Check for updates

Chemistry & Biodiversity

CHEMISTRY & BIODIVERSITY

RESEARCH ARTICLE OPEN ACCESS

Further Data on the Chemical Profile of Iberian *Calendula* Species by GC–MS, Including a First Report on *C. tripterocarpa*

Mariza B. Marques[1,2,3] | Silvana Ohse[2,3,4] | Paulo C. Silveira[2] | Mónica S. G. A. Válega[3] | Daniel Granato[5,6] | Artur M. S. Silva[3] | Diana C. G. A. Pinto[3]

[1]Department of Chemistry, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil | [2]CESAM-Centre For Environmental and Marine Studies and Department of Biology, University of Aveiro, Aveiro, Portugal | [3]LAQV-REQUIMTE and Department of Chemistry, Campus de Santiago, University of Aveiro, Aveiro, Portugal | [4]Department of Phytotechnics and Plant Health, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil | [5]Department of Food Science and Microbiology, Auckland University of Technology, Auckland, New Zealand | [6]AUT Centre for Future Foods, Auckland University of Technology, Auckland, New Zealand

Correspondence: Paulo C. Silveira (psilveira@ua.pt)

Received: 26 May 2026 | **Revised:** 18 October 2026 | **Accepted:** 3 November 2026

Keywords: *C. suffruticosa* subsp. *cinerea* | *C. suffruticosa* subsp. *lusitanica* | *C. suffruticosa* subsp. *tomentosa* | *C. arvensis* | PCA

ABSTRACT

This study aimed to trace the chemical profile of the genus *Calendula* L. (Asteraceae) in the Iberian Peninsula, including *Calendula arvensis*, three subspecies of *Calendula suffruticosa*, and *Calendula tripterocarpa*. In total, 28 compounds were identified in hexane extracts by GC–MS. Fatty acids, terpenoids, and alkanes were reported for the first time for *C. suffruticosa* subsp. *cinerea* and *C. suffruticosa* subsp. *tomentosa*. In *C. suffruticosa* subsp. *cinerea* alkanes (63.3%) were the most abundant class, followed by terpenoids (25.9%), while *C. suffruticosa* subsp. *tomentosa* presented alkanes (66.4%) and terpenoids (21.0%). *C. suffruticosa* subsp. *lusitanica* was the most differentiated sample because it was the only one to show alcohols (2.4%), terpenoids (56.8%), carboxylic acids (24.8%), and alkanes (16%). *C. arvensis* and *C. tripterocarpa* showed a smaller number of compounds, 11 and 9, respectively. Principal Component Analysis was able to separate the species *C. suffruticosa* from *C. arvensis* and *C. tripterocarpa*.

1 | Introduction

The genus *Calendula* L. (Asteraceae) is considered a taxonomically difficult genus, mostly due to difficulties in the interpretation of achene morphology variation within and between taxa. Depending on the chosen taxonomic criteria, it can be split into 10 to 27 species, and a variable range of infraspecific *taxa* [1]. Efforts have been made to improve classification using the number of chromosomes and nuclear DNA content [2], and phytochemical profile [3].

In the Iberian Peninsula, the genus *Calendula* includes four species, of which three are the subject of this study, and which grow in a variety of habitats. *Calendula arvensis* L. is fairly dispersed throughout the territory, while *Calendula suffruticosa* Vahl is present in the littoral of the North-West, West and South Iberian Peninsula. *Calendula tripterocarpa* Rupr. is restricted to Almería, Murcia and Alicante (Spain).

Calendula arvensis is a rather ubiquitous species, found in waste grounds and ruderal places. *C. tripterocarpa* grows in grasslands, in semi-desert to desert regions, mostly at low altitudes. Finally, *C. suffruticosa* Vahl comprises nine infraspecific *taxa*, three from Portugal (subsp. *algarbiensis* (Boiss.)) Nyman, subsp. *cinerea* (Ohle) P.Silveira and A.C.G.Gonç. and subsp. *lusitanica* (Boiss.) Ohle), and six from Spain (subsp. *carbonellii* Ohle, subsp. *greuteri*

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. All other rights reserved, including rights for text and data mining and training of artificial intelligence technologies or similar technologies.
© 2026 The Author(s). *Chemistry & Biodiversity* published by Wiley-VHCA AG.

Ohle, subsp. *marginata* (Willd.) Maire, subsp. *trialata* P.Silveira & A.C.G.Gonç., subsp. *tomentosa* (Ball) Murbeck, and subsp. *vejerensis* P.Silveira and A.C.G.Gonç.) [4]. These plants occur in limestone soils, sandy loams, and dunes, rocky cliffs, near the sea, from 0 to 200 m of elevation, or in limestone cliffs, hills, and coastal canyons up to 1400 m of altitude. *C. suffruticosa* subsp. *cinerea* showed a low elevation range because it is restricted, endemic, and poorly represented taxa [4].

Calendula officinalis L. is the most widespread and studied *Calendula* species [5], followed by *C. arvensis*, traditionally used in African and Asian folk medicine [6, 7].

Terpenoids, flavonoids, coumarins, quinones, volatile oils, carotenoids, polysaccharides, and amino acids are the majority of compounds classes identified in the genus [8]. Substances like arvensoside B isolated from *C. arvensis* and calenduloside A from *C. officinalis* have established hemolytic activity [9]. The study of the genus *Calendula* is still insufficient given the medicinal uses already set and potential new applications: as antibacterial, cytotoxic and antioxidant [10], anti-collagenase [11], anti-HIV [12], healing and anti-inflammatory [13], resistance to aphid attack [14], antifungal [15], and anti-leishmanial [16], attractor for hoverflies [17], and others.

There are no reports on the chemical composition of *C. tripterocarpa*, *C. suffruticosa* subsp. *cinerea* and *C. suffruticosa* subsp. *tomentosa*, and only two of *C. suffruticosa lusitanica* [3, 15]. Therefore, this study aimed to conduct hexane extract analysis by GC-MS and provide the profile of non-polar secondary metabolites of these taxa from Portugal and Spain, aiming to contribute to the knowledge of the genus in the Iberian Peninsula.

2 | Results and Discussion

2.1 | Extraction Yield and Water Content

The highest values of water content were obtained for samples collected in an extremely arid environment, in poorly fertile ground, subjected to high temperatures, a lot of sunlight, in addition to being quite far from the coast: *C. arvensis* (19,6%) and *C. tripterocarpa* (17,8%). The yields of the hexane extract of C. *suffruticosa* subsp. cinerea, *C. suffruticosa* subsp. *lusitanica, C. suffruticosa* subsp. *tomentosa, C. arvensis and C. tripterocarpa* ranged from 1.5 to 4.8% (Table 1). The values for *C. suffruticosa* subsp. *lusitanica* (2.7%) and C. arvensis (2.7%) are lower than those previously reported by this group, 8.1 and 5.5%, respectively [15]. Even lower for *C. arvensis* in other reports: 4% [18], 4.8% as the average value for leaves and flowers [19], and equal to media (2.7%) for stem, leaves, and flowers [20]. The difference between yields corroborates the indication that each species and subspecies, expresses quantitative and qualitative differences in the same extract [15, 21].

