recognized,^[11] hence *S. brachyodon* was also tested for growth inhibition using an NTM model strain, i.e., *Mycobacterium smegmatis*.^[12]

The aim of this study was to provide novelty regarding the diterpenes and phenolic compounds as well as an antimycobacterial activity of *S. brachyodon*.

EXPERIMENTAL

Plant Material

The samples were collected on the Pelješac peninsula, at its highest peak, Sv. Ilija (843 m.a.s.l.; 42°59'45" N, 17°09'29" E), in August 2019, when the plants were in full bloom. Herbarium voucher specimen is deposited in the Herbarium Croaticum (ZA) of the Faculty of Science, University of Zagreb (ID: 37083).

Isolation of Compounds from Dichloromethane Extract

Powdered airdried plant material (28.7 g) was extracted with dichloromethane in a Soxhlet apparatus for 6 h. The extract was brought to dryness by a rotary evaporator, resulting in 2.8 g dry residue. 2.25 g thereof were dissolved in 10 mL methanol of which 6 mL were separated in 3 portions of 2 mL on Sephadex LH-20 columns (column 1 and 2 23 × 1.5 cm, column 3 32 × 1 cm), fractions of 2.5 mL each were collected. According to TLC pattern (silica 60 F₂₅₄ plates, Merck, mobile phase toluene – ethyl formate – formic acid – hexane, 5 : 4 : 1 : 10, v/v ,^[13] modified) three combined fractions A – C (58, 49 and 45 mg, respectively) were obtained. As fraction A showed most promising

antimycobacterial activity it was further analysed by LC-PDA-MS. Analysis was carried out on a LC system (Ultimate 3000 RS; Thermo Fisher Scientific) which was coupled to a linear ion-trap mass spectrometer (LTQ XL; Thermo Fisher Scientific), using an electrospray ionisation (ESI) source and a Zorbax SB C18 column (Rapid Resolution HD, 100 × 2.1 mm, 1.8 μ m; Agilent Technologies) at 35 °C. The mobile phase consisted of 0.1 % formic acid in water (A) and acetonitrile (B) at a flow rate of 0.2 mL min⁻¹, starting at 8 % B, increasing to 25 % B within 10 min, and reaching 100 % B at 25 min, returning to 8 % B within 1 min, and equilibrating for 7 min. at 8 % B. PDA detection was performed in the 190 nm to 500 nm wavelength range. Ionization was performed in positive ion mode at 350 °C capillary temperature, 300 °C source temperature, 5 kV source voltage, as well as sheath gas and auxiliary gas flows of 40 and 10 arbitrary units, respectively. Mass spectra were recorded in the m/z range of 50 to 2000. High resolution mass spectrum (ESI positive mode) of compound **1** was obtained on a QExactive Hybrid Quadrupole Orbitrap MS (Thermo Scientific). In order to isolate the main compounds from fraction A, it was further separated by HPLC using a Lichrospher 100 RP-18 column (250 × 4 mm; Merck) at 25 °C column temperature. Separations were carried out on a Merck-Hitachi HPLC LaChrom system. The mobile phase consisted of acetonitrile (A) and water (B) starting at 50 % A, increasing to 100 % B within 40 min, returning to 50 % A within 1 min and using 10 min equilibration time, at a flow rate of 0.4 mL min⁻¹. Detection was done by a photodiode array detector (L-7455) between 200–500 nm. 60 μ L of the sample solution (20 mg mL⁻¹ in MeOH) were injected at each chromatographic run. Two main peaks were collected at 32.5 min (compound **1**, 6.8 mg) and 44.1 min (compound **2**, 2.2 mg), respectively.

