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Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)

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Sincerely,
Prof. Dr. Nazım ŞEKEROĞLU
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Slovak Chamomile Varieties and their Comparison of Natural Components

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Abstract

Chamomile, *Matricaria recutita* L., is one of the most important medicinal plants cultivated in the Central Europe. Primarily the dry flower anthodia, *Chamomillae Flos*, in its entirety has anti-inflammatory, antiseptic, healing, simulative, carminative, spasmolytic and sedative activity. Slovakia belongs to these European countries in which particular attention has been devoted to the research and development of chamomile in all its aspects including the breeding of this special crop. Prof. Dr. Robert Honcariv was the pioneer of medicinal plant breeding in Slovakia, and made the first selections in chamomile at the P. J. Safarik University in Kosice. The first variety '*BONA*' was registered in the National Book Variety in 1984. The first tetraploid variety '*LUTEA*' was accepted by the Slovak Central Control and Testing Institute in Agriculture in 1995. Both these varieties were from the old century. Their qualitative and quantitative parameters are very commonly inconvenient. On the present, the chamomile variety '*LIANKA*' was bred at the University of Presov, Slovakia, between the years 2008 – 2013. Currently, this variety obtains the Certificate by the Community Plant Variety Office (CPVO) in Angers, France in 2018. The GC/MS results confirm earlier reports that major volatile constituents obtained from the flower inflorescences are /-/-α-bisabolol (67.35 ± 2.82 %) and chamazulene (10.05 ± 1.04 %) and the low contents of /-/-α bisabolol oxides A and B (2.95 ± 0.32 %).

Key Words: Composition, Essential Oil, Chamomile, Quality, Sesquiterpenes, Slovakia, Varieties

1. Introduction

Chamomile, *Matricaria recutita* L., is most favored and used medicinal plant in Slovakia. Our folk saying indicates that individual should always bow when facing a chamomile plant. This respect resulted from hundred years' experience with curing in folk medicine of the country.

In former Czechoslovakia, the first recommendation for chamomile cultivation was published in the book '*Cultivation of Medicinal, Aromatic and Spice Plants*' by Dr. Vladimir Mycak in 1953. The author referred to the results of his own chamomile cultivation experience. It is very interesting information about a time comparison in days between using hand (390 days) and horsy (14 days) work per 1 ha. The seeds (2 or 3 kg) or seedlings (180,000 or 200,000 pcs.) used for a large-scale cultivation in this time. The dry flower yield per ha was determined from 400 to 600 kg. On the other hand, whole production of Czechoslovak chamomile originated in collecting the wild growing plants approximately till the half of the fifties of XX century, when large-scale cultivation began to introduce. In the first stage, the

seeds reproducing by self-seedling in country gardens in Bohemia and Moravia were firstly used for cultivation. In 1952, these seeds became the ground of the regional variety given as *Matricaria chamomilla* L., *forma culta provincialis Bohemica*, later known as the variety '*BOHEMIA*' (Jasicova and Felklova, 1979).

In Slovakia, the breeding activity became more intensified by involvement of the Department of Special Biology, P.J.Safarik University in Kosice, in the scientific project "*The Research of Drugs based on Medicinal Plants and Natural Substances*". From 1975 to 1995, under the leadership Prof. Dr. Robert Honcariv, the breeding works on varieties '*BONA*', '*GORAL*', '*NOVBONA*' and '*LUTEA*' were gradually accomplished. Also, the methods of conserving breeding of this plant material were elaborated (Salamon and Honcariv, 1994). In 2015 the new Slovak variety '*LIANKA*' was widely introduced into cultivation, which helped to supply large amounts of uniformed raw material of high quality for pharmaceutical purposes.

This study presents essential oil contents and their qualitative-quantitative composition of natural substances in two Slovakian varieties ('*LIANKA*' and '*LUTEA*'), which are cultivating under different field environments.

2. Material and Methods

A several years field experiment was initiated with a diploid variety '*LIANKA*' and tetraploid variety '*LUTEA*' at 2 localities in the Slovakia (Tab. 1): Trebisov (GPS: N 48° 38' 01"; E 21° 43' 02"; altitude: 109 m) – a warm climatic region of the Lowlands in East Slovakia with mildly acid black fertile soil having a high content of phosphorous, potassium, and magnesium; and Plavnica (GPS: N 49° 16' 28"; E 20° 46' 50"; altitude: 530 m) – with a mildly warm climatic montane region with neutral soil and good fertility No fertilizers were added to any of the soils at the experimental sites.

The essential oil was isolated from dry flower anthodia by repeated hydro-distillation (from 6 to 8 times depending on sample size) and the weight of oil was determined gravimetrically (Humphrey, 1992; Read, 1992). The modified distillation apparatus by Coocking & Middleton were used (9th ed. Ph. Eur., 2018).

The essential oil components were characterized using a GC-MSD system on a Varian 450-GC instrument together with a Varian 220-MS with Split-Splitless injection port, MSD detector. Two columns were used: RX-5MS (non-polar), 30 m × 0.25 mm with an inside diameter: 0.25 µm, carrier gas: helium (21 psi) with a flow rate of 1.50 ml.min-1 and a BPX-5MS column (polar), 50 m long with an inside diameter of 0.25 mm and a thickness stationary phase 0.25 µl. Temperature program: 50 °C - 0 min.; 3 ° C.min^{-1} to 250 °C; 250 °C - 15 min.. The identification of the individual components of the essential oil was made using the retention times of 40 authentic component standards supplied by the companies: Extrasynthese, Merck, Fulka and Sigma-Aldrich, Kovat's indexes (used C_5-C_{22} alkanes) and NIST 14 integrated library (version 2014). The spectra of the individual constituents of essential oil were compared to mass spectra for using literature (Adams, 2007).

Several statistical methods and biometric parameters (Palaniswamy, 2006) were used to evaluate biological material (n = 6, 8) and chemical identification results: arithmetic mean, weighted arithmetic mean, standard deviation, mean error, and confidence interval after transforming the percentage by angular transformation.

3. Results and Discussion

The aim of Slovakian's breeding program and requirements of new chamomile variety were: high content of active substances (essential oil, /-/-α-bisabolol, chamazulene), sufficient yield of dry flower drug,

morphological traits required for the harvest mechanization (increase of flower heads size and their number, synchronous flowering, long pedicle with leaves, high tubular flower frequency) and resistance to abiotic stress (drought and flood). For achievement of success in regard to the variety '*LIANKA*', it was chosen conventional breeding methods – selection and examination of posterity of selected plant by method of "the middle seedbed". In the case of individual selection there is necessary to take into account that it is the population of each other pollinating heterozygotes. Repeated long time lasting selection extreme individuals causes progressive decay of primary population to families (Rod, 1982).

The contents of chamomile oil isolated from samples of dry flowers grown in Trebisov (variety '*LIANKA*') and in Plavnica (variety '*LUTEA*') confirmed intervals of quantities ranging from 0.60 ± 0.20% to 0.64 ± 0.25% (Table 1).

The range of the confidence interval of the essential oil content shows that there are no significant differences between both varieties. Chamomile variety '*LUTEA*', as tetraploid plants, must present higher content essential oil, from 1.0 to 1.2 %, into the dry raw-material (Oravec Sr. and Oravec Jr., 2007). In regard to results from the field experiment this respect has not been validated.

Table 1. Geographic coordinates of experimental fields and content of essential oil in chamomile flowers

Locality / Variety	Geographic coordinate system Latitude / Longitude	Altitude above sea level	The yield of essential oil [%]
Trebisov variety '*LIANKA*'	N 48° 38' 01" E 21° 43' 02"	109 m	0.64 ± 0.20
Plavnica variety '*LUTEA*'	N 49° 16' 28" E 20° 46' 50"	530 m	0.60 ± 0.25

Full-scale of essential oil GC-MS profiles showed the identified 75 respectively 68 chemical parties in the essential oil isolated from the dry flowers both varieties (Table 2).

For the identification of individual chemical types, we can select only the main essential oil components, which are important not only for therapeutic properties but also for industrial processing in the pharmaceutical and cosmetic industries.

Dry chamomile flower heads of the variety '*LIANKA*', which were collected in 2018, contained in their essential oil the main components – /-/ - α-bisabolol: 67.35 ± 2.82 %, chamazulene: 10.05 ± 1.04 %, /-/ - α-bisabolol oxide A: 1.11 ± 0.28 %, /-/-α-bisabolol oxide B: 1.84 ± 0.32 %, cis-en-in-dicycloether: 8.88 ± 1.47 % and α-farnesene: 2.13 ± 0.44 % .

The essential oil of variety '*LUTEA*' involved /-/ - α-bisabolol: 42.53 ± 1.51 %, chamazulene: 18.34 ± 0.74 %, /-/ - α-bisabolol oxide A: 1.45 ± 0.31%, /-/ - α-bisabolol oxide B: 4.80 ± 0.81 %, cis-en-in-dicycloether: 20.55 ± 0.77 % and α-farnesene: 3.68 ± 0.53%. In regard to the results of essential oil composition, both varieties belong to the bisabolol chemotype (Lawrence and Reynolds, 1987); of course the variety '*LIANKA*' has higher content of /-/-α-bisabolol.

Table 2. Full-scale of GC-MS essential oil profiles of the variety '*LUTEA*' and '*LIANKA*'

			% area	
Natural components	***Rt* *[min.]**	**Kovat's Index**	**'*LUTEA*'**	**'*LIANKA*'**
trans-2-hexanal	10.28	850	0.05	0.12
tricyclene	10.35	885	0.07	0.14
α-pinene	13.44	933	0.18	0.10
camphene	14.42	941	0.05	0.17
sabinene	15.44	974	0.06	0.17
β-pinene	16.01	981	0.07	0.13
β-myrcene	17.49	986	0.05	0.08
p-cymene	18.36	1031	0.07	0.11
limonene	18.58	1034	0.08	0.13
cis-β-ocimene	19.04	1035	0.09	0.07
trans-β-ocimene	19.06	1038	0.06	0.15
cis, cis-allo-ocimene	19.19	1047	0.13	0.11
dihydro tageton	19.28	1061	-	0.06
γ-terpinene	19.36	1064	0.30	0.29
terpinolene	19.90	1093	0.10	0.12
nerol	19.98	1245	0.14	0.13
methyl geranate	20.03	1336	0.07	0.42
methyl acetate	20.46	1344	0.19	0.05
carvacryl acetate	20.49	1347	0.05	0.07
germacrene β	20.51	1349	0.05	0.07
neryl acetate	20.56	1371	-	0.04
α-copaene	20.69	1395	0.07	0.15
decanone	21.81	1406	0.07	0.03
decanoic acid	23.00	1413	0.51	0.35
longifolene	23.21	1440	0.05	0.11
β-caryophyllene	23.59	1443	0.25	0.04
α-guaiene	23.76	1452	0.06	0.08
trans-β-fernesene	23.91	1464	0.33	0.10
aromadendrene	24.68	1467	0.35	0.35
ε-cadinene	24.73	1475	0.17	0.13
α-caryophyllene	24.79	1481	0.21	0.17
allo-aromadendrene	24.96	1485	-	0.03
(ε,ε)-farnesyl acetate	25.01	1492	0.04	0.09
bicyklogermacrene	25.13	1499	0.12	0.08
germacrene D	25.21	1506	0.08	0.14
γ-muurolene	25.49	1509	0.05	0.27
α-farnesene	**25.83**	**1514**	**3.68**	**2.13**
α-muurolene	26.78	1520	0.04	0.29

α-bulnesene	27.11	1526	0.05	0.16
γ-cadinene	27.91	1540	0.05	0.04
calamenene	27.99	1545	0.01	0.06
α-acoradiene	28.02	1553	0.12	0.14
cadina-1,4-diene	28.24	1557	0.13	0.11
δ-cadinene	28.36	1564	0.62	0.13
α-amorphene	28.42	1566	0.08	0.07
α-calacorene	28.54	1568	0.09	0.11
trans-nerolidol	28.60	1571	0.09	0.40
epiglobulol	28.73	1585	0.06	0.12
junenol	28.81	1591	0.08	0.11
spatulenol	28.87	1602	0.48	0.09
β-eudesnol	29.07	1606	0.10	0.13
globulol	29.53	1610	0.15	0.11
tremetone	29.84	1612	0.12	0.09
α-bisabolon oxide A	30.26	1615	0.15	0.15
viridiflorene	30.29	1625	0.04	0.05
dillapiole	30.42	1642	0.03	0.08
cubebol	30.50	1655	0.07	0.06
β-bisabolol	30.56	1660	1.66	0.13
τ-muurolol	30.61	1668	0.15	0.11
α-bisabolol oxide B	**30.69**	**1678**	**4.80**	**1.84**
bulnesol	30.89	1683	0.01	0.03
valerianol	31.02	1687	-	0.01
α-farnesol	31.39	1691	0.08	0.07
α-bisabolone oxide A	32.27	1702	0.23	0.12
cadalene	33.13	1705	-	0.04
α-bisabolol	**33.52**	**1710**	**42.53**	**67.35**
chamazulene	**42.92**	**1772**	**18.34**	**10.05**
α-bisabolol oxide A	**44.20**	**1782**	**1.45**	**1.11**
guaiazulene	45.36	1806	0.14	0.06
(ζ, ε)-farnesyl acetate	46.09	1842	-	0.01
cis-en-in-dicykloether	**46.57**	**1928**	**20.55**	**8.88**
trans-en-in-dicykloether	47.99	1940	0.04	0.14
hexadekanoic acid	53.34	1965	0.08	0.22
eicosane	54.97	1999	-	0.03
total			**100.00**	**100.00**

*Rt *-retention times*

Dry chamomile flower heads of the variety '*LIANKA*', which were collected in 2018, contained in their essential oil the main components – /-/ - α-bisabolol: 67.35 ± 2.82 %, chamazulene: 10.05 ± 1.04 %, /-/ - α-bisabolol oxide A: 1.11 ± 0.28 %, /-/-α-bisabolol oxide B: 1.84 ± 0.32 %, cis-en-in-dicycloether: 8.88 ± 1.47 % and α-farnesene:

2.13 ± 0.44 %. The essential oil of variety '*LUTEA*' involved /-/ - α-bisabolol: 42.53 ± 1.51 %, chamazulene: 18.34 ± 0.74 %, /-/ - α-bisabolol oxide A: 1.45 ± 0.31%, /-/ - α-bisabolol oxide B: 4.80 ± 0.81 %, cis-en-in-dicycloether: 20.55 ± 0.77 % and α-farnesene: 3.68 ± 0.53%.

In regard to the results of essential oil composition, both varieties belong to the bisabolol chemotype (Lawrence and Reynolds, 1987); of course the variety '*LIANKA*' has higher content of /-/-α-bisabolol. According to Table 3 the chamomile essential oils after a laboratory hydro-distillation were presented the highest levels of the precursor sesquiterpenes –/-/-α-bisabolol and chamazulene (70.27 respectively 84.50 %), ethers – cis- and trans-en-in-dicykloethers (9.02 respectively 20.59 %), sesquiterpene oxides (3.22 respectively 6.63) and level of monoterpenes were observed very low (≥ 4.0 %).

Table 3. Groups of natural components [%] identified in essential oil of '*LUTEA*' and '*LIANKA*' varieties

Group and compounds/Slovakian varieties	'*LUTEA*'	'*LIANKA*'
Monoterpenes (chiefly: α-pinene, β-pinene, β-myrcene, *p*-cymene, limonene, ocimenes, methyl acetate, decanoic acid)	2.37	3.13
Monoterpenes alcohols (chiefly: nerol)	0.14	0.13
Ether (chiefly: cis-en-in-dicycloether, trans-en-in-dicycloether)	20.59	9.02
Sesquiterpenes (chiefly: α-bisabolol, chamazulene)	70.27	84.50
Sesquiterpene oxides	6.63	3.22

Based on the study of pharmacodynamics properties, /-/-α-bisabolol and chamazulene are considered to be the most valuable constituents of this plant species (Isaac, 1979). Formulation several liquid and ointment preparations were standardized to contain essential oil, high contents of both natural substances (Mann and Staba, 1986). These formulations were used on support of the skin's ability to repair it, strengthen the tissues of the mouth's natural defense, sooths the throat and airways, and have a calming effect on the skin and digestive system (Shilcher, 2004).

4. Conclusion

Breeding efforts were successful in developing the chamomile diploid variety '*LIANKA*' of better essential oil composition than tetraploid variety '*LUTEA*'. The total oil content and /-/-α-bisabolol content of oil were increased. The variety has plant populations, which are characterized by good yield, resistance to stress, bigger flower heads, and sufficient amount of essential oil of appropriate chemical composition. Cultivated raw material has totally displaced wild collection of chamomile flower heads. The chamomile variety '*LIANKA*' and its large-scale cultivation, extraction and distillation is very forward raw-material to the today's pharmaceutical industry.

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Conflict of Interest

I may declare that I have not any conflicts of interest.

References

1. Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th ed. , Allured Pub Corp: New York, NY, USA, 823 p., ISBN: 978-1932633214
2. *European Pharmacopoeia* (Ph. Eur.), 2018, 9th ed. Strasbourg, Council of the Europe Union, 5825 p.
3. Humphrey, A.M., 1992. Observation on esential oil distillation in the laboratory. In: The 23rd International Symposium on Essential Oils, Ayr, Scotland, UK, pp. IV-2
4. Isaac, O., 1979. Pharmacological investigation with compounds of chamomile * I. On the pharmacology /-/-α-bisabolol and bisabololoxides (Review). Planta Medica, Vol. 35, pp. 118-124
5. Jasičová, M., Felklová, M., 1976. Vliv oblasti púvodu na kvalitu úboru *Matricaria chamomilla* L. (*in Slovak*). Farmaceutický obzor. Vol. XLV, s. 317-322
6. Lawrence, B.M. and Reynolds, A.C., 1987. Progress in essential oils. Perfumer & Flavorist, Vol. 12, pp. 35-55
7. Mann, C. and Staba E. J., 1986: The Chemistry, Pharmacology and Commercial Formulation of Chamomile. In: L. E. Craker and J. E. Simon (eds.) Herbs, Spices, and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology. Vol.1, Phoenix, AZ: Oryx Press. pp. 235-280
8. Mycak, V., 1953. Pestovanie liečivých, aromatických a koreninových rastlín (*in Slovak*). 1. Vyd., Štátne pôdohospodárske nakladateľstvo: n.p. Praha, závod Bratislava, PIO 105100/52-III/1., 249 p.
9. Oravec, V., Sr., Oravec, V., Jr. 2007. Breeding of bisabolol diploid and tetraploid varieties of chamomile in Slovakia. Acta Horticulturae, 2007, No. 749, pp. 115-121
10. Palaniswamy, U.R. – Palaniswany, K.M.: 2006. *Handbook of Statistics for Teaching and Research in Plant and Crop Science*. 1st ed. , Food Products Press and The Haworth Reference Press, New York, London, Oxford. 624 p. ISBN: 978-1-56022-293-4
11. Read, Ch., 1992. Essential Oils. Focus on Herbs. Vol. 9, p. 2-8
12. Rod, J., 1982. *Šlechtění rostlin* (*in Czech*). 4. vyd., Státní zemnědelské nakladatelství : Praha. 368 s., 07-062-02
13. Salamon, I. and Honcariv, R., 1994. Growing Condition and Breeding of Chamomile (*Chamomilla recutita* (L.)Rauschert) Regarding the Essential Oil Qualitative-Quantitative Characteristics in Slovakia. Herba Polonica, Vol. XL, Iss. 1, pp. 69-74
14. Schilcher, H., 2004. *Wirkungweise und Anwendungsformen der Kamillenblűten – Handbuch fűr Apotheker, Ärzte, Heilpraktiker und weitere Heilberufe sowie fűr Medizin-, Pharmazie- und Biologiestudenten* (*in German*). BMV Berliner Medizinische Verlagsanstalt GmbH, 160 s., ISBN: 3880400067.

