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Phytochemical Screening of Bioactive Components of Medicinal Plant *Ajuga chamaepitys* subsp. *laevigata* (Banks & Sol.) P.H.Davis and *Ajuga bombycina* Boiss. by GC-MS Analysis

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Abstract

Herbal plants have been a source of food for human beings for many years; they have also frequently been used as an alternative to modern medicine. Because synthetic drugs have possible side effects and are often considerably expensive, understanding how various plants are used for the treatment of specific ailments has become increasingly important. Plant extracts contain multiple active constituents and this has led to the production of new drugs and chemicals derived from the various parts of plants. In Anatolian folk medicine, *Ajuga* L. (Lamiaceae) species are used by people in many villages and towns for the therapeutic value of their bioactive components. This study was thus designed to examine the possible bioactive components of *Ajuga bombycina* Boiss. (an endemic species) and *Ajuga chamaepitys* subsp. *laevigata* (Banks & Sol.) P.H.Davis. In the study the bioactive components of the GC-MS analysis of dried leaves and flower samples were screened using an Agilent 7890B GC-5977MSD model with hexane as solvent. Phytochemicals which have a wide range of biological applications and high therapeutic value were found in the samples.

Keywords: Medicinal plants, bioactive components, GC-MS analysis, *Ajuga* L.

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1.INTRODUCTION

For centuries, herbal medicine has been one of the bases of medical treatment, and such traditional medicine is still widely practiced today. The World Health Organization (WHO) has estimated that up to 80 % of people worldwide still rely on traditional medical remedies such as the use of plants [1-2]. Modern medicine recognizes herbal medicine as a form of alternative medicine because its practice is not strictly based on evidence gathered using the scientific method. On the other hand, modern medicine does use many plant-derived compounds as the basis for evidence-tested pharmaceutical drugs. Phytotherapy works to apply modern standards of testing to medicines that are derived from natural sources. Analyzing the bioactive compounds in plants has led to the discovery of new drugs which provide effective protection and treatment against various diseases [3-4].

The genus *Ajuga* L. consists of about 90 species, mostly scattered across the northern temperate zone. The genus is also seen in South Africa and Australia. Members of the genus *Ajuga* L., which belongs to the family Lamiaceae, grow naturally or are cultivated in Europe, Asia, Africa, Australia and North America [6]. The *Ajuga* genus is represented in Anatolia by 23 taxa, 13 species and 10 subspecies [7]. Most of the plants belonging to this genus are used as an anthelmintic, diuretic, anti-fungal, anti-inflammatory and antimycobacterial agents, and, in traditional medicine, for fever, toothache, dysentery, high blood pressure and gastrointestinal disorders. In addition, they are used to prevent pest growth. Phytoecdysteroids, neo-clerodane-diterpenes, diterpenes, triterpenes, sterols, anthocyanidin-glycosides, iridoid glycosides, withanolides, flavonoids, triglycerides and essential oils have been isolated from one of the members of the *Ajuga* genus. The biological, pharmacological and therapeutic properties of these compounds include anabolic, analgesic, antibacterial, antiestrogenic, anti-hypertensive, antitumor, antimalarial/antiplasmodial, antimycobacterial, antioxidant, antipyretic, larvae and insect antifeedant, cardiotonic, cytotoxic, hypoglycemic, vascular-relieving and insect growth effects. The *Ajuga* genus thus has both medical and economic significance. [6]

In the present study, phytochemical analysis of *A.bombycina* and *Ajuga chamaepitys*. subsp. *laevigata* was carried out to identify their therapeutic value. Prior to this study, it was found that there was only a limited number studies of the bioactive composition of *A. bombycina* and *Ajuga chamaepitys* subsp. *laevigata* in the scientific literature. This research was thus conducted to define the qualitative bioactive compositions of these traditionally used medicinal plants, to identify possible drug precursors and to point the way towards further studies.

2. MATERIAL AND METHOD

2.1. Collection and Identification of Plant Material

Fully matured leaves and flowers were collected from Konya Kent Ormanı, Konya, Turkey (*Ajuga bombycina*), and Batı Raman Campus, Batman, Turkey (*Ajuga chamaepitys* subsp. *laevigata*) (Figure 1). The botanical identity of the plant was confirmed by Dr. Alevcan Kaplan. This identification was made using Volume 7 of the Flora of Turkey [8]. The collected, disease-free leaves and flowers 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.

