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Biological and Chemical Comparison of Natural and Cultivated Samples of *Satureja macrantha* C.A.Mey.

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Abstract: In this study, investigation on the essential oils and ethanol extracts of naturally grown and cultivated *Satureja macrantha* samples were reported. The essential oil, flavour and terpenoid-steroid-flavonoid contents of *S. macrantha* samples were determined by GC-MS and their phenolic contents by LC-MS/MS. Besides, the biological activities of the samples were investigated for their antioxidant, anti-Alzheimer, antimicrobial, cytotoxic, antityrosinase, antiurease, antielastase and anticollagenase properties. The phenolic content and antioxidant capacity of the cultivated sample were higher than those of the naturally grown sample. According to the GC-MS results, terpinene-4-ol (30.9%) and *p*-cymene (56.7%) were determined as the major components in the essential oils of the naturally grown and cultivated *S. macrantha*, respectively. The flavour analysis results showed that *cis*-sabinene hydrate (20.7%) and carvacrol (42.2%) were found to be the major components in the naturally grown and cultivated samples, respectively. While the naturally grown sample was rich in abietane diterpenoids (ferruginol (17.5 mg analyte/g extract) and sugiol (4.2 mg analyte/g extract)), these components were not detected in the cultivated sample. The rosmarinic acid content (0.20 and 24.87 mg analyte/g extract, respectively) of the cultivated sample was found to be significantly higher than that of the natural sample. The biological activities of the samples were determined to be changed in parallel with their chemical contents that are due to factors such as climatic conditions, and soil structure.

Keywords: *Satureja macrantha;* essential oil; flavour; terpenoid content; biological activities; spectroscopic analyses.

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1. Introduction

The importance of medicinal and aromatic plants in the world is increasing day by day. Medicinal and aromatic plants are widely used in traditional and alternative medicine in many developed countries [1]. Lamiaceae is one of the families that contain essential oil used in perfumery and medicine [2]. Lamiaceae family is represented by 236 genera and 6900-7200 species all over the world [3], and it is represented by 45 genera and 758 taxa in Turkey, 45% of them are endemic [4].

Satureja species (Lamiaceae family), called as "kekik", are traded in Turkey. It is represented by 16 species and 17 taxa, and its rate of the endemism is 31% [5]. *Satureja* species are popularly known with names such as ‘‘thyme, pointed thyme, sword thyme, oregano, black thyme, catli, firubi and bee thyme”. They are economically very important plants due to their medicinal properties, and their use as spice, tea and export product [6]. *Satureja* species are mainly used in the food industry, and they are known to have antibacterial, antifungal, insecticidal, analgesic, antimicrobial and antioxidant effects. The common feature of *Satureja* species is that they contain high levels of essential oils which contain carvacrol, thymol, *p*-cymene and *γ*-terpinene as main components [7].

S. macrantha is popularly used in the treatment of various diseases such as diarrhea, wound healing, gastrointestinal inflammation, upper respiratory tract and urinary tract infections, in traditional medicine. Additionally, its leaf part is used as food flavoring [8]. In the literature, there are several studies on its essential oil content and biological activities [8-11]. However, this is the first study on the chemical contents and biological activities of the ethanol extract of *S. macrantha*, and also on the cultivated *S. macrantha*. Considering the use of the species in traditional medicine and its economic potential, it is important to determine the chemical content analysis and biological activities of *S. macrantha*.

The aim of the current investigation was to compare the biological activities and chemical contents of the agriculturally cultivated and naturally grown *S. macrantha*, which has rich chemical content and high biological activity potential. In this context, the essential oil and ethanol extracts of naturally grown and cultivated samples of *S. macrantha* were prepared. The essential oil, flavour and terpenoid-steroid-flavonoid contents of *S. macrantha* were determined by GC-MS, and the phenolic content of the ethanol extracts by LC-MS/MS. Additionally, the biological activities of the samples; antioxidant (ABTS, DPPH, CUPRAC, Total Phenolic-Flavonoid), toxic-cytotoxic properties HDF (Human dermal fibroblast cell line), HT-29 (colon cancer cell line) and MCF-7 (breast cancer cell line), antimicrobial (disc diffusion), anti-Alzheimer (against acetyl- and butyryl-cholinesterase enzymes), antiurease, antityrosinase, antielastase and anticollagenase activities were investigated in detail.

2. Materials and Methods

2.1. Plant Material

Natural samples of *Satureja macrantha* C.A.Mey. were collected from Hakkâri province (Turkey) in October 2016 and identified by Mehmet Firat. Cultivated samples were obtained in October 2015 by Dr. Fethullah Tekin from Diyarbakir (Turkey) (see Table 1 and Figure S1). The seeds of the species were collected in 2013 and its agricultural cultivation studies were performed in 2014. The cultivated samples of *S. macrantha* were collected in the flowering period of the species in 2015. The collected samples were shadow-dried, and their chemical and biological analyses were carried out in the same period.

Table 1. Information about *S. macrantha*

Samples	Abbreviations	Yield (%)	Herbarium number	Collection time	Collection location
Natural S. macrantha ethanol extract	SM-N	3.4	VANF-33303	October-2016	Hakkari
Natural S. macrantha essential oil	SM-NE	0.8			
Cultivated S. macrantha ethanol extract	SM-C	3.7	VANF-32644	October-2015	Diyarbakır
Cultivated S. macrantha essential oil	SM-CE	0.7			

2.2. Agricultural Cultivation Conditions

Cultivation studies were carried out in the GAP International Agricultural Research and Training Center (GAPUTAEM) experimental area in Diyarbakır. Seeds of *S. macrantha* C.A.Mey. were used as material in the field trial. The seeds have been obtained from *S. macrantha* growing naturally in Hakkâri. In August 2013, seeds were collected from their natural environment. These seeds were transferred into a water container, and left overnight. The seeds that rose above the water were not used, and those that remained underwater were used.

For the seedbed, a mortar consisting of burnt sheep manure (1/3), forest soil (1/3) and river sand (1/3) (Particle size: 0.02-2.00 mm), which was finely sieved, was prepared. The mortar was filled into the perforated plastic tubes (10×25 cm) that is purchased from Naksan Plastic, Turkey . The seeds were shown at a depth of 3-4 cm in November, with 3-4 seeds in each tube. The seedbed was irrigated with a strainer until the seeds germinated. After the seedlings reached a height of 5-10 cm, they were transplanted into the field in early spring (after February-March). For this, pits of 30×30×30 cm were dug in the field. Then, 2 L of water was poured into each pit, the seedlings were placed in the pit, filled with soil and pressed. Since the first year of the plantation is considered as the year of establishment, the harvest was made in the second year.The trial site is in the basement area on the banks of the Dicle (Tigris) River and is about 609 meters above sea level and was established at 37°56'29.36"N (north latitude) and 40°15'16.07"E (east longitude). The region's climate belongs to the Mediterranean climate. Generally, summer is hot and drought; but winter is cold and rainfall. Long-year climatically findings showed that there were 454 mm total rainfall and the average temperature was 15.8°C every year (Meteorology Directorship's average of many years of Diyarbakır). The general soil characteristics are the alluvial soils belonging to the large soil group formed on the material transported by the Dicle (Tigris) river waters. The field trial was set in 4 plots and the (area of each plot = 3 m (length of each row) × 2.8 (0. 7 interval rows × 4 number of rows) m=8.4 m^2. There is 3 m space between the parcels. In the experiment, the distance between rows was set to 70 cm, the spacing between two plants on the same row was 20 cm. Harvesting was carried out during the full flowering period (June) after leaving one plant from the beginning and the end of each plot and one row from each side. The field was irrigated every 15 days after May, with an average of 8 to 10 flood irrigation in a growing season. In addition, surface irrigation was used as the irrigation method [12].