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Characterization of *Origanum vulgare* SUBSP. *hirtum* (LINK) Iestwaart Population and Determination of A Clones

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Abstract

Identification of the high yielding and the good quality clones of one population (B population) of *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart was one of the purposes in this study and furthermore some agronomic and quality characteristics of individual plants were examined during two years (2014-2015). Annual performances were determined according to the minimum and maximum values of the first, second and non-flowering harvests of the population. Also for second year, minimum, maximum and mean values of the individual plants of the population were stated in the paper. The aim was to reveal if the high yield and quality characteristics of plants are based on the genotype. Individual plants of the B population, the mean plant height was found as 39 cm, canopy value as 30.5 cm, Fresh herb yield as 167.9 g/plant, Drog herb yield as 65.5 g/plant, Drog leaves yield as 32.2 g/plant and the mean rate of the essential oil as 4,01%. Considering the two years' results of the B population, A clones were created in 2016 by selecting the high yielding and of good quality genotypes. On A clones, the plant height, fresh herb yield, Drog herb yield, essential oil rate and essential oil yield values were determined between 13-52.5 cm, 8-624 g/plot, 4-218.4 g/plot, 1.6-100.1 g/plot, 1.08-7.92 % and 0.03-5.74 L/parcel, respectively.

Key Words: *Origanum vulgare* subsp. *hirtum*, Population, Selection, Yield, Essential Oil, Compound

1. Introduction

The importance of the medicinal plants and herbs increases day by day. In Turkey, a great majority of these plants are being picked up from the nature; consequently, environmental destruction and the loss of some endemic species are inevitable. The primary of these plants is oregano which is being picked from the flora and has a crucial role in export. In Turkey, the genus involving different types of oregano are *Origanum, Thymbra, Thymus, Coridothymus* and *Satureja*. The species which are exported most and used in the production of essential oils are *Origanum onites, Origanum vulgare* subsp. *hirtum*, *Origanum minutiflorum, Origanum majorana* and *Origanum syriacum* var. *bevanii*. Beside these, other traded species are *Coridothymus capitatus, Thymbra spicata, Thymbra sintenissii, Satureja cuneifolia*, *Satureja hortensis, Satureja montana, Satureja spicigera* and *Thymus eigii'*. The common quality of all these species

is containing essential oils and the main components of their essential oils are Carvacrol or Thymol (Başer, 2001; Hayta and Arabacı, 2011). Recently, Lukas et al. (2013) indicated that *Origanum dubium* and *Origanum majorana* should be better classified as different species even though they are morphologically similar. Marjoram, which has been known as *O. majorana* so far, actually belongs to the species *O. dubium*. In Turkey, the most valuable oregano species belong to the genus *Origanum* in economical and agricultural fields.

In Turkey, approximately 80% of the oregano which is still exported is cultivated under field conditions, 20% is picked up from the nature (Bayram et al. 2010). Oregano picked up from the nature belong to the various genuses and species such as *Coridothymus capitatus, Thymbra spicata, Origanum onites*, *Origanum syriacum*, *Origanum majorana*, *Origanum minutiflorum* and *Origanum vulgare* subsp*. hirtum* (TSI, 2004). Cultivated oregano species are *Origanum onites* and *Origanum vulgare* subsp. *hirtum* which are mainly grown in Marmara, Mediterranean and Aegean Regions.

This study is done with the purpose of the characterization of selected promising plants at the B clone of *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart and the development of new types with higher Drog yields and essential oil rates using the clone selection method.

2. Material and Method

The seeds of B population is used in study that is one of the five promising populations (A,B,C,D and E). All populations were selected from the *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart plants. The study carried out in Aydın ecological conditions. The B population is originated from the south east exposure of the Ida Mountains with the altitude of 559 m and the mapping coordinates of 545202-4423405.

The field study of the experiment was carried out on the area of the Research and Practice Farm of the Adnan Menderes University, Faculty of Agriculture. The individual plants were investigated during the years of 2013-2014 and 2014-2015 and A clones were cultivated during the year 2016. Study field has typical Mediterranean climate conditions, the total precipitation of long years is 636,7 mm and the average temperature is 17,7^{o}C. The soil texture of the study area was sandy-loam, pH value is 8.43 and the ratio of the organic substances were 1.30%.

The seeds of the B population were sown in 06/12/2013 on the seedbeds in a greenhouse and the germination was observed in 17/12/2013. When plants have proper maturity (8-10 cm seedlings), to create the individual plants, 648 seedlings were planted to an area of 129.6 m^2 with a planting level of 50x40 cm in 02/04/2014. The flowering individual plants of the population were harvested in 10-18/07/2014 and in 10/09/2014 during the first year. Some plants were harvested once and some of them were harvested twice. While the plants were harvesting (10-18/07/2014 and 10/09/2014), some of the individual plants of population B haven't flowered so the harvesting could not be performed on these plants. To determine the annual growth and yield performance of the individual plants for the first year, just after identifying the morphological characteristics of the plants without taking the flowering or nonflowering into account they were harvested between the dates of 14-20/10/2014 and 04-07/11/2014. During the harvest between these dates even some plants have reached to the maturity for a third harvest. In order to determine the individual plants those have good quality features, observations and measurements were repeated for two years. The harvestings of the second year were done on the dates 24/06/2015 and 07/07/2015.

The analyses of the essential oil were performed in the Medicinal Plants Laboratories of Adnan Menderes University and Ege University Agriculture Faculties. The essential oil rates of the study were determined volumetrically on the air dried leaves samples using the Neo Clevenger apparatus. The essential oil rates are stated as milliliter /100 g (%) on air dried leaves (Wichtl, 1971).

Among the plants on the field, the promising individual ones were identified from B popultions to form A clones considering the results of the yield of leaves and essential oil of two years and The superior 140 genotypes were selected.

From 140 selected genotypes, for each genotype the 10 cuttings planted in the greenhouse between 03-05/12/2015. Nevertheless, upon determining that four genotypes are not suitable for the vegetative reproduction so final number of the genotypes planted on the field was 136. After rooting of cuttings, between dates of 06/04/2016 and 07/04/2016, the A clone cuttings were planted to the 2 m^2 plots with a planting density of 50 x 40 cm and 10 plants belonging to the each row. So the A clones were created. On the third year of the trial, all the crop care was performed properly on the A clones. The observations and measurements were done firstly and after that the first and last plants of each clone were left as the border effect and the remaining plants were harvested on 27/06/2016.

The essential oil components were determined with GC-MS (Shimadzu 2010 Plus QP-5050 Quadrapole Detector) at the SDU Experimental and Observational Research and Application Center. GC-MS operating conditions were carried out as follows: CP-Wax 52 CB (50 m x 0.32 mm, 0.25 µm) capillary column was used, column temperature was initially 60°C, then gradually increased to 220°C at 10°C/min and waited for 10 minutes at 220 °C. The total analysis time was 60 minutes, the injector temperature was 240°C and the detector temperature was 250°C. Helium (20 mL/min, split 1:20) gas was used as the carrier gas. 7.5 µl of the sample was diluted by adding 1500 µl of dichloromethane. Wiley, Nist, Tutor, FFNSC libraries were used to identify the essential oil components.

The findings were evaluated statistically using the SPSS (SPSS 17.0 2008) and TOTEMSTAT (Acikgoz et al., 2004) programs.

3. Results

The statistical findings of the two years (2013-2015 growth years) which belong to the individual plants of the B population of *O. vulgare* subsp. *hirtum* (Link) Iestwaart grown under Aydın ecological conditions and A clones created from these population were stated below;

3.1. Individual plants

Within 648 individual plants, 581 plants were reached maturity for harvest. The first year of the study, flowering was viwed on the 208 plants of the B population and the harvesting was done for those individual plants. Some statistical analyses of the characteristics belonging to the first harvest of the B population are shown on Table 1. According to the mean values, plant height, canopy value, Fresh herb yield, drog herb yield, drog leaves yield and essential oil rate have been determined as 35.3, 17.8, 56 g/plant, 21 g/plant, 14. 6 g/plant and 4.15%, respectively. It was observed that after the first harvest, some of the individual plants of the B population kept growing and some plants have had second flowering so the second harvest were made with those 11 flowering individual plants. The plant height of the harvested plants was 28-67 cm, canopy value 20-36, fresh herb yield 8-83 g/plant, drog leaves yield 3.95- 47.58 g/plant and the essential oil rate varied between 3-4.7% (Table 2).

Table 1. Statistical values for the first year (2014) I. harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Chracteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	208	14.000	50.000	35.327	50.7235	7.1220	0.4938	20.1604
Canopy Value (cm)	208	3.000	45.000	17.827	51.6704	7.1882	0.4984	40.3222
Fresh Herb Yield (g/plant)	208	12.000	185.000	55.966	776.5931	27.8674	1.9323	49.7932
Drog Herb Yield (g/plant)	208	2.000	90.000	21.000	144.7826	12.0326	0.8343	57.2979
Drog Leaves Yield (g/plant)	208	1.110	42.240	14.636	58.5139	7.6494	0.5304	52.2653
Essential Oil Rate (%)	208	1.100	7.300	4.147	1.2583	1.1217	0.0778	27.0502

Table 2. Statistical values for the first year (2014) II. harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	11	28.000	67.000	44.273	178.4182	13.3573	4.0274	30.1706
Canopy Value (cm)	11	20.000	36.000	29.182	38.5636	6.2100	1.8724	21.2802
Fresh Herb Yield (g/plant)	11	17.000	200.000	97.091	4961.0909	70.4350	21.2370	72.5454
Drog Herb Yield (g/plant)	11	8.000	83.000	40.455	859.0727	29.3099	8.8373	72.4515
Drog Leaves Yield (g/plant)	11	3.950	47.580	24.239	311.8603	17.6596	5.3246	72.8557
Essential Oil Rate (%)	11	3.000	4.700	3.700	0.3000	0.5477	0.1651	14.8033

When table 3 was examined, it was seen that 530 individual non-flowering plants were harvested in the first year. On this harvest, plant height varied between 12-67 cm and the mean value has been 32.4 cm. The minimum and maximum canopy values were 20 cm and 73 cm, respectively and the mean canopy value was found as 44.7 cm. The yield of the fresh herb varied between 20-699 g/plant and the mean yield was 229.85. Minimum drog herb yield was 4 g/plant, maximum herb yield was 262 g/plant and the mean yield has been 88.75 g/plant whereas yield of drog leaves varied between 3.3-119.79 g/plant and the mean leaves yield has been found as 41.13 g/plant. Minimum and maximum essential oil rates of the plants were 0.2% and 2.5%, respectively and the mean value was stated as 1.26%.

Table 3. Statistical values for the first year (2014) non flowering harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	530	12.000	67.000	32.389	93.7050	9.6801	0.4205	29.8874
Canopy Value (cm)	530	20.000	73.000	44.740	78.4992	8.8600	0.3849	19.8034
Fresh Herb Yield (g/plant)	530	20.000	699.000	229.847	11591.6609	107.6646	4.6767	46.8418
Drog Herb Yield (g/plant)	530	4.000	262.000	88.745	1679.1430	40.9773	1.7799	46.1740
Drog Leaves Yield (g/plant)	530	3.300	119.790	41.132	395.9682	19.8989	0.8644	48.3780
Essential Oil Rate (%)	530	0.200	2.500	1.264	0.1375	0.3708	0.0161	29.3280

Table 4. Statistical values for the second year (2015) harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	581	9.000	85.000	40.738	219.2107	14.8058	0.6142	36.3435
Canopy Value (cm)	581	7.000	55.000	30.306	94.7715	9.7351	0.4039	32.1222
Fresh Herb Yield (g/plant)	581	7.000	925.000	208.098	25474.2093	159.6064	6.6216	76.6977
Drog Herb Yield (g/plant)	581	3.000	407.000	80.972	4027.9406	63.4661	2.6330	78.3798
Drog Leaves Yield (g/plant)	581	1.620	170.700	37.791	751.8826	27.4205	1.1376	72.5580
Essential Oil Rate (%)	581	1.800	8.500	4.991	0.9451	0.9722	0.0403	19.4777

On the second year of the study, 581 individual plants were harvested. Some statistical values of the second year are shown on table 4. The plant height varied between 9-85 cm and the mean value was determined as 40.7 cm. The mean canopy value was 30.3 cm, minimum and maximum values have been 7 and 55 cm, respectively. The mean values of fresh herb yield, drog herb yield and drog leaves yield has been 208.1 g/plant, 80.97 g/plant and 37.79 g/plant, respectively. The minimum essential oil rate was 1.8 % whereas the maximum value was 8.5 % and the mean value was 4.99% (Table 4).

Table 5. Mean harvest values (2014-2015) of the individual plants of B population *Origanum vulgare* subsp. *hirtum* for two years

Characteristics	1.Year I. Harvest	1.Year II. Harvest	1.Year Non Flowering Harvest	1.Year Mean Harvest Values	2.Year Harvest	Mean Values for Two Years
Plant Height (cm)	35.327	44.273	32.389	37.330	40.738	39.034
Canopy Value (cm)	17.827	29.182	44.740	30.583	30.306	30.445
Fresh Herb Yield (g/plant)	55.966	97.091	229.847	127.635	208.098	167.867
Drog Herb Yield (g/plant)	21.000	40.455	88.745	50.067	80.972	65.520
Drog Leaves Yield (g/plant)	14.636	24.239	41.132	26.669	37.791	32.230
Essential Oil Rate (%)	4.147	3.700	1.264	3.037	4.991	4.014

The mean values of the two years of the examined characteristics of B population were shown on table 5. The plant height was found as 39 cm, canopy value as 30.4 cm, fresh herb yield as 167.9 g/plant, drog herb yield as 65.5 g/plant, drog leaves yield as 32.2 g/plant and the mean rate of the essential oil has been 4%.

Table 6. Statistical values of some characteristics of A clone belonging to the B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	136	13.000	52.500	33.567	85.233	9.232	0.792	27.504
Fresh Herb Yield (g/plant)	136	8.000	624.000	186.965	22655.392	150.517	12.907	80.506
Drog Herb Yield (g/plant)	136	4.000	218.400	68.660	2706.207	52.021	4.461	75.767
Drog Leaves Yield (g/plant)	136	1.600	100.100	31.689	423.865	20.588	1.765	64.968
Rate of Leaves-Stem (%)	136	20.800	80.000	52.056	183.635	13.551	1.162	26.032
Essential Oil Rate (%)	136	1.080	7.920	4.529	1.674	1.294	0.111	28.568
Essential Oil Yield (g/plot)	136	0.030	5.740	1.534	1.286	1.134	0.097	73.942

Table 7. Essential oil Components of A clones in B Population (%)

Component Names and Ratios (%)										
Clone No	**TCC * (Quantity)**	**α-Pinene**	**2- β-Pinene**	**β-Myrcene**	**α-Terpinene**	**ρ-Cymene**	**γ-Terpinene**	**Thymol**	**Carvacrol**	**Essential Oil Ratio (%)**
B-4	11	0.85	1.53	-	-	3.55	14.44	0.04	76.91	6.83
B-20	12	1.45	2.14	-	1.54	3.68	11.32	0.16	76.64	6.25
B-70	16	0.37	1.06	-	0.74	2.93	6.36	0.12	84.45	5.50
B-102	13	-	1.64	-	1.32	3.47	13.59	0.28	76.52	6.92
B-183	18	0.62	0.04	1.18	0.89	2.80	5.99	0.07	83.48	5.75
B-201	15	1.29	-	1.55	0.70	3.42	3.85	0.17	84.95	5.75
B-296	14	1.16	-	1.43	0.87	3.26	3.72	0.16	86.02	5.50
B-342	11	2.80	-	2.53	2.72	8.54	16.67	0.71	62.17	5.75
B-345	15	1.39	2.09	-		3.87	13.25	0.02	75.97	6.00
B-368	14	0.93	-	1.43	1.21	3.30	9.67	0.11	80.89	7.25
B-413**	10	0.19	-	-	2.72	1.51	0.13	-	92.14	5.13
B-414	12	0.77	1.14	-	0.80	3.24	5.21	0.03	86.01	5.42
B-417	12	0.60	1.35	-	-	3.31	6.70	0.08	83.90	6.33
B-423	13	0.84	1.10	-	0.83	3.08	4.41	0.05	87.00	5.46
B-430	15	0.60	-	1.12	1.11	2.87	8.77	0.03	83.12	5.58
B-446	50	0.47	-	1.14	0.56	2.54	2.73	0.03	86.92	6.33
B-447	16	0.49	1.20	-	0.55	1.92	3.08	0.11	88.50	6.67
B-458	12	0.41	-	0.78	-	2.68	3.11	0.16	89.92	5.25
B-466	8	3.27	3.66	-	-	-	21.51	0.21	69.99	5.92
B-575	15	1.45	2.08	-	-	4.68	13.26	0.04	74.06	5.33

* Total Components Contents = TCC
** The B-413 clone also contains 2.05% Alloaromadendrene.

3.2. A clones

The findings of the variance, standard deviation, mean values and coefficient variation of the A clone were shown on table 6. According to the results of the statistical analyses, the minimum and maximum values have been determined for plant height 13-52.5, for fresh herb yield 8-624 g/plot, for drog herb yield 4-218.4 g/plot, for drog leaves yield 1.60-100.1 g/plot, for the ratio of leaf-stem 20.8-80%, for the essential oil rate 1.08-7.92% and for the essential oil yield 0.03-5.74 g/plot. On the other hand, coefficient variation has been found significantly high for the characteristics of fresh herb yield, drog herb yield, drog leaves yield and essential oil yield.

From each A clones, 20 clones have the maximum values or higher values than the average for the characteristics of fresh herb yield, drog herb yield, drog leaves yield and essential oil rate were selected for the further research.

4. Discussion

According to the years, some growth variations are observed on the individual plants. Second year of plant development, root system develop better and vegetation period is longer so an increased yield could be expected.

A wide variation was identified among the examined B population. A similar situation was stated on the studies of Ceylan et al. (2003) examined yield potential and essential oil composition of *Origanum onites* L. clones and individual plants and Arabacı et al. (2012) who studied on the ontogenetic variability of *Coridothymus capitatus* L. genotypes. Our results are in accordance with

the findings of the researchers. While the plant height values of the study are similar to the values of Žukauska (2001) as 47-53 cm from nature, 32-93 cm cultivated, Oflaz et al. (2002) as 75-80 cm, W'glarz et al. (2006) as 54-68 cm and Ahmad et al. (2008) as 25.45-55.58 cm. Our maximum plant height values are close to the cultivated plant heights of Žukauska (2001).