Each species secondary metabolites profile: The hexane extracts of *C. suffruticosa* subsp. *cinerea, C. suffruticosa* subsp. *lusitanica, C. suffruticosa* subsp. *tomentosa, C. arvensis and C. tripterocarpa* were analyzed through GC–MS, leading to the identification of 28 compounds, among which 23 different compounds in *C.*

TABLE 1 | Extraction yield of *C. taxa*.

	Dry mass content (%)	Extraction yield (%)
C. suffruticosa subsp. *cinerea*	86.7	1.6
C. suffruticosa subsp. *lusitanica*	85.7	2.7
C. suffruticosa subsp. *tomentosa*	84.8	1.5
C. arvensis	80.4	2.7
C. tripterocarpa	82.2	4.8

suffruticosa subsp. *tomentosa*, 19 in *C. suffruticosa* subsp. *cinerea*, 16 in *C. suffruticosa* subsp. *lusitanica*, 11 in *C. arvensis* and 9 compounds in *C. tripterocarpa*. The retention time, identification and content of each compound in each taxon are presented in Table 2. The compounds identified belong to carboxylic acids, terpenoids, alcohols and alkanes families.

Concerning *C. suffruticosa* subsp. *cinerea*, as in *C. suffruticosa* subsp. *tomentosa* the alkanes predominate in their chemical profile (39.1% and 33.5%) being from this family the three major constituents of each extract: untriacontane (1.72 and 1 mg/g of the dry plant), nonacosane (1.18 and 1.44 mg/g of the dry plant) and tetratriacontane (0.51 and 0.67 mg/g of the dry plant) respectively (Table 2). For *C. suffruticosa* subsp. *lusitanica*, it was only possible to quantify an alkane, heptacosane (0.73 mg/g of the dry plant), also as a constituent in higher concentration, followed by palmitic acid (0.43 mg/g of the dry plant) and stigmasterol (0.40 mg/g of the dry plant). The subsp. *lusitanica* was the only one to present the terpenoids β-eudesmol (0.21 mg/g of the dry plant), α-tocopherol (0.35 mg/g of the dry plant), and β-sitosterol (0.29 mg/g of the dry plant), as well as 1-octacosanol (0.11 mg/g of the dry plant), the only alcohol detectable in all samples.

Neophytadiene, phytol and heptacosane appear in higher concentrations in *C. suffruticosa* subsp. *lusitanica*, than in the other two subsp. of *C. suffruticosa*. On the other hand, α-amyrin was identified in *C. suffruticosa* subsp. *cinerea* (0.17 mg/g of the dry plant) and *C. suffruticosa* subsp. *tomentosa* (0.19 mg/g of the dry plant), but was not present in the hexane extract of *C. suffruticosa* subsp. *lusitanica*.

Calendula arvensis and *C. tripterocarpa* showed very similar chemical profiles, in which the alkanes untriacontane (0.34 and 0.57 mg/g of the dry plant) and nonacosane (0.31 and 0.50 mg/g of the dry plant), and the carboxylic acid α-linolenic (0.31 and 0.50 mg/g of the dry plant) were the constituents in higher concentration. Alkanes were also the most abundant class in *C. arvensis* (39.1%), followed by terpenoids (30.7%) and carboxylic acids (30.2%), while *C. tripterocarpa* showed 33,8% of terpenoids, 33.5% of alkanes, and carboxylic acids, respectively. *C. arvensis* and *C. tripterocarpa* were collected in soil under conditions of extreme drought, far from the sea coast (very low humidity) and at very high temperatures (desert of Tabernas, Spain), which probably contributed to the lower expression of

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 | Chemical composition of the hexane extracts from *Calendula taxa*.

			mg/g of the dry plant (mean ± SD, $n = 3$)				
$n°$	Rt[a] (min)	Identification	*C. suffruticosa* subsp. *cinerea*	*C. suffruticosa* subsp. *lusitanica*	*C. suffruticosa* subsp. *tomentosa*	*C. arvensis*	*C. tripterocarpa*
		Carboxylic Acids					
1	11.8	Myristic acid	—	0.06 ± 0.00	0.06 ± 0.01	—	—
2	16.7	Palmitic acid	0.21 ± 0.07	0.43 ± 0.03	0.27 ± 0.07	0.23 ± 0.01	0.34 ± 0.01
3	22.3	Linoleic acid	0.12 ± 0.04	0.27 ± 0.03	0.18 ± 0.01	0.17 ± 0.01	0.33 ± 0.02
4	22.6	α-Linolenic acid	0.13 ± 0.04	0.31 ± 0.04	0.18 ± 0.03	0.24 ± 0.02	0.37 ± 0.03
5	23.8	Stearic acid	0.04 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.04 ± 0.00	—
6	52.0	Lignoceric acid	−2	—	0.06 ± 0.02	—	—
7	67.4	Phytyl palmitate	0.15 ± 0.03	—	0.17 ± 0.01	0.13 ± 0.00	0.22 ± 0.03
		Terpenoids					
8	8.3	β-Eudesmol		0.21 ± 0.00			
9	11.6	Neophytadiene	0.09 ± 0.01	0.26 ± 0.02	0.11 ± 0.00	0.18 ± 0.00	0.28 ± 0.00
10	21.0	Phytol	0.09 ± 0.01	0.30 ± 0.03	0.10 ± 0.01	—	0.24 ± 0.01
11	59.1	α-Tocopherol	—	0.35 ± 0.12	—	—	—
12	61.0	Cholest-5-en-3-ol	—	—	0.08 ± 0.02	—	—
13	61,6	Stigmasterol	0.31 ± 0.07	0.40 ± 0.05	0.43 ± 0.02	0.21 ± 0.02	0.34 ± 0.01
14	62.6	β -Sitosterol	—	0.29 ± 0.02	—	—	—
15	62.8	β -Amyrin	0.15 ± 0.01	0.26 ± 0.05	0.16 ± 0.01	0.17 ± 0.00	—
16	63.2	Lanosterol	0.17 ± 0.01	—	0.08 ± 0.02	—	—
17	63.5	α -Amyrin	0.17 ± 0.03	—	0.19 ± 0.01	—	—
18	63.7	Lupeol	0.28 ± 0.06	0.26 ± 0.01	0.18 ± 0.07	—	—
19	65.4	Lup-20(29)-en-28-al	0.18 ± 0.04	0.26 ± 0.03	0.21 ± 0.01	—	—
20	66.1	Friedelan-3-one	0.12 ± 0.02	—	0.09 ± 0.03	—	—
		Alcohols					
21	59.7	1-Octacosanol	—	0.11 ± 0.04	—	—	—
		Alkanes					
22	46.3	Heptacosane	—	0.73 ± 0.32	0.26 ± 0.02	0.23 ± 0.00	—
23	54.2	Nonacosane	1.18 ± 0.30	—	1.44 ± 0.14	0.31 ± 0.01	0.50 ± 0.03
24	56.7	Triacontane	0.24 ± 0.03	—	0.21 ± 0.03	—	—
25	58.9	Untriacontane	1.72 ± 0.46	—	1.95 ± 0.27	0.34 ± 0.01	0.57 ± 0.01
26	60.9	Tritriacontane	0.16 ± 0.01	—	—	—	—
27	62.7	Tetratriacontane	0.51 ± 0.11	—	0.67 ± 0.03	—	—
28	66.4	Pentatriacontane	—	—	0.15 ± 0.00	—	—

[a]Rt = retention time.

phytocompounds when compared to the 3 subspecies of *C. suffruticosa*, grown and collected under more favorable conditions (Figure 1) [22].