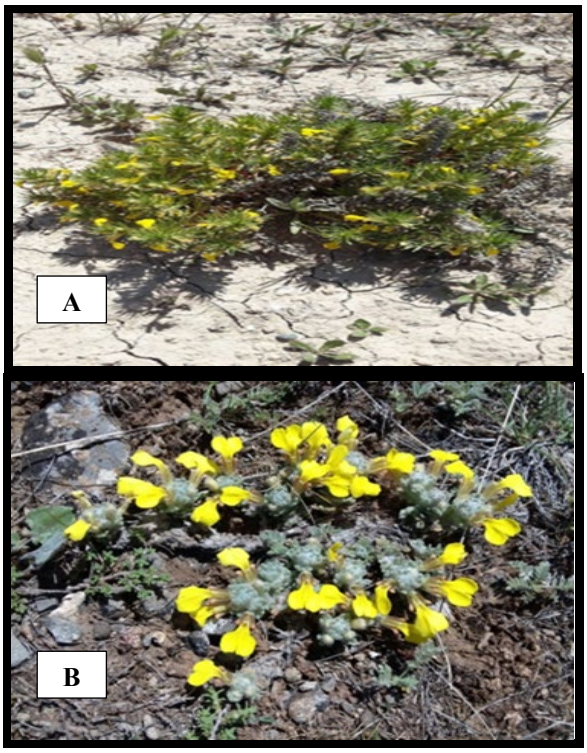


Figure 1 General view of plants (A: *Ajuga chamaepitys* subsp. *laevigata* B: *Ajuga bombycina*)
Photo: A. Kaplan

2.2. Plant Sample Extraction for GC-MS Analysis

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. Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis of n-hexane extracts of plants were 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 °C 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). Split flow was 2.4 ml / min and an injection volume of 1 µl was employed (split ratio of 2:1). The hexane extract of plants were injected with syringe manually for total bioactive components of leaf and flower samples. Total GC running time is 68 min.

2.4. Identification of Compounds

Interpretation of bioactive components on mass spectrum of GC-MS was carried out using spectrometric electronic libraries (W9N11.L, MPW2011.L and RTLPEST3.L). The mass spectrum of the unknown component was compared to the spectrum of the known components stored in these libraries. The name, the nature of the compound, molecular weight, molecular formula and structure of the components of the test materials have been confirmed.

3. RESULTS AND DISCUSSIONS

Medicinal plants a revaluable sources of treatments for the prevention of diseases and protection of human health [9]. Turkey has a long tradition and knowledge of folkloric medicine and an abundance of flora, and thus provides a rich source of research on this topic. Most Turkish people living in the countryside have traditionally used plants for therapeutic purposes, generally using herbs both for nutrition and as forms of medicine. In recent years, this traditional use of plants to combat disease in Turkey has attracted the attention of a number of researchers [10]. To study this usage, plant metabolites are extracted using various methods, including maceration, boiling, Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction and the ultrasound assisted extraction method [11]. As the Soxhlet extraction technique is theeasiest method, one that uses simple and inexpensive equipment and

that requires little effort, it is still routinely used in laboratories today [12]. The efficiency of extraction depends on various factors, such as the nature of the phytochemical components, the extraction method, the particle size, extraction time, temperature, pH, solute/solvent ratio, and solvent polarity [13]. Proper use of the solvent system is essential in order to achieve higher extract yields, polyphenols and bioactive compounds [14]. Hexane is used because it is a solvent that can be easily removed without leaving any residue, has a low moisture absorption and a relatively low boiling point (nonpolar solvent; dipole moment <0.1), and can easily penetrate into particles without toxicity in both liquid and vapor [15] .In this context, the bioactive composition and the main ingredients present in the *Ajuga chamaepitys* subsp. *laevigata* and *Ajuga bombycina* are shown in Tables 1 and 2, and chromatograms are presented in Figures 2 and 3, respectively.

The leaf and flower sample of *Ajuga chamaepitys* subsp. *laevigata* was air-dried and powdered and subjected to qualitative phytochemical analysis with hexane. Approximately seven bioactive compounds were identified from the sample (leaves and flowers together). The retention time of the bioactive compounds of sample varied from 11.650 to 65.225, and the area percentage varied from 1.26 to 47.57. A list of the bioactive components of the sample is given in the Table 1, with the name of the compound, molecular formula, molecular weight, retention time, peak area percentage, and the nature of the compound. The chromatogram information for the sample is given in Figure 2. It was found that main constituents of sample were 2-ethyl-1,3-hexanediol (6.27 %), neophytadiene (4.02 %), tricosane (1.26 %), pentacosane (1.86 %), heptacosane (47.57 %), eicosane (1.50 %), celidoniol (35.46 %). The compound 2-ethyl-1,3-hexanediol is known for its antiparasitic qualities andis used in ectoparasiticides, incl. scabicides, insecticides and repellents [16-17]. Neophytadiene has been reported to have antimicrobial, antioxidant, antiviral, antifungal activities, to be effective against lung cancer cells and to have good analgesic, antipyretic and anti-inflammatory effects [18-23]. Tricosane is known for to be effective against the foraging behavior of *Trichogrammatids*, cruciferous host plants and host larval bodies of *Plutella xylostella* and for behavioural manipulation of *Cotesia plutellae* [24-25-26]. Pentacosane is a volatile pheromone and induces avoidance responses in aphid parasitoids with varying host ranges [27-28]. Heptacosane has antioxidant, antibacterial, antimalarial, antitumor and antidermatophyticeffects [29-30-31]. Eicosane is known for its antioxidant and antitumor activity [32]. Celidoniol is antibacterial and anti-inflammatory, and is involved in chemical communication especially in the *Anopheles stephensi* mosquito and is a pheromone of *Orgyia leucostigma* [33-34-35-36]. Based on GC-MS studies, most of the chemical components appear to be biologically active compounds and have been found to have pharmacological activities that have therapeutic effects. The presence of different bioactive compounds justifies the use of the leaf for various ailments by traditional practitioners. In particular, the high percentage of celidoniol and heptacosane, which are major components with very different biological activities, is advantageous in using the plant. In addition, the results of the GC-MS profile can be used as a pharmacognostical tool for plant identification. [37] isolated a new clerodane diterpene and some other compounds from the *Ajuga chamaepitys* subsp. *laevigata* plant. Among the compounds they isolated were ajugalaevigatic acid, a diterpene, (13S) -15-hydroxylabd-8 (17) -en-19-oic acid, a steroidal glucoside, 3-O-β -D-glucopyranosyl-stigmasta-5,25- diene, and triterpenes, α- and β -amyrin and ursolic acid. They performed a structural elucidation of the compounds by NMR and MS spectroscopic analysis. [38] detected 19 bioactive components in leaf extracts and 13 in flower extracts of *Tagetes erecta* L. This is similar to present study, in which celidoniol was found to be among the dominant components. In addition, [39] determined the bioactive components of *Barleria courtallica* in which heptacosane was among the dominant compounds in their studies. This result is similar to that of the present study. Most of these phytocomponents were also identified from