Žukauska (2001) obtained a fresh herb yield of 82-915 g/m^2 from the natural area of Latvia, fresh herb yields of cultivated population of this plants have been for the first, second and third years 113-1050 g/m^2, 125-2135 g/m^2 and 516-2414 g/m^2, respectively. Our fresh herb yields have similarity to the results of the researcher.

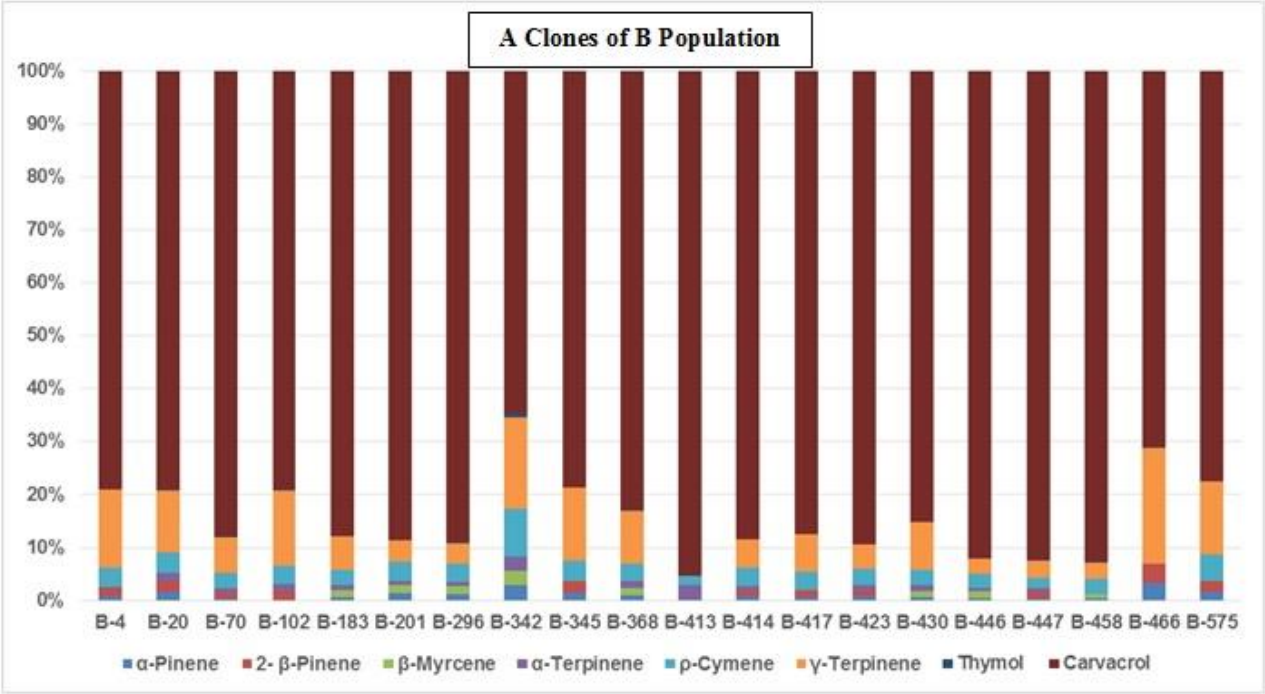

Figure 1. Distribution of Essential Oil Components of A Clones for Population B (%)

Leto and Salamone (1996) reported that the drog herb yield of the natural population varies between 105-1100 g/plant. W'glarz et al. (2006) stated that the values for this characteristic are between 243.1-636.6 g for a single plant. While the findings we obtained from our research are in the range of the findings of W'glarz et al. (2006), they have low values compared to the maximum values of Leto and Salamone (1996).

Kokkini (1996) reported that the essential oil yield of *O. vulgare* subsp. *hirtum* samples from different locations were between 1-1.6 ml/100g. Tınmaz et al. (2002) determined the quality characteristics of *O. vulgare* subsp. *hirtum* from various cities of Marmara Region and they observed that the essential oil rate increases when the plants are transferred to a cultivated area from nature. Study also has mentioned that the maximum essential oil yield was 12.3 kg/da. Said-Al Ahl et al. (2009) indicated that the essential oil rate is between 0.070-0.072 ml/plant. Our findings are in accordance with the results of Said-Al Ahl et al. (2009).

Tınmaz et al. (2002) identified that the essential oil rate varies between 1-6.1% on the *O. vulgare* subsp. *hirtum* samples picked up from the nature, whereas the natural plant samples of Leto and Salomone (1996) include 4% essential oil. Başer (2001) found a range for the essential oil rate between 1-7% and

Oflaz et al. (2002) found 3.6-4.4 %. They reported a value of 3.9% for a plant sample from Kaz Mountains. Veres et al. (2003) specified the essential oil rate of *O. vulgare* subsp. *hirtum* as 4.3 %. Oil rate of an individual plant was reported between 1.1-3.2% by W'glarz et al. (2006).

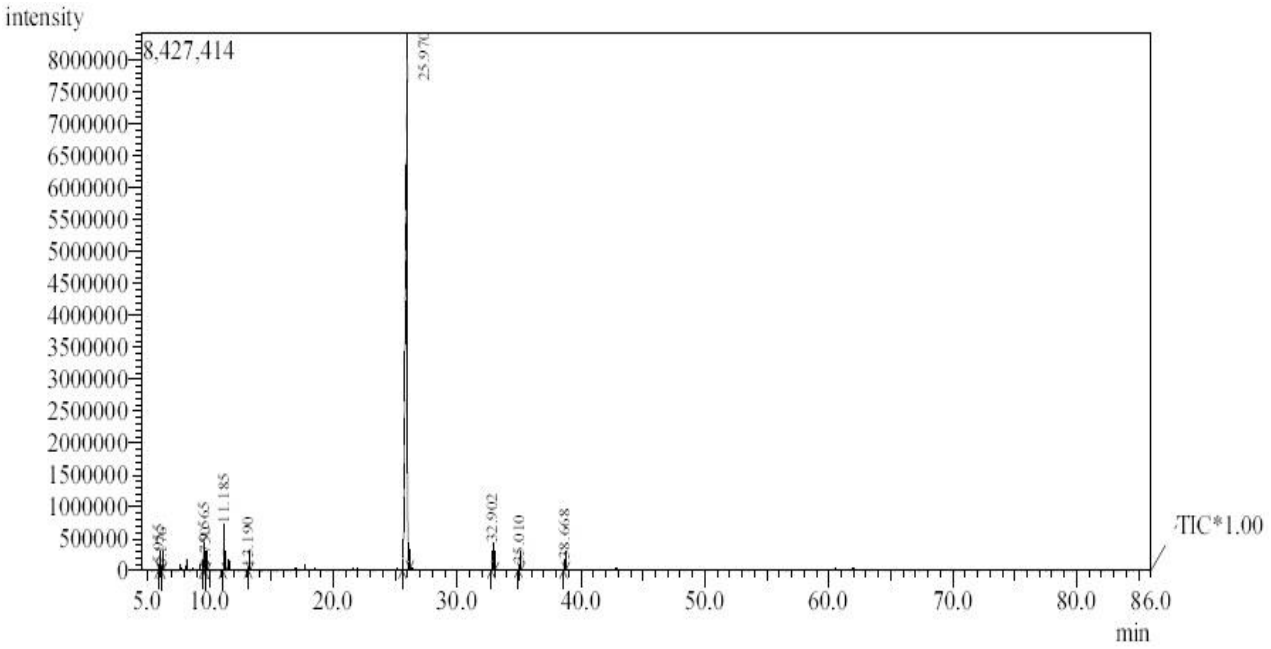

Figure 2. GC / MS Chromatogram of B-413 Clone

Sancaktaroğlu and Bayram (2011) determined the essential oil rate of cultivated plants between 3.78-4.59%. Karamanos and Sotiropoulou (2013) observed an increase of essentil oil rates which were 1.5% on the second season and 2% on the third. Lukas et al. (2015) examined the essential oil content of 502 *Origanum vulgare* individual plants from 17 different countries and 51 populations and reported that the values varies between 0.03-4.6%. Our findings are considerably higher than the results of the other researchers.

5. Conclusions

When all the results are generally evaluated, there was found wide genotypic variation among the examined population. For first year of the study, the values of agronomical features of first harvest were lower than the second and the non-flowering (third) harvest. However maximum essential oil rate values were obtained from the first harvest. For second year of the study, there were increase on values of plant height, canopy, fresh herb yield, drog herb yield, drog leaves yield and essential oil rate. Differences among genotypes in terms of yield and quality were determined in this study. The population was evaluated in the sense of agronomy and quality and a major variation was observed. According to the results, genotypes with higher yield and essential oil rates have been selected and A clones were created. Among A clones, 20 superior clones were selected for the further studies. It could be possible in the future to complete the breeding circle of clone selection with further studies.

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Conflict of Interest

The authors have declared that they have no conflict of interest.

References

1. Acikgoz, N., Ilker, E. and Gokcol, A., 2004. Evaluation of biological research on the computer. E.U. TOTEM No:2.
2. Ahmad, S., Kouka, S., Islam, M., Ahmad, K., Aslan, S. Aminullah and Gill, A., 2008. Germplasm evaluation of medicinal and aromatic plants in highland balochistan Pakistan. Pak. J. Bot. 40 (4): 1473-1479.
3. Arabacı, O., Ogretmen, N.G., Aslan, D.F. and Ozcan, I.I., 2012. Ontogenetical Variability of *Coridothymus capitatus* (L.) Genotypes. 13-15 September. Symposium of Medicinal Plants and Herbs; Tokat.
4. Baser, K.H.C., 2001. Healthy Oregano. Science and Technique. May. 74-77.
5. Bayram, E., Kırıcı, S., Tansı, S., Yılmaz, G., Arabacı, O., Kızıl, S. and Telci, I. 2010. Possibilities to Increase the Production of Medicinal Plants and Herbs. VII. Technical Congress of Agriculturel Engineers Book of Reports 1. Community of Agricultural Engineers. Ozdoğan Press. 437-456.
6. Ceylan, A., Bayram, E., Sahbaz, N., Otan, H. and Karaman, S., 2003. Yield Performance and Essential Oil Composition of Individual Plants and Improved Clones of *Origanum onites* L. Grown in Aegean Region of Turkey. Israel Journal of Plant Sciences. 51(4):285-290.
7. Hayta, E. and Arabacı, O., 2011. Determination of Different Germination Methods on the Seeds of Some Plants Named as Oregano. Journal of Adnan Menderes University Faculty of Agriculture 8(1):91-101.
8. Karamanos, A.J. and Sotiropoulou, D.E.K., 2013. Field Studies of Nitrogen Application on Greek Oregano (*Origanum vulgare* subsp*. hirtum* (Link) Iestwaart) Essential Oil During Two Cultivation Seasons. Industrial Crops and Products 46: 246-252.
9. Kokkini, S., 1996. Taxonomy, diversty and distribution of *Origanum* species. Promoting the conservation and use of under utilized and neglected crops. 14. Oregano. Proceedings of the IPGRI International Workshop on Oregano. 8-12 May. CIHEAM; Valenzano, Bari, Italy.
10. Leto, C. and Salamone, A., 1996. Bio-agronomical Behaviour in Sicilian *Origanum* ecotypes. Promoting the conservation and use of under utilized and neglected crops. 14. Oregano. Proceedings of the IPGRI International Workshop on Oregano. 8-12 May. CIHEAM; Valenzano, Bari, Italy.
11. Lukas, B., Samuel, R., Mader, E., Baser, K.H.C., Duman, H. and Novak, J., 2013. Complex Evolutionary Relationships in *Origanum* section *Majorana* (Lamiaceae). Botanical Journal of the Linnean Society. 171: 667-686.
12. Lukas, B., Schmiderer, C. and Novak, J., 2015. Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). Phytochemistry 119:32-40.
13. Oflaz, S., Kurkcuoglu, M. and Baser, K.H.C., 2002. Pharmacognosic Researches on *Origanum onites* and *Origanum vulgare* subsp. *hirtum*. 14. Meeting of Medicinal Raw materials. 29-31 May. ISBN 975-94077-2, Eskişehir.
14. Said-Al Ahl, H.A.H., Omer, E.A. and Naguib, N.Y., 2009. Effect of Water Stress and Nitrogen Fertilizer on Herb and Essential Oil of Oregano. Int. Agrophysics. 23:269-275.
15. Sancaktaroğlu, S. and Bayram, B., 2011. Researches on Determination of Yield and Quality Characteristics of Different Originated *Origanum vulgare* subsp. *hirtum* L. Journal of Ege University Faculty of Agriculture 48 (3): 265-276.
16. SPSS 17.0. 2008. SPSS 17.0 for Windows. Chicago. IL. SPSS Inc.
17. Sezik, E., Tümen, G., Kirimer, N., Ozek, T. and Baser, K.H.C., 1993. Essential Oil Composition of Four *Origanum vulgare* subspecies of Anatolian Origin. Journal of Essential Oil Research Vol. 5, Iss. 4.
18. Tınmaz, A.B., Kürkcüoglu, M., Baser, K.H.C. and Ozturk, M., 2002. Determination of the Quality Characteristics of *Origanum vulgare* subsp. *hirtum* Populations. 14. Meeting of Medicinal Raw materials Reports. 29-31.05.2002. Eskişehir. ISBN: 9759407728.
19. TSI, 2004. Turkish Statistical Institute. Export Statistics, Ankara.
20. Veres, K., Varga, E., Dobos, Á., Hajdú, Z., Máthé, I., Németh, E. and Szabo, K., 2003. Investigation of the Composition and Stability of the Essential Oils of *Origanum vulgare* ssp. *vulgare* L. and *O. vulgare* ssp. *hirtum* (Link) letswaart. Chromatographia 57 (1):95-98.
21. W'glarz, Z., Osidska, E., Geszprych, A. and Przybyb, J., 2006. Intraspecific Variability of Wild Marjoram (*Origanum vulgare* L.) Naturally Occurring in Poland. Rev. Bras. Pl. Med. Botucatu. 8:23-26.
22. Wichtl, M., 1971. Die pharmakogostich-Chemische Analyse Band. Frankfurt. pp. 479.
23. Žukauska, I., 2001. Agrobotanical Features and Productivity of Wild and Cultivated Populations of *Origanum vulgare*. Botany, Acta Biol. Daugavp. 1(2):107-109.

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Researches on Cultivation of Medicinal and Aromatic Plants in Kayseri

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Abstract

This study comprises the results of demonstrative studies on some medicinal and aromatic plants carried out by Kayseri Directorate of Provincial Agriculture and Forestry under the field conditions in 2015-2017 within the Project of Improving the Medicinal-Aromatic and Dye Plants Cultivations, funded by General Directorate of Plant Production (BÜGEM). In this project, five different plants consisting of lavandin (*Lavandula x intermedia* Emeric ex Loisel), lemon balm (*Melissa officinalis* L.), oregano (*Origanum onites* L.), salep (*Orchis sancta* and *Serapias womeraceae*), and black cumin (*Nigella sativa*) were studied in 11 counties for three years. Desired results were obtained from lavandin, lemon balm, black cumin and oregano plants under the ecological conditions of Kayseri. In terms of cultivation of black cumin, there were not any considerable problems except for weeds affecting sufficient yields. The weed problem was primarily orginated by not having herbicides authorized by the Ministry of Agriculture and Forestry. Although application of different planting times and mulching methods in salep plants, desired results couldn't be obtained because of the harsh winter conditions and frost damage.

Key Words: Cultivation, Field Conditions, Medicinal Plants

1. Introduction

Medicinal and aromatic plants have been used by mankind since ancient times in many fields such as food, medicine, cosmetics, perfumery and spices. Some of these plants are collected from nature and some of them are cultivated. Most of the plants used for therapeutic purposes are still collected from nature (Acibuca and Budak, 2018).

Although more than 40% of the drugs used at the beginning of the 20th century were of plant origin, this rate dropped to less than 5% in the mid-1970s. However, especially after the 1990s, the discovery of new areas of use of medicinal and aromatic plants and the increasing demand for natural products increase the use volume of these plants with each passing day (Kumar, 2009).

According to World Health Organization (WHO) data, approximately 20,000 plants are used for medical purposes (Baser, 1997). The number of medicinal and aromatic plants cultivated for commercial purposes worldwide is about 900 (Arslan at al., 2015).

Turkey, thanks to its geographic location, climate and plant diversity, agricultural

potential and broad space, is one of the leading countries in the trade of medicinal and aromatic plants. Turkey's importance in this matter stems from the presence of many plants in our country flora which constitute the raw materials to the industry of herbal medicines, drug chemicals, food and additives, cosmetics and the perfumery, in developed countries (Karik et al., 2018).

Therefore, these plants are mostly collected from nature and marketed. In recent years, there has been a significant increase in the use of medicinal and aromatic plants and products derived from them (Gezici, 2018). In order to meet the constantly increasing demand in the coming years, production of medicinal and aromatic plants needs to be increased in order to obtain a better quality standard product. Thus, these plant extracts will increase and the industries that process them will develop (Bayram et al., 2010).

This project has been carried out in the province of Kayseri since 2015 within the scope of the Project of Improving the Medicinal-Aromatic and Dye Plants Cultivations, sustained by General Directorate of Plant Production (BUGEM), carried out on 5 plants (Lavandin, Lemon Balm, Oregano, Salep, and Black Cumin) in 11 counties and furthermore it has been expanding yearly by means of adding new plants. By increasing plant diversity, to determine the alternative crops that can adapt to the ecological conditions of Kayseri and bringing high income from the unit area, to learn the growers the cultivation of these plants and to expand the production of these crops are aimed..

2. Materials and Method

2.1. Materials

The materials used in demonstrations were provided from the Directorate of Aegean Agricultural Research Institution/Menemen-IZMIR, the Directorate of Directorate of Fruit Research Institute/Egirdir-ISPARTA, The Directorate of Transitional Zone Agricultural Research Institute-ESKISEHIR, and the Directorate of Horticultural Central Research Institute-YALOVA. The species used in the study; *Lavandula x intermedia* Emeric ex Loisel, *Melissa officinalis* L., *Origanum onites* L., *Orchis sancta, Serapias vomeraceae, Nigella sativa.*

2.2. Methods

Lavandin and lemon balm seedlings were planted in May and oregano seedlings were planted in April. Plant densities were applied as 50x120-150 cm in lavandin, 30x70 cm in lemon balm and 20-30x40-60 cm in oregano plant. Black Cumin seed were sown in April as 15-20 kg ha^{-1}. Fertilizer application In lavandin, lemon balm, oregano and black cumin plants, almost similar to each other pure 4-5 kg / ha nitrogen and phosphorus, in addition, in some locations 1-2 tons / ha in the form of burned animal manure. Salep plants were planted in October in the first year. Salep tubers were planted within a burnt manure-enriched soil bed, 25-30 cm in height and 80-100 cm in width, in 15-15 cm soil depth and 10-20 cm row spacing. Unsuccessful results were obtained in the firsth year. Therefore, planting time was changed to 3 different planting times as October (a month later than the firsth planting time), Novomber and December to observe the result of different planting times. Also, different mulching method made of wheat straw and burnt manure, greenhouse and low tunnel methods were tried to see the developments under cover.