2.2 | Carboxylic Acids and Derivatives

Six carboxylic acids and derivatives were identified among all samples, comprising saturated and unsaturated fatty acids. Myristic, palmitic, linoleic, linolenic, and stearic acids have already been described for the genus *Calendula* [15]. *C. suffruticosa subsp. cinerea* differs from other *taxa* in that it is the only one to present lignoceric acid, in small quantities (0.06 mg/g of the dry plant). Lignoceric acid is also present in the dry material of flowers of *C. officinalis* [23]. Palmitic acid and the polyunsaturated essential fatty acids linoleic and α-linolenic are the three most abundant ones (Table 2). Palmitic acid is a saturated C16 acid that is present in higher quantities in *C. suffruticosa* subsp. *lusitanica* (0.43 mg/g of the dry plant) and *C. tripterocarpa* (0.34 mg/g of the dry plant). It showed a favorable effect on the increase in blood levels of

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

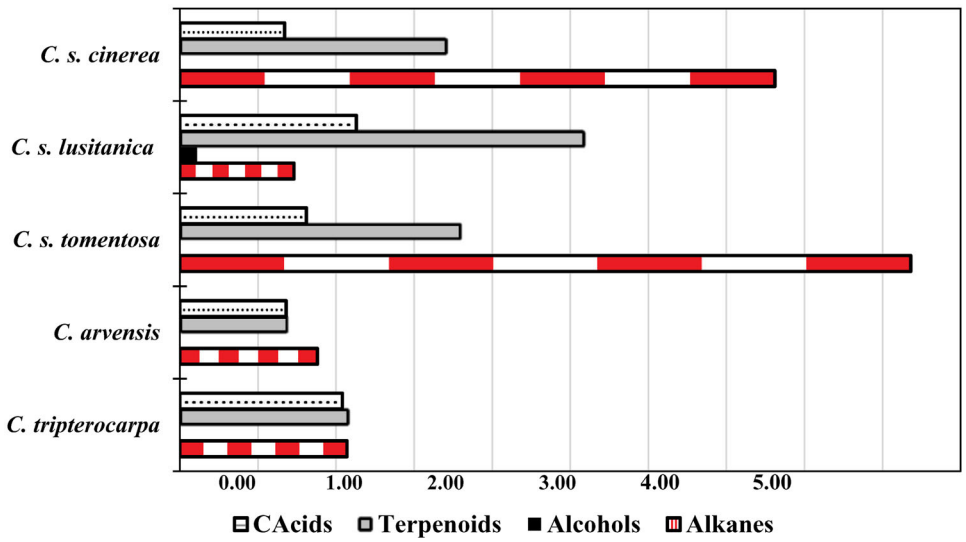


FIGURE 1 | Compound mass by class (mg/g of the dry plant) versus *Calendula* L. *taxa*.

HDL-C without significant changes in the TC/HDL-C ratio [24].

Dietary linoleic acid differentially influences brain fatty acids desaturases activities, inhibits inflammation, and promotes amyloid-β clearance in vitro, suggesting a potential use as anti-Alzheimer's medications [25]. Self-assembled β-lactoglobulin-linoleic acid (β-LG-LA) complexes killed HeLa and MCF7 tumor cells by inducing apoptosis [26]. These two polyunsaturated fatty acids were identified in all samples being the larger quantities present in *C. suffruticosa* subsp. *lusitanica* (0.27 and 0.31 mg/g of the dry plant), followed by *C. tripterocarpa* (0.33 and 0.37 mg/g of the dry plant), while the lowest concentration was found in *C. suffruticosa* subsp. *cinerea* (0.12 and 0.13 mg/g of the dry plant, respectively).

The samples collected in May 2019 showed lower amounts of carboxylic acids (palmitic, linoleic, α-linolenic and stearic), on average, 24.3% for *C. suffruticosa* subsp. *lusitanica* and 42.8% for *C. arvensis*, relative to those previously obtained for the same rate collected in the summer of 2015 [15]. It is possible that this decrease in the quantities of carboxylic acids and other classes has suffered a strong influence due to the exceptional warmth of the years 2015–2019, the warmest five years on record in the world [27].

2.3 | Terpenoids

Neophytadiene and stigmasterol were the only two terpenoids identified in all *Calendula taxa*, with amounts ranging from 0.09 to 0.28 and 0.21 to 0.43 mg/g of the dry plant, respectively. They have also been identified in *C. suffruticosa* subsp. *algarbiensis* and *C. officinalis*, and with similar values for neophytadiene in *C. suffruticosa* subsp. *lusitanica* (0.18 mg/g of the dry plant in both) and close to *C. arvensis* (0.31 × 0.26 mg/g of the dry plant), while stigmasterol showed half the amount in *C. arvensis* (0.80 × 0.40 mg/g of the dry plant) [15]. Neophytadiene showed anti-inflammatory activity [28] and stigmasterol has an effect on the host intestine and intratumoral immune cells, remodeling the intestinal microbiota of mice with hepatocellular carcinoma [29].

β-Eudesmol was identified and quantified (0.21 mg/g of the dry plant) only in *C. suffruticosa* subsp. *lusitanica*. This sesquiterpenol is the main compound contained in *Atractylodes lancea* (Thunb.) DC, which have been used as traditional Japanese-Sino medicine, to alleviate muscular pain [30]. It blocks the neuromuscular junction in mouse skeletal muscles [31]. Also inhibit the proliferation of the human tumors cells as HeLa, SGC-7901, BEL-7402 and HL60 [30, 32], β-eudesmol isomers can reduce cell proliferation and induce tumor cell death in HepG2 cells [33], can be used as antivirals in COVID-19 [34] and is a potential promising candidate for further development as an anti-cholangiocarcinoma (anti-CCA) [35].