various plant extracts by [40] from *Hugonia mystax* L.; [41] from *Lawsonia inermis* Linn.; [42] from *Aplotaxis auriculata*.

Table 1
GC-MS analysis of bioactive components in hexane extract of *Ajuga chamaepitys* subsp. *laevigata*

No	Name of the compound	Molecular formula	Molecular weight	RT	Peak area(%)	Nature of the compound	Chemical structure
1	2-Ethyl-1,3hexanediol	$C_8H_{18}O_2$	146.23 g mol^{-1}	11.650	6.27	Aliphatic alcohol	
2	Neophytadiene	$C_{20}H_{38}$	278.5 g mol^{-1}	27.534	4.02	Aliphatic acyclic compound	
3	Tricosane	$C_{23}H_{48}$	324.6 g mol^{-1}	42.273	1.26	N-Alkane	
4	Pentacosane	$C_{25}H_{52}$	352.7 g mol^{-1}	50.157	1.86	N-Alkane	
5	Heptacosane	$C_{27}H_{56}$	380.7 g mol^{-1}	57.083	47.57	N-Alkane	
6	Eicosane	$C_{20}H_{42}$	282.5 g mol^{-1}	61.190	1.50	N-Alkane	
7	Celidoniol	$C_{29}H_{60}O$	424.8 g mol^{-1}	65.225	35.46	N-Alkane	

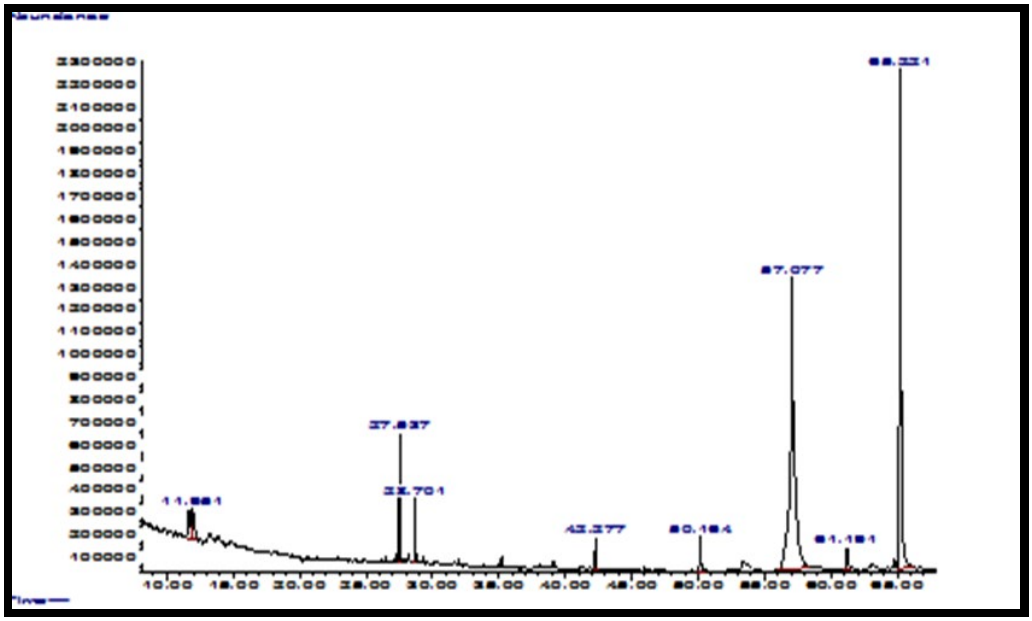
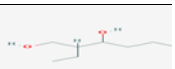