3. Results and Discussion

3.1. Lavandin (Lavandula x intermedia Emeric ex Loisel)

Lavanding seedlings were obtained in the years of 2015 and 2016. Demonstrative activity researches were carried out at totally 14 locations with a total of 7 locations firsth year and 7 locations second year. Harvestings were carried out in 2016-2017. Accordingly,

while good results were taken from some locations in all plants, it was not possible to get any results from some of them since just firsth year or in the other years because of the reasons originated from the growers. In Lavandin, demonstrations were estabilished in 3,35 decares in 7 locations in 6 counties in 2015, in 5,2 decares in 7 locations in 7 counties in 2016, totally in 8,55 decares.

The harvest wasn't made in the firsth year because of plant growth being slow, however to enhance the tillering in flowering plants, flowers were dissevered from their stems.

In lavandin demonstrations, constituted in 2015, however even if just a bit desiccations in plants originated from cold wheather and frost in some locations in 2015-2016, plants recovered again by tillering with the warming wheather in Spring. In three designated locations below, the results of dry flowers yield, essential oil rate and essential oil yield could be obtained.

Table 1. According to the 2. Years (2016) Harvests in Some Locations, Dry Flowers Yields, Essential Oil Rates and Essential Oil Yields

Location Name	Dry Flowers Yield (kg ha^{-1})	Essential Oil Rate (%)	Essential Oil Yield (L ha^{-1})
Yahyali/Mustafabeyli	1270	6,1	77
Yesilhisar/Merkez	300	5,7	17
Yesilhisar/Kayadibi	680	6,0	48

When examining in Table 1, dry flowers yields, essential oil rates and essential oil yields are seen regarding Lavandin trials results on the grower basis in Mustafabeyli/Yahyali, Yesilhisar and Kayadibi/Yesilhisar. The highest dry flowers yield became in Yahyali, Mustafabeyli Location as 1270 kg ha-1, the lowest dry flowers yield became in Yesilhisar location as 300 kg ha^{-1}. Essential oil rates in dry flower were very close to eachother between 5,7-6,1%. While essential oil yields were 77 L ha^{-1} because of high dry flowers yield in Mustafabeyli/Yahyali location, it was stayed at a lower level of 17 L ha^{-1} because of low dry flowers yield in Yesilhisar. The reasons why dry flowers rate was so low were those grower retarted the harvest becasuse of his beekeeping and shadows of the trees around flower yields increased more in the following years, but these values could not be measured. As examined in Table 2, the essential oil ratios of the locations in the dry flower ranged between 2,82-9,00%. The highest Felahiye Center is located at the lowest Kocasinan Yazir location. Average essential oil ratio of the locations in dry flowers was 5,72%. The emergence of a essential oil rate of 9% in the Felahiye Center is an important indicator for this location. The altitude of this location is 1330 m, the south open face, the sun is good and the climatic open days are high. It is a known fact that sunbathing increases the rate of essential oil. In Kocasinan Yazir and Tomarza Isıklar, essential oil ratios were lower than other locations because of the mixing of some lavender flower stalks in dry flower sample and lack of good sample preparation. These essential oil ratios in dry flowers are a good indicator. In the scope of the support given to young farmers in the province, the producers removed the essential oils from the products they obtained in the essential oil distillation system and placed them in small glass bottles and marketed them. Arabaci and Bayram (2005), 1340-4430 kg ha^{-1}, and Arslancan et al. (2014) (1620-3410 kg ha^{-1}) reported that low yields of lavender were lower in producers of lavender. the inadequacy, harvest method and the methods of removing the dry flowers from stalks, the lack of sufficient infrastructure in this issue has been due to. Deaf oil yields were also low.

Table 2. The Amount of Essential Oils Obtained in Dry Flower in 2017 Year Harvest in Lavandin Locations (%)

Location Name	Essential Oil Rate (%)	Location Name	Essential Oil Rate (%)
Develi/Sindelhöyük	5,67	Yahyali/Karakoy	6,00
Felahiye/Merkez	9,00	Yahyali/Mustafabeyli	6,80
İncesu/Garipce	5,20	Yesilhisar/Merkez	5,70
Kocasinan/Akcatepe	5,80	Tomarza/Isıklar	4,50
Kocasinan/Yazir	2,82	Average Value	5,72

Otherwise, when we look at the essential oil ratios, we see a rate of 5.7% which is much higher than the values (1.54-2.34%) indicated by Arabaci and Bayram (2005).

3.2. Lemon Balm (Melissa officinalis L.)

In 2015, 4 activities were carried out in 0.9 decares area, in 4 locations in 4 counties, in 2016 in 2.05 decares, in 3 locations and in 3 counties. In total, 2,95 decare area demonstrations have been established and followed up.

In the demonstrations established in 2015, a harvest was made in the autumn plants before entering the winter, but there was not much yield. In the winter, although the above-ground parts of the plants completely desiccated in all locations with the arrival of the spring with the new shoots occurred in the plant development was not a problem. Due to winter and cold weather in Kayseri, there was no negative situation. Plant growth and yields were very good after the first year. A harvest in the flowering period in June, and another harvest before the winter in September-October, plants was harvested twice per year. While a high herbage yield was obtained in the first in June, a lower herbage yield was obtained in the second.

In Table 3, while the total dry herbagea yield was 5250 kg ha-1, the essential oil rate was 0.08% and the essential oil yield was 4,2 L ha-1 in the Yahyali Mustafabeyli location, these were 6100 kg ha^{-1}, 0.07% and 4,2 L ha^{-1} in the Kocasinan Yazir location at the 2nd year harvest. The dry and fresh herbage yields for the second year were higher than in the first year, but these data could not be recorded since the yield values could not be weighed. According to this, it can be said that the lemon balm plant is in compliance with the ecological conditions in Kayseri and it has no climatic, disease or harmful problems.

Table 4 shows the rates of essential oil in four locations in the lemon balm plant. According to this, the highest essential oil ratio was found at İncesu Garipce location with the highest rate of 0,24% and the lowest value was found at 0,09% Tomarza Isıklar location. Values are close (0,1-0,35%), to the values reported by Uzun et al. (2014), some higher than the values reported by (Koc, 2002) % 0,01-0,25 and Uyanık and Gurbuz (2014) 0.03-0.08%. According to the results of Abdellatif et al. (2014) 1,54-2,34%, it is slightly lower. Essential oil ratios are close to the findings obtained in our country.

Table 3. Total Dry Herbage, Essential Oil Ratio and Essential Oil Yield Values of Some Locations According to the Harvest Results of the Lemon Balm

Location Name	Dry Herbage Yield (kg ha^{-1})	Essential Oil Rate (%)	Essential Oil Yield (L ha^{-1})
Yahyali/Mustafabeyli	5250	0,08	4,2
Kocasinan/Yazir	6100	0,07	4,2

3.3. Oregano (Origanum onites L.)

Demonstrations in oregano were established in 2016 in 5 locations and 5 counties in a total area of 2.5 decares. Plant growth was very good in locations. A superficial harvest was made before the first year of winter and no significant yield was obtained. In the second year flowering period, the herbage yields of the plants harvested in June were taken but could not be measured. In some locations 1 and 2 reaps were taken in 2 years, in second reaps in September and October, a very low herbage yield was mentioned. No disease, no harmful organism or no cold and frost damage in winter in the plants was observed.

Table 4. Essential Oil Ratio of Dry Leaf in Lemon Balm According to 2017 Harvest

Location Name	Essential Oil Rate (%)	Location Name	Essential Oil Rate (%)
Yahyali/Mustafabeyli	0,17	İncesu/Garipce	0,24
Tomarza/Isıklar	0,09	Kocasinan/Yazir	0,13

Producers in Yahyali Kopçu and İncesu Garipce locations sold their products in the markets and generated certain amounts of income. In the İncesu Garipce location, the amount of essential oil detected in the 2nd year (2017) *Origanum onites* L. leaves was 4.64%. Since our producer is an organic farming producer, it has been able to pack and sell medicinal and aromatic plants (lavandin, lemon balm, oregano and sage) in the organic market. He is still doing this job.

3.4. Salep (Orchis sp.)

Demonstrations in the Salep plant were established in 2015 in 3 counties in 0.9 decares area, in 2016 in 6 locations in 6 counties in 3.2 decares, in a total of 4.1 decares. The first year plantings were made in October. In autumn there was no outflow of tubers. In the spring, there was some output in the Kocasinan location as in March, but they also disappeared with the effect of late frosts in spring. It was planned to investigate different applications such as different planting time, mulching methods and production conditions under cover for 2 years. In order to see the results of different planting time in the 2nd year, 3 different planting time applications were made in a way that the suturing times of the tubers were one month after the first planting time, from September to October-November. Different mulching methods were tried. To keep the pillows warm, burnt farm manure was laid in straw-straw and some were planted in the greenhouse and some were planted in the form of a low tunnel cover. After planting, demonstrative follow-ups were performed regularly. No improvement was observed in plants in autumn and winter Only in the greenhouse and under the cover planted some output was observed in winter, but in the later stage they disappeared. A few plants were found in the Felahiye location, which overlooks the southern slope of the open area.

It is thought that the winter conditions in Kayseri are hard and long, the damages of late frosts in spring and the salep species used in the spring are unsuccessful due to the cold and frost sensitive species *Orchis sancta* and *Serapias vomeraceae*. Salep plant is a difficult plant culture. (Arabacı et al., 2014), in their study on the Effect of Different Cultural Practices in Salep Orchids, reported that many of the studies conducted for culturing Salep orchids were in vitro studies and that the plants were failing at the stage of adaptation to outdoor conditions. We are of the opinion that in the conditions of Kayseri cultivation of salep plants can be made in other species compatible with the Central Anatolia Region. Tutar et al. (2012) in our opinion in accordance with the salepte each

region of their own species and work with their ecological conditions can be achieved with successful results stated.

3.5. Black Cumin (Nigella sativa L.)

The demonstrations in Black Cumin were implemented in 3 locations (13 decares) in 3 counties in 2015, and in 5 locations (14 decares) in 4 counties in 2016, totally in 27 decares. Seed of 15-20 kg per hectare were used for seed decantation in April and sprinkling irrigation was done. Sprouts and exits occurred in locations. However, due to the large number of weed populations, black cumin did not show any improvement. Since the Ministry is not a licensed herbicide and mechanically it is not suitable for anchoring, it is not possible to obtain a product at the level of economic efficiency in black cumin. Because the yield values of the decay remained at very low levels of 100-200 kg ha^{-1}, farmers did not even need to harvest because they could not remove them.

4. Conclusions

The following conclusions can be drawn from these studies conducted in 45.1 decares area in 43 locations in 11 counties in 5 different medicinal and aromatic plant species (Lavandin, Lemon Balm, Oregano, Salep and Black Cumin) for 3 years between 2015-2017.

As the studies were conducted under farmer conditions, the desired results could not be obtained from each demonstration depending on the farmers. In the trials and demonstrations carried out in the farmer's conditions, the choice of the wrong farmers, the lack of equipment and equipment infrastructure at the desired level, the willingness to appear at first, and then the difficulty in raising their work intensity or cultivation in these plants, the lack of interest and the lack of enough results could not be achieved. For these reasons, some demonstrations were disabled at the first stage. However, a certain result has been reached in the studies. We tried to reduce this risk by establishing the demonstrations at more locations. Under the conditions of Kayseri, these plants can be cultivated economically and they have an idea about their adaptation to Kayseri ecology, their efficiency and quality. These results shed light on local people for those who will work or invest in these plants. As a matter of fact, the Provincial Directorate of Agriculture and Forestry has a lot of people who are interested or not interested in agriculture and want to invest in these matters. These outputs are important in informing and referring them to the right direction.

Under the conditions of farmers, making and conducting these demonstrations cause the producers to recognize and learn these plants, and to recognize, see and be interested in the farmers of the neighboring farmers and other nearby villages. In the next stage, these plants gradually enter the crop rotation in this area and make it become agricultural. There are many examples of this, but this can take many years. Before the start of this project, none of the medicinal and aromatic plants in Kayseri were cultivated and produced. Now some of the producers still continue to grow existing plants, new producers, new plants are added, they are included in municipalities under the leadership of Provincial Directorate of Agriculture and Forestry.

According to this study; In the ecological conditions of Kayseri, positive results were obtained in the cultivation of lavandin, Lemon Balm and oregano plants. Although we do not see a problem in plant growth in the sowing of Black Cumin plants, weed problem has emerged as an important problem. Due to the lack of a medicinal product licensed from the Ministry of Agriculture and Forestry, it was not possible to obtain sufficient yield. In order to be successful in the agriculture of black cumin in Kayseri situations, we think it is necessary to

plant more broadly (40-50 cm) in order to allow the weeding of the weeds mechanically, it would be appropriate to make different sowing times, including autumn, winter and early spring.

Due to the fact that the winter conditions in Kayseri are hard and long, and the species used are not compatible with this region, appropriate results could not be obtained. Salep plant is a plant that is difficult to culture, but we believe that other types of studies compatible with the Central Anatolia region can be done.

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Conflict of Interest

None

References

1. Abdellatif, F., A. Boudjella Hadjira Zitouni and A. Hassani. 2014. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian Melissa officinalis L. Exclı Journal. 13: 772-781.
2. Acibuca. V., D. Bostan Budak. 2018. Place and Importance of Medicinal and Aromatic Plants in the World and Turkey, Cukurova J. Agric. Food Sci. 33(1): 37-44.
3. Arabaci. O. and E. Bayram. 2005. The Effect of Nitrogen Fertilization and Plant Density on SomeAgronomic and Quality Traits of Lavender (Mill.) Under Ecological Conditions of Aydin. Adu Journal of the Faculty of Agriculture, 2: 2: 13-19.
4. Arabaci. O., M. Tutar, N.G. Ogretmen, F. Yasar and U. Tan. 2014. Effects of Different Cultural Practices in Salep Orchids, II. Symposium of Medicinal and Aromatic Plants, Ataturk Horticultural Central Research Institute Directorate, 23-25 September, Yalova, pp. 479-487.
5. Arslan, N., Baydar, H., Kizil, S., Karik, U., Sekeroglu, N., Gumuscu, A. 2015. Tıbbi ve Aromatik Bitkiler Üretiminde Değişimler ve Yeni Arayışlar. VII. Türkiye Ziraat Mühendisliği Teknik Kong. s:483-507.
6. Aslancan. H., R. Sarıbas, C. Dagıstanlioglu, A. B. Tinmaz and D. Arslan. 2014. Determination of Agricultural Properties of Some Lavender (Lavandula x Intermedia Emeric Ex Loisel.) Cultivars in Isparta Conditions, II. Symposium of Medicinal and Aromatic Plants, Ataturk Horticultural Central Research Institute Directorate, 23-25 September, Yalova, pp. 344-347.
7. Baser, K.H.C. 1997. Tıbbi ve Aromatik Bitkilerin İlaç ve Alkollü İçki Sanayinde Kullanımı, İstanbul Ticaret Odası Yayın No: 39.
8. Bayram. E., S. Kirici. S. Tansi. G. Yilmaz. O. Arabaci. S. Kizil and I. Telci. 2010. Tıbbi ve Aromatik Bitkiler Üretiminin Arttırılması Olanakları, TMMOB Ziraat Mühendisleri Odasi, Ziraat Mühendisligi VII. Teknik Kongresi, Ankara, Türkiye, 11-15 Ocak 2010, s. 437-456.
9. Gezici. S. 2018. Promising Anticancer Activity of Lavender (*Lavandula angustifolia* Mill.) Essential Oil Through Induction of both Apoptosis and Necrosis. Annals of Phytomedicine, 7(2), 38-45.
10. Karik. U, Cinar. O, Tunçtürk. M, Şekeroğlu. Gezici. S. 2018. Essential Oil Composition of Some Sage (*Salvia* spp.) Species Cultivated in İzmir (Turkey) Ecological Conditions. 52(4), 102-107.
11. Koc. H. 2002. Bitkilerle Saglıklı Yaşam: Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü, Tokat.
12. Kumar. S. A. 2009. Plants-based Medicines in India. http://pib.nic.in/feature/feyr2000/fmay2000/f240520006.html
13. Tutar. M., F. Cicek. A. O. Sari. A. Bilgiç and O. Yildiz. 2012. The Culture of Some Orchid Species Distributed İn Aegean Region. Turkey 2. Orchids and Sahlep Workshop Proceedings, Aegean Agricultural Research Institute, 25-26 April, İzmir, pp. 301-320.
14. Uyanık. M. and B. Guurbuz. 2014. A Study on Determination of Dıurnal Variability in Lemon Balm (*Melissa officinalis* L.), II. Symposium of Medicinal and Aromatic Plants, Ataturk Horticultural Central Research Institute Directorate, 23-25 September, Yalova, pp. 432-437.
15. Uzun. A., K. Kevseroglu and B. Gurbuz. 2014. Determination of Some Vegetal and Chemical Traits of Lemon Balm (*Melissa officinalis* L.) Ecotypes Presented in the Flora of Middle and East Black Sea Region, II. Symposium of Medicinal and Aromatic Plants, Ataturk Horticultural Central Research Institute Directorate, 23-25 September, Yalova, pp. 377.

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The Determination of the Effects of Wild Thyme (*Thymbra spicata L.*) and Cumin (*Cuminum cyminum L.*) Extracts on SCE (Sister Chromatid Exchange) in Human Lymphocyte Chromosomes

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Abstract

The purpose of this work is to determine of *T. spicata* L. and *C. cyminum* L. plant extracts on sister chromatid exchange rate in human peripheral lymphocyte culture as *in vitro*. Human blood lymphocyte cells, *T. spicata* L. and *C. cyminum* L. plant extracts were allowed to interact with doses of 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL for 24 hours. When trial groups where plant extracts were applied were compared with negative control and mitomycin-C (MMC) which was used as positive control, it was determined that the extract doses applied led to an increase in sister chromatid exchange rate. Also, it was found that increasing concentrations of plant extracts caused cell replication index to decrease. Between *C. cyminum L.* doses and RI, a negative correlation (r = -0.95) was observed and between *T. spicata* L. doses and RI a negative correlation was observed (r = -0.94) respectively. As a result of this study; *T. spicata* L. and *C. cyminum* L. plant extracts were found to have genotoxic and clastogenic effects on human peripheral lymphocytes.

Key Words: Mitomycin-C, Replication Index, Genotoxic, Clastogenic.

1. Introduction

In the genetic information of the living species, hereditary variations that occur suddenly due to factors other than gene recombination are called mutations. In addition to spontaneous mutations in nature, mutagen can also be caused by physical and chemical factors. Mutagenes in DNA molecules cause many damages and some of these damages are tried to be repaired in the cell by special mechanisms. Various damages to DNA can sometimes be incorrectly repaired so that the cell can survive death. Damages in DNA may remain unrepaired in the presence of mutations in the genes controlling the functioning of the repair mechanisms, or by adverse effects such as age, disease, nutrition, and heat. As a result, that cell also causes a mutation, which results in various disorders. Cancer and cell death are among these disorders (Galloway et al., 1998; İpek et al., 2003)

Mutations in the DNA of living things are determined by *in vitro* mammalian cell gene mutation test and back mutation tests using bacteria. *In vitro* mammalian, Sister

Chromatide Exchange, Chromosomal Aberration and Micronucleus tests are among the most widely used methods for cytogenetic investigation of mutations and genotoxic effects in DNA (Natarajan and Obe, 1982).