LC-PDA-ESI-MS Analysis of Methanolic Extract

The residual plant material (25.9 g) after dichloromethane extraction was subjected to extraction with methanol using a Soxhlet apparatus for 3 h, yielding 3.7 g of dry extract. 1.14 g of extract were dissolved in 5.0 mL methanol, 2.0 mL were further separated on Sephadex LH-20/MeOH. Fractions C_m and D_m (eluting between 37.5–47.5 mL) which were suspected to contain flavonoids after TLC analysis (silica 60 F₂₅₄ plates, Merck, mobile phase ethyl acetate – ethyl methyl ketone – formic acid – water, 5 : 3 : 1 : 1, v/v ,^[14] detection by spraying with Naturstoffreagens A (Neu) and PEG 400, UV 366 nm) were subjected to LC-PDA-MS analysis with the instrumentation mentioned above. A Luna phenyl-hexyl column (250 × 2 mm, 5 μ m; Phenomenex) was used at 35 °C column temperature, The mobile phase consisted of 0.1 % formic acid in water (A) and acetonitrile (B) at a flow rate of 0.2 mL min⁻¹, starting at 8 % B, increasing

to 100 % B within 25 min, isocratic 100 % B for 5 min, returning to 8 % B within 0.5 min, and equilibrating for 5.5 min at 8 % B. Ionization was performed in negative ion mode at 350 °C capillary temperature, 300 °C source temperature, 3.5 kV source voltage, as well as sheath gas and auxiliary gas flows of 40 and 10 arbitrary units, respectively. Mass spectra were recorded in the m/z range of 50 to 2000. Compounds were identified due the UV and mass spectral data as well as chromatographic and spectroscopic comparison with reference compounds, i.e., caffeic acid, isoquercitrin, luteolin 7-O-glucoside, rosmarinic acid (Roth).

NMR Spectroscopy

A proton spectrum and a set of 2D NMR spectra (COSY, HSQC, and HMBC) were recorded in deuterio chloroform for compounds **1** and **2** with an Avance II spectrometer (Bruker), operating at 700 MHz proton frequency and equipped with a cryo-probe. In addition, a carbon spectrum was recorded for compound **1**. Chemical shifts are expressed in δ (ppm) with TMS used as internal standard, the sample temperature was 25 °C.

Antimycobacterial Activity

MIC determination of the crude dichloromethane extract, fractions A – C and compound **1** was conducted for a fast-growing mycobacterium, *Mycobacterium smegmatis* mc² 155 (ATCC 700084) by a broth dilution method in Mueller-Hinton Broth (MHB) as described previously.^[15] Test samples were dissolved in DMSO and diluted in MHB to reach particular start concentrations (512 mg L⁻¹ for the extract, 256 mg L⁻¹ for fractions and 128 mg L⁻¹ for compound **1**, final assay concentration). A bacterial inoculum was adjusted equal to the McFarland turbidity standard 0.5 and diluted to yield a final bacterial density of 5×10^5 cfu mL⁻¹. 0.125 mL aliquots of the bacterial suspension were transferred into the wells containing 0.125 mL aliquots of MHB with the two-fold serially dilutions of each test compound. Plates were incubated at 37 °C for 72 h and the MIC was registered after adding MTT (20 μ L, 5 mg mL⁻¹) and further incubation at 37 °C for 30 min. MIC was defined as the lowest concentration that inhibited visible bacterial growth. Isoniazid (INH) was used a positive control (MIC 8 mg L⁻¹).

RESULTS AND DISCUSSION

S. brachyodon has been scarcely investigated for its constituents except for essential oil composition.^[9,10] In a study by Antolić et al., the total content of flavonoids and of phenolic acids has been determined.^[2] As the genus *Salvia* is known to be a rich source of phenolic and terpenoid compounds,^[16] a more detailed phytochemical study of the leaves of *S. brachyodon* was undertaken. The plant material was extracted successively with

dichloromethane and methanol, and the extracts analysed by TLC and LC-PDA-MS. Furthermore, the extracts were checked for a potential antimycobacterial effect using a model strain of non-tuberculous mycobacteria, *M. smegmatis*.