Figure 2 Chromatogram of *Ajuga chamaepitys* subsp. *laevigata*

The leaf and flower sample of *Ajuga bombycina* was also air-dried and powdered and subjected to qualitative phytochemical analysis with hexane. Approximately five bioactive compounds were identified from the sample (leaves and flowers together) extracts. The retention time of the bioactive compounds of sample varied from 11.621 to 65.225 and the area percentage varied from 2.46 to 44.52. A list of bioactive components from the sample is given in the Table 2 with the name of the compound, molecular formula, molecular weight, retention

time, peak area percentage, and the nature of the compound. The chromatogram information for the sample is given in Figure 3. It was found that main constituents of the sample were 2-ethyl-1,3hexanediol (2.46 %), trans-caryophyllene (5.80 %), docosane (45.34 %), eicosane (1.45 %), celidoniol (44.52 %). From these bioactive ingredients, docosane is report to aid in host egg parasitization, and can be used as a biocontrol agent and for antimicrobial, antioxidant and functional food nutraceutical applications [43-44-45]. Trans-caryophyllene is known for its anti-inflammatory, analgesic, antipyretic, and platelet-inhibitory activities. It acts by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, the precursors of prostaglandins [46-47-48]. In particular, the high percentage of celidoniol and docosane, which are major components with very different biological activities, is advantageous in using the plant in the same way. [49] screened water-distilled essential oil from *Ajuga bombycina* analyzed by GC-MS. GC-MS analysis of extract of *Ajuga bombycina* aerial parts revealed the presence of various chemical component and the prevailing components in water extract were β-pinene (28.2 %), α-pinene (18.5 %), germacrene D (8.5 %), and β-phellandrene + limonene (6.9 %). [50] screened the bioactive components of *Plectranthus amboinicus* leaves using GC-MS. GC-MS analysis of extract of *Plectranthus amboinicus* leaves found that they also contained celidoniol (nonacosane) as the dominant component. While [41] and [51] found that the plant extracts used in their studies contain less docosane (0.17 % and 0.29 %, respectively), a high amount of docosane was obtained in the present study (45.34 %). This suggests that using the plant in areas where the docosane molecule is frequently used will be advantageous. Similarly, a GC-MS analysis of the bioactive components of *Evolvulus alsinoides* (L.) was performed by [52]. They identified 16 bioactive compounds from whole plant ethanolic extracts and reported that the bioactive compounds contained in Evolvulus alsinoides had a wide range of benefits. The study thus supported their traditional use for various disorders.

Table 2
GC-MS analysis of bioactive components in hexane extract of *Ajuga bombycina*

No	Name of the compound	Molecular formula	Molecular weight	RT	Peak area (%)	Nature of the compound	Chemical structure
1	2-Ethyl-1,3hexanediol	$C_8H_{18}O_2$	146.23 g mol^{-1}	11.621	2.46	Aliphatic alcohol	
2	trans-Caryophyllene	$C_{15}H_{24}$	204.35g mol^{-1}	11.936	5.80	Aliphatic heteropolycyclic compound	
3	Docosane	$C_{22}H_{46}$	310.6 g mol^{-1}	57.083	45.34	N-Alkane	
4	Eicosane	$C_{20}H_{42}$	282.5 g mol^{-1}	61.190	1.56	N-Alkane	
5	Celidoniol	$C_{29}H_{60}O$	424.8 g mol^{-1}	65.225	44.52	N-Alkane	

