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Research Article

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS IN THE WHOLE PLANT PARTS OF HEXANE EXTRACT OF SOME TRADITIONAL MEDICINAL PLANTS BY GC-MS ANALYSIS

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Abstract: *The aim of this study was to determine the phytochemical components from the hexane extract of some medicinal plants (Salvia palaestina Bentham, Alkanna trichophila var. mardinensis Hub-Mor. (an endemic* variety*), Scutellaria orientalis L. and to evaluate its biological activity by GC-MS analysis. The chemical components in this hexane extract were subjected to the Agilent 7890B GC-5977MSD model Gas Chromatography - Mass Spectrometric analysis. Thirty-two chemical compounds have been identified in the plant extracts. This definition is based on the peak area, retention time, molecular weight, and molecular formula. In this research, Bis (2-ethylhexyl) phthalate, tricosane, docosane, transcaryophyllene, nonacosane, beta-Cubebene, Trichlorfon, Naphthalene, 1,2,3,4,4a, 5,6,8a-octahydro-4a, 8-dimethyl -2- (1-methylet henyl) -, [2R, 3-Aminophenol were found predominantly compounds in extracts. These compounds, which we identified in our study, have a wide range of biological activity and have been found to have high therapeutic value. They are candidate plants to be medicines with various active ingredient content.*

Keywords: *GC-MS analysis, medicinal plants, phytochemical constituents, therapeutic*

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1.Introduction

Turkey is among the richest countries in the world in terms of plant diversity. Although it is about 15 times smaller than Europe in terms of surface area, it has plant diversity as much as European continental flora in terms of flora. The main reasons for this wealth are as follow; a variety of climates, topographical diversity with marked changes in ecological factors over short distance, geological and geomorphic variation, a range of aquatic environments such as seas, lakes, and rivers, altitude variations from sea level to 5000 [1]. The total number of species and subspecies taxa in our country is 11 707 including the foreign origin and cultivated plants [2]. It is estimated that the type of plants used for medicinal purposes is around 1000 [3]. Medicinal plants are part of the nature pharmacy. Despite important advances in medicine, people have sought healing from time to time and have never stopped using medicinal plants as a result of their experience for centuries. Especially the emergence of side effects of synthetic and chemical drugs has increased the use of medicinal plants. Medicinal plants spice, pharmaceutical industry, soft drink, perfume, soap, confectionery, cosmetics, toothpaste, chewing

gum, healing and relaxing tea manufacture, essential oil, aroma, etc. it is used in many fields [4]. In addition, natural products, pure compounds or standard herbal extracts offer unlimited opportunities to obtain new medicines due to the unique availability of chemical diversity. The knowledge gained about the chemical constituents of plants will be more useful in discovering the true value of folk remedies [5].

In the present study, it was aimed to determine the preliminary phytochemical analysis of *Salvia palaestina* Bentham, *Alkanna trichophila* var. *mardinensis* Hub.-Mor. (an endemic variety), *Scutellaria orientalis* L. which has not been studied before, which grows in the untouched Mount Raman, Batman. For this reason, phytochemical screening of the species in question during current research is carried out in order to analyze the presence of chemical compounds, which are secondary metabolites, in order to suggest their application in the pharmaceutical industry.

2. Materials and Methods

2.1. Collection and identification of plant samples

The plant samples were collected from the natural habitat of the Batı Raman campus in Batman University. Voucher specimens have been deposited at the Batman University (voucher no. 2020/015, 2020/016, and 2020/014, respectively) (Figure 1). The taxonomical identity of the plant was confirmed by Dr. Alevcan Kaplan. This identification was made using, Flora of Turkey, Volume 6-7. [6,7]. The collected, whole parts of plants were washed to remove dust and other plant materials and were shade dried at room temperature. The dried leaves and flowers were then ground to a powder using an electric grinder and kept separately for future research in lidded containers.

