Chapter

Seed Propagation and Constituents of the Essential Oil of *Stevia serrata* Cav. from Guatemala

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Abstract

Stevia serrata Cav. (Eupatorieae, Asteraceae) grows in Central America and Mexico usually over 1500 m. In this study, essential oils of aerial parts from three populations of western Guatemala were obtained yielding 0.17–0.27% of oil by hydrodistillation. Chamazulene (42–62%) was the most abundant compound in the oil analyzed GC/MS, also presenting germacrene D (4.4–15.3%), caryophyllene oxide (3.2–11.8%), (*E*)-nerolidol (3.9–7.1%), spathulenol (2.3–7.9%), and (*E*)-caryophyllene (2.5–6.6%). Besides, a propagation trial was carried out on seeds of plants collected in Santa Lucía Utatlán, as the first step for the domestication of the plant, obtaining approximately 75% survival in the transplanting of the germinated seedlings. After the flowering of the individuals, a greenish essential oil was obtained from the roots yielding 0.2% of oil. This oil did not present chamazulene, but α-longipinene (23.5%), germacrene D (22.2%), santolina triene (12.6%), and (*E*)-caryophyllene (8.1%) as major components. As conclusion, it was confirmed that the aerial parts of the essential oil of *S. serrata* from western Guatemala presents a high content of chamazulene and that there is feasibility for the domestication of the plant through the germination of seeds.

Keywords: α-longipinene, chamazulene, Guatemala, sesquiterpenes, *Stevia serrata*

1. Introduction

The high biodiversity of Guatemala, caused by the great variety of microclimates and the convergence of the flora of North and South America, presents plants that have developed a large number of secondary metabolites to fulfill functions of defense and interaction with the environment. Many of these metabolites have biological and pharmacological activities that are used by communities, through the use of plants for the treatment of different diseases [1]. In this way, many investigations have been carried out aimed at determining the composition and biological activity of the metabolites of different medicinal plants used in Guatemala [2–5].

One of the biodiverse plants of Guatemala, which also grows in neighboring countries and for which no medicinal uses have been reported in Guatemala, is *Stevia serrata* Cav. [6] whose essential oil presents chamazulene in high proportions. Chamazulene is a substance of intense blue color of high economic value, which has been shown to have a high anti-inflammatory activity [7].

The genus *Stevia* belongs to the Asteraceae family within the Eupatorieae tribe [8]. It is a New World genus distributed from the south of the United States to Argentina and the highlands of Brazil, passing through Mexico, the Central American countries, and the South American Andes [9, 10]. The records indicate that the genus is not represented in the Antilles or the Amazon. The members of the *Stevia* genus are found mainly at altitudes between 500 and 3500 m. Although they usually grow in semidry mountainous terrain, their habitats range from meadows, leafy forests, forested mountain slopes, coniferous forests, to subalpine vegetation [8].

The genus *Stevia* consists of between 220 and 230 accepted species. Of these, only about 34 (15%) have some type of ethnobotanical record that relate uses with common names of the species. Of these 34 species, only the South American species *Stevia rebaudiana* (Bertoni) Bertoni presents records of outstanding use because its sweet leaves are used for imparting sweetness to beverages and foods [8, 12]. Due to this, *S. rebaudiana* is of great economic importance internationally, given its intensive commercialization due to its use as a natural low calorie sweetener [8].

The sesquiterpenoids are by far the majority and characteristic constituents of the aerial parts and roots of the *Stevia* genus. The overwhelming majority of these compounds belong to the guaiane, longipinane, and germacrene groups [8]. Derivatives of longipinene have been isolated and elucidated mainly in roots of *S. eupatoria*, *S. porphyria*, and *S. pilosa* in Mexico, in *S. triflora* from Venezuela, and in *S. lucida* of Colombia [13–18]. Diterpene glycosides have been isolated from commercial extracts of *S. rebaudiana* leaves in Malaysia [19, 20]. The composition of the essential oil of plants of the genus *Stevia* has been determined in leaves of *S. urticifolia* in Brazil being the main components found the oxygenated sesquiterpene α-cadinol (8.6%) and the sesquiterpene hydrocarbon germacrene D (10.4%) [21].