2.3. Sample Preparation

All of the samples used in the extraction process were shadow-dried. To prepare the extracts for the biological activity and chemical content analyses, the aerial parts of *S. macrantha* were macerated with ethanol (50 mL, 3 × 8 h). Ten grams of each sample were extracted with 99% ethanol (1/5 w/v). Crude extracts were obtained after filtration and solvent evaporation processes. Stock solutions at a concentration of 4000 µg/mL were prepared from the dry extracts. New stock solutions were prepared at different concentrations (such as 2000 and 1000 µg/mL) using the dilution method according to the process applied from these stock solutions. The yields and abbreviations of the studied species were given in Table 1. The extraction yields were calculated on the total amount of dry extract and the amount of dry plant (10 g).

2.4. Instrumental Methods

2.4.1. GC-MS Analysis for Essential Oil and Flavour Content

In this study, the essential oil and flavour contents of the samples were analysed using Agilent brand 7890A Model GC/FID gas chromatograph and Agilent brand 5977B model mass spectrometer (MS). Components of essential oils (from shadow-dried aerial parts of the species) obtained by hydrodistillation method using Clevenger apparatus were determined by GC-MS/FID [13,14]. HP-5MS UI capillary column (30 m–0.25 mm and 0.25 µm film thickness) was used. The injector temperature was adjusted to 250°C. Split flow and split ratios were 25 mL/min and 25:1, respectively. The injection volume was 1.0 µL Mass spectra were detected at 70 eV and the mass range was *m/z* 40–500 amu. GC oven temperature started at 50°C and held at this temperature for 4 min and then ramped to 240°C by a rate of

3°C per minute and held at final temperature for 5 min. Helium gas as a carrier has a flow rate of 1 mL/min. The MS and FID detectors' temperatures were 230°C and 300°C, respectively. Data were collected from both MS and FID detectors at the same time with the help of a separator installed at the exit of the column. While qualitative identification of the components was completed with the MS data, quantitative and percentage results were made with the data collected from the FID detector. For this reason, all parameters, except for the temperatures of MS and FID detectors, were the same in the analyses.

For flavour analysis, one gram of the dried plant material was directly placed into a 20 mL headspace vial and was incubated for 15 min at 40°C. Compounds in the flavour were extracted using solid-phase microextraction (SPME) fibers made from StableFlex carboxen-polydimethylsiloxane (CAR-PDMS) in 85µm, which were purchased from Supelco (Bellefonte, PA, USA) [13, 14]. The chromatographic conditions both for flavour and essential oil analyses were the same.

Alkanes (C7-C40) were used as reference points in the calculation of Retention Indices (RI) by the same conditions. The compounds were identified by comparing their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as data from the published literature.

2.4.2. GC-MS Analysis for Terpenoid-Steroid-Flavonoid Content

Analysis was performed using Agilent 7890A Model GC/FID gas chromatograph coupled to an Agilent 5977B model mass spectrometer (MS), equipped with a split injection port. Chromatographic separation was performed using an apolar HP-5MS column (30 m × 0.25 mm × 0.25 mm film thickness). Helium was used as the carrier gas with a constant flow (1 mL/min). GC oven temperature was started at 150°C and then ramped to 300°C by a rate of 5°C per minute and held at final temperature for 20 min. Samples were injected in splitless mode. The injection volume was 2.0 µL. Injection block and transfer line temperatures were held at 300°C. The mass spectrometer was operated in the electron impact (EI) mode with ionization energy of 70eV. The temperature of the ion source was 230°C. The mass spectrometer (MS) data were obtained in full scan mode and the scanning mass range *m/z* was set to 100-600 atomic mass units (amu). The compounds were identified by comparing their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as data from the literature. For the quantification, the reconstructed ion chromatograms were used, where usually two fragment ions with greater intensities were selected. Molecular ions and two specific fragment ions with relative intensities of investigated compounds are presented in Table S1. Terpenoid-steroid-flavonoid contents were investigated by using the method developed by Bakir et al.[13].

2.4.3. LC-MS/MS Analysis for Phenolic Content

The phytochemical content of the extracts was quantitatively identified by a previously developed and validated LC-MS/MS method [15, 16]. In this method, a Shimadzu-Nexera model ultrahigh performance liquid chromatography (UHPLC) coupled with a tandem mass spectrometer was used to accomplish a quantitative evaluation of 53 phytochemicals. The reversed-phase UHPLC was equipped with an auto sampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and a degasser (DGU- 20A3R model). The chromatographic conditions were optimized in order to achieve optimum separation for 53 phytochemicals and overcome the suppression effects. Consequently, the chromatographic separation was performed on a reversed-phase Agilent Poroshell 120 EC-C18 model (150 mm × 2.1 mm, 2.7 µm) analytical column. The column temperature was set to 40°C. The elution gradient was composed of eluent A (water + 5 mM ammonium formate + 0.1% formic acid) and eluent B (methanol + 5 mM ammonium formate + 0.1% formic acid). The following gradient elution profile was used: 20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min). Furthermore, the solvent flow rate and injection volume were settled as 0.5 mL/min and 5 µL, respectively.

The mass spectrometric detection was carried out using a Shimadzu LCMS-8040 model tandem mass spectrometer equipped with an electrospray ionization (ESI) source operating in both negative and positive ionization modes. LC-ESI-MS/MS data were acquired and processed by LabSolutions software (Shimadzu). The MRM (multiple reaction monitoring) mode was used for the quantification of the

phytochemicals. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on the screening of specified precursor phytochemical-to-fragment ion transitions. The collision energies (CE) were optimized in order to generate optimal photochemical fragmentation and maximal transmission of the desired product ions. The MS operating conditions were applied as: drying gas (N_2) flow, 15 L/min; nebulizing gas (N_2) flow, 3 L/min; DL temperature, 250°C; heat block temperature, 400°C, and interface temperature, 350°C.