The dichloromethane extract showed moderate activity (MIC 512 mg L⁻¹) whereas the methanolic extract was devoid of activity. Hence, the investigations focused on the dichloromethane extract, supported by the fact that striking yellow spots were visualized on TLC plates after detection with diphenylboric acid 2-aminoethyl ester (Naturstoffreagens A (Neu), UV 366 nm). The combined fractions A – C obtained after separation on Sephadex LH-20/MeOH were analysed by TLC and LC-PDA-MS. Fraction A contained most of the yellowish compounds, and turned out to be the fraction with a significant antimycobacterial effect (16 mg L⁻¹), whereas fractions B and C were not active (MIC > 256 mg L⁻¹). LC-PDA-MS analysis of fraction A revealed two major peaks at $R_t = 15.59$ (compound **1**, $[M+H]^+$ m/z 331; HRMS m/z 331.1906 $[M+H]^+$ C₂₀H₂₇O₄; calculated for C₂₀H₂₇O₄ m/z 331.1904) and 18.60 min (compound **2**, $[M+H]^+$ m/z 345), respectively. Both compounds could be isolated by HPLC on a RP-18 column and subjected to structure elucidation by NMR. For both compounds a complete assignment of NMR resonances was performed. Compound **2** was found to be the known diterpene agastanol^[17] the carbon shift values are in a very good agreement with the published data, however, we provide also a complete set of assigned proton resonances (Table 1), the chemical structures can be depicted from Figure 1. In compound **1** a free OH group replaced the methoxy group that was found at position C-12 in **2**, hence, the structure of **1** was determined as 3-methyl-4-methylen-11,12,14-trihydroxy-8,11,13-abietatrien-7-one. The similar carbon shift values and the observed homonuclear coupling constants of the aliphatic ring protons indicate the same relative configuration at the stereogenic centers C-5 and C-10. The clear differences of chemical shift values in the aromatic ring in comparison to compound **2** are a direct

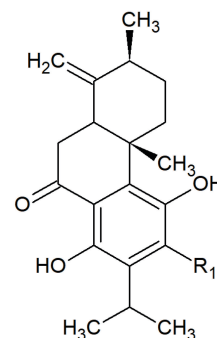


Figure 1. Diterpenoids from *Salvia brachyodon*. Compound **1**, R₁ = OH; Compound **2** (Agastanol), R₁ = OCH₃

result of the demethylation of OH-12. Compound **1** is a new natural product and has not been described in literature yet. Compound **2** (agastanol) is a new report for *S. brachyodon*, but has been identified before in two *Salvia* species, namely *S. corrugata* Vahl^[18] and *S. hydrangea* DC. ex Benth.^[19] as well as *Hyptis verticillata* Jacq.^[18] and *Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze^[17] and showed cytotoxic and antiviral (HIV-1) effects.^[17,20,22]

Similar compounds as **1** and **2** have been found in other *Salvia* species like *S. candelabrum* Boiss., *S. clevelandii* (Gray) Greene, *S. pachyphylla* Epling ex Munz, *S. leucantha* Cav. and *S. palaestina* Benth.,^[23–27] differing in position and number of keto-, hydroxy groups and double bonds, respectively.

Compound **1** was active against *M. smegmatis* at an MIC of 32 mg L⁻¹. Due to lack of substance compound **2** could not be tested. Comparison of antimycobacterial effects of fraction A and compound **1** suggests the

Table 1. ¹H (700 MHz) and ¹³C (175 MHz) NMR spectroscopic data of the compounds **1** and **2** in CDCl₃ (25 °C).