On the other hand, the composition of the essential oil of *S. rebaudiana* leaves analyzed in Nigeria showed carvacrol (67.89%), caryophyllene oxide (23.50%), spathulenol (15.41%), cardinol (5.59%), α-pinene (3.75%), ibuprofen (1.79%), isopinocarveol (1.26%), and α-caryophyllene (1.15%) as the main components found [22].

Other types of compounds isolated in plants of this genus include four flavonoids isolated from the aerial parts of *S. urticifolia* in Brazil [23], two triterpenes isolated from the roots of *S. viscida* and *S. eupatoria* from Mexico [24], the breviarolide and guaianolide isolated from the aerial parts of *S. breviaristata* from Argentina [25], and the stephalic acid isolated from the whole plant of *S. polycephala* from Mexico [26]. Nineteen hydroxycinnamic acid derivatives were successfully characterized in *S. rebaudiana* leaves: three monocaffeoylquinic acids (Mr354), seven dicaffeoylquinic acids (Mr516), one *p*-coumaroylquinic acid (Mr338), one feruloylquinic acid (Mr368), two caffeoyl-feruloylquinic acids (Mr530), three caffeoylshikimic acids (Mr336), and two tricaffeoylquinic acids (Mr678) [12].

Likewise, two new *stevia* amino acid sweeteners have been synthesized from natural stevioside: *stevia* glycine ethyl ester and *stevia* L-alanine methyl ester. The sweetness intensity rate of the new sweeteners was higher than sucrose, and they also had a clean sweetness without the unpleasant bitter aftertaste of stevioside [27]. *Stevia* products have been elaborated as an infusion with suitable organoleptic characteristics using a formulation of 80–85% of leaves + dried flowers of anise (*Tagetes filifolia* Lag.) and 15–20% of dried *stevia* leaves (*S. rebaudiana*) [28].

As for the *S. serrata* plant, it is distributed from southern Arizona, New Mexico and Texas to northern Oaxaca, and from Chiapas to Honduras, Colombia, Venezuela, and Ecuador. In Guatemala it is found in the departments of Chimaltenango, Guatemala, Huehuetenango, Quetzaltenango, El Quiché, Sacatepéquez, and Sololá [6, 11].

The species grows along pastures and roadsides in various habitats from *Yucca-Opuntia* scrub, sand pine woods, steep rock outcrops in *Quercus-Acacia* grasslands, and pastured slopes, usually between 900 and 2800 m. The plants prefer sunny, stony, well-drained places but also grow in moist pastures and other flat areas [6, 11]. They grow as erect perennial herbs to 0.6–1 m, the stems single to many, puberulent to densely pilose. Leaves alternate, scattered and often crowded, sessile to subsessile, serrate toward the apex, 2.5–6.5 cm long, 0.2–1.5 cm wide, apex rounded to acute. Capitula 5–9 mm, phyllaries 3.5–6 mm long, 0.7–1 mm wide, puberulent with numerous glandular dots. Corollas white, 3–5 mm long, often gland-dotted, lobes 1–1.5 mm long, puberulent. Achenes are usually heteromorphic, 2.2–4.2 mm long, hispid. Pappus of the four adelphocarps with 3–5 awns equaling the corolla and alternating with 3–5 scales, 0.2–0.7 mm long [6, 11].

As for the chemistry of *S. serrata*, five new derivatives of longipinene have been isolated and elucidated from the roots of the plant in Mexico, these being 7β,9α-diangeloyloxy-8α-hydroxylongipinan-1-one; 8β,9α-diangeloyloxy-9α-hydroxylongipinan-1-one; 7β,9α-diangeloyloxy-8α-acetyloxylongipinan-1-one; 7β,9α-diangeloyloxy-8α-acetyloxylongipin-2-en-1-one; and 7β-angeloyloxy-8α-isobutyryloxylongipin-2-en-1-one [29]. Likewise, in Mexico, two new prochamazulene sesquiterpene lactones from the dried leaves of *S. serrata* from Mexico were isolated and identified: steviserrolide A and steviserrolide B [30]. The presence of the R enantiomer of chamazulene carboxylic acid (**Figure 1**) of *S. serrata* from Central America was determined [31].