Analytical validation parameters of the utilized method comprises inter-day (values range between 0.69 and 2.51) and intra-day precision (0.51-2.13) (repeatability), inter-day (0,9922-1,0072) and intra-day (0.9946-1.0083) accuracy (recovery), linearity, limits of detection (1.3 µg/L-214.2 µg/L) and quantification (2.0 µg/L-365.9 µg/L) (LOD/LOQ), and relative standard uncertainty (0.0083-0.0396) (U% at 95% confidence level (k=2)). Table S2 shows the parameters related to the LC-MS/MS method validation studies.

2.5. Biological Activities

2.5.1. Total Phenolic and Flavonoid Contents

The total phenolic [17] and flavonoid [18] contents of the studied samples were calculated as equivalent to pyrocatechol and quercetin, respectively. The following equations were used for the total phenolic and flavonoid contents of the extracts:

Absorbance = 0.0370 + 0.0551 quercetin (µg) (r^2= 0.9965)

Absorbance = 0.0408 + 0.0453 pyrocatechol (µg) (r^2= 0.9961)

2.5.2. Antioxidant Activities

ABTS cation radical [19], DPPH free radical [20] and CUPRAC (Copper (II) ion reducing antioxidant capacity) [21] methods were used to determine the antioxidant properties of the samples. In these three antioxidant test methods, α-tocopherol and BHT (Butylated hydroxytoluene) were used as standards. IC_{50} calculations were performed by using the samples with 100, 50, 25, 10 and 1 µg/mL concentrations.

2.5.3. Cytotoxic Activities

The MTT method for using the toxic and cytotoxic effects of the samples was developed by Mojarraba et al. [22]. MTT Assay with minor modifications was used for the determination of the toxic and cytotoxic effects of the samples. The toxic effects of the samples were studied against HDF (Human dermal fibroblast cell line) cell line, while their cytotoxic effects were studied against HT-29 (colon cancer cell line), and MCF-7 (breast cancer cell line) cell lines [23]. Ethanol used as the extraction solvent was utilized with the same volume as the control sample.

2.5.4. Anticholinesterase Activity

Spectrophotometric method based on the inhibitory effects of AChE and BChE developed by Ellman et al. [24] was used to determine the anticholinesterase activity. Galanthamine was used as the standard substance in this test method. In all enzyme inhibition methods, inhibition% values of the samples were calculated at a concentration of 50 µg/mL. Besides, the same volume of ethanol was used in the enzyme inhibition methods.

Antiurease Activity

Method developed by Hina et al. [25] was used to determine the urease inhibition activities of the samples. Thiourea was used as the reference material for the urease activity test method.

2.5.5. Anti-aging Activity

Tyrosinase [26], elastase [27], and collagenase [28], inhibitory activities were used to determine the anti-aging potentials of the samples. In these three anti-aging test methods, kojic and oleanolic acids, and epicatechin gallate compounds were used as standards, respectively.

The following equation was used to calculate the acetyl-cholinesterase (AChE), butyryl-cholinesterase (BChE), urease, tyrosinase, elastase, and collagenase enzyme inhibition.

Inhibition (%) = 100-(OD test well/OD control) × 100.

2.5.6. Antimicrobial Activity

Antimicrobial activity was determined against Gram-negative (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853), Gram-positive (*Staphylococcus aureus* 25923, *Streptococcus pyogenes* ATCC19615) bacteria and yeast (*Candida albicans* ATCC10231) by disc diffusion method. Ampicillin and fluconazole were used as a positive control for the bacteria and the yeast, respectively. All tests were done in triplicate.

2.6. Statistical Analysis

The results of the activity assays were shown as means ± standard error meaning. The results were evaluated using an unpaired *t*-test and one-way analysis of variance ANOVA. The differences were regarded as statistically significant at $p < 0.05$.

3. **Results and Discussion**

3.1. Essential oil, Flavour and Terpenoid-Steroid-Flavonoid Contents

Q8The essential oil and flavour contents of the naturally grown and cultivated samples were determined by GC-MS. According to the GC-MS results, the essential oil of the naturally grown sample had 23 components (95.6%) and that of the cultivated sample included 21 constituents (98.0%) (Table 2, Figure S2). While terpinene-4-ol (30.9%), *p*-cymene (14.9%), linalool (9.1%), borneol (8.1%), and spathulenol (4.3%) were determined as the major components in the naturally grown sample, and *p*-cymene (56.7%), carvacrol (11.0 %), thymol (7.8%), *γ*-terpinene (3.7 %), and borneol (3.5%) in the cultivated sample. When the essential oil contents of the naturally grown and cultivated samples were evaluated in detail, the major components of the samples were found to be very different. Although terpinene-4-ol, linalool and borneol were detected in the naturally grown sample (30.9%, 9.1% and 8.1%, respectively), it is quite striking that they were found in the cultivated sample (0.6%, 1.1% and 3.5%, respectively). While carvacrol (11.0%) and thymol (7.8%) were detected in the cultivated sample, carvacrol (3.0%) was determined in the naturally grown sample, but thymol was not found. Besides, the amount of *p*-cymene in the naturally grown and cultivated samples (14.9%, 56.7%, respectively) was not similar.

The previous studies on *S. macrantha* essential oils indicated that the major components vary considerably according to the locality where they were collected [8-11]. In the study conducted by Aghbash *et al.*[8], carvacrol (42.7-48.2 %), thymol (0.2-16.5%) and *p*-cymene (10.1-14.7%) were determined as the main constituents, and similarly, carvacrol (64.4%) and *p*-cymene were found in another study [9]. However, in a study reported by Sefidkon and Jamzad [10], *p*-cymene (25.8%), limonene (16.3%) and thymol (8.1%) were identified as major components. In our study, the essential oil compositions of the naturally grown and cultivated samples of *S. macrantha* varied greatly. In the light of the data both in this study and the literature, it can be said that the main components of the essential oil of *S. macrantha* were highly influenced by variables such as climate conditions and soil structure in which the species grows.

Table 2. Chemical compositions of the essential oil and flavour of *S. macrantha samples*