Position	Compound 1		Compound 2 (Agastanol)	
	<i>d_c</i>	<i>d_H</i> (<i>J</i> -values in Hz)	<i>d_c</i>	<i>d_H</i> (<i>J</i> -values in Hz)
1	36.3	3.07 brd (13.4) 1.66 td (13.4, 3.5)	35.7	3.26 brd (13.4) 1.55
2	32.6	1.83 brd (13.4) 1.37		1.79 brd (13.4) 1.36
3	38.2	–	38.3	–
4	151.7	–	152.0	–
5	47.6	2.64 brd (14.8)	47.7	2.64 brd (15.0)
6	37.7	2.75 t (15.4) 2.50 dd (16.3, 2.3)	38.3	2.77 t (15.4) 2.54 dd (16.2, 2.5)
7	203.7	–	205.2	–
8	109.1	–	112.6	–
9	135.3	–	133.9	–
10	40.8	–	41.0	–
11	133.9	OH 4.79 s	139.3	OH 5.65 s
12	151.9	OH 6.14 s	152.1	–
13	118.5	–	126.3	–
14	159.0	OH 13.75 s	158.2	OH 13.27 s
15	24.4	3.45 sept (7.1)	26.1	3.31 m
16	20.5	1.37 d (7.1)	20.4	1.40 d (7.0)
17	20.5	1.37 d (7.1)	20.4	1.40 d (7.0)
18	105.4	4.91 brs 4.64 brs	105.1	4.89 brs 4.61 brs
19	18.1	1.10 d (6.5)	18.2	1.10 d (6.5)
20	16.0	1.18 s		
12-OCH ₃			62.1	3.80 s

conclusion that additional antimycobacterial compounds are present in fraction A, or synergistic effects increase the antimycobacterial potency of compound **1** within fraction A compared to the isolated compound **1**. Nevertheless, diterpenes like compound **1** represent a promising group of antibacterials.^[28–30]

The methanolic extract of the pre-extracted plant material afforded two fractions after separation on Sephadex LH-20/MeOH which were suspected to contain flavonoids and phenolic acids according to TLC analysis and spraying with diphenylboric acid 2-aminoethyl ester. LC-

Table 2. LC-PDA-MS data of identified compounds in *Salvia brachyodon* leaves.

Compound	Full scan MS, <i>m/z</i>	MS ² , <i>m/z</i> (rel. int.)	MS ³ , ^(a) <i>m/z</i> (rel. int.)	UV, λ _{max} / nm
1	331 [M+H] ⁺	315 (30), 313 (45), 295 (40), 271 (50), 253 (100), 219 (65)	238 (45), 225 (100), 211 (60), 197 (15), 183 (10)	353, 292, 245sh, 225sh
Caffeic acid	179 [M-H] ⁻	135 (100)	135 (100), 107 (10), 91 (15)	325, 300sh, 240, 221
Isoquercitrin	463 [M-H] ⁻	301 (100), 300 (20)	179 (100), 151 (70)	356, 265sh, 258
Kaempferol 3-O-hexoside	447 [M-H] ⁻	327 (20), 285 (100), 284 (75), 255 (10)	243 (60), 241 (100), 217 (55), 199 (70), 175 (75)	352, 268
Kaempferol 3-O-desoxyhexosylhexoside	593 [M-H] ⁻	447 (3), 285 (100)	243 (60), 241 (100), 217 (55), 199 (70), 175 (65)	349, 267
Luteolin 7-O-glucoside	447 [M-H] ⁻	285 (100)	241 (100), 217 (60), 199 (70), 175 (70)	352, 267, 257
Rosmarinic acid	359 [M-H] ⁻	223 (15), 197 (25), 179 (30), 161 (100)	161 (10), 133 (100)	330, 300sh, 240sh, 222

^(a) Fragmentation of most intensive ion of MS² spectrum.