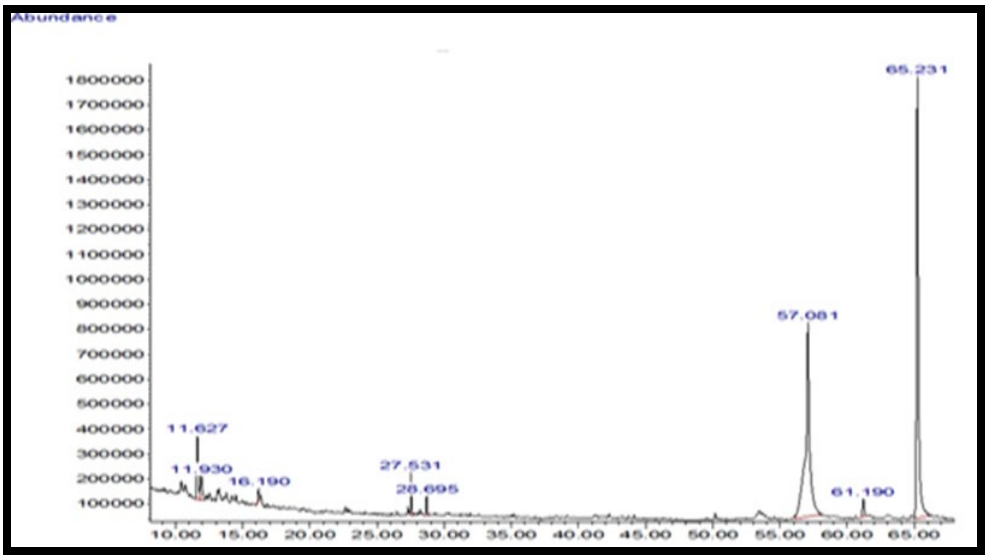


Figure 3 Chromatogram of *Ajuga bombycina*

This study subjected the hexane extracts of *Ajuga bombycina* and *Ajuga chamaepitys* subsp. *laevigata* to GC-MS analysis and various major phytocompounds were identified. Both species contain significant amounts of compounds with biologically significant activity. The physical and chemical properties of vegetable oils largely depend on the percentages and types of fatty acids they contain, and the fatty acid composition of plants is not constant. The synthesis and content of fatty acids can vary depending on genetic, ecological, morphological, physiological and cultural practices, as well as on other factors [53]. The presence of various bioactive chemical compounds supports the use of this plant by traditional medicine practitioners for various ailments [9]. Studies have been carried out in this field for a considerable period of timeand this research continues today [54-61]. The present study investigated the potential for using specific plants rich in bioactive chemical components for their therapeutic effects.

4. CONCLUSION

This current investigation of *Ajuga bombycina* and *Ajuga chamaepitys* subsp. *laevigata* samples (leaf and flower) revealed that they contain a wide range of bioactive phytochemicals with high therapeutic values. In particular, celidoniol, heptacosane and docosane molecules were found in large amounts, showing that these plants are a candidate drug plant that can be used for antibacterial, anti-inflammatory, antitumor, pheromone, antimalarial, antioxidant, antidermatophytic purposes and as nutraceutical and functional food nutraceutical ingredients. On the other hand, the remaining phytochemicals can be used for their antiparasitic and platelet-inhibitory activities, as insecticides, and are analgesics and antipyretics etc. More research on these phytochemicals will lead to lower-cost drug interventions with fewer side effects. At the same time, it will be necessary to further purify and analyze these major chemical components that play biologically active roles and to investigate them in greater details. Further research will also be necessary in order to develop these plants for use in treating specific illnesses.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the author.

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The author declares that this document does not require an ethics committee approval or any special permission.

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REFERENCES

[1] L. Tripathi and JN. Tripathi, "Role of Biotechnology in Medicinal Plants", Tropical Journal of Pharmaceutical Research, vol. 2, no. 2, pp.243-253, 2003.

[2] SO. Odesanmi, RA. Lawal, SA. Ojokuku, "Effects of ethanolic extract of Tetrapleura tetraptera on Liver Function Profile and Histopathology in Male Dutch White Rabbits", International Journal of Tropical Medicine, vol. 4, no. 4, pp.136-139, 2009.

[3] K. Sheeja, C. Karthar, "Activation of cytotoxic Tlymphocyte responses and attenuation of tumor growth in vitro by *Andrographis paniculata* extract and andrographolide", Immunopharmacology and Immunotoxicology, vol. 29, pp.81-93, 2007.

[4] PK. Mukherjee, V. Kumar, PJ. Houjhtor, "Serenity of Indian medicinal Plants for acetyl cholinesterase inhibitory activity", Phytotherapy Research, vol.21 no. 12, pp. 1142-5, 2007.

[5] YB. Köse, S. Alan, MB. Mutlu, T. Güner, Batı Anadolu Bölgesinde Yayılış Gösteren *Ajuga* L. Cinsine Ait Taksonların Taksonomik ve Ekolojik Özellikleri", Bilimsel Araştırma Projesi, Anadolu Üniversitesi, Eskişehir, 2011.

[6] ZH. Israili, B. Lyoussi, "Ethnopharmacology of the Plants of Genus *Ajuga*", Pakistan Journal of Pharmaceutical Sciences, vol.22, no.4, pp. 425-462, 2009.

[7] A. Güner, S. Aslan, T. Ekim, M. Vural, M. Babaç, "Türkiye Bitkileri Listesi Damarlı Bitkiler", Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, 2012.

[8] PH. Davis, "Flora of Turkey and the East Aegean Islands, Edinburgh", Edinburgh University Press, vol.7, pp. 42- 52, 1982.

[9] S. Padmavathy, DN. Nair, and T. Shanthi, "GC-MS Analyses of Bioactive Components in *Gaultheria fragratissima* Wall, Asian Journal of Pharmaceutical and Clinical Research, vol. 7, no. 1, pp. 83-85, 2014.

[10] M. Nadiroğlu, L. Behçet, and Çakılcıoğlu U, "An ethnobotanical survey of medicinal plants in Karlıova (Bingöl-Turkey)", Indian Journal of Traditional Knowledge, vol.18, no. 1, pp. 76-87, 2019.

[11] NN. Azwanida, "A review on the extraction methods use in medicinal plants, principle, strength and limitation", Medicinal & Aromatic Plants, vol. 4, no.3, pp. 196, 2015.

[12] SE. Büyüktuncel, "Gelişmiş Ekstraksiyon Teknikleri I", Hacettepe Üniversitesi

Eczacılık Fakültesi Dergisi, vol. 32, no. 2, pp. 209-242, 2012.