The α-Tocopherol was identified for the first time in *C. suffruticosa* subsp. *lusitanica* (0.35 mg/g of the dry plant), but it is also present in *C. officinalis* (25.5 and 19.2 µg/10 g of tissue in chloroplasts and mitochondria, respectively) [36]. The different tocotrienols perform numerous biological functions, already classically described, such as antioxidant capacity. Still, a high number of new functions have been elucidated: regulation of cellular signaling and gene expression, prevention of cancer-related processes, and amelioration of neurodegeneration [37]. It is likely that the other vitamers are also present, perhaps in minimal quantities, which does not prevent suggesting the potential use of *C. suffruticosa* subsp. *lusitanica* and the other *Calendula taxa* in folk medicine, and corroborate uses described by folk medicine of *C. arvensis*, *C. tripterocarpa* and *C. officinalis* [8, 10, 22, 38], and functional food, as *C. officinalis* [39].

Lanosterol was detected in *C. suffruticosa* subsp. *cinerea* (0.17 mg/g of the dry plant) and *C. suffruticosa* subsp. *tomentosa* (0.08 mg/g of the dry plant). The treatment with lanosterol diminishes aberrant proteotoxic aggregation and mitigates its cytotoxicity and can probably offer an efficient therapeutic approach against misfolding of different disease-causing proteins in neurodegenerative disorders, including cataractous human lens [40, 41]. On the other hand, the lanosterol coating (1000 µg = 0.02 kg/m2) inhibits bacterial biofouling on PVC plates when they were subjected to seawater for up to one year, suggesting its use as an eco-friendly protector [42].

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Phytosterols have been reported to exhibit anti-inflammatory, antibacterial, antifungal, and antitumor activities that have become popular as a health supplement in reducing plasma TC and LDL-C [43]. In the intestinal lumen, they displace cholesterol from mixed micelles and inhibit cholesterol absorption [44].

The β-Sitosterol was detected only in *C. suffruticosa* subsp. *lusitanica* (0.29 ± 0.02 mg/g dry plant) by GC–MS. LC–MS–MS showed that the most abundant phytosterol in many samples of edible oils was β-sitosterol [45]. β-sitosterol could similarly suppress the hypercholesterolemia induced by both dietary free cholesterol (FC) and cholesteryl palmitate (CP) in the hamster model [46].

Lupeol was identified in three subspecies of *C. suffruticosa* (0.28, 0.26 and 0.18 mg/g of the dry plant, Table 2). Previous data indicate the presence of greater amounts of this compound in *C. suffruticosa* subsp. *lusitanica* (0.36 mg/g of the dry plant), identical behavior to that verified for lup-20(29)-en-28-al (0.56 × 0.26 mg/g dry plant) [15]. Climatic and physical-chemical variations may have contributed to the variation in the quantity of the two phytochemicals, as obtained for the seasonal variation of essential oils α-cadinol and δ-cadinene in *C. arvensis* [47].

Lupeol exhibits healing properties, acting principally on inflammatory reactions and free radicals, enhancing the formation of extracellular matrix by regulating collagen, and presents analgesic properties [48]. Evidence supports the therapeutic benefits of lupeol against a wide variety of human diseases, namely cancer, diabetes, obesity, cardiovascular diseases, neurological disorders, kidney and liver problems, and skin diseases that are attributed to its antioxidant, anti-inflammatory, and immunomodulatory effects [49]. Furthermore, antileishmanial and immunomodulatory activities have been attributed to lupeol isolated from *Sterculia villosa* Roxb [50].

C. suffruticosa subsp. *cinerea* and *C. suffruticosa* subsp. *tomentosa* presented α-amyrin (0.17 and 0.19 mg/g of the dry plant, respectively), and only *C. tripterocarpa* did not present β-amyrin, whose amounts varied between 0.15–0.26 mg/g of the dry plant. Amyrin amounts ranging from 0.10 to 0.42 mg/g of the dry plant had previously been reported for *C. arvensis* and *C. suffruticosa* subsp. *lusitanica* [3], β-Sitosterol and β-amyrin extracted from pitaya (*Hylocereus polirhyzus* Weber) inhibited proliferation of PC3, Bcap-37, and MGC-803 cancer cell lines. However, α-amyrin displayed weak activity on them [51].

Phytyl palmitate was not identified and quantified in *C. suffruticosa* subsp. *lusitanica*, reinforcing what was found in previous reports [3], while the other taxa showed small variations in the quantities (0.13–0.22 mg/g of the dry plant).

2.4 | Alcohols and Alkanes

It's interesting to note that the only alcohol found was octacosanol, and only in *C. suffruticosa* subsp. *lusitanica* (0.11 mg/g of the dry plant) (Table 2 and Figure 1). In addition to *C. suffruticosa* subsp. *cinerea and C. suffruticosa* subsp. *tomentosa, C. arvensis and C. tripterocarpa*, neither was identified in *C. suffruticosa* subsp. *algarbiensis* or *C. officinalis* [3]. Octacosanol is a long-chain aliphatic alcohol present in various vegetable oils, being the main component of a natural product wax extracted from plants. Amongst the reported health benefits, are cholesterol-lowering effects, antiaggregatory properties, cytoprotective use, and ergogenic properties [52]. Moreover, it is effective in an animal model of parkinsonism [53], reduces liver injury by increasing glutathione (GSH) levels, and restores stress-affected sleep central nervous system in mice [54]. Octacosanol isolated from the leaves of *Sabicea grisea* Cham. and Schlitdl var. *grisea*, which is used in the Amazon for treatment of fever and malaria, showed antinociceptive and anti-inflammatory effects in mice [55].

C. suffruticosa subsp. *lusitanica* maintains the phytochemical profile differentiated from the other taxa also concerning the number of alkanes present. Since only the heptacosane was identified (0.73 mg/g of the dry plant) and in an amount three times higher than previously quantified by this group (0.27 mg/g of dry plant) [3]. It was also the one that presented the lowest proportion of this class in relation to the others, contributing only 16%, against 63.3% for *C. suffruticosa* subsp. *cinerea*, 66.4% for *C. suffruticosa* subsp. *tomentosa*, 39.1% for *C. arvensis*, and 33.5% for *C. tripterocarpa*.

C. arvensis and *C. tripterocarpa* showed a very homogeneous distribution among the classes: carboxylic acids 39.1% × 33.5%, terpenoids 30.7% × 33.9%, and alkanes 30.2% × 32.6%, respectively, associated with a smaller number of compounds (**11** and **9**). It is known that seasonality, water availability, temperature, and UV radiation are factors that directly influence the phytochemical profile of species, and in this case the influence can be enhanced by the extreme characteristics of the collection sites [10, 56, 57]. Likewise, extraction methods and analytical variables also contribute to the secondary metabolite profile [56].

2.5 | Principal Component Analysis

The relationship of the 5 *taxa* of *Calendula* with the chemical compounds quantified by GC–MS was explored using the PCA (Figure 2). The two-dimensional projection accounts for up to 79.51% of the data variability, with PC1 and PC2 explaining 47.93% and 31.58%, respectively. Variables (compounds) with a correlation above 0.6, in absolute value, with the Principal Components were considered the most strongly correlated with each component and the most important for the distinction between samples (Table 3).