PDA-MS analysis on a phenyl-hexyl stationary phase and comparison with authentic reference compounds confirmed the presence of caffeic acid ($R_t = 10.12$ min), isoquercitrin ($R_t = 15.15$ min), luteolin 7-O-glucoside ($R_t = 15.90$ min) and rosmarinic acid ($R_t = 19.42$ min), the compound at $R_t = 17.23$ min was identified as kaempferol 3-O-hexoside, the one at 14.87 min as kaempferol 3-O-desoxyhexosylhexoside, mass spectral and UV data are presented in Table 2. None of these compounds has been reported before for *S. brachyodon*. However, they are frequently occurring in other *Salvia* species.^[31]

CONCLUSION

S. brachyodon is a promising source of antimycobacterial diterpenes which should be studied more in detail. One of the isolated diterpenes has been reported as a new natural product. The investigated species also contains flavonoids and phenolic acids that can contribute to the antimycobacterial effect.

Although the wide use of *S. brachyodon* and the availability of its bioactive compounds is limiting and questionable due to its endangerment in nature, growing the plants in culture could very likely avoid this.

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REFERENCES

- [1] T. Vissi, R. Zelkó, R. Földesi, I. Túri, *Heliyon* **2021**, 7(10):e08114. <https://doi.org/10.1016/j.heliyon.2021.e08114>
- [2] A. Antolić, Ž. Maleš, M. Tomičić, M. Bojić, *Food Technol. Biotechnol.* **2018**, 56(2), 265–269. <https://doi.org/10.17113/ftb.56.02.18.5474>
- [3] S. Jedidi, H. Sammari, H. Selmi, K. Hosni, K. Rtibi, F. Aloui, O. Adouni, H. Sebai, *J. Funct. Foods* **2021**, 79, 104406. <https://doi.org/10.1016/j.jff.2021.104406>
- [4] M. M. Sabry, R. F. Abdel-Rahman, S. M. El-Shenawy, A. M. Hassan, S. H. El-Gayed, *J. Ethnopharmacol.* **2022**, 282, 114579. <https://doi.org/10.1016/j.jep.2021.114579>
- [5] O. Pelkonen, K. Abass, J. Wiesner, *Regul. Toxicol. Pharmacol.* **2013**, 65, 100–107. <https://doi.org/10.1016/j.yrtph.2012.11.002>
- [6] Z. Liber, S. Bogdanovć, I. Radosavljević, M. Pruša, M. Filipović, D. Stešević, Z. Šatović, *Agric. Conspec. Sci.* **2014**, 79, 71–76.
- [7] I. Radosavljević, O. Antonić, D. Hruševar, J. Križan, Z. Šatović, D. Turković, Z. Liber, *Plants* **2020**, 9, 828. <https://doi.org/10.3390/plants9070828>
- [8] T. Nikolić, **2023**, University of Zagreb, Faculty of Science, Department of Biology. Available from: <http://hirc.botanic.hr/fcd/>. (accessed: January 3rd 2023).
- [9] M. Maksimović, D. Vidić, M. Miloš, M. E. Šolić, S. Abadžić, S. Šiljak-Yakovlev, *Biochem. Syst. Ecol.* **2007**, 35, 473–478. <https://doi.org/10.1016/j.bse.2007.02.005>
- [10] O. Tzakou, M. Couladis, V. Slavkovska, N. Mimica-Dukić, R. Jančić, *Flavour. Fragr. J.* **2003**, 18(1), 2–4. <https://doi.org/10.1002/ffj.1132>
- [11] C. N. Ratnatunga, V. P. Lutzky, A. Kupz, D. L. Doolan, D. W. Reid, M. Field, S. C. Bell, R. M. Thomson, J. J. Miles, *Front. Immunol.* **2020**, 11. <https://doi.org/10.3389/fimmu.2020.00303>
- [12] DePas William H., Bergkessel Megan, Newman Dianne K., *mBio* **2019**, 10, e01715-19. <https://doi.org/10.1128/mBio.01715-19>
- [13] H. Wagner, S. Bladt, E.M. Zgainski, *Drogenanalyse*, Springer, Berlin-Heidelberg-New York, **1983**, p. 164.
- [14] E. Stahl, *Dünnschichtchromatographie*, Springer, Berlin-Heidelberg-New York, **1967**, p. 662.
- [15] B. Gröblacher, O. Kunert, F. Bucar, *Bioorg. Med. Chem.* **2012**, 20, 2701–2706. <https://doi.org/10.1016/j.bmc.2012.02.039>
- [16] J. Xu, K. Wei, G. Zhang, L. Lei, D. Yang, W. Wang, Q. Han, Y. Xia, Y. Bi, M. Yang, M. Li, *J. Ethnopharmacol.* **2018**, 225, 18–30. <https://doi.org/10.1016/j.jep.2018.06.029>
- [17] H.-K. Lee, S.-J. Byon, S.-R. Oh, J.-I. Kim, Y.-H. Kim, C.-O. Lee, *Saengyak Hakhoechi* **1994**, 25, 319.
- [18] R. A. Kentsop, V. Iobbi, G. Donadio, B. Ruffoni, N. de Tommasi, A. Bisio, *Molecules* **2021**, 26. <https://doi.org/10.3390/molecules26216681>
- [19] S. Zare, G. Hatam, O. Firuzi, A. Bagheri, J. N. Chandran, B. Schneider, C. Paetz, S. Pirhadi, A. R. Jassbi, *J. Mol. Struct.* **2021**, 1228, 129447. <https://doi.org/10.1016/j.molstruc.2020.129447>
- [20] R. B. R. Porter, D. A. C. Biggs, W. F. Reynolds, *Nat. Prod. Commun.* **2009**, 4, 15.
- [21] B. S. Min, M. Hattori, H. K. Lee, Y. H. Kim, *Arch. Pharmacol. Res.* **1999**, 22, 75–77.
- [22] B.-S. Min, H. Miyashiro, M. Hattori, *Phytother. Res.* **2002**, 16, S57. <https://doi.org/10.1002/ptr.808>
- [23] J. Hohmann, G. Janicsak, P. Forgo, D. Redei, I. Mathe, T. Bartok, *Planta Med.* **2003**, 69, 254–257.
- [24] I. C. Guerrero, L. S. Andres, L. G. Leon, R. P. Machin, J. M. Padron, J. G. Luis, J. Delgadillo, *J. Nat. Prod.* **2006**, 69, 1803–1805.