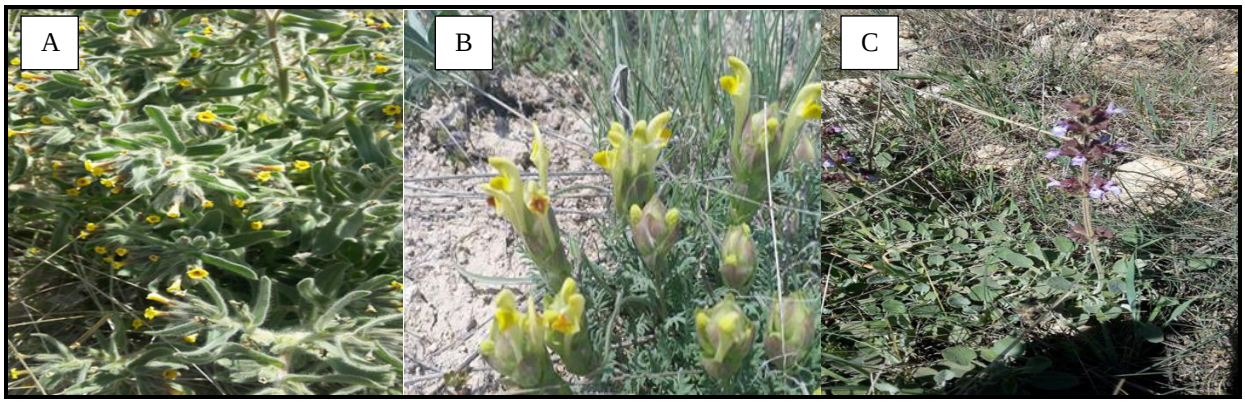

Figure 1. General view of plants (A: *Alkanna trichophila* var. *mardinensis* B: *Scutellaria orientalis* C: *Salvia palaestina*) Photo: A. Kaplan

2.2. Plant Sample Preparation for GC-MS

The n-hexane extract of the plants was obtained using the Soxhlet extractor. 10 g of powdered plant samples were put into the Soxhlet extractor and the required amount was obtained by repeatedly using 100 ml of n-hexane (boiling point about 40 - 60 °C) as solvent extract for four (4) hours. The oil was kept in a refrigerator without further processing until required for analysis.

2.3. GC-MS Data Analysis Condition

Gas chromatography-Mass spectrometry (GC-MS) analysis of n-hexane extracts of plants was performed using Agilent 7890B GC- 5977MSD model with the column length (30 m), diameter (250

μm), and film thickness (0.25 μm) was used with Helium (99.9995 % purity) as the carrier gas, operating in electron impact mode at 70 eV. and the GS-MS condition during the research is following conditions. Injector temperature was 250 ºC, ion-source temperature 200 ºC split flow was 2.4 ml/min. The oven temperature was programmed 120 ºC (5 ºC / min, 7 min), 150 ºC (5 ºC / min, 7 min), 200 ºC (5 ºC / min, 7 min), 220 ºC (5 ºC / min, 7 min), 240 ºC (5 ºC / min, 7 min), 250 ºC (5 ºC / min, 7 min). The split flow was 2.4 ml/min and an injection volume of 1 μ l was employed (split ratio of 2:1). The hexane extract of plants was injected with syringe manually for total bioactive components of leaf and flower samples. Total GC running time is 68 min.

2.4. Identification of constituents

The identity of the constituents in the extract is assigned by comparison of retention times and mass spectra with those stored in the computer fragmentation models library and also with electronic libraries (W9N11.L, MPW2011.L and RTLPEST3.L). Electronic Libraries sources were also used to match the components identified from the plant material. The name, the nature of the compound, molecular weight, molecular formula of the components of the test materials have been confirmed.