Therefore, linoleic acid (**3**), α-tocopherol (**11**), β-sitosterol (**14**), 1-octacosanol (**21**), β-eudesmol (**8**), neophytadiene (**9**), α-linolenic acid (**4**), heptacosane (**22**), phytol (**10**), palmitic acid (**2**) and myristic acid (**1**), were, in a descendant order of importance, the most relevant in the positive side of PC1, while phytyl palmitate (**7**), friedelan-3-one (**20**), triacontane (**24**), tetratriacontane (**26**), lanosterol (**16**), untriacontane (**25**) and nonacosane (**23**), were, in a crescent order of importance, the most relevant, in the negative side of PC1. The variables (compounds) that showed higher positive correlations with PC2 were, in a descending order of importance, α-amyrin (**17**), lupeol (**18**), stearic acid (**5**), lup-20(29)-en-28-al (**19**), myristic acid, stigmasterol (**13**),

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

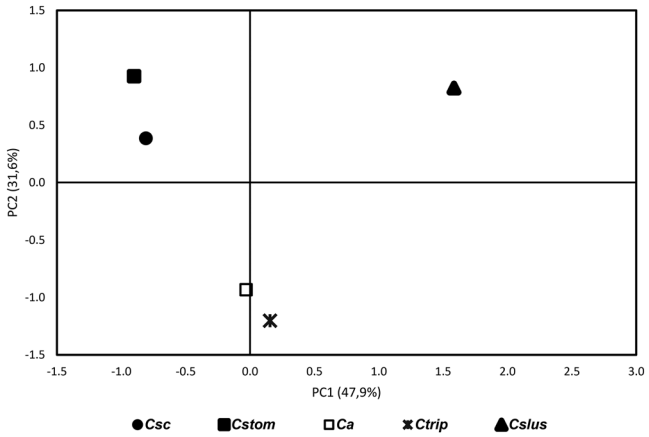


FIGURE 2 | Score plot of PC1 versus PC2 from a PCA based on 28 compounds identified on five *C. taxa*. Csc –*C. suffruticosa* subsp. *cinerea*, Cstom—*C. suffruticosa* subsp. *tomentosa*, Ca- *C. arvensis*, Ctrip- *C. tripterocarpa*, Cslus- *C. suffruticosa* subsp. *lusitanica*.

friedelan-3-one, tetratriacontane and lanosterol. None of the compounds presented negative correlations above the absolute value of 0.6 with PC2. Having this in consideration, one can see that, for example, *C. arvensis* and *C. tripterocarpa* were the only taxa where α-amyrin, lupeol and lup-20(29)-en-28-al were not detected.

Finally, heptacosane also justifies the isolation of *C. suffruticosa* subsp. *lusitanica* in the positive quadrant of PC 1 and PC 2, as it is the only alkane identified for this taxon.

3 | Conclusions

Results allow us to separate *C. suffruticosa* from *C. arvensis* and *C. tripterocarpa* species, and the subsp. *lusitanica* from the subsp. *cinerea* and subsp. *tomentosa*, since this was the only one to minimally express the class of alcohols together with three terpenoids that weren't found in the other taxa. However, further study is needed to confirm these results, both by increasing the number of populations studied and by using other extraction and analysis methods, before final conclusions can be drawn. *C. tripterocarpa, C. suffruticosa* subsp. *cinerea* and *C. suffruticosa* subsp. *tomentosa*, whose data is reported for the first time, showed secondary metabolites such as lanosterol, α-amyrin, β-amyrin and stigmasterol. This suggests that both may also present potential medicinal and gastronomic uses.

4 | Experimental Section

4.1 | Standards, Solvents and Reagents

Several pure compounds were used as standards in order to carry out the quantification and to ensure the correct identification of the extract's constituents. Tetradecanol, palmitic acid, and 5α-cholestan-3-β-ol, were purchased from Sigma–Aldrich (St. Louis, USA); tetracosane and octadecane from Supelco (Bellefonte, USA). For the extraction, hexane was used, while dichloromethane was employed to dissolve the extracts. Pyridine,

TABLE 3 | Correlations between the initial variables (compounds) and the principal components of the PCA, Eigenvalues, percentage of total variance.

Compounds	PC 1	PC 2	PC 3
Myristic acid	0.646	0.713	0.242
Palmitic acid	0.704	−0.060	0.641
Linoleic acid	0.927	0.083	0.309
α-Linolenic acid	0.769	−0.433	0.440
Stearic acid	0.293	0.859	−0.159
Lignoceric acid	−0.504	0.518	0.623
Phytyl palmitate	−0.743	−0.508	0.364
β-Eudesmol	0.886	0.460	−0.058
Neophytadiene	0.824	−0.456	0.303
Phytol	0.711	0.174	0.381
α-Tocopherol	0.886	0.460	−0.058
Cholest-5-en-3-ol	−0.504	0.518	0.623
Stigmasterol	0.119	0.712	0.617
β-Sitosterol	0.886	0.460	−0.058
β-Amyrin	0.545	0.467	−0.602
Lanosterol	−0.781	0.610	−0.010
α- Amyrin	0.252	0.955	−0.078
Lupeol	−0.007	0.919	−0.276
Lup-20(29)-en-28-al	0.415	0.801	−0.335
Friedelan-3-one	−0.770	0.625	0.111
1-octacosanol	0.886	0.460	−0.058
Heptacosane	0.766	0.525	0.057
Nonacosane	−0.903	0.374	0.149
Triacontane	−0.773	0.576	−0.147
Untriacontane	−0.884	0.425	0.087
Nonatriacontane	−0.452	0.215	−0.701
Tetratriacontane	−0.774	0.623	0.081
Pentatriacontane	−0.504	0.518	0.623
Eigenvalue	13.420	8.842	3.833
% of Var.	47.927	31.577	13.689
Cum. %	47.927	79.504	93.193

N, O-bis(trimethylsilyl) (BSTFA), and trimethylsilyl chloride (TMSCl) (Sigma–Aldrich, USA) were used in the sample's derivatization by silylation.

4.2 | Plant Collection

The aerial parts of *C. suffruticosa* subsp. *cinerea, C. suffruticosa* subsp. *lusitanica, C. suffruticosa* subsp. *tomentosa, C. arvensis and C. tripterocarpa*, were collected in one population each (mixing aerial parts of several individuals), in different localities of Portugal and Spain in May 2019 (Table 4) and identified by the taxonomist Paulo Silveira. A voucher for each taxon

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 | Collection sites of *C. taxa* in Portugal and Spain.