Regarding studies of the essential oil of the plant, the distillation of 178 g of flowers of *S. serrata* from Mexico provided 700 mg of the blue essential oil, which yielded 320 mg of chamazulene [32]. The compounds found in highest concentration in the essential oil of *S. serrata* from Guatemala were the sesquiterpenes chamazulene (60.1%), (*E*)-nerolidol (7.3%), caryophyllene oxide (6.3%), and germacrene D (5.4%) [33], which are shown in **Figure 2**. Chamazulene is produced from prochamazulenic sesquiterpenlactones. Among these precursors, matricine (**Figure 2**) and the carboxylic acid of chamazulene, among others, have been identified, which are present in the plant and are transformed into chamazulene by the action of the temperature during the steam extraction process [31]. Other compounds isolated from the plant include the methyl-ripariochromene A from the dried leaves of *S. serrata* cultivated in Japan [34].

The plant, known in Mexico as "tlachichinole," was used in decoction of the aerial parts for the washing of infected pimples [8], while the "donkey chili" or "sheep tail" is used as medicine to treat intestinal discomforts in Honduras [35]; the

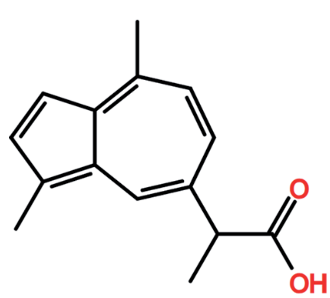

Figure 1.
Chamazulene carboxylic acid.

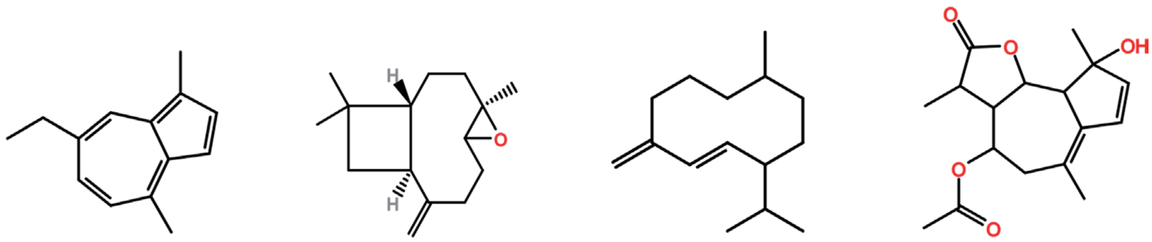

Figure 2.
From left to right, structures of chamazulene, caryophyllene oxide, germacrene D, and matricine.

decoction of the "October flower" is used by the midwives to accelerate the contractions of the parturients during childbirth [36]. Oral administration of *S. serrata* essential oil from Guatemala produced a marked antinociceptive activity in mice in the formalin test [33].

The purpose of the study was to determine the composition of the essential oil of aerial parts of *S. serrata* from different localities of the Guatemalan highlands, to evaluate the variability of the content of chamazulene. The capability of propagation of plants of *S. serrata* was also determined by a seed propagation trial. Finally, the composition of the essential oil of the roots of the propagated plants was determined to compare it with the composition of the oil extracted from aerial parts of the plant.

2. Methodology

2.1 Collection and preparation of plant material

Aerial parts of *S. serrata* were collected from populations in different localities (**Table 1**) during 2018. The plant material was dried in a solar dryer at a temperature between 30 and 35°C and immediately extracted. **Figure 3** shows pictures of the population in Santa Cruz del Quiché, Quiché, and details of floral button of the plant.

2.2 Seed germination

Seeds of *S. serrata* were collected in the surroundings of Santa Lucia Utatlán, Sololá (N 14° 46 40.4″ W 091° 14 41.5″/2430 m), in December 2015. Seeds were stored in trays inside a solar dryer at a temperature between 30 and 35°C for 2 months.

After drying, seeds were manually removed from the flower receptacles and subsequently placed for germination in peat moss previously moistened into plastic strainers (**Figure 4**).

2.3 Transplantation of seedlings and root obtention

The seedlings obtained were transplanted to 4-gallon flowerpots containing potting soil. The plants were placed in direct sunlight and watered daily. After the seed production by the individuals grown in pots, their roots were removed, washed, and dried in a solar dryer. Then, the roots were pulverized in a forage mill for the extraction of the essential oil.