The goal of this work is to research the effects of plant extracts of cumin and wild thyme on sister chromatid Exchange (SCE) in human lymphocyte chromosomes *in vitro*.

2. Material and Method

The method developed by Speit and Haupter (1985) to provide different staining (Sister Chromatid Differentiation = SCD) of a sister chromatid belonging to a chromosome was modified and used.

In our study, peripheral blood from 10 females (20-25 years old) and 10 males (20-25 years old) was used as material. Wild thyme (*T. spicata*) and Cumin (*C. cyminum*) plant essential oils were used as test substances. Mitomycin-C (MMC) was used as positive control and Acetone (C_3H_6O) was used as test control (Uzun, 2007).

Plant extracts of wild thyme (*T. spicata)* and cumin (*C. cyminum*) were extracted from the leaves and seeds of the plants. NON ASBESTOS brand Clevenger device was used for extraction. For 40 g of plant, 400 mL of distilled water was added to remove essential oil in about 3 hours. This process was repeated until sufficient essential oil was obtained. 100 µL of the obtained essential oil was mixed with 900 µl of acetone. As a result, 1 part of oil and 9 parts of acetone were mixed.

2.1. Test Materials and Solutions

In this study, cumin and wild thyme essential oils were used as test substances.

Acetone

Merck acetone was used in this study. Acetone to be added to 5 mL of medium was calculated as 2.5 µL/mL.

Chromosome Medium

Chromosome Medium B (Cat. No. F5023) from Biochrom was utilized as cell culture in this work.

5"-Bromo-2"-deoxyuridine (BrdU)

BrdU from Sigma (Cat. No. B 5002) was dissolved in 10 ml Chromosome Medium B (50 µg/10 mL medium). 10 µg/mL of SCE was added to the prepared solution (100µL) for SCE study.

Mitomisin-C (MMC) 2 mg (Sigma, M 0503)

MMC (Mitomycin-C) was used as a positive control by dissolving bidistyl in water. Mitomycin-C, weighing 0.6 mg, was dissolved in distilled water to a volume of 1.5 mL.

2.2. Experiments

The method developed by Speit and Haupter (1985) to provide different staining (Sister Chromatid Differentiation = SCD) of a sister chromatid belonging to a chromosome was modified and used. 12 drops of heparinized blood samples taken from 10 healthy, close aged and non-smoking 10 women and 10 men were added to the chromosome medium (5mL) under sterile conditions. 10 µg/mL (100 µL of the prepared BrdU solution) from each BrdU solution prepared under sterile conditions were mixed thoroughly under sterile conditions (50 µg BrdU/100 µL medium). The prepared cell culture was incubated in an oven at 37°C for 72 hours.

Solvent Control Group (Acetone)

Acetone to be added to 5 ml of medium was calculated as 2.5 µL/mL. Acetone was put in medium tubes during the last 24 hours of incubation. (72 hours).

Negative Control Group

Both experiment groups were compared with this group. No additions were made to this group. Incubation of 72 hours was performed.

Positive Control Group

0,3 µg/mL of Mitomiycin-C was put in to the medium tubes during the last 24 hours of incubation. (72 hours).

Experiment 1: Essential oil of C. cyminum (72 hours incubation)

Group 1. 0.05 µL/mL Cumin
Group 2. 0.10 µL/mL Cumin
Group 3. 0.15 µL/mL Cumin
Group 4. 0.20 µL/mL Cumin

Experiment 2: Essential oil of T. spicata (72 hours incubation)

Group 1. 0.05 µL/mL Wild Thyme
Group 2. 0.10 µL/mL Wild Thyme
Group 3. 0.15 µL/mL Wild Thyme
Group 4. 0.20 µL/mL Wild Thyme

At the 70th hour of culture (2 hours before the end of the culture period), the solution of colchicine was put in each tube (0.06 µg/mL) and the tubes were slowly shaken to mix well. For 2 hours at 37°C, the cells were pre-treated with colchicine. The culture tubes were centrifuged at 2000 rpm for 10 min at the end of the culture period (at the end of 72 hours). The supernatant was eliminated on the centrifuged culture tubes. After stirring 0.5-0.7 ml of liquid remaining at the lower part of the tube, the hypotonic solution maintained in the oven at 37°C was added to the tubes.

To prevent clustering of the cells, 5 ml of colchicine were added to the tubes and tubes were placed in the incubator in a reserved way after turning upside-down. The tubes with hypotonic solution were kept in the oven at 37°C for 30 minutes. At the end of the period, the supernatant was discarded by centrifugation at 2000 rpm for 10 min. 5 ml of cold fixative were added to each tube with gentle stirring. Cells treated with fixative at 2000 rpm for 10 min were centrifuged and the supernatant was discarded. This procedure was repeated 3 times to completely clear the liquid remaining in the tube. At the end of the centrifugation, the supernatant was discarded so that 0.5-0.7 mL of liquid remained at the lower part of the tube and the slides were prepared with the remaining liquid. Cells collected at the bottom of the tube with a Pasteur pipette were homogenized by mixing and 4-5 drops were drawn from this cell suspension to the pasteur pipette. On the previously cleaned slides, 1 drop of the pasteur pipette was dropped to different areas from 50 cm height (3-4 drops). During the dropping of the cell suspension on the slides, it was ensured that the cells and thus chromosomes were spread on the slide by taking care of not overlapping the drops. Preparations were kept at room temperature for 24 hours to dry.

2.3. Staining of Preparations

The method developed by Speit and Haupter (1985) in order to provide different staining (Sister Chromatid Differentiation = SCD) of sister chromatids belonging to a chromosome was used by modifying it. The dried preparations were put into the irradiation vessel and covered with Sorenson buffer to cover them like a film. Irradiation solution (pH = 6.8) was prepared by taking 5 ml of buffer A and 5 ml of buffer B and this mixture was covered to 100 ml with distilled water. The difference in contrast between the sister chromatids was found to be significantly influenced by the low or high irradiation solution. For this reason, a thin layer of the preparations was covered with irradiation solution. These preparations were irradiated with a single ultraviolet lamp from a height of 15 cm for 30 minutes in the dark which could emit light at a wavelength of 254 nm at 30 W. At the end of irradiation, the preparations were incubated in a 1xSSC solution for 60 minutes at 58-60°C in an oven. The 5% Giemsa dye solution was prepared by mixing 5 mL of Giemsa, 5 ml of buffer A and 5 mL of buffer B 15 minutes before the completion of the incubation period (pH =

6.8). This prepared dye was filtered through filter paper in a vertical trough. When the incubation period was completed, the preparations in 1xSSC solution were taken directly into the giemsa dye solution and left for 20 minutes. At the end of the period, the preparations extracted from the dye were passed through the pure water, put into three different containers and the excess dye on the preparations was allowed to flow and dry. The dried preparations were closed with entellan to make them permanent. With the drying of Intella, the preparations were examined under a microscope.

2.4. Microscopic Examination

Permanent preparations were examined under the Binocular light microscope (10×100 = 1000 magnification) with an immersion objective.

2.5. Replication Index (RI) Detection

RI was calculated to determine the effects of wild thyme (*T. spicata)* and cumin (*C. cyminum*) plant extracts on DNA replication. For these calculations, 100 randomly selected cells were examined in each preparation. By counting the cells in the first, second and third metaphase circuits seen during these investigations, RI was calculated by the following formula.

RI = (1×M1 + 2×M2 + 3×M3) / 100
M1: 1. Number of cells in mitosis
M2: 2. Number of cells in mitosis
M3: 3. Number of cells in mitosis

2.6. Taking Photo wih a Microscope

Photos were taken with an Olympus microscope at 1000X magnification. Photos of SCE samples were taken.

3. Results and Discussion

3.1. Effect of Cumin (C. cyminum) and Wild Thyme (T. spicata) Plant Essential Oils on SCE in Acetone Mixed Human Peripheral Lymphocytes

The mean sister chromatid numbers of *C. cyminum* and *T. spicata* essential oils in human peripheral lymphocytes treated with doses of 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL are shown in table 1. When compared with both controls, in the 100 cells counted, *C. cyminum* and *T. spicata* essential oils increased the number of SCE in human lymphocytes (Tables 1 and 2). R = 0.94 positive correlation was found between cumin dose and SCE, and r = 0.99 positive correlation was found between wild thyme dose and SCE (Graphs 1 and 2).

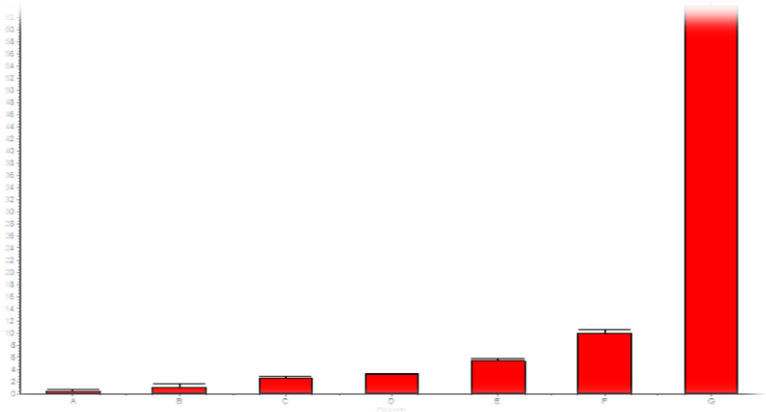
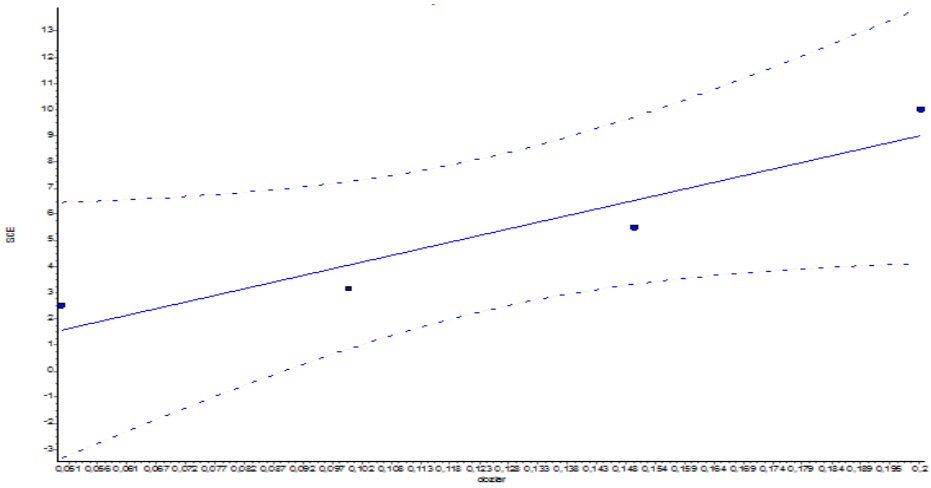


Chart 1. Average number of sister chromatid exchange (SCE) in human peripheral lymphocytes treated with a mixture of Acetone Mixture of Cumin Extracts at different doses for 24 hours. *(A: Negative Control, B: Acetone (2.5 µL/mL), C: Cumin (0.05 µL/mL), D: Cumin (0.10 µL/mL), E: Cumin (0.15 µL/mL), F: Cumin (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg / mL).*



Graph 1. A positive regression was found between cumin dose and SCE (r = 0.94).

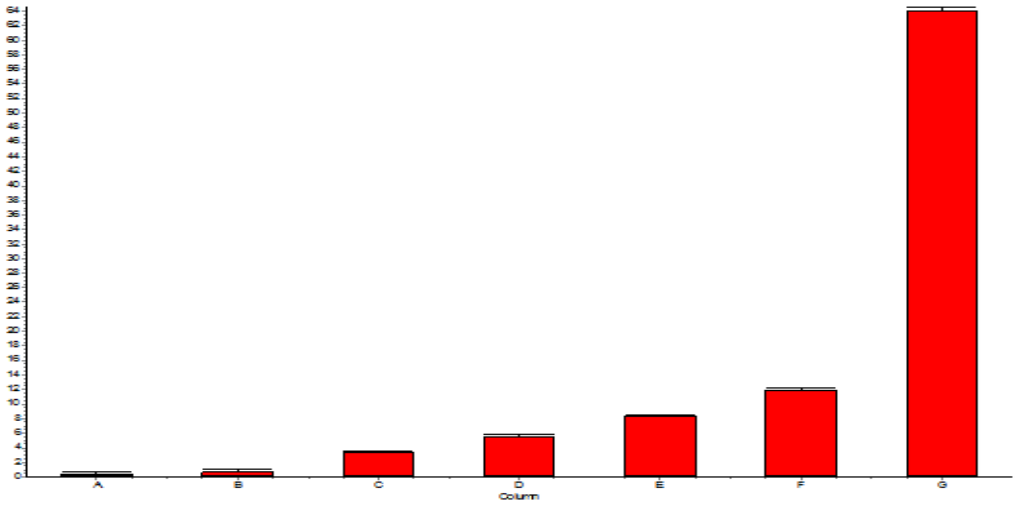
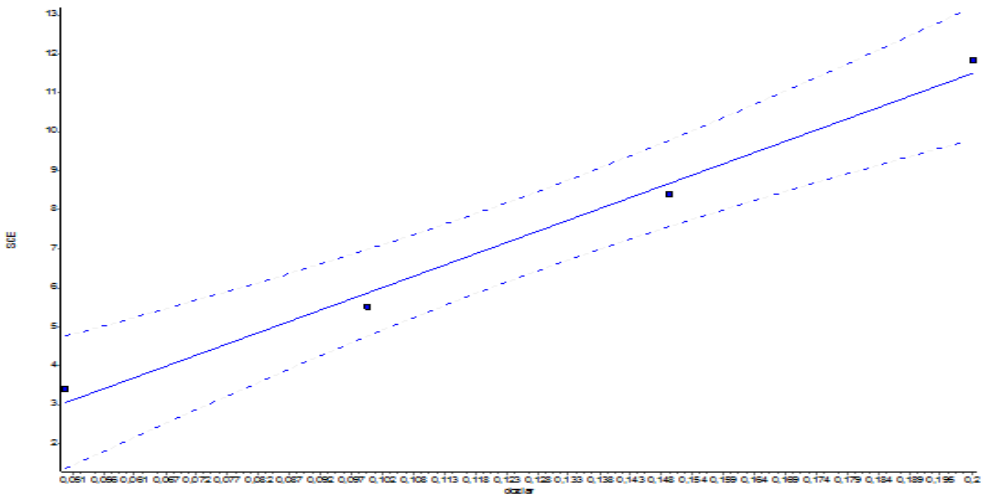


Chart 2. Average number of sister chromatid exchange (SCE) changes in human peripheral lymphocytes treated with Acetone mixture of Wild Thyme Extracts at different doses for 24 hours. *(A: Negative Control, B: Acetone (2.5 µL/mL), C: thyme (0.05 µL/mL), D: thyme (0.10 µL/mL), E: thyme (0.15 µL/mL), F: thyme (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg / mL).*



Graph 2. A positive regression was found between thyme dose and SCE (r = 0.99).

Table 1. Different doses of cumin and wild thyme essential oils treated for 24 hours with a mixture of acetone average human peripheral lymphocytes number of sister chromatid exchange (SCE)

Groups	Concentration (µl/ml)	Treatment Time (hour)	Total Cells	Number of SCE	±SEM (%)
Negative Control	-	-	100	2.57	±0.02
Acetone	2.5 µL/mL	24	100	1	±0.57
	0.05 µL/mL	24	100	2.5*	±0.28
Cumin	0.10 µL/mL	24	100	3.16*	±0.16
Essential Oil	0.15 µL/mL	24	100	5.5*	±0.28
	0.20 µL/mL	24	100	10*	±0.57
	0.05 µL/mL	24	100	3.4*	±0.20
Wild Thyme	0.10 µL/mL	24	100	5.5*	±0.28
Oil	0.15 µL/mL	24	100	8.4*	±0.20
	0.20 µL/mL	24	100	11.83*	±0.44
Positive Control (MMC)	0.3 µg/mL	24	100	64	±0.57

*Compared with Dunnett T test. No difference between negative control and solvent control (p> 0.05). * Important compared to control and solvent control (p<0.01).*

Table 2. Replication ındex of control and wild thyme essential oil groups in human lymphocyte cells

Groups	Concentration (µl/ml)	Treatment Time (hour)	Total Cells	M1	M2	M3	Number of RI	±SEM (%)
Negative Control	-	-	100	10	25	65	2.57	±0.02
Acetone	2.5 µL/mL	24	100	35	20	45	2.25*	±0.08
Wild	0.05 µL/mL	24	100	12	38	50	2.44	±0.05
Thyme	0.10 µL/mL	24	100	48	12	40	1.90*	±0.05
Essential	0.15 µL/mL	24	100	59	11	30	1.75*	±0.02
Oil	0.20 µL/mL	24	100	60	17	23	1.58*	±0.02
Positive Control (MMC)	0.3 µg/mL	24	100	89	5	6	1.20	±0.02

*Compared with Dunnett T test. No difference between negative control and solvent control (p> 0.05). * Important compared to control and solvent control (p<0.01).*

3.2. Effect of Wild Thyme (T. spicata) Essential Oil on SCE in Human Peripheral Lymphocytes Treated with Acetone Mixture

Replication index (RI) and M1, M2 and M3 ratios of human peripheral lymphocytes treated with doses of 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL of *T. spicata L.* essential oil are given in Table 2. When compared with both controls in 100 cells count, *T. spicata* extract appears to reduce RI in human lymphocytes (Chart 3).

The doses of 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL of *T. spicata* extract were found significant when compared with control and solvent control ($p < 0.01$). There was a negative correlation between wild thyme dose and RI ($r = -0.94$) (Graph 3).