No	RI[a]	Constituents[b]	Essential oil[c]		Flavour[c]		95% RI range in literature[f]
			SM-NE[d]	SM-CE[e]	SM-NA[d]	SM-CA[e]	
1	927	*α*-Thujene	-	0.9	-	0.3	905–938
2	935	*α*-Pinene	0.2	1.2	1.3	0.3	912–948
3	951	Camphene	0.4	1.5	1.0	0.2	928–964
4	967	Nonane, 3- methyl	-	-	-	0.2	-
5	975	Sabinene	-	-	1.0	-	953–982
6	980	*β*-Pinene	-	0.3	-	-	962–989
7	991	*β*-Myrcene	-	1.2	1.5	1.2	975–998
8	995	Mesitylene	-	-	-	0.4	-
9	1000	Decane	-	-	-	1.1	-
10	1007	*α*-Phellandrene	0.2		0.4	-	989–1014
11	1018	*α*-Terpinene	2.0	0.5	2.4	1.1	1003–1026
12	1022	Decane, 4-methyl	-	-	-	0.2	-
13	1026	*p*-Cymene	14.9	56.7	15.3	10.3	1010–1034
14	1030	D-Limonene	-	-	-	0.3	1014–1040
15	1031	*β*-Phellandrene	-	0.5	2.6	-	1005–1043
16	1037	*β*-*trans*-Ocimene	-	-	0.7	4.1	1022–1049
17	1047	*β*-*cis*-Ocimene	-	0.9	0.7	4.6	1035–1058
18	1060	*γ*-Terpinene	4.3	3.7	4.6	11.7	1043–1073
19	1068	Terpineol, *cis*-beta	1.6	-	-	-	1132–1150
20	1069	*cis*-Sabinene hydrate	-	-	20.7	0.3	1044–1076
21	1074	*cis*-Linaloloxide	0.7	-	-	-	1057–1087
22	1091	Terpinolene	2.1	-	1.8	-	1070–1098
23	1100	Undecane	-	-	-	0.7	-
24	1101	Linalool	9.1	1.1	16.1	-	1086–1112
25	1141	*p*-Menth-2-en-1-ol	3.0	-	-	-	1115–1138
26	1148	Camphor	0.9	-	0.1	-	1126–1159
27	1157	Menthone	-	0.6	-	-	1125–1174
28	1169	Borneol	8.1	3.5	1.4	2.0	1152–1182
29	1182	Terpinene-4-ol	30.9	0.6	13.8	-	1154–1189
30	1187	*p*-Cymene-8-ol	2.9	0.6	-	-	1178–1191
31	1194	*α*-Terpineol	3.5	-	4.0	-	1163–1207
32	1243	Pulegone	-	2.0	-	0.3	1210–1253
33	1256	Thymoquinone	-	-	-	0.8	-
34	1258	Piperitone	-	0.4	-	-	1245–1266
35	1291	Thymol	-	7.8	-	13.0	1272–1304
36	1294	Dihydro edulan	1.1	-	-	-	-
37	1301	Carvacrol	3.0	11.0	1.8	42.2	1284–1314
38	1383	Copaene	-	-	0.2	-	1360–1393
39	1392	*β*-Bourbonene	0.7	-	0.8	-	1347–1412
40	1428	Caryophyllene	0.3	-	0.8	1.1	1396–1444
41	1436	*β*-Cubebene	-	-	0.1	-	1360–1394
42	1448	Aromadendrene	-	-	0.2	-	1419–1464
43	1470	Alloaromadendrene	-	-	0.1	0.4	1442–1478
44	1489	Germacrene D	-	-	0.2	-	1463–1499
45	1505	Bicyclogermacrene	-	-	0.5	-	1472–1502
46	1530	*δ*-Cadinene	-	-	0.1	-	1505–1551
47	1587	Spathulenol	4.3	1.6	-	0.7	1554–1587
48	1594	Caryophyllene oxide	0.9	1.4	-	0.7	1560–1596
49	1602	Viridiflorol	0.5	-	-	-	1569–1604
Total identified (%)			95.6	98.0	94.2	98.2	

a) Kovats index on HP–5MS fused silica column, **b)** A nonpolar Agilent HP-5MS fused silica column, **c)** Percentage concentration, **Co-GC:** Co-injection with authentic compounds, **RI:** Retention Index literature comparison, **d) SM-NE** and **SM-NA**; Natural *S. macrantha* essential oil and flavour, **e) SM-CE** and **SM-CA**: Cultivated *S. macrantha* essential oil and flavour, **f)** Retention indices for most frequently (95%) reported essential oil compounds [29].

According to the flavour analysis results of the samples, 94.2% of the flavour content of the naturally grown samples and 28 components of them were determined, while 98.2 % of the flavour content of the cultivated samples and 25 components of them were defined (Table 2 and Figure S2). *cis*-Sabinene hydrate (20.7%), linalool (16.1%), *p*-cymene (15.3%), terpinene-4-ol (13.8%) and γ-terpinene (4.6%) were detected as the major components in the naturally grown sample, carvacrol (42.2%), thymol (13.0%), γ-terpinene (11.7%), *p*-cymene (10.3%) and *β-cis*-ocimene (4.6%) were identified as the main compounds of the cultivated sample. In terms of flavour contents, the naturally grown sample was detected to be richer in the number of components than those of the cultivated sample. It was also observed that the naturally grown and cultivated samples differed considerably in terms of the first 5 major components and their percentages. Although there was especially *cis*-sabinene hydrate (20.7%), linalool (16.1%) and terpinene-4-ol (13.8%) in the naturally grown sample, *cis*-sabinene hydrate (0.3%) was only found in the cultivated sample. Carvacrol and thymol (42.2 and 13.0%, respectively) were detected in the cultivated sample, while in the naturally grown sample, only carvacrol (1.8%) was detected, and thymol was not found.

There is no study in the literature about the flavour content of the cultivated and naturally grown samples of *S. macrantha*. The current research exhibited that the flavour content, as well as the essential oil content, was highly influenced by variables such as the climatic conditions and soil structure in which the species grows. As a result, the natural and cultivated samples of *S. macrantha* differed considerably in terms of both essential oil and flavour contents.

As shown in Table S1 and Figure S3, the terpenoid-steroid-flavonoid contents of the natural samples were rich in terms of component diversity. While ferruginol (17.5 mg/g extract), sugiol (4.2 mg/g), salvigenin (7.7 mg/g), and *β*-sitosterol (18.7 mg/g) were detected in the naturally grown sample, *β*-sitosterol (3.1 mg/g) was the compound only detected in the cultivated sample. As a result, the abietane diterpenoids in the cultivated species were under the detectability limit of the method. There are no previous quantitative studies on the terpenoid-steroid-flavonoid contents of *S. macrantha* in the literature.

Although there are many studies on a small number of *Satureja* species, there are few researches on their secondary metabolites. In the previous studies, naringenin, eriodictyol, and luteolin were determined in *S. obovata* [30]; eriodictyol, luteolin, ursolic and oleanolic acids were found in *S. parvifolia* [31]; keshonin, saturejin (3′-(2,5-dihydroxy-*p*-cymene) 5,7,4′-trihydroxyflavone), ponciretin, 5,6-dihydroxy-3′,4′,7′-trimethoxyflavone, 5,6-dihydroxy-4′,7-dimethoxyflavone, and acacetin were identified in *S. khuzistanica* [32]; 5,7,3',5'-tetrahydroxy flavanone and 5,4'-dihydroxy-3'-methoxyflavanone-7-(6"-O-*α*-L-rhamnopyranosyl) -glucopyranoside, nubigenol, thymol, carvacrol, *β*-sitosterol, ursolic and oleanolic acids were determined in *S. spicigera* [33]. This study was the first research to demonstrate that the *Satureja* genus contain the abietane diterpenes, ferruginol and sugiol, and the flavonoid, salvigenin.