2.4 Extraction of essential oil

The oil from 50.0 g of aerial parts of *S. serrata* was extracted by hydrodistillation using a Clevenger-type apparatus for 2 h. It was then weighed with an analytical scale. The extraction of the essential oil of 100 g of powdered roots was carried out

Localities and dates of collection of individuals of *S. serrata*

Locality	Sample code	Organ	Geographic position	Altitud e (m)	Collectio n date	Phenologic al stage
San Miguel Ixtahuacán, San Marcos.	SS3	aerial parts	N 15^{0} 14'21.7" W 091^{0} 41'31.4"	2093	21/08/201 8	Flowering
Santa Cruz del Quiché, Quiché	SS4	aerial parts	N 14^{0} 59'03.7" W 091^{0} 07' 15.0"	2013	10/07/201 8	Floral button
Santa María Chiquimula. Totonicapá n	SS5	aerial parts	N 14^{0} 58'30.4" W 091^{0} 25'59.2"	2830	13/06/201 8	Vegetative
Santa Lucía Utatlán, Sololá*	S-SLU	roots	N 14^{0} 46'40.4" W 091^{0} 14'41.5"	2430	/05/2017	Vegetative

*The roots were obtained from the first generation of plants cultivated in Guatemala city using seeds from this locality.

Table 1.
Localities and dates of collection of individuals of S. serrata.

Figure 3.
Population of S. serrata in Santa Cruz del Quiché, Quiché, on the left and details of S. serrata in floral button stage on the right.

Figure 4.
Germinated seeds of S. serrata on the left, seedlings in peat moss in the middle, and transplanted plants on the right.

in the same Clevenger-type apparatus for 2 h. The essential oils of the aerial parts and of the roots were collected in pentane which was later removed in a rotatory evaporator at 40°C. All the extractions were made in triplicate, and the reported yield corresponds to the average of the three extractions.

2.5 Gas chromatography coupled to mass spectrometry analyses (GC/MS)

GC/MS analyses were performed using a chromatograph Shimadzu 2010 Plus system coupled with a Shimadzu QP-2010 Plus selective detector (MSD) and equipped with a DB5-MS capillary fused silica column (60 m, 0.25 mm I.D., 0.25 μm film thickness). The oven temperature program initiated at 60°C, then was raised by 3°C/min to 246°C, and then was held for 20 min. Other operating conditions were as follows: carrier gas, He (99.999%), with a flow rate of 1.03 mL/min; injector temperature, 220°C; split ratio of 1:50; and injection volume of 1 μL. Mass spectra were taken at 70 eV. The m/z values were recorded in the range of m/z 40–700 Da.

3. Results

Tables 2 and **3** present the results of yields and chemical composition of the essential oils of the three sampled populations of *S. serrata* and roots of plants obtained by seed propagation, respectively. Chamazulene was the major component of the essential oils of the aerial parts meanwhile α-longipinene was the compound found in major proportion in the essential oil of the roots.

4. Discussion

4.1 Essential oil of aerial parts of *S. serrata*

Table 2 shows the yield and composition results of the intense blue essential oil obtained from the aerial parts of individuals of *S. serrata* collected in three different populations distinct of the population sampled in a previous study of the chemical composition of oil of *S. serrata* from Guatemala [33]. The three populations are located in the highlands of western Guatemala. Extraction yields were between 0.2 and 0.3% (w/w) (**Table 3**), corresponding the highest yield to the SS4 oil from Santa Cruz del Quiché. A probable explanation for the difference in yields among the sampled populations is that the production of essential oil depends on the phenological stage, so that there is a greater production of oil in the flowering stage and lower production in the fruiting stage.

Another probable explanation could be edaphic factors affecting the production of secondary metabolites in general, but only after new investigations could the relationship between these factors and the production of essential oil and other metabolites be determined.