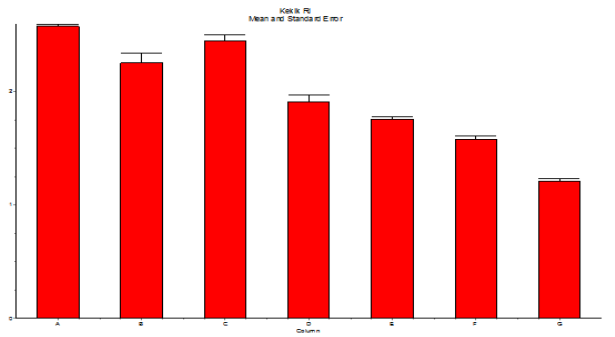


Chart 3. Replication Index Rates of Human Lymphocyte Cells of Control and Wild Thyme Extract Groups *(A: Negative Control, B: Acetone (2.5 µl/mL), C:Oregano (0.05 µL/mL), D:Thyme (0.10 µL/mL), E:Thyme (0.15 µL/mL), F:Thyme (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg/mL).*

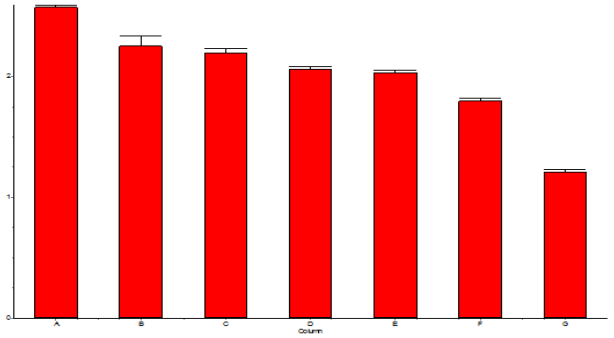
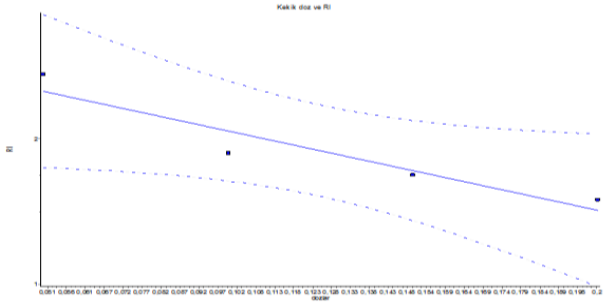
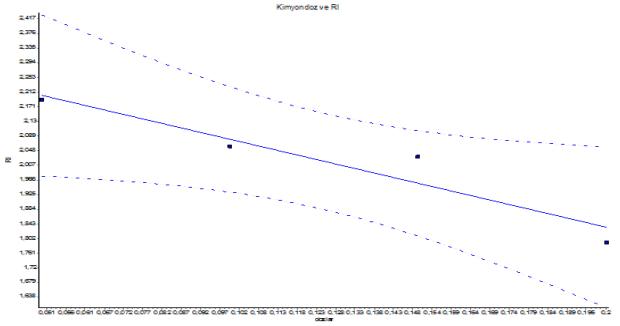


Chart 4. Replication Index Ratios of Control and Cumin Extract Groups in Human Lymphocyte Cells *(A: Negative Control, B: Acetone (2.5 µL/mL), C: Cumin (0.05 µL/mL), D: Cumin (0.10 µL/mL), E: Cumin (0.15 µL/mL), F: Cumin (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg/mL).*



Graph 3. A negative regression was found between wild thyme dose and RI (r = -0.94).



Graph 4. A negative regression was found between the cumin dose and RI (r = -0.95).

3.3. Effect of Cumin (C. cyminum) Essential Oil on SCE in Human Peripheral Lymphocytes Treated with Acetone Mixture

C. cyminum extract 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL doses of 24 hours treated with human peripheral lymphocytes determined in the replication index (RI) and M1, M2 and M3 rates are seen in Table 3. When compared with both controls in 100 cells counted, *C. cyminum* extract appears to reduce RI in human lymphocytes (Chart 4). Doses of *C. cyminum* L. extract 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL were found significant when compared to control and solvent control ($p < 0.01$). A negative correlation was found between cumin dose and RI (r = -0.95) (Graph 4).

In the literature, in two separate studies conducted with Thymol, the essential oil component of thyme was used as an antiseptic in some diseases such as bronchitis, cough, catarrh and eczema. At the same time, the genotoxic effect of Thymol was determined by the SCE test and Tyhmol significantly increased the number of SCE at high doses, but this result was not found to be statistically significant (Büyükleyla, 2007; Becarano and Emden, 1946). In the study conducted by Yavuz (2005), whether benzol peroxide used as bleaching agent in flour has genotoxic effects on human peripheral lymphocytes or not via *in vitro* SCE test were investigated; benzene peroxide generally increased SCE, but this increase was observed to be statistically significant over 48 hours of administration with only the highest concentration of benzene peroxide (100 µg / ml).

Table 3. Replication index of control and cumin extract groups in human lymphocyte cells

Groups	Concentration (µl/ml)	Treatment Time (hour)	Total Cells	M1	M2	M3	Number of RI	±SEM (%)
Negative Control	-	-	100	10	25	65	2.57	±0.02
Acetone	2.5 µL/mL	24	100	35	20	45	2.25*	±0.08
	0.05 µL/mL	24	100	36	15	49	2.19*	±0.03
Cumin	0.10 µL/mL	24	100	43	10	47	2.06*	±0.01
Essential Oil	0.15 µL/mL	24	100	42	14	44	2.03*	±0.01
	0.20 µL/mL	24	100	40	36	24	1.79*	±0.02
Positive Control (MMC)	0.3 µg/mL	24	100	89	5	6	1.20	±0.02

Compared with Dunnett T test. No difference between negative control and solvent control ($p > 0.05$). * Important compared to control and solvent control ($p < 0.01$).

In another study using SCE method; as a result of evaluating the role of genomic instability in patients with different types of leukemia; he considered the low incidence of SCE to be an expected condition in patients with leukemia and suggested that it would not be informative in the study of genomic instability (Sevinç, 2006).

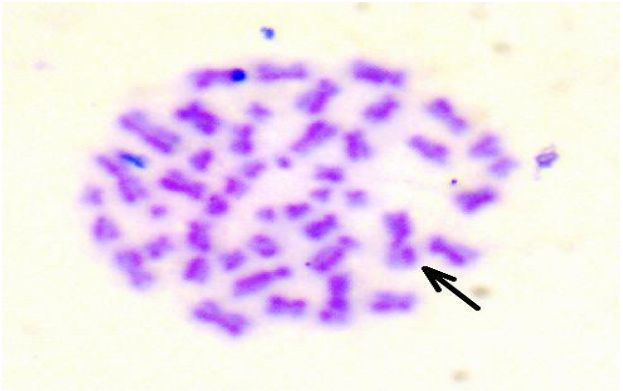


Photo 1. Metaphase plate with sister chromatid exchange.

Genotoxicity tests; In this study, we aimed to determine the role of genomic instability in chromosome aberration, micronucleus and sister chromatid exchange methods and myelodysplastic syndrome (MDS) and to investigate the relationship between these methods. Significant increase in the incidence of MN and SCE in patients with MDS and MN and SCE for genomic instability seem to be an informative biological marker (Nazlıgül, 2009).

By looking at histopathological effects of different doses of TSL (*T. spicata*) grown in Isparta, also known as thyme; and atorvastatin (ATS) on liver, heart and kidney tissues of rats; it was seen that TSL and ATS did not impair routine biochemical tests in general. It was found that TSL may be effective in hypercholesterolemia and 300 mg/kg TSL and ATS show antihypercholesterolemic effect at approximately the same rate. TSL and ATS have very low renal damage, but the amount of damage increases with increasing dose (Demiralay, 2010).

In the study on cinnamon, cumin and sumac which are widely used in our country; water, ethanol-water, methanol and chloroform extracts of each species of spice samples were obtained using appropriate methods to the literature. Extracts' antioxidant activity, phenolic compound amounts and reducing ability were determined; it was concluded that antioxidant activity levels were the highest in cinnamon and cumin extracts, the lowest in chloroform extracts and medium in all other extracts. The total amount of phenolic compounds of the extracts; sumac was the highest in methanol and ethanol-

water extracts, the lowest in chloroform extracts and at varying levels in all other extracts. According to the measurements, the reductive power of extracts was found to be the highest in sumac methanol extract, the lowest in chloroform extracts and at varying levels in all other extracts (Aydın, 2011).

4. Conclusion

In accordance with our *in vitro* study, we demonstrated the genotoxic effects of Wild Thyme (*T. spicata*) and Cumin (*C. cyminum)* plants by SCE test.

As a result, four different doses (0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL) of Cumin plant extract were applied in human lymphocyte culture for 24 hours.

a) There is a negative regression between the replication index and the different doses that reduce the replication index (r = -0.95).
b) Each dose is significant compared to the control and solvent control ($p < 0.01$).
c) When compared to negative control, it increased SCE number.

Wild Thyme (*T. spicata)* Extract was applied by applying four different doses (0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL) of it in human lymphocyte culture for 24 hours.

a) There was a negative regression between different doses and replication index (r = -0.94) and it was found to decrease the replication index.
b) Doses of 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL are significant compared to the control and solvent control ($p < 0.01$).
c) Positive regression was found between doses and SCE number (r = 0.99).

According to the data of our study, when the experimental groups treated with Cumin and Wild Thyme Extract were compared with the negative control, it was seen that the ratio of SCE increased due to the increase in the extract doses applied. In addition, it was observed that the replication index (RI) decreased with increasing extract concentration. As a result of this study; *T. spicata* and *C. cyminum* plant extracts were found to have genotoxic and clastogenic effects on human peripheral lymphocytes. This *in vitro* study may not fully explain the effects of Cumin and Wild Thyme extracts, so *in vivo* studies are also recommended.

Acknowledgments

This study is summarized from the master thesis supported by KAU Scientific Research Projects Commission (Project No: 2013-FEF-90). In addition to, in order to carry out our study, approval of the study numbered as 29.06.2011/6 was obtained by Kafkas University Faculty of Medicine Ethics Committee.

Conflict of Interest

None

References

1. Aydın, Ö., 2011. 'Tarçın, Kimyon Ve Sumak Adlı Baharat Türlerinden Elde Edilen Su, Etanol-Su, Metanol ve Kloroform Ekstraktlarının In Vitro Antioksidan Özelliklerinin Belirlenmesi' Atatürk University, Institute of Health Sciences, Pharmacy-Biochemistry Department, Erzurum.
2. Becarano, S. J., Emden, C., 1946. "Pratik Tıbbi Formüller". İsmail Akgün Printing Hause. İstanbul.
3. Büyükleyla, M., 2007. 'Thymol'ün İnsan Lenfositlerinde Kardeş Kromatid Değişimi, Kromozom Anormalliği ve Mikronükleus Oluşumu Üzerine Etkileri'. Çukurova University, Graduate School of Natural and Applied Sciences, Department of Biology, Adana.
4. Demiralay, H., 2010. "Thymbra Spicata Labiatae Oil'in (Karabaş Kekik Yağı) Ve Atorvastatin'in Antihiperkolesterolemik Etkinliğinin Araştırılması' Süleyman Demirel Üniversitesi, Sağlık Bilimleri Enstitüsü Biyokimya Anabilim Dalı Isparta.
5. Galloway, S.M., Miller, J.E., Armstrong, M.J., Bean, C.L., Skopek, T.R., Nıchols, W.W., 1998. "DNA Synthesis İnhibition As An İndirect Mechanism Of Chromosome Aberrations: Comparison Of DNA-Reactive And Nondna-Reactive Clastogens". J. Mutat. Res. 400,169-186.
6. İpek, E., Tüylü, B.A., Zetinoğlu, H., , 2003. "Effects Of Carvacrol On Sister Chromatid Exchanges İn

Human Lymphocyte Cultures". J. Cytotechnology. 43,1-3:145-148.
7. Natarajan, A. T., Obe, G., 1982. "Mutagenicity Testing With Cultured Mammalian Cells: Cytogenetic Assays". In: Heddle JA(ed) Mutagenicity. New Horizons in Genetic Toxicology. Academic Pres, New York, 1-213.
8. Nazlıgül, E., 2009. 'Miyelodisplastik Sendromlu Olgularda Genomik İnstabilitenin Farklı Sitogenetik Yöntemlerle (Kromozom Aberasyonu, Kardeş Kromatid Değişimi, Mikronukleus) Araştırılması', Istanbul University, Institute of Health Sciences, Department of Genetics / Genetics Master Thesis, İstanbul.
9. Sevinç, B., 2006. 'Hematolojik Malign Hastalıklarda Genomik İnstabilitenin Farklı Sitogenetik Yöntemlerle (Kromozom Aberasyonu, Kardeş Kromatid Değişimi, Mikronukleus) Araştırılması'. Istanbul University Institute of Health Sciences, Department of Medical Genetics, Master Thesis, İstanbul.
10. Speit, G., Haupter, S., 1985. On the mechanism of differantial giemsa staining of bromodeoxyuridine substitued chromosomes. II. Differences Between the demonstration of sister chromatid differantiation and replication patterns. J. Hum. Genet. 70, 126-129.
11. Uzun, S., 2007. 'Sodyum Hipoklorite Maruz Kalmış Kişilerin Lenfositlerindeki Mikronükleus Sıklığının Araştırılması', Master Thesis, Erciyes University, Institute of Health Sciences, Department of Medical Biology, Kayseri.
12. Yavuz, A., 2005. 'Benzol Peroksit'in İnsan Periferal Lenfositlerinde *in vitro* Genotoksik Etkileri'. Çukurova University, Graduate School of Natural and Applied Sciences, Department of Biology, Adana.

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Identification and Authentication of Microbes Causing Urinary Tract Infection and Detection of Antibacterial Activity for Methanolic Extract of *Senna alexanderina* against these Pathogenic Bacteria in Khartoum State, Sudan

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Abstract

The aim of this study is to isolate and identify the microbes causing urinary tract infection and antibacterial of those microbes by used methanolic extract of plant and antibiotics. One hundred samples were collected for both genders in Khartoum State. From sixty-three out of one hundred samples obtained on different types of microbes are *Staphylococcus aureus* (33.3%), *Enterococcus fecales* (9.5%), *Escherichea coli* (19%), *Klebsiella pneumonae* (7.9%), *Protus mirabilis*, *Pseudomonas aeuroginosa* (9.5%), *Citrobacter* ssp (4.7%), *Candida albicans* (7.9%). The antibacterial results against isolated microorganisms using methanolic extract of *Senna alexanderina* showed resistance to these microbes except *S. aureus* was sensitive; also, most microbes were sensitive to antibiotics.

Key Words: Urinary Tract Infection, Methanolic Extract, *Senna alexanderina*, Pathogenic Bacteria, Microbes

1. Introduction

Urinary Tract Infection (UTI) represents one of the most common diseases occurring from the neonate to the geriatric age groups encounters in medical practice today. It is estimated that about 35% of healthy women suffer symptoms of UTI at some stages in their life. The incidence of UTI is greater in women as compared to men, which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors. An antimicrobial compound is a substance that kills or inhibits the growth of bacteria (Sharma et al., 2009).

Some plants have been investigated significantly for their antimicrobial activity and as large numbers of plant products have been shown to inhibit the growth of pathogenic microorganism (Kanwar et al., 2019).

In recent years, works in medical field return back to nature particularly in the use of medical plants treat human ailments, this now trend was supported by the recent World Health Organization, orientation strategy that embarked on examination of historical position of modernization of biochemically based health care (WHO, 1988; Dole, 2004).

Consequently, medical practitioners are also prescribing herbal medicine teas and herbal extracts as a supplementary type of treatment in everyday problems caused by our modern civilization (Gomez et al., 2007, Shida et al., 2019). Sudan has an immense diversity and variation in vegetation and is one of the richest countries with regard to phytopharmaca. Although herbal remedies are often perceived as being natural and, therefore, safe, they are not principally free from adverse effects. While many investigations of the quality values of medicinal plants are being reported in the current literature, less emphasis has been made on the metal content of herbal products. Such as renal failure, symptoms of chronic toxicity and liver damage (Gomez et al., 2007; Gezici and Sekeroglu, 2019). The aim of this study is to investigate the antimicrobial activity of Alexandrian senna (leaves) to ascertain the rationale for its use in traditional medicine.

2. Materials and Method

2.1. Study Area

This study was conducted at Khartoum State, Sudan. Samples were taken from patients admitted at Khartoum Teaching Hospital and Omdurman Teaching Hospital.

2.2. Samples Collection

Hundred individuals were recruited, for taking urine randomly irrespective of sex and age.

2.3. Data Collection

Data were collected from the patients using structured questionnaire involving ; age , sex.

2.4. Identification of Isolated Bacterial

The colonial morphology and fermentation of lactose were examined macroscopically on Cystine lactose Electrolyte Deficient (CLED), this was observed by yellow color. Then uses staining test (gram stain) and biochemical test such as (Indole, Citrate Utilization, Urease, H_2S production)

2.5. Plant Material

The leaves of *Alexandrian senna* were collected from the local market in Khartoum. The plant was identified in the microbiology department, Faculty of Pure and Applied Sciences, International University of Africa by Ahmed Elshikh and by comparison with herbarium of the department. The dried plant samples were cleaned from dust and grass then they were separately crushed to a powder by using sterilized mortar and pestle.

2.6. Preparation of Plant Extracts

Fifty grams from *Alexandria senna* were extracted into 500 ml methanol. Resulting extraction in the solvent was evaporated and concentrated using the rotary evaporator at 50 °C (Abeysinghe, 2010).

2.7. Preparation of Bacterial Suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 °C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108-109 C.F.U./ml. The suspension was stored in a refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes on drop of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension,

expressed as the number of colony forming units per ml suspension (C.F.U. /ml). Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

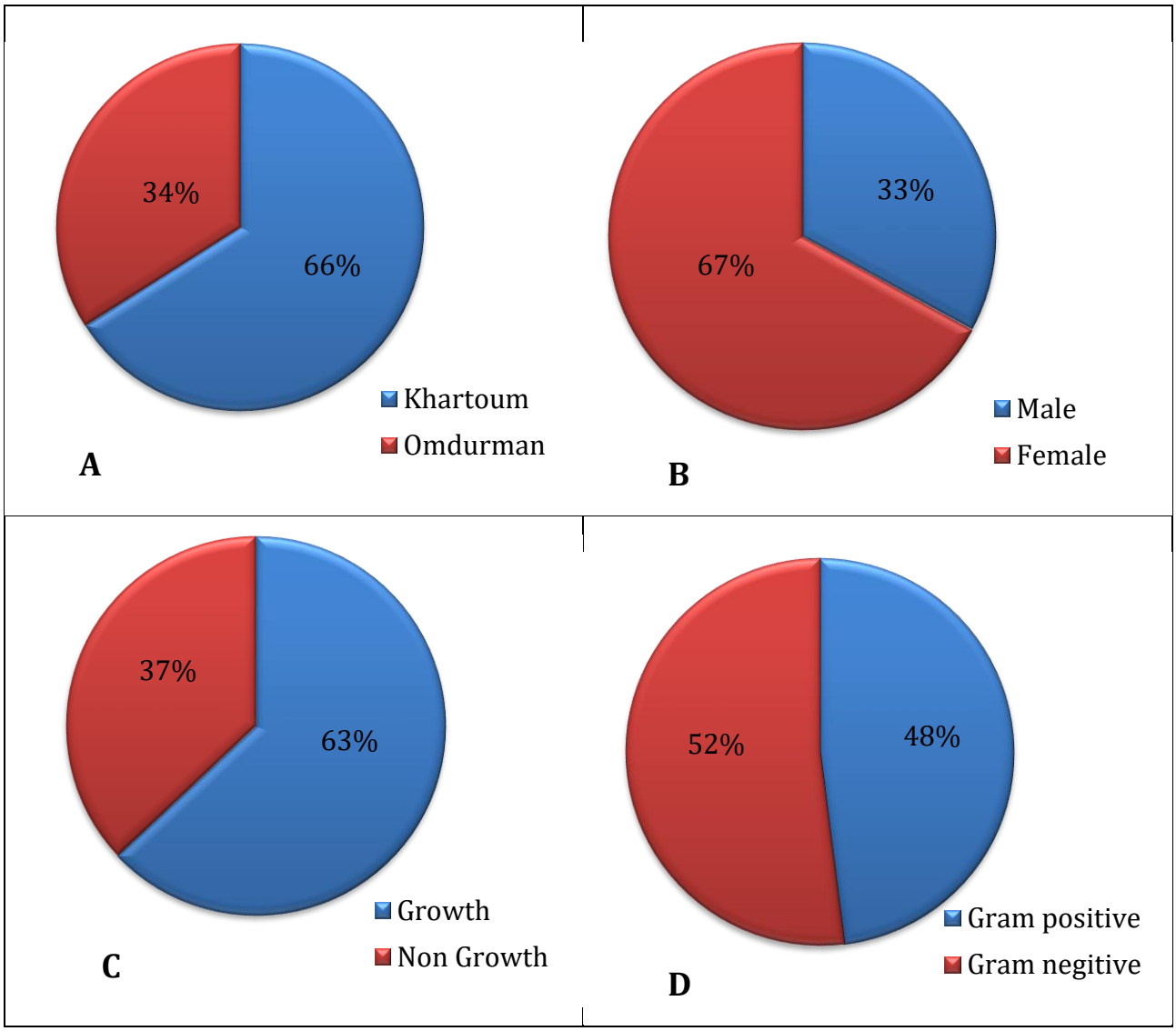

Figure 1. Distributions of specimens and bacteria

2.8. In vitro Testing of Extracts for Microbial Activity

The cup-plate agar diffusion method was adopted according to (Kavanagh, 1972) with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 108–109 C.F.U/ml were thoroughly mixed with 100ml of sterile molten nutrient agar which was maintained at 45°C. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar were left to dry and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No.4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic Microlitrepipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 18 hours. Two replicates were

carried out for each extracts against each of the test organisms. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the resultants and growth inhibition zones were measured averaged and the mean values were tabulated.