3. Results and Discussion

Traditional medicine and food in many plants used Turkey is one of the world's richest countries in terms of genetic diversity. The main reasons for the richness of plant species in Turkey are different climates and soil types and topography as a result of other environmental conditions. In Anatolia folk medicine, medicinal plants have an important place in the field of health both in the world and in our country. Therefore, it is important to conduct research on such plants containing a variety of phytochemicals as it has a high potential to result in cost-effective drug intervention with fewer side effects.

In this study, phytochemical constituents of *Alkanna trichophila* var. *mardinensis*, *Scutellaria orientalis* and *Salvia palaestina* were identified and characterized respectively. In this respect, nine compounds were identified in *Alkanna trichophila* var. *mardinensis* by GC-MS analysis (Table 1). The list of phytochemical compounds from the plant sample is tabulated in Table 1 with retention time, area, and area percentage. The chromatogram information for the sample is given in Figure 2. The retention time taken by the bioactive compounds of the plant sample varied from 31.183 to 65.168. It was found that main constituents of sample were 13-Octadecenal (1.84 %), Tetradecanal (0.65 %), Dinocap II (3.32 %), Bis(2-ethylhexyl) pht-halate (60.60 %), alpha-Amyrin (3.43 %), Metolcarb (1.58 %), Tricosane (12.44 %), Octacosane (7.50 %), Docosane (8.64 %). The compound phenol 13-Octadecenal is known for its sex pheromone, antimicrobial activity [8,9]. Tetradecanal is known for immunotoxicity activity. bacterial bioluminescence, sex pheromone *Heliothis virescens* (F.) females [10, 11, 12]. Dinocap II has been reported to contact fungicide used to control powdery mildew on many crops and is also used as a non-systemic acaricide [13]. Bis (2-ethylhexyl) pht-halate has a role as an apoptosis inhibitor, an androstane receptor agonist and a plasticiser. Moreover, it has an anti-leukaemic, antimutagenic, antimicrobial, and cytotoxic activity [14,15]. Alpha-Amyrin is known for attenuates orofacial pain, reducing hyperalgesia, anti-inflammatory effects [16,17]. Metolcarb is an insecticide [18]. Tricosane has been reported to bear antimicrobial activity and influence host egg parasitisation by Trichogramma [19,20]. Octacosane is known to possess antimicrobial, antioxidant, and anti-inflammatory [21,22]. Docosane is reported to aid in host egg parasitization that can be used as a biocontrol agent, antimicrobial, antioxidant, and functional food nutraceutical applications [23,24,25]. In

light of this information, it has been determined that the plant constituting our experimental material contains different amounts of bioactive components with various therapeutic effects. The potential of the plant to be used in the treatment of diseases such as antileukemia, which is the most common disease of today, has been revealed, especially due to its high content of Bis (2-ethylhexyl) pht-halate. [15] reported that Bis (2-ethylhexyl) pht-halate isolated from *Aloe vera* L. showed anti-leukemic and anti-mutagenic effects (*Salmonella typhimurium* TA98 and TA100 strains). [26] found that di- (2-ethylhexyl) phthalate (DEHP) isolated from *Calotropis gigantea* L. flowers had an antitumor effect. We determined that our plant in question has a feature that can be used in the treatment of these diseases.

Table 1. Phytochemical constituents of *Alkanna trichophila* var. *mardinensis* by GC-MS

No	Name of the compound	Molecular formula	Molecular weight	RT	Peak area(%)	Nature of the compound
1	13-Octadecenal,	$C_{18}H_{34}O$	266.5 g/mol	31.183	1.84	-
2	Tetradecanal,	$C_{14}H_{28}O$	212.37 g/mol	31.884	0.65	fatty aldehyde
3	Dinocap II,	$C_{18}H_{24}N_2O_6$	364.39 g/mol	42.215	3.32	enoate ester
4	Bis(2-ethylhexyl) phthalate,	$C_{24}H_{38}O_4$	390.6 g/mol	50.157	60.60	phthalate ester
5	alpha-Amyrin,	$C_{30}H_{50}O$	426.7 g/mol	53.434	3.43	pentacyclic triterpenoid
6	Metolcarb	$C_9H_{11}NO_2$	165.19 g/mol	54.608	1.58	carbamate ester
7	Tricosane	$C_{23}H_{48}$	324.6 g/mol	56.754	12.44	N-Alkanes
8	Octacosane	$C_{28}H_{58}$	394.8 g/mol	57.055	7.50	N-Alkanes
9	Docosane	$C_{22}H_{46}$	310.6 g/mol	65.168	8.64	N-Alkanes