Regarding the chemical composition analyzed by GC/MS, 22 compounds were identified in the SS3 (94.7% of the total area) and SS4 (97.6% of the total area) oils and 18 compounds in the SS5 oil (98.4% of the total area). A chromatogram of the essential oil of SS4 is shown in **Figure 5**. The most abundant compound was the chamazulene in area percentages between 42 and 62%, with the highest percentage corresponding to the SS5 essential oil. The mass spectrum of chamazulene from the essential oil of sample SS4 is shown in **Figure 6**. The other compounds found in high percentage in the oil were germacrene D (4.4–15.3%), caryophyllene oxide (3.2–11.8%), (*E*)-nerolidol (3.9–7.1%), spathulenol (2.3–7.9%) and (*E*)-caryophyllene (2.5–6.6%). The α-longipinene, frequently found in *Stevia* genus plants [8] that had not been reported in the essential oil of *S. serrata*, was found in the SS4 oil in 0.4%.

RI	**Compound**	**SS3**	**SS4**	**SS5**
	yield	**0.2**	**0.29**	**0.17**
939	α-pinene	0.6	--	--
1353	α -longipinene	--	0.4	--
1388	β-bourbonene	0.4	--	--
1419	*(E)*-caryophyllene	6.6	2.5	4.0
1441	aromadendrene	--	0.2	--
1455	α -humulene	1.2	0.6	0.6
1480	γ-muurolene	0.7	1.1	0.4
1485	germacrene D	4.4	8.7	15.3
1493	bicyclogermacrene	1.7	0.6	0.2
1500	α -muurolene	1.6	0.8	2.3
1502	epizonarene	0.5	--	--
1512	NI	0.3	0.3	--
1514	γ-cadinene	0.6	0.7	0.3
1523	δ-cadinene	1.8	2.4	1.0
1539	α -cadinene	--	0.2	--
1555	NI	0.5	0.2	--
1563	*(E)*-nerolidol	7.1	4.1	3.9
1572	aromadendrene oxide	0.4	0.8	0.3
1575	NI	0.4	--	--
1578	spathulenol	7.9	6.0	2.3
1583	caryophyllene oxide	11.8	9.0	3.2
1587	isoaromadendrene epoxide	--	0.3	--
1601	guaiol	0.6	--	--
1608	humulene epoxide II	0.5	0.4	0.2
1616	NI	--	0.3	--
1624	10-epi-γ-eudesmol	1.0	--	--
1634	NI	0.3	--	--
1640	epi- α -cadinol	0.6	0.5	0.2
1646	NI	--	0.3	0.3
1648	NI	--	0.2	--
1651	NI	0.4	--	--
1654	α -cadinol	1.1	1.3	1.0
1660	caryophyllene<14-hydroxy-9-epi-(*E*)->	--	0.2	0.4
1672	NI	0.9	0.6	--
1685	NI	--	0.3	0.2
1693	NI	--	0.4	--
1698	Eudesm-7(11)-en-4-ol	0.7	0.7	0.3
1732	chamazulene	42.9	56.1	62.5
1780	NI	--	0.6	0.6
		94.7	97.6	98.4

NI: Not identified

Table 2.
Composition of the essential oil of the aerial parts of S. serrata from three localities.

The results confirm that essential oil of *S. serrata* with high content of chamazulene can be obtained from the different populations of the Guatemalan highlands. The authors consider that although the extraction yield in all the samples has been lower than 0.3%, the plant presents economic potential for its domestication for oil production in view of its high content of chamazulene and the presence in it of other components for which pharmacological activity has been reported.

RI	Compound	Area %
	yield	0.2
909	santolina triene	12.6
939	α-pinene	1.7
979	β-pinene	0.2
1003	α-phellandrene	0.6
1030	β-phellandrene	0.7
1037	NI	0.2
1238	trans-chrysanthenyl acetate	0.3
1261	NI	0.2
1267	NI	0.3
1290	lavandulyl acetate	4.9
1337	NI	1.1
1353	α-longipinene	23.5
1362	neryl acetate	1.9
1374	longicyclene	1.4
1375	α-ylangene	4.8
1380	NI	0.2
1401	β-longipinene	1.4
1408	longifolene	0.5
1419	*(E)*-caryophyllene	8.1
1451	α-himachalene	0.6
1457	*(E)-b*-farnesene	3.5
1483	γ-himachalene	1.3
1485	germacreno D	22.2
1500	bicyclogermacrene	1.0
1505	β-himachalene	1.7
1506	β-bisabolene	0.3
1583	caryophyllene oxide	1.2
1609	NI	1.1
1623	NI	0.5
1637	NI	0.3
1651	vulgarone B	0.5
1654	himachalol	0.3
1730	NI	0.4
1743	Cedr-8(15)-en-9-alpha-ol acetate	0.6
		95.8

NI: Not identified

Table 3.
Composition of the essential oil of roots of propagated S. serrata.