2.9. Analysis of Data

Data were collected from the patients using structured questionnaire involving; age, sex. Data were analyzed using Microsoft Office Excel 2007.

3. Results

Sixty six patients (66%) were collected from Khartoum Hospital and thirty four (34%) from Omdurman Hospital as shown in Figure 1A (distribution of specimens according to hospital). In addition, the sex ratio was determined for both sexes, where the proportion of females was 67% and the proportion of males 33% as shown in Figure 1B (showed gender males and females distribution). The frequency of bacterial growth was determined for the samples collected, where the percentage of bacterial growth (63%) and ratio of samples that did not grow (37%) as shown in Figure 1C (the frequency of bacterial growth). The gram reaction of isolated bacteria revealed that (30) isolates were gram positive and (33) were gram negative shown in Figure 1D (frequency the Gram reaction for isolated bacteria).

The isolated causative agents were identified to be *Staphylococcus aureus* (33.3%), *Klebsiella pneumonae* (7.9%), *Protus mirabilis* (11.1%), *Enterococcus fecales* (9.5%), *Escherichea coli* (19%), *Pseudomonas aeuroginosa* (9.5%), *Candida albicans* (7.9%) and *Citrobacter* (4.7%), given as Table 1 and Figure 2.

Table 1. Type of isolated bacteria and the percentage

Bacteria	Frequency	Percentage
Staphylococcus aureus	21	33.3%
Enterococcus fecales	4	6.3%
Escherichea coli	12	19%
Klebsiella pneumonae	5	7.9%
Protus mirabilis	7	11.1%
Pseudomonas aeuroginosa	6	9.5%
Citrobacter spp	3	4.7%
Candida albicans	5	7.9%
Total	**63**	**100%**

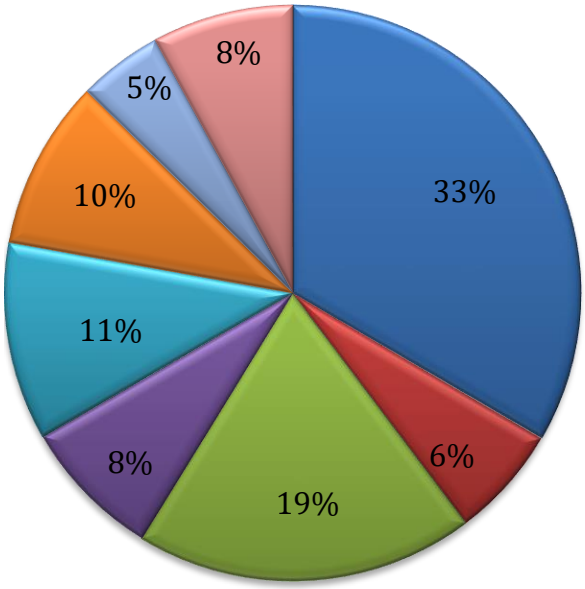

Figure 2. Type of microbial isolated and their percentage

Antibacterial activity of Senna alexandrina of isolated bacteria organisms is shown in (Table 2) using different concentration of the methanol extracts (100, 50, 25, 12.5, 6.2 mg/ml), The highest activity of the methanolic extract was in the concentration (100%) against *S. aureus* (15 mm), while no good activity was shown against other organisms and other concentrations used, compared with reference drugs The results were presented in the Table 3 and Figure 3. Most of them were of active than methanolic extract of *Senna alexandrina*, except for amoxicillin, where *E. coli*, *K. pneumonae*, *Ps. aeroginosa* and *Citrobacter* were resistant to it, whereas *E. fecales* and Prot. mirablis were half-sensitive to it. In addition, *Ps. aeruginosa* and *Citrobacter* showed resistance against Co-trimoxa, while *K. pneumoniae* was half-resistant.

Table 2. Number of isolated bacteria and their Minimum Inhibitory Concentration (MIC) of methanolic extract of *Senna alexandrina* leaves at different concentrations

Bacteria isolated	Concentrations of extract (mg/ml) and MDIZ			
	100	50	25	12.5
Staphylococcus aureus	15	4	2	-
Enterococcus fecales	4	-	-	-
Escherichea coli	3	5	4	-
Klebsiella pneumonae	6	-	-	-
Protus mirabilis	4	3	-	-
Pseudomonas aeuroginosa	2	3	-	-
Citrobacter spp	2	1	-	-

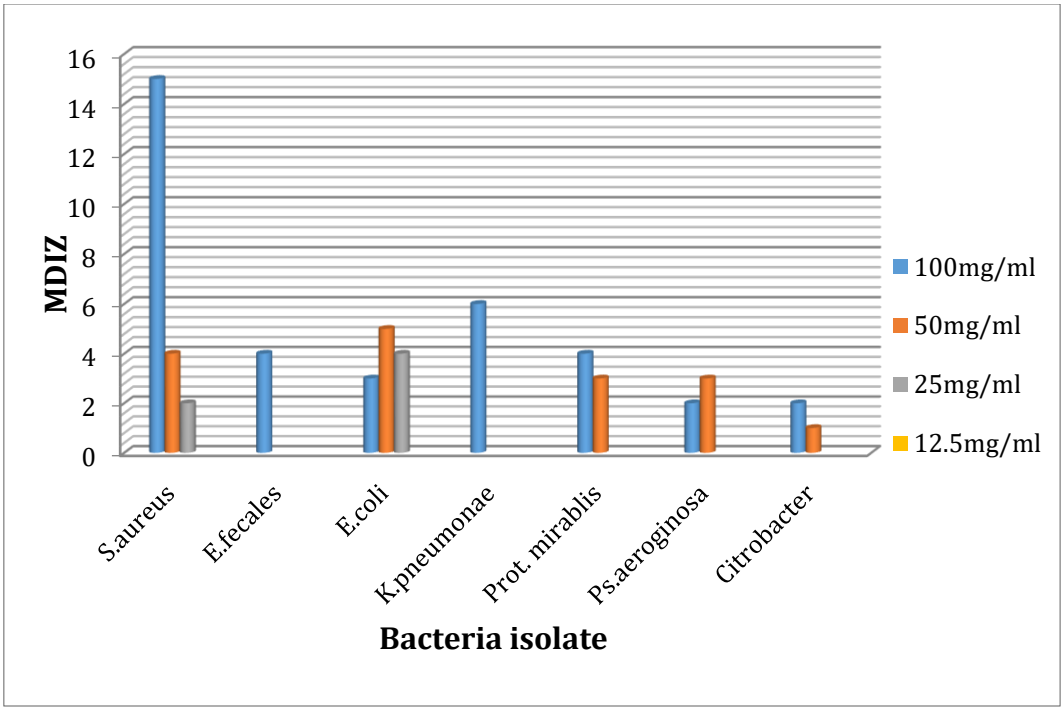

Figure 3. Antibacterial activity of methanolic extract of *Senna alexandrina* (leaves) at different concentrations

Table 3. Antibacterial activity of reference drugs against isolated bacteria

Bacteria	Ciprofloxacin	Gentamicin	Amoxacillin	Co-trimoxa
Staphylococcus aureus	S	S	S	S
Enterococcus fecales	S	S	S/R	S
Escherichea coli	S	S	R	S
Klebsiella pneumonae	S	S	R	S/R
Protus mirabilis	S	S	S/R	S
Pseudomonas aeuroginosa	S	S	R	R
Citrobacter spp.	S	S	R	R

** S= Sensitive, R= Resistant*

4. Discussion

Bacterial infection is one of the most serious global health issues in 21st century. The emergence of bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanism of action to overcome these problems (Sharma et al., 2009). In the modern world, multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Saranraj and Sivasakthivelan, 2012). According to result the percentage of *S. aureus, E. coli* and *P. mirabilis* was higher in the studied samples. These results differed from those obtained by (Sharma et al., 2009). The antibacterial results of methanolic extract of Senna alexandrina showed a poor activity against isolated bacteria. These results are similar to those of (Selim et al., 2013), who used different solvents of *Senna alexandrina* against some of the standard bacteria used in this study. While (Viswanathan and Nallamuthu, 2012) reports have shown the effectiveness of the methanolic extract of *Senna alexandrina* against *E. coli* and *P. aeruginosa,* these results are different from those of the present study. Results of antibiotic susceptibility showed that nearly all the isolates were sensitive and other resistant against most of the antibiotics tested during the present investigation. The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements/plasmids (Sharma et al., 2009).

5. Conclusion

This study revealed some microbes causing urinary tract infection that were isolated and identified and activity of methanolic extract of *Senna alexanderina* was evaluated for these microbes, as well as their sensitivity to antibiotics.

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References

1. Abeysinghe P.D. (2010). Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. Indian Journal of Pharmaceutical Science, 72, 167-72
2. Dole C. (2004). In the shadows of medicinal and modernity medicinal integration and secular histories of religious healing in turkey journal culture. Medicinal Psychiatry, 28(3),255-80.
3. Gezici S. and Sekeroglu N. (2019). Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 19(1), 101-111.
4. Gomez MR, Cerutti S, Sombra LL, Silva MF and Martı´nez LD. (2007). Determination of heavy metals for the quality control in Argentinian herbal medicines by ETAAS and ICP-OES. Food Chem Toxicol 45:1060–1064.

5. Kanwar K., Pandey R., Gezici S., Azmi W. (2019). Enzymes as competent tool for efficient management of pathogen's biofilms. Annals of Phytomedicine, *8*(1), 70-81.
6. Kavanagh F. (1972). Analytical Microbiology, Vol II, Academic Press, New York and London, pp. 11.
7. Miles A. A. and Misra S. S., (1938). Estimation of bacterial power of blood, J Hygiene (Lond) 38: 732-49.
8. Saranraj P. and Sivasakthivelan P. (2012). Screening of Antibacterial Activity of the Medicinal Plant Phyllanthus amarus Against Urinary Tract Infection Causing Bacterial Pathogens, Applied Journal of Hygiene, 1(3), 19-24.
9. Selim S. A., El Alfy S. M., Abdel Aziz M. H., Mashait M. and Warrad M. F. (2013). Antibacterial activity of selected Egyptian ethnomedicinal plants, Malaysian Journal of Microbiology, 9(1), pp. 111-115.
10. Sharma A., Verma R. and Ramteke P. (2009). Antibacterial Activity of Some Medicinal Plants Used by Tribals Against Uti Causing Pathogens, World Applied Sciences Journal, 7(3), 332-339.
11. Shida W., Tateishi H., Tahara, Y. Fujita, M. Husham Majeed Alsaadi D., Watanabe M., Koga R., Radwan M.O., Ciftci H.I., Gezici S., Kurauchi Y., Katsuki H., Otsuka M., Sugimura K., Wada M., Sekeroglu N., Watanabe T. (2019). Antileukemic Activity of Twig Components of Caucasian Beech in Turkey. Molecules, 24(21), 3850.
12. Viswanathan S. and Nallamuthu T. (2012). Phytochemical screening and antimicrobial activity of leaf extracts of Senna alexandrina Mill. against human pathogens, International Journal of Current Science, 2, 51-56.
13. World Health Organization (WHO), (1988). The international pharmacopoeia, 3rd ed. Vol. 3. Quality specifications. Geneva.

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Bioapigyn® Ointment of Pelvic Muscle Tonus Versus Pelvic Floor Muscle Training for the Treatment of Urinary Incontinence

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Abstract

Objective/Purpose: The purpose of this work was the assessment of the clinical efficacy and safety of Bioapigyn® vaginal ointment for pelvic muscle tonus compared to pelvic floor muscle training in alleviating the symptoms of stress, urge and mixed urinary incontinence and vulvo-vaginal disorders in child-bearing and menopausal & postmenopausal women. **Materials and Methods:** The experimental group consisted of 66 women was treated 28 days with Bioapigyn® ointment for pelvic muscle tonus (2.5 mL/day). The control group also consisted of 66 participants was subjected to pelvic floor muscle training during 28 days (five times a day). ICIQ-UI SF score, the residual urine volume, perineometry, the total score of vulvo-vaginal symptoms and vaginal pH were determined before and after the treatment or training. **Results:** Following the treatment with Bioapigyn® ointment ICIQ-UI-SF score decreased 54.9%, perineometry parameters increased between 31.5 and 34.3%, residual urine decreased for 76.9% and vaginal pH for 14.2%. All the symptoms of vulvo-vaginal disorders disappeared completely in all participants. The control group showed no changes in vaginal pH or the improvement concerning the vulvo-vaginal complaints. ICIQ- UI-SF score decreased for 4.3%, residual urine volume for 9.1% while perineometry parameters increased between 4.3 and 8.3%. **Conclusion/Discussion:** Bioapigyn® vaginal ointment for pelvic muscle tonus alleviate the symptoms of incontinence by tightening and firming of the smooth muscles of the pelvic floor thanks to the ingredients with smooth muscles contraction/relaxation and astringent properties. Low pH, high osmolarity, viscosity, greasiness and coating effect of the ointment eradicated vulvo-vaginal complaints.

Key Words: Urinary Incontinence, Vulvo-Vaginal Disorders, Honey, Herbal Macerates

1. Introduction

Urinary incontinence (UI) is the complaint of involuntary leakage of urine (Aoki et al., 2018). It is a common chronic condition that affects millions of persons around the world (Minassian et al., 2008). Although UI tend to increase with age it could affect people of all ages. Urinary incontinence occurs when the pressure within the bladder exceeds the total urethral resistance and urine flows involuntarily beyond the urinary sphincter

(Rovner and Wein, 2004; Aoki et al., 2018). It occurs as a result of abnormalities of the urethra or both that may result in either overfunction or underfunction of the bladder and/or urethra, resulting in the development of urinary incontinence.

Chronic UI is classified into five types: stress, urge, mixed, overflow and functional (Rovner and Wein, 2004; Khandelwal and Kistler, 2013; Aoki et al., 2018).

Stress urinary incontinence (SUI) is defined as the complaint of involuntary leakage during effort or exertion, or on sneezing or coughing (Luber, 2004; Khandelwal and Kistler, 2013; Aoki et al., 2018). It is characterized by loss of small amount of urine during physical activity or intra-abdominal pressure (coughing, sneezing jumping, lifting, exercise).

Urge urinary incontinence (UUI) is characterized by loss of urine preceded by a sudden and severe desire to pass urine in which patient typically loses urine on the way to the toilet (Khandelwal and Kistler, 2013). Unlike the physical changes associated with stress urinary incontinence, UUI involves physiological perturbations to bladder function. There are three main etiologies essential to the bladder that leads to urge incontinence: detrusor over activity, poor detrusor compliance and bladder hypersensitivity (Rovner and Wein, 2004; Khandelwal and Kistler, 2013; Aoki et al., 2018; Radzimińska et al., 2018).

Mixed UI presents the combination of stress and urge incontinence which is characterized by involuntary leakage associated with symptoms of urgency as well as loss of urine with exertion, effort, sneezing, or coughing (Rovner and Wein, 2004; Khandelwal and Kistler, 2013; Radzimińska et al., 2018). Although UI increases with age, its prevalence varies widely. The median prevalence of any type of UI in women based on 35 studies (Minassian et al., 2003) was 27.6% (with a range of 4.8–58.4%).

Among 1700 French women employed in academic hospital, 12.4% of them reported SUI (Peyrat et al., 2002). The pregnancy, particularly previous vaginal delivery and hysterectomy represented the significant risk factors. The prevalence of stress, urge, mixed, and any UI among 2,875 adult women were 23.7%, 9.9%, 14.5%, and 49.2%, respectively (Minassian et al., 2008). The obtained significant risk factors were age, ethnic background, weight, parity and hysterectomy.

Among 83,355 American nurses at the age range from 37 to 54 years 43% of them reported incontinence. Identified risk factors were age, race/ethnicity, body mass index, parity, smoking, type 2 diabetes mellitus, and hysterectomy (Danforth et al., 2006).

Among 20,000 Chinese women in the age range from 20 to 99 years the prevalence of UI was 30.9%. Among them 18.9%, 2.6%, and 9.4% were diagnosed with SUI, UUI and mixed incontinence, respectively (Zhu et al., 2009). The authors identified age, vaginal delivery, multiparty, alcohol consumption, central obesity, constipation, chronic pelvic pain, history of respiratory disease, gynecological events, pelvic surgery, and perimenopause and postmenopause status as the significant risk factors. Buchsbaum et al. (2002), estimated the prevalence of urinary incontinence among a group of nulliparous nuns which was app. 50%. Among them 30% had stress incontinence, 24% had urge incontinence, 35% had mixed incontinence, and 11% had urine loss unrelated to stress and urge. Identified covariates were BMI, multiple urinary tract infections, and depression.

Higher prevalence of UI (Brown et al., 1999) was obtained among postmenopausal women (56%). Luber (2004), reported the prevalence of SUI which ranged between 4% and 35% depending on the country with age, obesity, and smoking as the most significant risk factors. Nygaard and Heit (2004),

reported that SUI occurs at least weekly in one third of adult women.

A world wide survey conducted by McPhil (2004), revealed the highest percentage of women with SUI in UK (41%) and Canada (42%) and the lowest percentage were obtained in Spain (23%) while the mean value for all considered countries was 32%. Two-thirds of the symptomatic women were younger than 50 years.