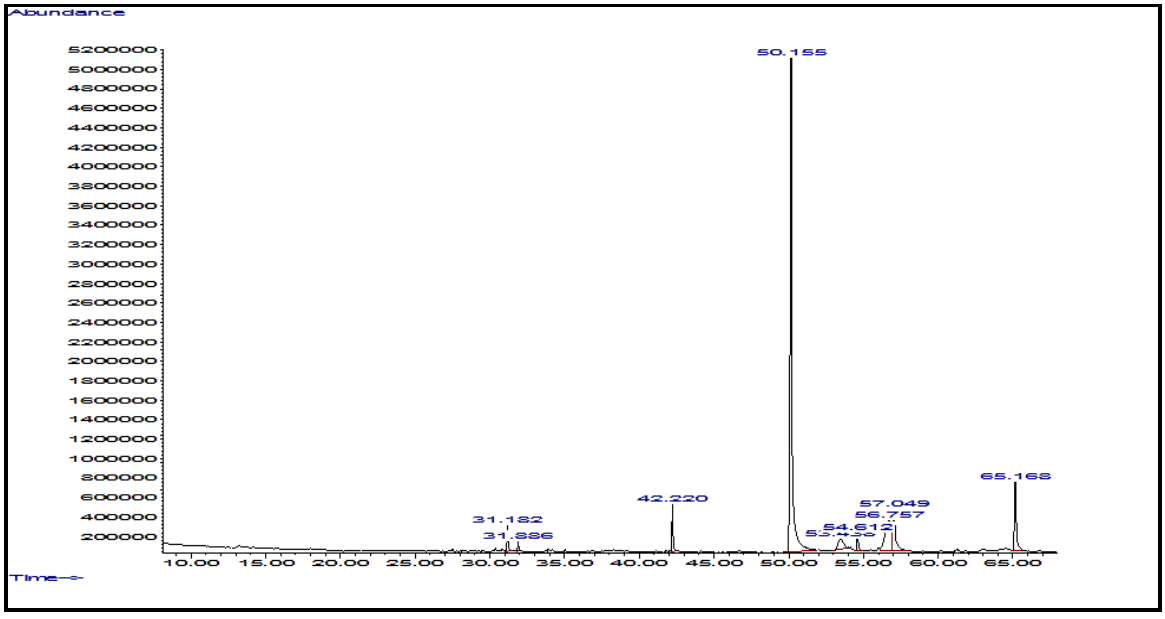

Figure 2. GC- MS chromatogram of the hexane fraction of compounds from *Alkanna trichophila* var. *mardinensis*

Secondly, eleven compounds were identified in *Scutellaria orientalis* by GC-MS analysis (Table 2). The list of phytochemical compounds from the plant sample is tabulated in Table 2 with retention time, area, and area percentage. The chromatogram information for the sample is given in Figure 3. The retention time taken by the bioactive compounds of the plant sample varied from 0.93 to 28.83. It was found that main constituents of sample were 2-Ethyl-1,3-hexanediol (2.97 %), Tryclopyrbutoxy-ethyl (1.66 %), trans-Caryophyllene (28.83 %), beta-Cubebene (6.46 %), Tris(2-butoxyethyl) phosphate (0.93 %), Diisobutyl phthalate (0.65 %), Eicosane (2.44 %), 1-Triacontanol (3.48 %), Heptaco-