[13] QD. Do, AE. Angkawijaya, PL. Tran-Nguyen, LH. Huynh, FE. Soetaredjo, S. Ismadji, YH. Ju, "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*", Journal of Food and Drug Analysis, vol. 22, no. 3, pp. 296-302, 2014.

[14] SB. Iloki-Assanga, LM. Lewis-Lujan, CL. Lara-Espinoza, AA. Gil-Salido, D. Fernandez-Angulo, JL. Rubio-Pino, DD. Haines, "Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*", Complementary and Alternative Medicine BMC Research Notes, vol. 8, no. 1, pp. 396, 2015.

[15] O. Sevindik, S. Selli, "Üzüm çekirdek yağı eldesinde kullanılan ekstraksiyon yöntemleri", Gıda, vol. 42, no. 1, pp. 95-103, 2017.

[16] J. Drapeau, M. Verdier, D. Touraud, U. Kröckel, M. Geier, A. Rose, W. Kunz, "Effective insect repellent formulation in both surfactantless and classical microemulsions with a long-lasting protection for human beings", Chemistry & Biodiversity, vol. 6, no.6, pp. 934-47, 2009.

[17] P. Fowler, K. Smith, J. Young, L. Jeffrey, D. Kirkland, S. Pfuhler, P. Carmichael, "Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays. I. Choice of cell type", Mutation Research, vol. 742, no. 1-2, pp.11-25, 2012.

[18] R. Jeyadevi, T. Sivasudha, A. Ilavarasi, and N. Thajuddin, "Chemical Constituents and Antimicrobial Activity of Indian Green Leafy Vegetable *Cardiospermum halicacabum*", Indian Journal of Microbiology, vol. 53, pp. 208-213, 2012.

[19] S. Santoyo, L. Jaime, M. Plaza, M. Herrero, I. Rodriguez-Meizoso, E. Ibañez, and G. Reglero, "Antiviral compounds obtained from microalgae commonly used as carotenoid sources", Journal of Applied Phycology, vol. 24, pp. 731-741, 2012.

[20] A. Maxia, D. Falconieri, A. Piras, S. Porcedda, B. Marongiu, M. Assunta Frau, MJ. Gonçalves, C. Cabral, C. Cavaleiro and L. Salgueiro, " Chemical Composition and Antifungal Activity of Essential Oils and Supercritical CO_2 Extracts of *Apium nodiflorum* (L.) Lag.", Mycopathologia, vol. 174, no. 1, pp. 61-67, 2012.

[21] RB. Venkata, LA. Samuel, M. Pardha Saradhi, B. Narashimha Rao, A. Naga Vamsi Krishna, M. Sudhakar, TM. Radha Krishnan, "Antibacterial, antioxidant activity and GC- MS analysis of *Eupatorium odoratum*", Asian Journal of Pharmaceutical and Clinical Research, vol. 5, no.2, pp. 104-105, 2012.

[22] JSM. Richardson, G. Sethi, G. Serm Lee, and SN. Abdul Malek, "Chalepin: isolated from *Ruta angustifolia* L. Pers induces mitochondrial mediated apoptosis in lung carcinoma cells", BMC Complementary and Alternative Medicine, vol. 16, pp. 389, 2016.

[23] N. Balpınar and G. Okmen, "Biological Activities and Chemical Composition of *Senecio vernalis* Growing in the Lakes Region of Turkey", International Journal of Environmental Science and Technology, vol. 16, pp. 5205-5212, 2018.

[24] T. Seenivasagan, AV. Paul, "Gas-chromatography and electroantennogram analysis of saturated hydrocarbons of cruciferous host plants and host larval body extracts of *Plutella xylostella* for behavioural manipulation of *Cotesia plutellae*", Indian Journal of Experimental Biology, vol. 49, no. 5, pp. 375-86. 2011.

[25] S. Mathur, A. Zayeem, S. Kanameni, M. Tibrewal, N. Wadhwa, P. Arora, and A. Kumar, "Effect of Various Concentration of Octacosane, Pentacosane and Tricosane on Foraging Behavior of Trichogrammatids", International Journal of Scientific and Research Publications, vol. 2, Issue 6, 2012.

[26] N. Samadi, A. Manayi, M. Vazirian, M. Samadi, Z. Zeinalzadeh, Z. Saghari, N. Abadian, VO. Mozaffarian, M. Khanavi, "Chemical composition and antimicrobial activity of the essential oil of *Anthemis altissima* L. var. *altissima*", Natural Product Research, vol. 26, no. 20, pp.1931-4, 2012.

[27] Y. Nakashima, MA. Birkett, BJ. Pye, and W. Powell, "Chemically Mediated Intraguild Predator Avoidance by Aphid Parasitoids: Interspecific Variability in Sensitivity to Semiochemical Trails of Ladybird Predators", Journal of Chemical Ecology, vol. 32, pp. 1989-1998, 2006.