Species and subspecies	Voucher n°	Collection site (country)-year
C. suffruticosa cinerea	AVE 6626	Cabo de São Vicente (PT) 37°01'26"N, 8°59'44"W - 2019
C. suffruticosa lusitanica	AVE 6614	Serra de Aire e Candeeiros (PT) 39°38'02"N, 8°45'29"W- 2019
C. suffruticosa tomentosa	AVE 6627	Tarifa (SP) 36°01'38"N, 5°35'12"W - 2019
C. arvensis	AVE 6616	Tabernas (SP) 37°03'51,9"N, 2°20'41"W - 2019
C. tripterocarpa	AVE 6615	Tabernas (SP) 37°03'51,9"N, 2°20'41"W - 2019

was deposited in the Herbarium of the Department of Biology, University of Aveiro, Portugal (AVE).

4.3 | Extract Preparation and Derivatization

The vegetal material was washed in running water and dried in an oven at 60°C for two days. The resulting material was ground to a powder with a grinder. Approximately 25 g of powdered solid from each sample, except *C. suffruticosa* subsp. *lusitanica* (45 mg) was extracted with hexane (three cycles of 250 mL in 10 days) at room temperature using a magnetic stirrer. The mixture was further filtered and the solvent evaporated to dryness with a rotary vacuum evaporator at 40°C. For the silylation procedure, 250 μL of pyridine, 250 μL of BSTFA, and 50 μL of TMSCl were added to a screw glass tube with 20 mg of the extract dissolved in 1 mL of dichloromethane and an internal standard (tetracosane, 0.3 mg). The mixture was placed in a water bath at 70°C for 45 min. Three aliquots of each sample were prepared in the same way, and then each one was injected into the GC–MS apparatus.

4.4 | Gas Chromatography–Mass Spectrometry Analysis

GC–MS analyses of each silylated hexane extract were performed using a GC–MS QP2010 Ultra Shimadzu equipped with a DB-5- J&W capillary column (30 m x 0.25 mm i.d. and a film thickness of 0.25 μm). Samples were injected with a split ratio of 1:50 and helium as carrier gas with a flux of 1.13 mL/min. The temperature of the column was maintained at 130°C for 1 min and then increased at 7°C/min. until 190°C (8.57 min.), followed by 1.2°C/min. until 235°C (37.5 min.) and lastly 4°C/min. until achieved 300°C (16.25 min.), which was maintained for 5 min. Injector temperature was 320°C, and the transfer-line temperature was 200°C. The mass spectrometer was operated in the electronic impact (EI) mode with an energy of 70 eV, and data were collected at a rate of 1 scan/s over a range of m/z 50–1000. The chromatography lasted a total of 68.32 min, and the standards were analyzed separately by GC–MS under the same chromatographic conditions. The phytocomponents of the extracts from the different species were identified based on a direct comparison with the library entries of mass spectra database (NIST14 Mass Spectral and WILEY Registry TM of Mass Spectra Data) and by comparing the retention times and mass spectra data of the standard compounds injected under the same chromatographic conditions. The quantitative analysis was achieved by calibration curves which were obtained using standards representative of each chemical family present in the samples. The determination coefficient (R2) was higher than 0.99 for all compounds.

4.5 | Statistical Analysis

For each sample, three GC–MS independent analyses were carried out, and the results are expressed as mean values ± standard deviation. A *p*-value lower than 0.05 was considered statistically significant. A data set comprising five samples and 28 chemical compounds was organized into rows and columns, respectively, and auto-scaled to unit variance before applying principal component analysis (PCA). PCA was based on correlation analysis, variances were computed as sums of squares/n-1, and it was performed using the software TIBCO Statistica v.13.3.0 (TIBCO—StatisticaTM Ltda, USA).

Author Contributions

M.B.M., S.O., P.C.S., and D.C.G.A.P. conceived and designed the experiment, performed the experiments, analyzed the data, and wrote the article. M.S.G.A.V. conducted validation, investigation, and project administration. D.G. formal analysis. A.M.S.S. resources, funding acquisition. P.C.S. and D.C.G.A.P. also contributed samples, reagents, materials, analysis tools, and analyzed the data. All authors have read and approved the final version of the manuscript.

Acknowledgments

Thanks are due to the financial support from FCT (MCTES) to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds. Acknowledgement is also due to FCT/MCT for the financial support for the QOPNA Research Unit (UID/QUI/00062/2019) and the LAQV-REQUIMTE (UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. We thank the "Instituto da Conservação da Natureza e das Florestas" (ICNF) for allowing the collection of the samples of *C. suffruticosa* subsp. *cinerea* (Cabo de São Vicente) and *C. suffruticosa* subsp. *lusitanica* (Serra de Aire e Candeeiros). Likewise we thank to "Consejeria de Agricultura, Ganaderia, Pesca y Desarrollo Sostenible-Junta de Andalucia" for the collection of *C. suffruticosa* subsp. *tomentosa* (Tarifa).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

References

1. P. Silveira, A. C. Gonçalves, C. Santos, and J. Paiva, "Two Lectotypifications and a New Combination in *Calendula* (Asteraceae) for Flora Iberica," *Phytotaxa* 145 (2013): 47–53.

2. S. Nora, S. Castro, J. Loureiro, et al., "Flow Cytometric and Karyological Analyses of Calendula Species From Iberian Peninsula," *Plant Systematics and Evolution* 299 (2013): 853–864.

3. M. V. Faustino, L. A. M. Seca, P. Silveira, A. M. S. Silva, and D. C. G. A. Pinto, "Gas Chromatography–Mass Spectrometry Profile of Four *Calendula L.* taxa: A Comparative Analysis," *Industrial Crops and Products* 104 (2017): 91–98.

4. A. C. Gonçalves, S. Castro, J. Paiva, C. Santos, and P. Silveira, "Taxonomic Revision of the Genus *Calendula* (Asteraceae) in the Iberian Peninsula and the Balearic Islands," *Phytotaxa* 352 (2018): 1–91.

5. B. P. Muley, S. S. Khadabadi, and N. B. Banarase, "Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae): A Review," *Tropical Journal of Pharmaceutical Research* 8 (2009): 455–465.

6. G. S. Ćetković, S. M. Djilas, J. M. Čanadanović-Brunet, and V. T. Tumbas, "Antioxidant Properties of Marigold Extracts," *Food Research International* 37 (2004): 643–650.

7. Q. Yuan, Q. Ma, L. Ye, and G. Piao, "The Traditional Medicine and Modern Medicine From Natural Products," *Molecules* 21 (2016): 559.

8. D. Arora, A. Rani, and A. Sharma, "A Review on Phytochemistry and Ethnopharmacological Aspects of Genus *Calendula*," *Pharmacognosy Reviews* 2013, 7, 179.

9. W. Seebacher, R. Weis, J. Jurenitsch, K. Rauchensteiner, and E. Haslinger, "Synthesis and Hemolytic Properties of Arvensoside B Isomers," *Monatshefte für Chemie/Chemical Monthly* 131 (2000): 985–996.