3.2. LC-MS/MS Measurements

In this study, the phytochemical contents of ethanol extracts prepared from the naturally grown and cultivated samples of *S. macrantha* were determined by LC-MS/MS (Table 3 and Figure 1). The quantitative results of the compounds, and their parent and fragment ions in the LC-MS/MS method were given in Table 3. As a result, it was determined that the cultivated sample contained quite high amounts of phenolic compounds compared to those of the naturally grown sample.

Especially, it was determined that quinic acid (1.06 and 13.93 mg analyte/g extract, respectively), vanillic acid (1.60 mg/g and ND), caffeic acid (0.30 and 1.97 mg/g), vanillin (0.67 mg/g and ND), hesperidin (0.30 and 4.25 mg/g), rosmarinic acid (0.20 and 24.87 mg/g), astragalin (0.06 and 2.34 mg/g), naringenin (0.10 and 5.76 mg/g) and luteolin (0.04 and 1.43 mg/g) contents of the natural and cultivated samples were quite different. In addition, while chlorogenic acid, coumarin, salicylic acid, rutin, isoquercitrin, quercetin, kaempferol and chrysin were detected in the cultivated sample, these compounds were not determined in the natural sample. While vanillic acid and vanillin were detected in the natural sample, they were not found in the cultivated sample. These results indicated that the climatic conditions and soil structure in which the species was grown could cause changes on its phenolic content.

Table 3. Identification and quantification of phytochemicals in the extracts of *S. macrantha* by LC-MS/MS

No	Analytes[a]	RT[b]	M.I. (m/z)[c]	F.I. (m/z)[d]	Ion. mode	*r2*[e]	LOD/LOQ (µg/L)[f]	*U*[g]	SM-N	SM-C
									Quantification (mg analyte/g extract)[h]	
1	Quinic acid	3.0	190.8	93.0	Neg	0.996	25.7/33.3	0.0372	1.06	13.93
2	Fumaric aid	3.9	115.2	40.9	Neg	0.995	135.7/167.9	0.0091	0.73	0.77
6	Protocatechuic acid	6.8	152.8	108.0	Neg	0.957	21.9/38.6	0.0350	0.38	0.45
9	Chlorogenic acid	8.4	353.0	85.0	Neg	0.995	13.1/17.6	0.0213	ND	0.04
10	Protocatechuic aldehyde	8.5	137.2	92.0	Neg	0.996	15.4/22.2	0.0396	0.11	0.25
16	Vanillic acid	11.8	166.8	108.0	Neg	0.999	141.9/164.9	0.0145	1.60	ND
17	Caffeic acid	12.1	179.0	134.0	Neg	0.999	7.7/9.5	0.0152	0.30	1.97
19	Vanillin	13.9	153.1	125.0	Poz	0.996	24.5/30.4	0.0122	0.67	ND
24	*p*-Coumaric acid	17.8	163.0	93.0	Neg	0.999	25.9/34.9	0.0194	0.03	0.09
28	Coumarin	20.9	146.9	103.1	Poz	0.999	214.2/247.3	0.0383	ND	0.01
29	Salicylic acid	21.8	137.2	65.0	Neg	0.999	6.0/8.3	0.0158	ND	0.04
30	Cynaroside	23.7	447.0	284.0	Neg	0.997	12.1/16.0	0.0366	0.02	0.41
33	Rutin	25.6	608.9	301.0	Neg	0.999	15.7/22.7	0.0247	ND	0.05
34	Isoquercitrin	25.6	463.0	271.0	Neg	0.998	8.7/13.5	0.0220	ND	0.07
35	Hesperidin	25.8	611.2	449.0	Poz	0.999	19.0/26.0	0.0335	0.30	4.25
38	Rosmarinic acid	26.6	359.0	197.0	Neg	0.999	16.2/21.2	0.0130	0.20	24.87
40	Cosmosiin	28.2	431.0	269.0	Neg	0.998	6.3/9.2	0.0083	0.04	0.18
42	Astragalin	30.4	447.0	255.0	Neg	0.999	6.6/8.2	0.0114	0.06	2.34
47	Quercetin	35.7	301.0	272.9	Neg	0.999	15.5/19.0	0.0175	ND	0.10
48	Naringenin	35.9	270.9	119.0	Neg	0.999	2.6/3.9	0.0392	0.10	5.76
49	Hesperetin	36.7	301.0	136.0/286.0	Neg	0.999	7.1/9.1	0.0321	0.03	0.14
50	Luteolin	36.7	284.8	151.0/175.0	Neg	0.999	2.6/4.1	0.0313	0.04	1.43
52	Kaempferol	37.9	285.0	239.0	Neg	0.999	10.2/15.4	0.0212	ND	0.02
53	Apigenin	38.2	268.8	151.0/149.0	Neg	0.998	1.3/2.0	0.0178	0.30	0.40
55	Chrysin	40.5	252.8	145.0/119.0	Neg	0.999	1.5/2.8	0.0323	ND	0.01
56	Acacetin	40.7	283.0	239.0	Neg	0.997	1.5/2.5	0.0363	0.46	0.12

[a]Numbers on the far left raw indicate the standard phytochemical compounds. Undetected components are given as follows by the applied method:3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 7: Catechin. 8: Gentisic acid. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-Dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 18: Syringic acid. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 26: Ferulic acid. 27: Sinapic acid. 31: Miquelianin. 32: Rutin. 36: o-Coumaric acid. 37: Genistin. 39: Ellagic acid. 41: Quercitrin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 51: Genistein. 54: Amentoflavone.[b]R.T.: Retention time, [c]MI (*m/z):* Molecular ions of the standard analytes (m/z ratio), [d]FI (*m/z):* Fragment ions[e]r^2: Coefficient of determination, [f]*LOD*/*LOQ* (µg/L): Limit of detection/quantification, [g]*U* (%): percent relative uncertainty at 95% confidence level ($k = 2$). The abbreviations for natural *S. macrantha* ethanol extracts is SM-N and the abbreviation for ethanol extract of *S. macrantha* cultivated sample is expressed as SM-C. [h]Values in mg/g (w/w) of plant ethanol extract, ND: not detected

Many researches were conducted on the genus *Satureja*, but there was only a study investigating the phenolic contents of the species. In that study conducted by Shekarchi et al. [34], many species belonging to the Lamiaceae family were evaluated only in terms of their rosmarinic acid content. Therefore, this study is the most comprehensive work on the phenolic content of this species. There are few works in the literature on other species belonging to the genus *Satureja* [35, 36].