sane (5.19 %), 1-Heptacosanol (5.13 %), Nonacosane (16.14 %). 2-Ethyl-1,3-hexanediol has an antiparasitic, insecticidal, and repellent, ectoparasiticidal effect [27]. Tryclopyrbutoxyethyl is known to pesticide [28]. Trans-caryophyllene is also known as antibacterial and antifungal [29]. Beta-Cubebene has been reported that neuroprotective effects [30]. Tris (2-butoxyethyl) phosphate (TBEOP) is an organophosphate [31]. Diisobutyl phthalate also known proliferation and differentiation of primary osteoblasts, antioxidant, and free radical scavenging activities [32,33]. Eicosane has been reported that an antitumor activity [34]. 1-Triacontanol (TRIA) is a growth regulator for plants [35]. Heptacosane has shown antioxidant, antibacterial, antimalarial, antidermatophytic effects [36,37,38]. 1-Heptacosanol is known for its antimicrobial activity [39]. Nonacosane has also known as celidoniol deoxy antibacterial and anti-inflammatory [40,41]. It is clear that the plant in question contains bioactive components with broader effects. Our study results support the potential of the plant to be used ethnobotanically in the region.

Table 2. Phytochemical constituents of *Scutellaria orientalis* by GC-MS

No	Name of the compound	Molecular formula	Molecular weight	RT	Peak area (%)	Nature of the compound
1	2-Ethyl-1,3-hexanediol	$C_8H_{18}O_2$	146.23 g/mol	9.160	2.97	Aliphatic alcohol
2	Tryclopyrbutoxyethyl	$C_{13}H_{16}Cl_3NO_4$	356.6 g/mol	10.405	1.66	-
3	trans-Caryophyllene	$C_{15}H_{24}$	204.35 g/mol	11.607	28.83	Bicyclic sesquiterpene
4	beta-Cubebene	$C_{15}H_{24}$	204.35 g/mol	11.879	6.46	Tricyclic sesquiterpene
5	Tris(2-butoxyethyl) phosphate	$C_{18}H_{39}O_7P$	398.5 g/mol	14.469	0.93	-
6	Diisobutyl phthalate	$C_{16}H_{22}O_4$	278.34 g/mol	27.062	0.65	Phthalate ester
7	Eicosane	$C_{20}H_{42}$	282.5 g/mol	50.143	2.44	N-Alkanes
8	1-Triacontanol	$C_{30}H_{62}O$	438.8 g/mol	56.010	3.48	Fatty alcohol
9	Heptacosane	$C_{27}H_{56}$	380.7 g/mol	57.055	5.19	N-Alkanes
10	1-Heptacosanol	$C_{27}H_{56}O$	396.7 g/mol	64.152	5.13	Fatty alcohol
11	Nonacosane	$C_{29}H_{60}$	408.8 g/mol	65.154	16.14	N-Alkanes