When comparing this source of essential oil with chamazulene content in the oil of *Matricaria recutita* L. (Asteraceae), which is obtained only from the flowers of this species [31], *S. serrata* is shown as a promising species because all aerial parts (leaves, stems, and flowers) produce essential oil with high chamazulene content.

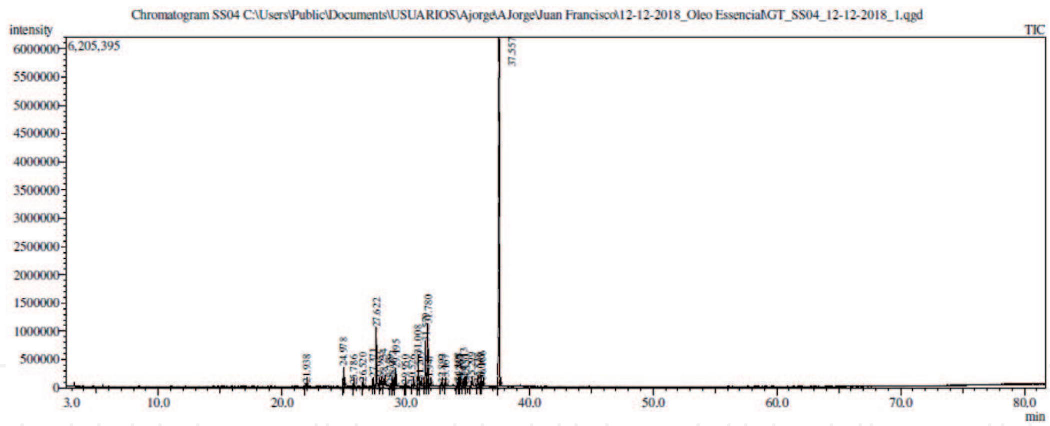

Figure 5.
Chromatogram of the essential oil of S. serrata from SS4 sample obtained by GC/MS.

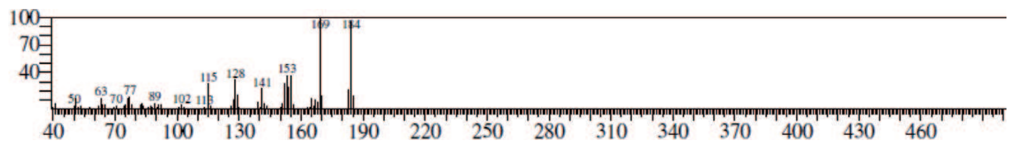

Figure 6.
Spectrum of chamazulene corresponding to the essential oil of sample SS4.

It is worth noting that the composition of the three oils is in congruence with the composition obtained by Simas et al. [33] of *S. serrata* from a population in the department of Sololá, presenting the same major compounds with some percentage variations and the majority of compounds such as sesquiterpenoids.

4.2 Essential oil of roots of propagated plants of *S. serrata*

A seed propagation trial was carried out with seeds of plants of *S. serrata* collected from a population of Santa Lucía Utatlán, Sololá, from where the composition of essential oil with a high content of chamazulene had been previously reported [33]. The purpose of the trial was to evaluate the capability of propagation of the plants, generate new seeds, and extract and analyze the essential oil from the root. The interest in analyzing the root oil was due to the fact that in interviews with residents of the region, the authors had received information that previously the root of the plant had been used in traditional medicine for the treatment of stomach pain [33]. The seeds were germinated in peat moss, and then seedlings were transplanted to pots where they developed well with approximately 75% survival reaching 1 m height after 6 months. It is important to note that the cultivation experiment was carried out in Guatemala City, at an altitude of 1495 m, this being a lower altitude than in the region where the plant grows naturally.