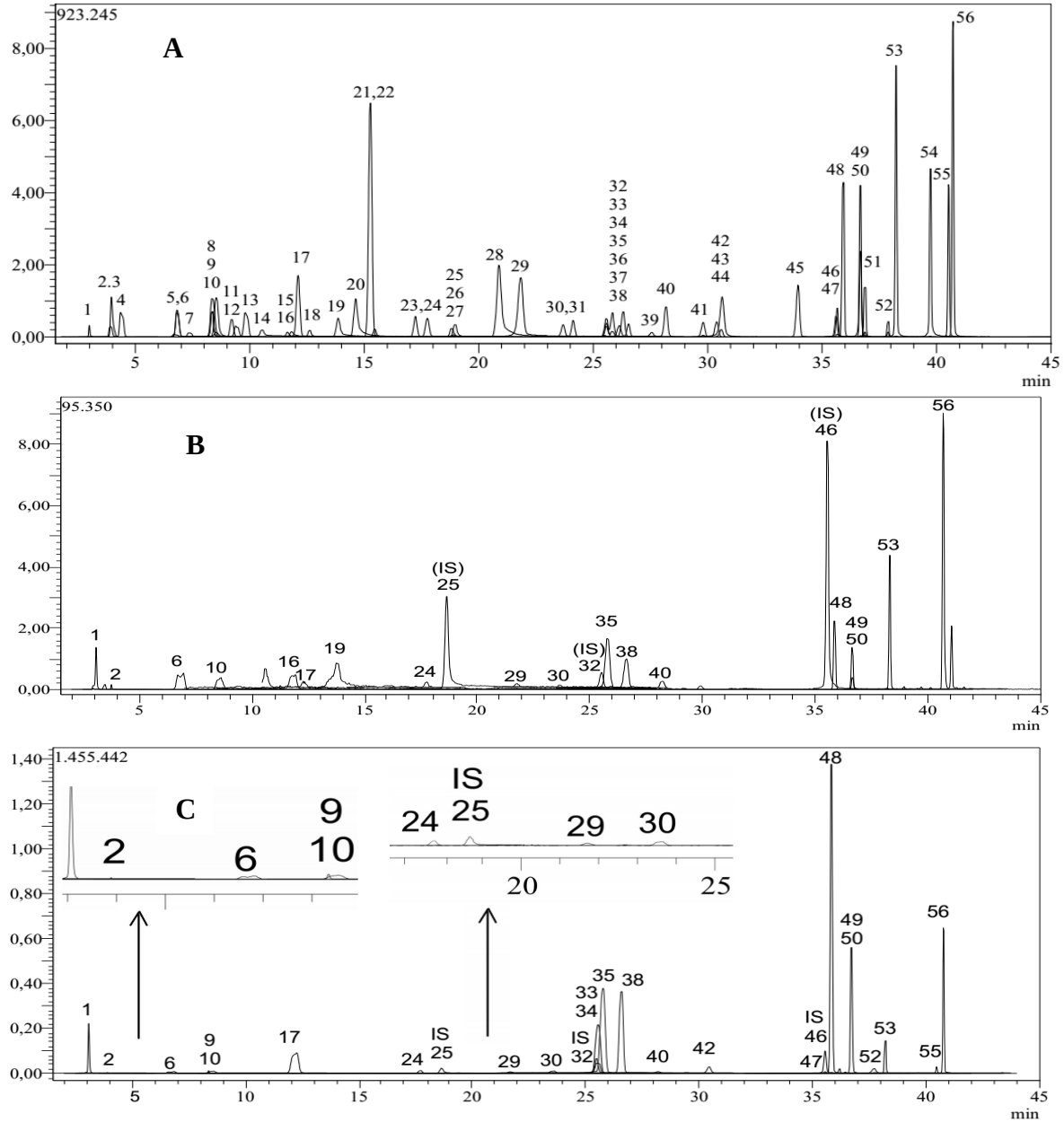

Figure 1. LC-MS/MS chromatograms **A:**TIC chromatogram of standard chemicals analyzed by LC-MS/MS method.**B:**LC-MS/MS chromatogram of the natural *S. macrantha*, **C:**LC-MS/MS chromatogram of the cultivated *S. macrantha*.IS: Internal Standard (Ferulic acid-D3: 25, Rutin-D3: 32 and Quercetin-D3: 46)

3.3. Biological Activities

In this study, the total phenolic and flavonoid contents of the ethanolic extracts of the naturally grown and cultivated *S. macrantha* were determined in terms of pyrocatechol and quercetin equivalents (Table 4). It was observed that both naturally grown and cultivated samples possessed moderate amount of phenolics. The cultivated sample was richer (52.1± 1.2 µg PEs/mg extract) than the naturally grown sample (16.3 ± 1.4 µg PEs/mg extract) in terms of phenolic content. However, the flavonoid contents of both naturally grown and cultivated samples were found to be very low. In parallel with the LC-MS/MS results, the cultivated sample of the species was rich in total phenolics and flavonoids.

The antioxidant activities of the ethanol extracts and essential oils of the species were determined by DPPH, ABTS and CUPRAC methods (Table 4). It was determined that the cultivated sample had higher antioxidant potential than the naturally grown sample in three used methods, parallel to the total phenolic and LC-MS/MS results. It was also observed that the essential oil of the naturally grown sample showed low activity in the ABTS method, and was inactive in the other two methods. Additionally, the essential oil of the cultivated sample exhibited good effect in all three methods.

According to the ABTS results, it was determined that the cultivated sample (IC_{50}: 8.7±0.1 µg/mL) showed higher antioxidant activity than the α-TOC (IC_{50}: 9.5±0.2 µg/mL) which was used as a standard, while the naturally grown sample (IC_{50}: 53.4±0.4 µg/mL) possessed low activity compared to that of the standard. The DPPH results indicated that the cultured sample had good effect (IC_{50}: 69.5±1.1 µg/mL), while the natural sample showed moderate activity (IC_{50}: 201.2±1.8 µg/mL).

The CUPRAC results showed that the ethanol extract of the cultivated sample exhibited the highest activity ($A_{0.5}$: 35.4 ± 1.6 µg/mL) among the tested samples. Both the essential oil and the ethanolic extract of the cultivated samples possessed higher antioxidant property than that of the naturally grown samples of the species. This high antioxidant property of the cultivated samples was in parallel with both total phenolic and LC-MS/MS results. In the results of the previous studies, there has a lot of information about the antioxidant properties of *S. macrantha* extracts, especially compounds such as rosmarinic acid, caffeic acid, hesperidin, astragalin, naringenin and luteolin were determined as very high antioxidative compounds [37, 38]. Therefore, the high antioxidant potential of the culture samples could be related with these phenolic compounds.

A literature survey indicated that Aghbash reported the moderate antioxidant activity of *S. macrantha* essential oil [8]. However, there were many studies in the literature regarding the antioxidant properties of other *Satureja* species [36, 39].

Toxic-cytotoxic effects of the ethanol extracts and essential oils of *S. macrantha* naturally grown and cultivated samples were determined by the MTT method against healthy cell lines (HDF) and cancer cell lines (HT-29, MCF-7). The viability % values of the samples were determined at 200 µg/mL. It was determined that the samples were generally not toxic to healthy cell lines, and only the ethanol extract of the naturally grown sample and the essential oil of the cultivated sample showed low toxicity (Vitality%: 73.7 ± 0.2 and 75.5 ± 1.2 respectively), the ethanol extract of the cultivated samples did not have cytotoxic effects against HT-29 and MCF-7 cell lines. However, the ethanol extract of the naturally grown sample and the essential oil of the cultivated sample exhibited moderate activity against the cancerous cell line HT-29 (Viability%: 59.9 ± 0.4 and 46.0 ± 1.1, respectively), and these two extracts indicated high activity against MCF-7 cancer cell line (Viability%: 24.5 ± 0.1 and 26.1 ± 1.3, respectively).