[28] X. Sun, X. Zhang, G. Wu, X. Li, F. Liu, Z. Xin, and J. Zhang, "n-Pentacosane Acts as both Contact and Volatile Pheromone in the tea Weevil, *Myllocerinus aurolineatus*", Journal of Chemical Ecology, vol. 43, pp. 557- 562, 2017.

[29] JR. Kuiate, JM. Bessière, PH. Zollo, SP. Kuate, "Chemical composition and antidermatophytic properties of volatile fractions of hexanic extract from leaves of *Cupressus lusitanica* Mill. from Cameroon", Journal of Ethnopharmacology, vol. 16, no.103(2), pp. 160-5, 2006.

[30] S. Kumar, R. Malhotra, D. Kumar, "*Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities", Pharmacognosy Reviews, vol. 4, no. 7, pp.58-61, 2010[a].

[31] MB. Jemia, C. Formisano, S. Bancheva, M. Bruno, F. Senatore, "Chemical composition of the essential oils of *Centaurea formanekii* and *C. orphanidea* ssp. *thessala*, growing wild in Greece", Natural product communications, vol. 7, no.8, pp. 1083-6, 2012.

[32] R. Sivasubramanian and P. Brindha, "*In-vitro* cytotoxic, antioxidant and GC-MS studies on *Centratherum punctatum* cass.", International Journal of Pharmaceutical Sciences, vol. 5, no. 3, pp. 364-367, 2013.

[33] GG. Grant, D. Frech, L. MacDonald, KN. Slessor, and King GGS. "Copulation releaser pheromone in body scales of female whitemarked tussock moth, *Orgyia leucostigma* (Lepidoptera: Lymantriidae): identification and behavioral role", Journal of Chemical Ecology, vol. 13, pp. 345-356, 1987.

[34] B. Brei, JD. Edman, B. Gerade, and JM. Clark, "Relative Abundance of Two Cuticular Hydrocarbons Indicates Whether a Mosquito Is Old Enough to Transmit Malaria Parasites", Journal of Medicinal Entomology, vol. 41, no. 4, pp. 807-809, 2004.

[35] MB. Zakariaa, Z. Vijayasekarana Ilhama, and NA. Muhamad, "Anti-Inflammatory Activity of *Calophyllum inophyllum* Fruits Extracts", Procedia Chemistry, vol. 13, pp. 218-22, 2014.

[36] YB. Köse, G. Iscan, and B. Demirci, "Antimicrobial Activity of the Essential Oils Obtained from Flowering Aerial Parts of *Centaurea lycopifolia* Boiss. et Kotschy and *Centaurea cheirolopha* (Fenzl) Wagenitz from Turkey", Journal of Essent Oil Bear Plants, vol. 19, no. 3, pp. 762-768, 2016.

[37] G. Topçu, G. Kökdil, Z. Türkmen, W. Voelter, E. Adou, and G. David, "A new Clerrodane Diterpene and Other Constituents from *Ajuga chamaepitys* ssp. *laevigata*", Zeitschrift für Naturforschung B, vol.59, pp.1, 2014.

[38] R. Devika and Justin K, "Screening and Evaluation of Bioactive Components of *Tagetes erecta* L. By GC-MS Analysis", Asian Journal of Pharmaceutical and

Clinical Research, vol. 7, no.2, pp. 58-60, 2014.

[39] A. Ponmathi Sujatha, R. Michael Evanjaline, S. Muthukumarasamy, V. Mohan, "Determination of Bioactive Components of *Barleria Courtallica* Nees (Acanthaceae) By Gas Chromatography-Mass Spectrometry Analysis", Asian Journal of Pharmaceutical and Clinical Research, vol. 10, no. 6, pp. 273-283, 2017.

[40] G. Rajeswari, M. Murugan, VR. Mohan, " GC-MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae)", Research Journal of Pharmaceutical, Biological and Chemical Sciences, vol. 3, no. 4, pp. 301-308, 2012.

[41] SN. Chandra Dev, De and D. Kantishree, MW. Khan, "GC-MS Analysis of Phytochemicals of Methanolic Extract of Leaves of *Lawsonia inermis* Linn.", Indian Journal of Medical and Pharmaceutical Sciences, vol. 3, no. 6, pp. 77-82, 2016.

[42] C. Arunmathi and T. Malarvili, "Analysis of bioactive compounds in methanol extract of *Aplotaxis auriculata* rhizome using GC-MS", Journal of Pharmacognosy and Phytochemistry, vol.6, no. 3, pp. 243-247, 2017.

[43] AVN. Paul, S. Singh, and AK. Singh, "Kairomonal effect of some saturated hydrocarbons on the egg parasitoids, *Trichogramma brasiliensis* (Ashmead) and *Trichogramma exiguum* (Hymenoptera: Trichogrammatidae)", Journal of Applied Entomology, vol.126, pp. 409-416, 2002.