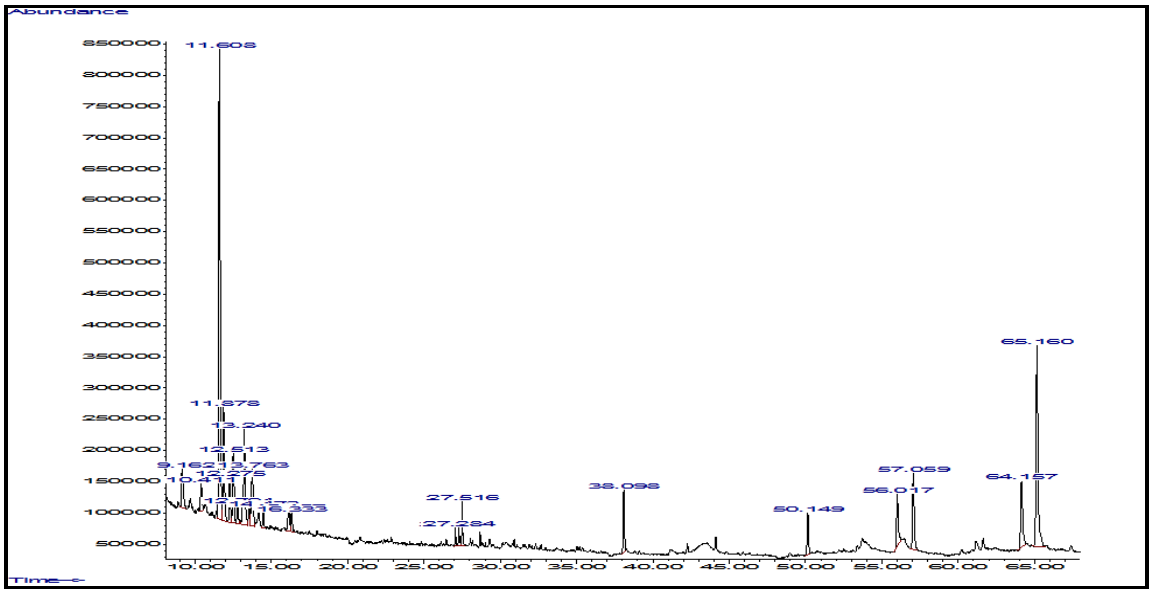

Figure 3. GC- MS chromatogram of the hexane fraction of compounds from *Scutellaria Orientalis*

Thirdly, twelve compounds were identified in *Salvia palaestina* by GC-MS analysis (Table 3). The list of phytochemical compounds from the plant sample is tabulated in Table 3 with retention

time, area, and area percentage. The chromatogram information for the sample is given in Figure 4. The retention time taken by the bioactive compounds of the plant sample varied from 0.22 to 79.15. It was found that main constituents of sample were trans-Caryophyllene (1.43 %), Neophytadiene (0.22 %), Sclareoloxide (0.61 %), geranyl-p-cymene (0.22 %), Caryophyllene (0.96 %), 3-Aminophenol (1.47 %), Trichlorfon (79.15 %), Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R (5.84%), Allidochlor (0.25 %), Isobornyl thiocyanoacetate (1.03 %), Heptacosane (0.64 %), Nonacosane (0.71 %). Neophytadiene is an antipyretic, analgesic, anti-inflammatory, anti-microbial, antioxidant [42]. Sclareoloxide is known to demonstrate good antimicrobial activity, antioxidant and antiviral activities [43]. Geranyl-p-cymene has been reported that an antimicrobial, antioxidant, and antiproliferative effect [44]. Caryophyllene is also known as anticancer, antioxidant, and antimicrobial [45]. 3-Aminophenol is an antibacterial [46]. Trichlorfon is known for its insecticidal effects [47]. Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R is an insecticide, repellant [48]. Allidochlor is the more active herbicide and has been introduced into agricultural use [49]. Isobornyl thiocyanoacetate is an insecticide and used to control ants, houseflies, and head lice [50]. In this study, it was clearly demonstrated that Salvia palaestina has the potential to be used as an insecticidal because of the high rate of triclorfon in addition to the various therapeutic substances it contains.