Son et al. [40] found that ferruginol, a diterpene containing phenol ring, was effective against breast, colon, and lung cancers. In this study, the activity of the naturally grown sample against both colon (HT-29) and breast (MCF-7) cancer cell lines may be related to its ferruginol content. There is no study on the cytotoxicity of this species in the literature. However, many studies showed that other *Satureja* species had cytotoxic potential [41-43].

Anticholinesterase activities of the extracts were determined against acetyl- and butyryl-cholinesterase enzymes (Table 4). It was determined that the extracts of both naturally grown and cultivated samples were generally showed low acetylcholinesterase enzyme inhibitory activity but high activity against butyrylcholinesterase enzyme. The ethanol extract of the naturally grown sample indicated low activity (Inhibition%: 13.4 ± 0.1) against the enzyme acetylcholinesterase, while it showed very high effect (Inhibition%: 82.0 ± 1.8) against the butyrylcholinesterase enzyme. Likewise, the ethanol extract of the cultivated sample showed low activity (Inhibition%: 18.2 ± 0.5) against the acetylcholinesterase enzyme, while it was observed moderate activity against the butyrylcholinesterase enzyme (Inhibition%: 65.8 ± 1.3). There is no study on the anticholinesterase activity of this species in the literature.

There were studies on the anticholinesterase capacity of the essential oils of other *Satureja* species in the literature [44-45]. The results of the previous studies and the current work indicated that *Satureja* species had anticholinesterase enzyme inhibitory potential.

Antiurease, antityrosinase, antielastase and anticollagenase enzyme activities of the ethanol extracts and essential oils were determined. According to the antiurease activity, it was determined that the ethanol extracts and essential oil of the naturally grown samples had no activity, whereas the ethanol extract of the cultivated sample showed moderate inhibition (Inhibition%: 34.8 ± 1.0). According to the

antityrosinase activity results, there was a reverse situation and it was determined that the cultivated samples did not show inhibitory activity, whereas the ethanol extract of the naturally grown sample exhibited moderate activity. The essential oils of the two samples had no antielastase and anticollagenase activity. The ethanol extract of the naturally grown sample exhibited high antielastase and anticollagenase capacity compared to those of the cultivated samples. In particular, the ethanol extract (Inhibition%: 30.2 ± 0.5) of the naturally grown sample possessed antielastase activity close to that of oleanolic acid (Inhibition%: 39.6 ± 0.3), which is used as the reference.

Table 4. Antioxidant activities with total phenolic-flavonoid contents and cholinesterase, urease, tyrosinase, elastase and collagenase enzymes inhibitory effects of *S. macrantha* extracts and essential oils

			Antioxidant activity[1]		
			IC_{50} (µg/mL)		**$A_{0.5}$(µg/mL)**
Samples	**Phenolic content (µg PEs/mg extract)[2]**	**Flavonoid content (µg QEs/mg extract)[3]**	**ABTS**	**DPPH**	**CUPRAC**
SM-N	16.3±1.4[a]	1.4 ± 0.2[a]	53.4±0.4[a]	201.2±1.8[a]	75.8±2.0[a]
SM-NE	-	-	49.1±1.0[b]	NA[b]	NA[b]
SM-C	52.1±1.2[b]	9.7±0.8[b]	8.7±0.1[c]	69.5±1.1[c]	35.4±1.6[c]
SM-CE	-	-	35.4±0.3[d]	112.5±2.5[d]	68.4±1.7[d]
α-TOC[4]	-	-	9.5±0.2[e]	17.2±0.7[e]	14.5±0.1[e]
BHT[4]	-	-	10.4±0.2[f]	60.2±1.4[f]	7.7±0.0[f]

	Enzyme activity (Inhibition(%), at 50 µg/mL)[1]					
Samples	**AChE**	**BChE**	**Urease**	**Tyrosinase**	**Elastase**	**Collagenase**
SM-N	13.4±0.1[a]	82.0±1.8[a]	NA[a]	26.8±0.3[a]	30.2±0.5[a]	21.0±0.1[a]
SM-NE	15.8±0.9[b]	61.8±1.0[b]	NA[a]	NA[b]	NA[b]	NA[b]
SM-C	18.2±0.5[c]	65.8±1.3[c]	34.8±1.0[b]	NA[b]	11.9±0.2[c]	12.1±0.2[c]
SM-CE	9.1±0.6[d]	54.1±0.6[d]	NA[a]	NA[b]	NA[b]	NA[b]
Galanthamine[4]	89.5±1.4[e]	79.7±0.5[e]	-	-	-	-
Thiourea[4]	-	-	95.7±1.7[c]	-	-	-
Kojic acid[4]	-	-	-	93.6± 1.2[c]	-	-
Oleanolic acid[4]	-	-	-	-	39.6±0.3[d]	-
Epicatechin gallate[4]	-	-	-	-	-	78.7±0.8[d]

The natural *S. macrantha* ethanol extract and essential oil: SM-N and SM-NE. The cultivated *S. macrantha* ethanol extract and essential oil: SM-C and SM-CE.

[1]Values represent averages ± standard deviations for triplicate experiments and values were calculated according to negative control, Values with different letters in the same column were significantly different ($p< 0.05$)

[2]PEs, pyrocatechol equivalents (y=0.0408 + 0.0383 pyrocatechol (µg) (r^2= 0.9951)

[3]QEs, quercetin equivalents (y=0.0355 + 0.0673 quercetin (µg) (r^2= 0.9975)

[4]Standard compound

[NA]Not active

Table 4. Antioxidant activities with total phenolic-flavonoid contents and cholinesterase, urease, tyrosinase, elastase and collagenase enzymes inhibitory effects of *S. macrantha* extracts and essential oils