10. A. M. Abudunia, M. Ansar, J. Taoufik, et al., "Evaluation of Antibacterial Activity of Extracts From *Calendula arvensis* Flowers," *Journal of Chemical and Pharmaceutical Research* 6 (2014): 156–161.

11. C. Nicolaus, S. Junghanns, A. Hartmann, R. Murillo, M. Ganzera, and I. Merfort, "In Vitro Studies to Evaluate the Wound Healing Properties of *Calendula officinalis* Extracts," *Journal of Ethnopharmacology* 196 (2017): 94–103.

12. Z. Kalvatchev, R. Walder, and D. Garzaro, "Anti-HIV Activity of Extracts From *Calendula officinalis* Flowers," *Biomedicine & Pharmacotherapy* 51 (2017): 176–180.

13. L. M. L. Parente, R. S. Lino Jr., L. M. F. Tresvenzol, M. C. Vinaud, J. R. de Paula, and N. M. Paulo, "Wound Healing and Anti-Inflammatory Effect in Animal Models of *Calendula officinalis* L. Growing in Brazil," *Evidence-Based Complementary and Alternative Medicine* 2012 (2012): 375671.

14. A. D. Baciu, L. Mihalte, A. F. Sestras, and R. E. Sestras, "Variability of Decorative Traits, Response to the *Aphis fabae* Attack and RAPD Diversity in Different Genotypes of *Calendula*," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38 (2010): 265–270.

15. M. V. Faustino, D. C. G. A. Pinto, M. J. Gonçalves, L. Salgueiro, P. Silveira, and A. M. S. Silva, "*Calendula* L. Species Polyphenolic Profile and In Vitro Antifungal Activity," *Journal of Functional Foods* 45 (2018): 254–267.

16. B. Nikmehr, H. Ghaznavi, A. Rahbar, S. Sadr, and S. Mehrzadi, "In Vitro Anti-Leishmanial Activity of Methanolic Extracts of *Calendula officinalis* Flowers, *Datura stramonium* Seeds, and *Salvia officinalis* Leaves," *Chinese Journal of Natural Medicines* 12 (2014): 423–427.

17. J. Barbir, F. R. Badenes-Pérez, C. Fernández-Quintanilla, and J. Dorado, "The Attractiveness of Flowering Herbaceous Plants to Bees (Hymenoptera: Apoidea) and Hoverflies (Diptera: Syrphidae) in Agro-Ecosystems of Central Spain," *Agricultural and Forest Entomology* 17 (2015): 20–28.

18. C. M. Messina, A. Troia, R. Arena, et al., "Species-Specific Antioxidant Power and Bioactive Properties of the Extracts Obtained from Wild Mediterranean *Calendula* Spp. (Asteraceae)," *Applied Sciences* 9 (2019): 4627.

19. T. Ercetin, F. S. Senol, I. Erdogan Orhan, and G. Toker, "Comparative Assessment of Antioxidant and Cholinesterase Inhibitory Properties of the Marigold Extracts From *Calendula arvensis* L. and *Calendula officinalis* L," *Industrial Crops and Products* 36 (2012): 203–208.

20. N. Abutaha, F. A. Nasr, A.-Z. Mohammed, A. Semlali, F. A. Al-Mekhlafi, and M. A. Wadaan, "*Calendula arvensis* L. as an Anti-Cancer Agent Against Breast Cancer Cell Lines," *Molecular Biology Reports* 46 (2019): 2187–2196.

21. M. R. A. Rodrigues, L. C. Krause, E. B. Caramão, J. G. dos Santos, C. Dariva, and J. V. de Oliveira, "Chemical Composition and Extraction Yield of the Extract of *Origanum vulgare* Obtained From Sub- and Supercritical CO_2," *Journal of Agricultural and Food Chemistry* 52 (2004): 3042–3047.

22. C. C. Eberle, F. Forcella, R. Gesch, D. Peterson, and J. Eklund, "Seed Germination of *Calendula* in Response to Temperature," *Industrial Crops and Products* 52 (2014): 199–204.

23. M. Miguel, L. Barros, C. Pereira, et al., "Chemical Characterization and Bioactive Properties of Two Aromatic Plants: *Calendula officinalis* L. (Flowers) and *Mentha cervina* L. (leaves)," *Food & Function* 7 (2016): 2223–2232.

24. C. Agostoni, L. Moreno, and R. Shamir, "Palmitic Acid and Health: Introduction," *Critical Reviews in Food Science and Nutrition* 56 (2016): 1941–1942.

25. G. M. Cole, Q.-L. MA, B. Teter, J. Mychica, and S. A. Frautschy, "Dietary Linoleic Acid Differentially Influences Brain FADS Activities Increasing an n-6 Metabolite That Inhibits Inflammation and Promotes Amyloid-β Clearance," *Alzheimer's & Dementia* 13 (2017): 982.

26. B. Fang, M. Zhang, M. Tian, and F. Z. Ren, "Self-Assembled β-Lactoglobulin–Oleic Acid and β-Lactoglobulin–Linoleic Acid Complexes With Antitumor Activities," *Journal of Dairy Science* 98 (2015): 2898–2907.

27. European State of the Climate, Copernicus Climate Change Service, Full report: climate.copernicus.eu/ESOTC/2019, 2019, https://doi.org/10.24381/zw9t-hx58.

28. M. Shah, B. V. Maran, S. Shaleh, W. Zuldin, C. Gnanaraj, and Y. Yong, "Therapeutic Potential and Nutraceutical Profiling of North Bornean Seaweeds: A Review," *Marine Drugs* 20 (2022): 101.

29. R. Huo, W.-J. Yang, Y. Liu, et al., "Stigmasterol: Remodeling Gut Microbiota and Suppressing Tumor Growth Through Treg and CD8+ T Cells in *Hepatocellular carcinoma*," *Phytomedicine* 129 (2024): 155225.

30. H. Tsuneki, E. Ma, S. Kobayashi, et al., "Antiangiogenic Activity of β-Eudesmol In Vitro and In Vivo," *European Journal of Pharmacology* 512 (2005): 105–115.

31. M. Kimura, H. Nojima, M. Muroi, and I. Kimura, "Mechanism of the Blocking Action of β-eudesmol on the Nicotinic Acetylcholine Receptor Channel in Mouse Skeletal Muscles," *Neuropharmacology* 30 (1991): 835–841.

32. Y. Li, T. Li, C. Miao, J. Li, W. Xiao, and E. Ma, "β-Eudesmol Induces JNK-Dependent Apoptosis Through the Mitochondrial Pathway in HL60 Cells," *Phytotherapy Research* 27 (2013): 338–343.

33. D. S. Bomfim, R. P. C. Ferraz, N. C. Carvalho, et al., "Eudesmol Isomers Induce Caspase-Mediated Apoptosis in Human Hepatocellular Carcinoma H ep G 2 Cells," *Basic & Clinical Pharmacology & Toxicology* 113 (2013): 300–306.