After obtaining the seeds during a plant vegetative stage, the roots were collected from which an essential oil with a light green color was obtained with a yield of 0.2% (w/w), and 25 compounds representing 95.8% of the total chromatographic area were identified (**Table 3**). The chromatogram of the essential oil of the roots is shown in **Figure 7**. Due to the green coloration of the oil, it was supposed that the chamazulene was absent in the oil, which was confirmed after the analysis by GC/MS. The major components of the root oil corresponded to α-longipinene (23.5%), germacrene D (22.2%), santolina triene (12.6%), and (*E*)-caryophyllene (8.1%). The mass spectrum of α-longipinene is shown in **Figure 8**.

The common components between the root and the aerial parts oils were germacrene D and (*E*)-caryophyllene. The α-longipinene (**Figure 9**) was only found in one of the oils of the aerial parts in low percentage (0.4%), while the santolina triene (**Figure 9**) was not found in any of the oils of the aerial parts. As in the oil of aerial parts, sesquiterpenoids predominated in the root oil. Since the plant has been used in the past for the treatment of stomach pain, the authors consider it of value to carry out pharmacological activity tests with this oil in the near future.

5. Conclusions

It was found in this study that the essential oil of aerial parts of wild *S. serrata* from different populations of the highlands of Guatemala showed high concentrations of chamazulene. In addition, the essential oil of roots of the plant was analyzed for the first time, which presented a composition very different from that of the aerial parts, as it did not present chamazulene and presented α-longipinene as the major component. It was also verified that the seeds of *S. serrata* present a high viability and that the seedlings obtained from seeds also have a high percentage of survival. Therefore, *S. serrata* can be considered as a plant with high potential for domestication and cultivation for the production of essential oil with high content of chamazulene.

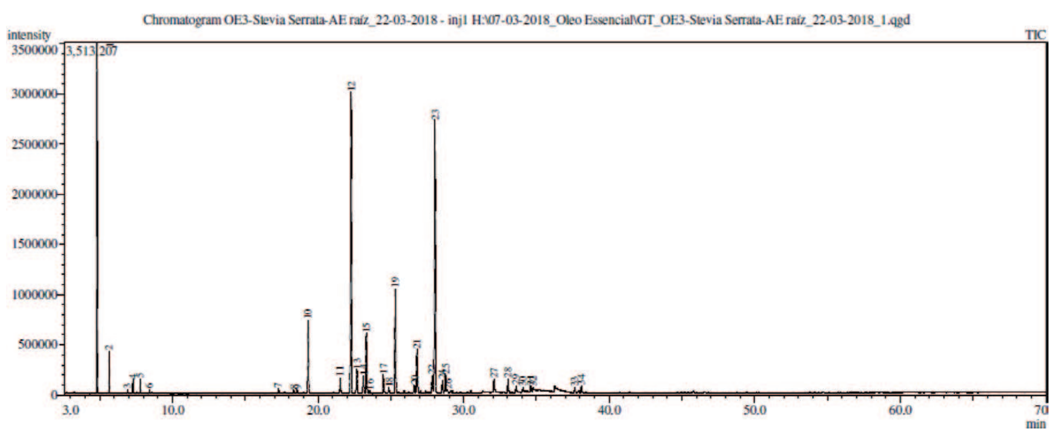

Figure 7.
Chromatogram of the essential oil of roots of S. serrata.

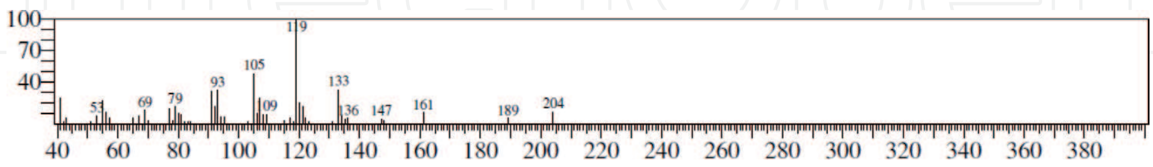

Figure 8.
Mass spectrum of α-longipinene corresponding to the essential oil of roots of S. serrata.

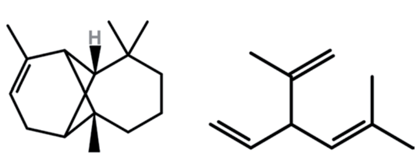

Figure 9.
Structures of α-longipinene and santolina triene, major components of the essential oil of roots of S. serrata.

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Conflict of interest

The authors declare that they have no conflict of interest with respect to this publication.

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