			Antioxidant activity[1]			**Enzyme activity (50 µg/mL)[1]**					
			IC_{50} (µg/mL)		**$A_{0.5}$(µg/mL)**	**Inhibition(%)**					
Samples	**Phenolic content (µg PEs/mg extract)[2]**	**Flavonoid content (µg QEs/mg extract)[3]**	**ABTS**	**DPPH**	**CUPRAC**	**AChE**	**BChE**	**Urease**	**Tyrosinase**	**Elastase**	**Collagenase**
SM-N	16.3±1.4[a]	1.4 ± 0.2[a]	53.4±0.4[a]	201.2±1.8[a]	75.8±2.0[a]	13.4±0.1[a]	82.0±1.8[a]	NA[a]	26.8±0.3[a]	30.2±0.5[a]	21.0±0.1[a]
SM-NE	-	-	49.1±1.0[b]	NA[b]	NA[b]	15.8±0.9[b]	61.8±1.0[b]	NA[a]	NA[b]	NA[b]	NA[b]
SM-C	52.1±1.2[b]	9.7±0.8[b]	8.7±0.1[c]	69.5±1.1[c]	35.4±1.6[c]	18.2±0.5[c]	65.8±1.3[c]	34.8±1.0[b]	NA[b]	11.9±0.2[c]	12.1±0.2[c]
SM-CE	-	-	35.4±0.3[d]	112.5±2.5[d]	68.4±1.7[d]	9.1±0.6[d]	54.1±0.6[d]	NA[a]	NA[b]	NA[b]	NA[b]
α-TOC[4]	-	-	9.5±0.2[e]	17.2±0.7[e]	14.5±0.1[e]	-	-	-	-	-	-
BHT[4]	-	-	10.4±0.2[f]	60.2±1.4[f]	7.7±0.0[f]	-	-	-	-	-	-
Galanthamine[4]	-	-	-	-	-	89.5±1.4[e]	79.7±0.5[e]	-	-	-	-
Thiourea[4]	-	-	-	-	-	-	-	95.7±1.7[c]	-	-	-
Kojic acid[4]	-	-	-	-	-	-	-	-	93.6± 1.2[c]	-	-
Oleanolic acid[4]	-	-	-	-	-	-	-	-	-	39.6±0.3[d]	-
Epicatechin gallate[4]	-	-	-	-	-	-	-	-	-	-	78.7±0.8[d]

The natural *S. macrantha* ethanol extract and essential oil: SM-N and SM-NE. The cultivated *S. macrantha* ethanol extract and essential oil: SM-C and SM-CE.

[1]Values represent averages ± standard deviations for triplicate experiments and values were calculated according to negative control, Values with different letters in the same column were significantly different ($p< 0.05$)

[2]PEs, pyrocatechol equivalents (y=0.0408 + 0.0383 pyrocatechol (µg) (r^2= 0.9951)

[3]QEs, quercetin equivalents (y=0.0355 + 0.0673 quercetin (µg) (r^2= 0.9975)

[4]Standard compound

[NA]Not active

There is no study on the antiurease, antityrosinase, antielastase and anticollagenase activities of *S. macrantha* in the literature. While researches on the antiurease [46] and antityrosinase [47] effects of other *Satureja* species were reported, this is the first one on their antielastase and anticollagenase potential.

The essential oils and extracts exhibited different levels of antimicrobial activity (Table 5). The activity of essential oils was found to be higher than the extracts. The highest activity was exhibited by the essential oil of the cultivated sample against *C. albicans* (inhibition zone 42 mm), this value was higher than the activity of the positive control. Our findings complied with Aghbash et al.[8] report that was expressing the high antibacterial activity of *S. macrantha* essential oil at different growth stages.

Table 5. Antimicrobial (inhitibion zone diameter in mm) and cytotoxic activities of *S.macrantha* extracts and essential oils

	Microorganisms[a]					**Cytotoxic activity (200 µg/mL)[a]**		
	Gram negative		**Gram positive**		**Yeast**	**Viability(%)**		
	E. coli	***P. aeruginosa***	***S. aureus***	***S. pyogenes***	***C. albicans***	**PDF**	**HT-29**	**MCF-7**
SM-N	8±0.6	8±0.1	9±0.1	8±0.4	11±0.3	73.7±0.2	59.9±0.4	24.5±0.1
SM-NE	14±0.5	14±0.2	16±0.3	14±0.7	20±0.7	98.8±1.8	85.3±1.0	87.4±1.4
SM-C	9±0.5	9±0.3	11±0.4	9±0.6	12±0.3	97.4±1.4	94.8±1.8	95.1±2.4
SM-CE	16±0.6	16±0.4	20±0.3	16±0.8	42±0.8	75.5±1.2	46.0±1.1	26.1±1.3
PC	20±0.2	NA	35±0.3	19±0.5	30±0.4	-	-	-

The natural *S. macrantha* ethanol extract and essential oil: SM-N and SM-NE. The cultivated *S. macrantha* ethanol extract and essential oil: SM-C and SM-CE. [a]Values represent averages ± standard deviations for triplicate experiments and values were calculated according to negative control. PC: positive controls that are ampicillin for bacteria and fluconazole for yeast

4. Conclusion

In the present study, all parameters of ethanol extract and essential oil of cultivated *S. macrantha* and the ethanol extract of naturally grown species were studied for the first time.

According to the GC-MS results, terpinene-4-ol (30.9%) was found as the major component in the essential oil of the naturally grown sample, and *p*-cymene (56.7%) in the cultivated sample. The flavour analysis results showed that *cis*-sabinene hydrate (20.7%) was determined as the major component in the naturally grown sample, and carvacrol (42.2%) in the cultivated sample. Additionally, the GC-MS analyses indicated that the naturally grown sample was rich in abietane diterpenoids (such as ferruginol and sugiol), while the diterpenoid components in the cultivated sample was found to be under the detectability limit of the method. The results of LC-MS/MS indicated that the cultivated sample contained quite high amounts of phenolic compounds compared to those of the naturally grown sample, and the rosmarinic acid content (24.9 mg/g extract) in the cultivated sample significantly increased compared to that of the natural sample (0.2 mg/g extract). The antioxidant capacity of the cultured sample was found to be generally higher than that of the naturally grown sample in parallel with their total phenolic contents and LC-MS/MS results. In parallel with the GC-MS results (ferruginol and sugiol contents), the cytotoxic activity of the ethanol extract of the naturally grown sample was higher than that of the cultivated sample, and their antityrosinase, antielastase and anticollagenase activities varied depending on the changes in the chemical contents of the samples.

As a result, it was observed that cultivating the species changed its chemical content, major components and biological activities in quite different ways. While the natural sample was collected from Hakkari province, cultural studies were conducted under the conditions of Diyarbakır province. While temperatures are high and little rainfall in Diyarbakır in most of the year, Hakkari province is cold and very rainy. Therefore, the chemical content of *S. macrantha* was very influenced by variables such as climatic conditions and soil structure. Therefore, it was concluded that these conditions should be taken under control if it is planned to cultivate the species.

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Conflict of interest

We declare that we have no conflict of interest.

Author Contribution Statement

Firat M. performed collection and identification of natural specimens, Resitoglu B. prepared the extracts, Yener I. and Akdeniz M. prepared the samples for analysis, and performed the antioxidant, antiurease and antityrosinase activities, Hasimi N. performed the antimicrobial activity, Irtegun-Kandemir S. carried out the cytotoxic activity, Yilmaz M.A. studied LC-MS/MS analysis, Akdeniz M. and Barla Demirkoz A. studied flavour and essential oil analyses, Ertas A. performed the elastase and collagenase activities, Ertas A., Barla Demirkoz A., Kolak U. and Oksuz S. designed and wrote the study.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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