[44] NM. Gumgumjee and SA. Hajar, "Antibacterial activities and GC-MS analysis of phytocomponents of *Ehretia abyssinica* R.Br. ex *fresen*", International Journal of Applied Biology Pharmaceutical Technology, vol. 6, no.2, pp. 236-241, 2015.

[45] D. Saïdana, MA. Mahjoub, O. Boussaada, J. Chriaa, I. Chéraif, Daami M, Z. Mighri, AN. Helal, "Chemical composition and antimicrobial activity of volatile compounds of *Tamarix boveana* (Tamaricaceae)", Microbiological Research, vol. 163, no. 4, pp. 445-55, 2008.

[46] B. Bakır, A. Him, H. Özbek, E. Düz, M. Tütüncü, "Investigation of the Anti-inflammatory and Analgesic Activities of β-caryophyllene", International Journal of Essential Oil Therapeutics, vol. 2, pp. 41-44, 2008.

[47] JP. Pinho, AS. Silva, BG. Pinheiro, I. Sombra, C. Bayma Jde, S. Lahlou, PJ. Sousa, PJ. Magalhães, "Antinociceptive and antispasmodic effects of the essential oil of *Ocimum micranthum*: potential anti-inflammatory properties", Planta Medica, vol. 78, no. 7, pp.681-5, 2012.

[48] Anonymous.[internet]. Availablefrom https://pubchem.ncbi.nlm.nih.gov/compound/trans-Caryophyllene, [cited 2020 April 09], 2020.

[49] KHC. Baser, M. Kürkcüoğlu, and FZ. Erdemgil, "The Essential Oil of *Ajuga bombycina* from Turkey", Chemistry of Natural Compounds, vol.37, pp. 3, 2001.

[50] MK. Swamy, G. Arumugam, R. Kaur, A. Ghasemzadeh, MM. Yusoff, and UR. Sinniah,"GC-MS. Based Metabolite Profiling, Antioxidant and Antimicrobial Properties of Different Solvent Extracts of Malaysian *Plectranthus amboinicus* Leaves", Evidence-Based Complementary and Alternative Medicine, 2017.https://doi.org/10.1155/2017/1517683.

[51] A. Yaşar, O. Üçüncü, C. Güleç, H. İnceer, S. Ayaz, and N. Yaylı, "GC-MS Analysis of Chloroform Extracts in Flowers, Stems, and Roots of *Tripleurospermum callosum*", Pharmaceutical Biology, vol. 43, no. 2, pp. 108-112, 2005.

[52] D. Gomathi, M. Kalaiselvi, G. Ravikumar, K. Devaki, C. Uma, "GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L.", Journal of Food Science and Technology, vol. 52, no. 2, pp.1212- 1217, 2015.

[53] Ö. Kılıç, "*Marrubium parviflorum* subsp. *parviflorum* Bitkisinin Yağ Asidi ve Uçucu Yağ Kompozisyonu", Mus Alparslan University Journal of Science, vol. 6, no. 1, pp. 487-491, 2018.

[54] P. Kumar, PS. Kumaravel, and C. Lilitha, "Screening of Antioxidant, Total Phenolic and GC-MS study of *Vitex negundo*", Africa Journal of Biochemistry and Research, vol. 4, no.7, pp.191-195, 2010[b].

[55] P. Kalaisezhiyen, V. Sasikumar, "GC-MS evaluation of Chemical constituents from methanolic leaf extract of *Kedrostis foetidissima* (Jacq.) Cogn.", Asian Journal of Pharmaceutical and Clinical Research, vol. 5, no. 4, pp. 77-81, 2012.

[56] M. Sermakkani and V. Thangapandian, "GC-MS analysis of *Cassia italica* leaf methanol extract", Asian Journal of Pharmaceutical Clinic Research, vol. 5, no. 2, pp. 90-94, 2012.

[57] MI. Bello, SB. Ishidi, and IY. Sudi, "Phytochemical Screening and Antimicrobial Activity of Ethanolic and Aqueous Stembark Extracts of *Boswellia dalzielli* (Hutch)", Asian Journal of Biochemical and Pharmaceutical, vol. 1, no. 3, pp. 194- 198, 2013.

[58] K.Shibula, S. Velavan, "Determination of Phytocomponents in Methanolic Extract of *Annona muricata* Leaf Using GC-MS Technique", International Journal of Pharmacognosy and Phytochemical Research, vol. 7, no.6, pp. 1251-1255, 2015.

[59] P. Powar and D. Gaikwad, "GC-MS Analysis of Bioactive Compounds of *Aegiceras corniculatum* Bark.", Indian Journal of Plant Sciences, vol. 5, no. 3, pp. 13-17, 2016.

[60] A. Omoregbeee, M. Idu, "GC-MS analysis of ethanolic extract of *Boswellia dalzieliihutch* (Burseraceae) root from Nigeria", Chemistry Research Journal, vol. 2, no. 2, pp. 33-38. 2017.

[61] P. Yamuna, P.Abirami, P. Vijayashalini, and M. Sharmila, "GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq.", Journal of Medicinal Plants Studies, vol. 5, no.3, pp. 31-37, 2017.