- [25] A. A. Hussein, B. Rodriguez, *Z. Naturforsch., B: Chem. Sci.* **2000**, *55*, 233.
- [26] J. Dang, Y. Cui, J. Pei, H. Yue, Z. Liu, W. Wang, L. Jiao, L. Mei, Q. Wang, Y. Tao, Y. Shao, *Molecules* **2018**, *623*. <https://doi.org/10.3390/molecules23030623>
- [27] L.-W. Li, Y.-Y. Qi, S.-X. Liu, X.-D. Wu, Q.-S. Zhao, *Fitoterapia* **2018**, *127*, 367–374.
- [28] E. Mendes, J. L. Marco, B. Rodriguez, M. L. Jimeno, A. M. Lobo, S. Prabhakar, *Phytochemistry* **1989**, *28*, 1685–1690.
- [29] J.-J. Cui, W.-J. Li, C.-L. Wang, Y.-Q. Huang, W. Lin, B. Zhou, J.-M. Yue, *Phytochemistry* **2022**, *201*, 113278. <https://doi.org/10.1016/j.phytochem.2022.113278>
- [30] A. Ulubelen, S. Oeksuez, G. Topcu, A. C. Goeren, W. Voelter, *J. Nat. Prod.* **2001**, *64*, 549. <https://doi.org/10.1021/np0004956>
- [31] B. Avula, J.-Y. Bae, A. G. Chittiboyina, Y.-H. Wang, M. Wang, R. Srivedavyasari, Z. Ali, J. Li, C. Wu, I. A. Khan, *J. Pharm. Biomed. Anal.* **2022**, *209*, 114520. <https://doi.org/10.1016/j.jpba.2021.114520>