34. I. R. Melani, M. F. Wafi, M. R. Riandinata, P. A. R. Halim, R. Muti'ah, and D. Santosaningsih, "Potential Inhibition of *Melaleuca leucadendron* L. Compounds Against the NSP5 SARS CoV-2 Protein," *Indonesian Journal of Cancer Chemoprevention* 13, no. 3 (2022): 195–206.

35. T. Plengsuriyakarn, J. Karbwang, and K. Na-Bangchang, "Anticancer Activity Using Positron Emission Tomography-Computed Tomography

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and Pharmacokinetics of β -Eudesmol in Human Cholangiocarcinoma Xenografted Nude Mouse Model," *Clinical and Experimental Pharmacology and Physiology* 42 (2015): 293–304.

36. W. Janlszowska, W. Michuki, and Z. Kasprzy, "Polyprenyl Quinones and α-tocopherol in *Calendula officinalis*," *Phytochemistry* 15 (1976): 125-127.

37. R. Brigelius-Flohé, "Bioactivity of Vitamin E," *Nutrition Research Reviews* 19 (2006): 174–186.

38. B. Powell, A. Ouarghidi, T. Johns, M. I. Tattou, and P. Eyzaguirre, "Wild Leafy Vegetable Use and Knowledge Across Multiple Sites in Morocco: A Case Study for Transmission of Local Knowledge?," *Journal of Ethnobiology and Ethnomedicine* 10 (2014): 34.

39. G. B. Escher, L. D. C. C. Borges, J. S. Santos, et al., "From the Field to the Pot: Phytochemical and Functional Analyses of *Calendula officinalis* L. Flower for Incorporation in an Organic Yogurt," *Antioxidants* 8 (2019): 559.

40. X. J. Chen, L. D. Hu, K. Yao, and Y. B. Yan, "Lanosterol and 25-Hydroxycholesterol Dissociate Crystallin Aggregates Isolated From Cataractous Human Lens via Different Mechanisms," *Biochemical and Biophysical Research Communications* 506 (2018): 868–873.

41. A. Upadhyay, A. Amanullah, R. Mishra, A. Kumar, and A. Mishra, "Lanosterol Suppresses the Aggregation and Cytotoxicity of Misfolded Proteins Linked With Neurodegenerative Diseases," *Molecular Neurobiology* 55 (2018): 1169–1182.

42. V. Balasubramanian, R. Rajaram, S. Palanichamy, G. Subramanian, K. Mathivanan, and A. Pugazhendhi, "Lanosterol Expressed Bio-Fouling Inhibition on Gulf of Mannar Coast, India," *Progress in Organic Coatings* 115 (2018): 100–106.

43. S. Klingberg, L. Ellegård, I. Johansson, et al., "Inverse Relation Between Dietary Intake of Naturally Occurring Plant Sterols and Serum Cholesterol in Northern Sweden," *Clinical Nutrition* 87 (2008): 993–1001.

44. E. D. Smet, R. P. Mensink, and J. Plat, "Effects of Plant Sterols and Stanols on Intestinal Cholesterol Metabolism: Suggested Mechanisms From Past to Present," *Molecular Nutrition & Food Research* 56 (2012): 1058–1072.

45. S. Mo, L. Dong, W. J. Hurst, and R. B. van Breemen, "Quantitative Analysis of Phytosterols in Edible Oils Using APCI Liquid Chromatography–Tandem Mass Spectrometry," *Lipids* 48 (2013): 949–956.

46. Y. Liu, L. Lei, X. Wang, et al., "Plasma Cholesterol-Raising Potency of Dietary Free Cholesterol versus Cholesteryl Ester and Effect of β-sitosterol," *Food Chemistry* 169 (2015): 277–282.

47. J. Paolini, T. Barboni, J.-M. Desjobert, N. Djabou, A. Muselli, and J. Costa, "Chemical Composition, Intraspecies Variation and Seasonal Variation in Essential Oils of *Calendula arvensis* L," *Biochemical Systematics and Ecology* 38 (2010): 865–874.

48. B. Adzu, B. A. Chindo, F. D. Tarfa, O. A. Salawu, and O. J. Igoli, "Isolation and Analgesic Property of Lupeol From *Diospyros Mespiliformis* Stem Bark," *J Med Plants Res* 9 (2015): 813–819.

49. A. A.-M. Sohag, T. Hossain, A. Rahaman, et al., "Molecular Pharmacology and Therapeutic Advances of the Pentacyclic Triterpene Lupeol," *Phytomedicine* 99 (2022): 154012.

50. A. Das, J. J. Jawed, M. C. Das, et al., "Antileishmanial and Immunomodulatory Activities of Lupeol, a Triterpene Compound Isolated From *Sterculia villosa*," *International Journal of Antimicrobial Agents* 50 (2017): 512–522.

51. H. Luo, Y. Cai, Z. Peng, T. Liu, and S. Yang, "Chemical Composition and in Vitro Evaluation of the Cytotoxic and Antioxidant Activities of Supercritical Carbon Dioxide Extracts of Pitaya (Dragon Fruit) Peel," *Chemistry Central Journal* 8 (2014): 1.

52. J. C. Taylor, L. Rapport, and G. B. Lockwood, "Octacosanol in Human Health," *Nutrition* 19 (2003): 192–195.

53. T. Wang, Y. Liu, N. Yang, C. Ji, P. Chan, and P. Zuo, "Anti-Parkinsonian Effects of Octacosanol in 1-methyl-4-Phenyl-1,2,3,6 Tetrahydropyridine-Treated Mice," *Neural Regeneration Research* 7 (2012): 1080–1087.

54. M. K. Kaushik, K. Aritake, A. Takeuchi, M. Yanagisawa, and Y. Urade, "Octacosanol Restores Stress-Affected Sleep in Mice by Alleviating Stress," *Scientific Reports* 7 (2017): 8892–8899.

55. A. M. D. Oliveira, L. M. Conserva, J. N. S. Ferro, F. D. A. Brito, R. P. L. Lemos, and E. Barreto, "Antinociceptive and Anti-Inflammatory Effects of Octacosanol From the Leaves of *Sabicea grisea* var. *grisea* in Mice," *International Journal of Molecular Sciences* 13 (2012): 1598–1611.

56. L. Gobbo-Neto and N. P. Lopes, "Medicinal Plants: Factors of Influence on the Content of Secondary Metabolites," *Química Nova* 30 (2007): 374–381.

57. L. F. R. de Almeida, R. D. O. Portella, J. Bufalo, M. O. M. Marques, R. Facanali, and F. Frei, "Non-Oxygenated Sesquiterpenes in the Essential Oil of *Copaifera langsdorffii* Desf. Increase During the Day in the Dry Season," *PLoS ONE* 11 (2016): e0149332.

16121880, 2026, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.71921 by Ministry Of Health, Wiley Online Library on [19/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License