Table 3. Phytochemical constituents of *Salvia palaestina* by GC-MS

No	Name of the compound	Molecular formula	Molecular weight	RT	Peak area(%)	Nature of the compound
1	trans-Caryophyllene	$C_{15}H_{24}$	204.35g/mol	11.607	1.43	Bicyclic sesquiterpene
2	Neophytadiene	$C_{20}H_{38}$	278.5 g/mol	27.519	0.22	Terpenes
3	Sclareoloxide	$C_{18}H_{30}O$	262.4 g/mol	28.049	0.61	Diterpene
4	geranyl-p-cymene	$C_{18}H_{26}$	242.4 g/mol	30.081	0.22	Homoditerpenes
5	Caryophyllene	$C_{15}H_{24}$	204.35 g/mol	32.986	0.96	Bicyclic sesquiterpene
6	3-Aminophenol	C_6H_7NO	109.13 g/mol	33.701	1.47	Aminophenol
7	Trichlorfon	$C_4H_8Cl_3O_4P$	257.43 g/mol	38.481	79.15	Phosphonic ester
8	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R	$C_{15}H_{24}$	204.3511 g/mol	39.311	5.84	Polycyclic hydrocarbon
9	Allidochlor	$C_8H_{12}ClNO$	173.64 g/mol	39.525	0.25	Carboxamide
10	Isobornyl thiocyanoace-tate	$C_{13}H_{19}NO_2S$	253.36 g/mol	44.333	1.03	Thiocyanoacetic acid ester
11	Heptacosane	$C_{27}H_{56}$	380.7 g/mol	57.069	0.64	N-Alkanes
12	Nonacosane	$C_{29}H_{60}$	408.8 g/mol	65.168	0.71	N-Alkanes

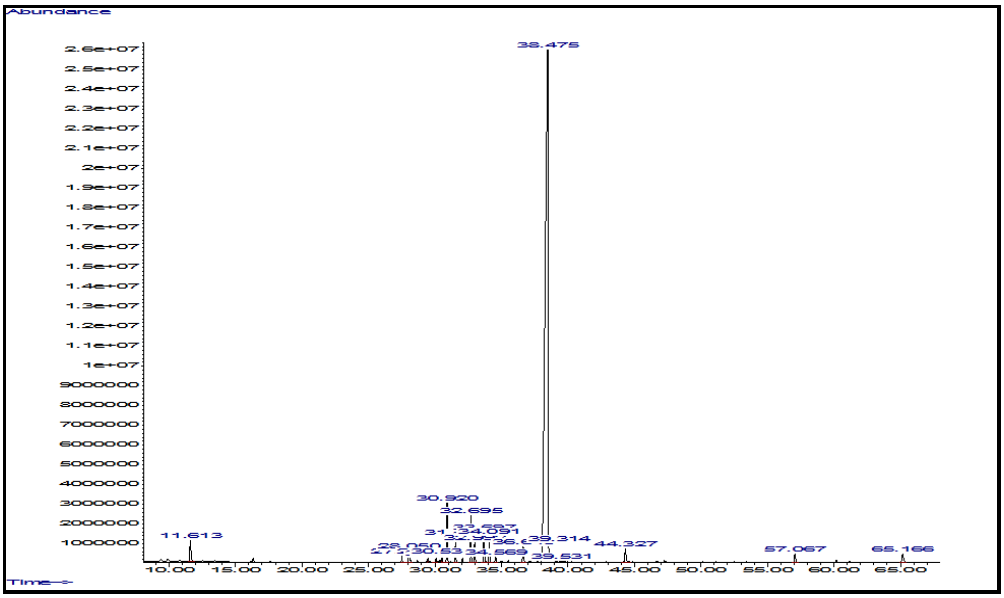

Figure 4. GC- MS chromatogram of the hexane fraction of compounds from *Salvia palaestina*

4. Conclusion

Today, more than 5000 phytochemicals have been discovered, although it varies according to plant species, most of them have not been identified yet. From current research, *Salvia palaestina*, *Alkanna trichophila* var. *mardinensis*, *Scutellaria orientalis* samples (whole plant parts) revealed that they constitute a wide range of bioactive phytochemicals with high therapeutic value. Especially, triclorfon, bis (2-ethylhexyl) pht-halate (DEHP), trans-caryophyllene molecules were found in large amounts, showing that these plants are candidate drug plant that can be used for a apoptosis inhibitor, an androstane receptor agonist and a plasticizer, anti-leukaemic, anti-mutagenic, antimicrobial and cytotoxic activity, insecticide, antibacterial and antifungal purposes. However, we are of the opinion that isolation of individual phytochemical components and subjecting them to pharmacological activity would certainly yield fruitful results.

The compliance to the Research and Publication Ethics: This study was carried out in accordance with the rules of research and publication ethics.

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