The treatment approaches depending on the type of incontinence and its severity. The most common treatment approaches are various behavioral techniques, pelvic floor muscle training (PFMT), electrical stimulation, medical devices, medication, surgical procedures, laser treatment (Nygaard and Heit, 2004; Aoki et al., 2018). Better results were obtained by surgical and laser treatment. However, those methods were associated with more risk compared to the conventional treatment.

The purpose of this study was to assess the efficacy and safety of Bioapigyn® vaginal ointment for pelvic muscle tonus for the local treatment of incontinence and vulvo-vaginal disorders in adult female population in comparison of pelvic floor muscle training due to similar mode of action of those two approaches.

2. Materials and Method

2.1. Study design

The study was designed as prospective, randomized, controlled clinical trial. The study protocol was approved by the Ethics Committee of Findri Gustek Health Care Center with EudraCT number 2019-001053-23.

The investigator recruited the patients based on their medical history, following the predefined inclusion and exclusion criteria. In total, 132 patients were included of 160 patients screened. After the informed consent has been signed at Visit 1, Day 1 all the patients completed International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF), and subjected to the measurement of post voiding residual urine volume, perineometry (maximal and average pressure (mm Hg) and the mean duration of the contractions), vaginal pH and self-assessment of vulvo-vaginal complaints. The questionnaire data and the clinical study results were recorded in source data. The patients were subjected to semi-quantitative urine analysis and full gynaecological examination in order to exclude other disease and conditions.

Patients were included in the study only if they meet all of the following criteria: stress, urge or mixed urinary incontinence and vulvo-vaginal disorders; history of vaginal delivery; non-existence of other gynecological problems; negative urine test; no injuries and bleeding in the vaginal canal, introitus and vestibule; in the investigator's judgment, the patients should receive local treatment only; signed informed consent. The patients who met all the criteria were divided into experimental and control group by nurse based on the randomization code.

The patients of the experimental group were given two tubes of Bioapigyn® vaginal ointment for pelvic muscle tonus with an applicator and instructions for use. Control group was subjected to pelvic floor muscle training and received training instructions.

66 patients were treated with Bioapigyn® vaginal ointment for pelvic muscle tonus for 28 days. 2.5 mL of ointment was self-administered by the patients once daily, between 21:00 and 24:00 hours using appropriate applicator. The second group also consisted of 66 patients was scheduled to the pelvic floor muscle training for 28 days according to the instruction given by the nurse. In short the patient mast contracts the pelvic floor muscles, holding the contractions for 10 seconds and then relaxes for 10

seconds. It must be repeated ten times five times daily. The purpose of PFMT was to improve the pelvic floor muscle function. Follow-up period for both groups was from Day 29 to Day 60.

2.2. Description of investigational product

Bioapigyn® vaginal ointment for pelvic muscle tonus is homogeneous, greasy, viscous mass of characteristic herbal odor and olive green color with pH of 4.93, density of 0.9801 g/cm^3 and viscosity of 20732 cP. It consists of the following ingredients: honey; beeswax (*Cera flava*); glycerol; oil extracts of the plant species: areal parts of shepherd 's purse (*Capsella bursa-pastoris* L.), nettle leaves (*Urtica diodica* L.), oak bark (*Quercus robur* L.), sage leaves (*Salvia officinalis* L.), areal parts of yarrow (*Achillea millefolium* L.), lady's mantle leaves and steam (*Alchemilla vulgaris* L.), marigold flowers (*Calendula officinalis* L.), cammomile flowers (*Matricaria chamomilla* L.), plantain leaves (*Plantago major* L.), olive leaves (*Olea europaea* L.); essential oils: Australien tea tree (*Melaleuca alternifolia*), thyme (*Timus vulgaris* ct. thymol), oregano (*Origanum vulgare*). Detailed description of the ointment production was published previously (Oreščanin et al., 2018).

2.3. Statistical analysis

Statistical analysis was performed using STATISTICA 12.0 software package. The required sample size was calculated by Power analysis method. Considering the medium effect size (0.35), the power goal of 80% and the Type I error significance level of 0.05, the required sample size is 66 participants per group, amounting to the total of 132 participants. Frequencies and percentages were calculated for each categorical variable using frequency tables. Potential differences between the groups of categorical variable were determined using $\chi 2$ Test. Basic statistical parameters were determined for each continuous variable. The Shapiro-Wilk W-Test was used to determine the normality of distribution of continuous variables, while Levene's Test was used for homogeneity of variances. The t test was used to determine the difference between mean values of two groups with normally distributed continuous variables, while the analysis of variance and Newman-Keuls test were used for the assessment of difference among three and more groups. Before carrying out the above-mentioned statistical analyses of data, logarithmic transformation was used to deal with the variables which deviate from the normal distribution. To determine the dependence of the dependent variable on the chosen predictor variables, multiple regression analysis was applied. The p-value of less than 0.05 ($p<0.05$) will be considered statistically significant in all measurements.

3. Results

3.1. Description of the population

The control population ranged from 50 to 73 (56.8±6.2) years and experimental group from 34 to 80 years (58.7±9.3). T-test showed no significant difference ($t=1.4$; $p=0.153$) between these two groups. There was no significant difference ($t=0.2$; $p=0.665$) in the number of childbirth between control (2.1±0.9) and experimental group (2.0±1.1). Both groups showed similar mean values and standard deviations for body mass index (26.3±4.8 for experimental and 25.7±4.1 for the control group). There was no significant difference between those two values ($t=0.6$; $p=0.434$).

Among 66 participants of the experimental group 20 of them are reproductive age woman while 46 of them are either menopausal or postmenopausal woman. Similar distribution was also found in the control group consisted of 28 reproductive age participants and 38 of those of menopausal & postmenopausal status. According to the results of $\chi 2$ test there was no significant difference between the

percentages of either reproductive age or menopausal & postmenopausal woman between the experimental and control group.

3.2. Treatment efficiency of vulvo-vaginal disorders

Table 1 presents the basic statistical parameters and the results of t-test for the symptoms of vulvo-vaginal disorders like unpleasant odor, itching, burning, vaginal discharge, vaginal dryness and dyspareunia graded from 0 to 3 and total score of those complaints in the control group subjected to pelvic floor muscle training and experimental group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the training or treatment.

Table 1. Treatment efficiency of vulvo-vaginal disorders

Group	Stat. parameter	Odor		Itching		Burning	
		Initial	Final	Initial	Final	Initial	Final
Control	$\bar{X}$	0.4	0.4	1.3	1.3	1.5	1.5
	SD	0.7	0.7	0.7	0.7	0.5	0.5
	M	0.0	0.0	1.0	1.0	1.5	1.5
	Min.	0	0	0	0	1	1
	Max.	2	2	3	3	3	3
	t-test	t=0; p=1.000		t=0; p=1.000		t=0; p=1.000	
Experimental	$\bar{X}$	0.5	0.0	1.3	0.0	0.0	1.2
	SD	0.6	0.0	0.5	0.0	0.0	0.5
	M	0.0	0.0	1.0	0.0	0.0	1.0
	Min.	0	0	0	0	0	0
	Max.	2	0	3	0	0	2
	t-test	t=5.7; p<0.0000*		t=20.3; p<0.0000*		t=7.6; p<0.0000*	

Table 1. Continued.

Group	Stat. parameter	Vaginal		Vaginal dryness		Dyspareunia		Total score	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	$\bar{X}$	1.4	1.4	1.0	1.0	1.2	1.2	6.7	6.7
	SD	1.1	1.1	0.9	0.9	1.0	1.0	1.9	1.9
	M	2.0	2.0	1.0	1.0	1.0	1.0	6.0	6.0
	Min.	0	0	0	0	0	0	3	3
	Max.	3	3	3	3	3	3	12	12
	t-test	t=0; p=1.000		t=0; p=1.000		t=0; p=1.000		t=0; p=1.000	
Experimental	$\bar{X}$	0.5	0.0	2.0	0.0	0.0	2.0	7.6	0.0
	SD	0.5	0.0	0.6	0.0	0.0	0.5	1.6	0.0
	M	0.0	0.0	2.0	0.0	0.0	2.0	7.0	0.0
	Min.	0	0	0	0	0	0	5	0
	Max.	2	0	3	0	0	3	11	0
	t-test	t=28.4; p<0.0000*		t=31.4; p<0.0000*		t=31.4; p<0.0000*		t=38.6; p<0.0000*	

The total score of vulvo-vaginal complaints in the control group ranged from 3 to 12 (6.7±1.9). The number of the symptoms at baseline ranged from 3 to 5. The highest mean values were obtained for burning, vaginal discharge and itching. Expectedly, following the four weeks of the training there was no reduction in the number or severity of the symptoms since there was no concomitant treatment of vulvo-vaginal disorders during the course of PFMT.

In the experimental group the total score of vulvo-vaginal complaints ranged from 5 to 11 (7.6±1.6) at baseline. Quite opposite to the control group, all the patients treated four weeks with the Bioapigyn® ointment for pelvic muscle tonus showed no symptoms of vulvo-vaginal disorders following the treatment. During the application of the ointment and monitoring period none of the patient experienced side-effects or new symptoms of vulvo-vaginal disorders.

Table 1. Basic statistical parameters and the results of t-test for the symptoms of vulvo-vaginal disorders graded from 0 to 3 and total score of the patients with incontinence and vulvo-vaginal disorders in control (C) group subjected to pelvic floor muscle training and experimental (E) group treated with Bioapigyn ointment for pelvic muscle tonus before and after the treatment or training. X̄-mean; SD-standard deviation; M-median; *-significantly different at p<0.05.

Vaginal pH value (Table 2) in the control group at baseline ranged from 5 to 7 (5.8±0.6) and showed the same value following the pelvic floor muscle training.

The experimental group showed similar values at baseline ranging from 4.8 to 7.5 (6.0±0.7). Four weeks of the application of Bioapigyn® ointment for pelvic muscle tonus resulted in statistically significant (t=7.4; p<0.0000) decrease of vaginal pH ranging from 4.3 to 6.5 (5.2±0.6).

Table 2. Basic statistical parameters for vaginal pH of the patients

Stat. parameter	Control		Experimental	
	Initial	Final	Initial	Final
X̄	5.8	5.8	6.0	5.2
SD	0.6	0.6	0.7	0.6
M	5.8	5.8	6.0	5.0
Min.	5.0	5.0	4.8	4.3
Max.	7.0	7.0	7.5	6.5
t-test	t=0.0; p=1.0		t=7.4; p<0.0000*	

Table 2. Basic statistical parameters for vaginal pH of the patients with incontinence and vulvo-vaginal disorders in control group subjected to pelvic floor muscle training and experimental group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the treatment or training. X̄- mean; SD-standard deviation; M-median; *-significantly different at p<0.05.

3.3. The results of urinary incontinence treatment

Based on the results of the total values of International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF) and additional information obtained from the patients when included in the study it was determined that among 66 participants of the experimental group 39 of them suffered from stress UI, 17 from mixed UI and 10 from urge UI (Table 3).

Stress urinary incontinence also prevailed in the control group with 45 of 66 participants. 11 of them had urge and 10 mixed urinary incontinence. Obtained results were in agreement with previous research confirming the highest incidence of SUI, followed by mixed and UUI (Buchsbaum et al., 2002; Minassian et al., 2008; Zhu et al., 2009).

Table 3. Frequencies and percentages of the subject of experimental and control group with stress (SUI), urge (UUI) and mixed urinary incontinence.

Type of incontinence	Experimental		Control	
	N	%	N	%
SUI	39	59.1	45	68.2
UUI	10	15.2	11	16.7
Mixed	17	25.8	10	15.2

The severity of the symptoms in both groups were determined at baseline and after four weeks of the treatment or training on the bases of the total value of ICIQ-UI SF score and the results were presented in Table 4. At baseline among 66 participants of the experimental group, 9 of them experienced only mild symptoms, 29 moderate, 26 severe and 2 very severe symptoms. Among the participants of the control group 11 of them had slight, 27 moderate and 28 of them severe symptoms of UI.

Following the treatment with Bioapigyn® ointment for pelvic muscle tonus, none of the patients reported very severe symptoms of incontinence while 18 of them (27.3%) were completely dry. Complete disappearance of the symptoms was obtained in the patients with prevalently stress urinary incontinence with initial ICIQ-UI SF score ranging from 3 to 10.

The percentage of the patients with severe symptoms decreased from 39.4% to only 6.1%. Those results were in line with the previous research results following two weeks of the application of Bioapigyn® ointment for pelvic muscle tonus (Orescanin et al., 2018). After the treatment, 4.56% of the patients were completely dry while very severe symptoms decreased from 10.61% to 0 and severe symptoms from 42.42% to 25.75%. Better results obtained in the current study were the function of two times longer treatment period.

Table 4. Frequencies and percentages of the subject of experimental (E) and control (C) group before and after the treatment or training based on the severity of the symptoms of urinary incontinence.

Severity	Initial				Final			
	E		C		E		C	
	N	%	N	%	N	%	N	%
Non (0)	0	0	0	0	18	27.3	2	3.0
Slight (1-5)	9	13.6	11	16.7	22	33.3	13	19.7
Moderate (6-12)	29	43.9	27	40.9	22	33.3	26	39.4
Severe (13-18)	26	39.4	28	42.4	4	6.1	25	37.9
Very severe (19-21)	2	3.0	0	0	0	0	0	0

Some improvement was also obtained in the control group subjected to PFMT. Two patients were completely dry, 13 of them experienced slight, 26 moderate and 25 severe symptoms. Similar to the experimental group complete disappearance of the symptoms was obtained in the patients with stress urinary incontinence with initial ICIQ-UI SF score of 3. When comparing the treatment with Bioapigyn® ointment for pelvic muscle tonus with PFMT applied for the same time-period the ointment treatment was found superior compared to PFMT. The total ICIQ-UI-SF score in the control group (Table 5) at baseline ranged from 3 to 18 (10.8±4.3). Slight decrease was obtained following the training to 9.7±4.6. However, this decrease was not statistically significant ($t=1.4$; $p=0.1770$). In the previous study (Oreščanin

et al., 2018), following the six months of PFMT the mean value of ICIQ-UI SF score slightly decreased from 8.34±4.21 to 7.92±4.71, which was not statistically significant. In the experimental group the initial values of ICIQ-UI-SF score ranged from 3 to 21 (11±4.5). Those values decreased significantly following the treatment (t=7.87; p<0.0000) with the range from 0 to 17 while mean values and standard deviation decreased to 4.9±4.5.

Table 5. Basic statistical parameters for total ICIQ-UI-SF score of the patients with incontinence and vulvo-vaginal disorders in control group subjected to pelvic floor muscle training and experimental group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the treatment or training. $\bar{X}$- mean; SD-standard deviation; M-median; *- significantly different at p<0.05

Stat. parameter	Control		Experimental	
	Initial	Final	Initial	Final
$\bar{X}$	10.8	9.7	11.0	4.9
SD	4.3	4.6	4.5	4.3
M	10.5	9.5	12.0	5.0
Min.	3.0	0.0	3.0	0.0
Max.	18.0	18.0	21.0	17.0
t-test	t=1.4; p=0.1770		t=7.87; p<0.0000*	

The results of perineometry and post voiding residual urine volume before and following the training or treatment with the ointment were presented in Table 6.

In the control group the initial values of the maximum pressure ranged from 0 to 32 (18.5±10.9), the average pressure from 0 to 30 (15.1±9.7) and duration of pressure from 0 to 32 (15.5±10.9). Following the PFMT all three parameters increased slightly and reached the mean values and standard deviations of 19.3±11.4, 16.0±10.3 and 16.8±11.5 for maximum, average and duration of pressure, respectively. Based on the results of t-test none of these values showed statistically significant increase compared to the initial values. In the experimental group the initial values of the maximum pressure ranged from 0 to 28 (11.0±9.0), the average pressure from 0 to 24 (8.1±7.5) and duration of pressure from 0 to 32 (9.4±9.9). Following the treatment with Bioapigyn® ointment all three parameters increased and reached the mean values and standard deviations of 14.6±9.8, 10.7±8.4 and 12.7±10.4 for maximum, average and duration of pressure, respectively.

Based on the results of t-test all three parameters showed statistically significant increase compared to the initial values. Those results confirmed that Bioapigyn® ointment for pelvic muscle tonus increased significantly the pelvic muscle strength following four weeks of the application. Previous study (Oreščanin et al., 2018) also showed increase in the pelvic muscle strength parameters. However, due to two times shorter treatment period this increase was not statistically significant. The initial values of post voiding residual urine (Table 6) in the control group ranged from 0 to 30 mL (11.2±8.9 mL) and showed slight but not significant (p=0.5042) decrease to 10.1±8.4 mL (range 0 to 27.7 mL).

On the contrary, the experimental group showed statistically significant decrease (t=4.1; p<0.0000) of the volume of residual urine from 6.9±10.1 mL (range 0 to 61.3 mL) to 1.6±2.3 mL (range from 0 to 10.5 mL) which could be explained by increase of pelvic floor muscle performance increasing intravesical pressure high enough to enable empting the bladder almost completely. Only two weeks of the treatment with Bioapigyn® ointment (Orescanin et al., 2018) reduced significantly (p=0.0002) post voiding residual urine volume from 8.73±11.18 to 2.78±5.93 mL.

Table 6. Basic statistical parameters and the results of t-test for the perineometry and residual urine volume of the patients with incontinence and vulvo-vaginal disorders in control (C) group subjected to pelvic floor muscle training and experimental (E) group treated with Bioapigyn® ointment for pelvic muscle tonus before and after the treatment or training. X- mean; SD-standard deviation; M-median; *-significantly different at p<0.05

Group	Stat. parameter	Max. pressure (mm Hg)		Average pressure (mm Hg)		Duration of pressure (s)		Residual urine (mL)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	$\bar{X}$	18.5	19.3	15.1	16.0	15.5	16.8	11.2	10.1
	SD	10.9	11.4	9.7	10.3	10.9	11.5	8.9	8.4
	M	21.0	22.0	15.5	16.5	14.5	15.5	9.7	9.3
	Min.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max.	32.0	33.0	30.0	30.0	32.0	34.0	30.3	27.7
	t-test	t=0.4; p=0.6851		t=0.5; p=0.5489		t=0.7;p=0.5113		t=0.7;p=0.5042	
Experimental	$\bar{X}$	11.0	14.6	8.1	10.7	9.4	12.7	6.9	1.6
	SD	9.0	9.8	7.5	8.4	9.2	10.4	10.1	2.3
	M	11.0	14.5	7.0	9.0	7.0	8.5	3.0	0.7
	Min.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max.	28.0	32.0	24.0	28.0	32.0	37.0	61.3	10.5
	t-test	t=2.21;p=0.0286*		t=2.04; p=0.0475*		t=2.09;t=0.0407*		t=4.1; p<0.0000*	

Table 7. The results of multiple regression analysis testing for the influence of selected predictor variables on the initial values of ICIQ-UI-SF score, perineometry (maximum and average pressure and duration of pressure) and post voiding residual urine. *-significantly different at p<0.05

Predictor variable	ICIQ-UI-SF		Maximum pressure		Average pressure		Duration of pressure		Residual urine volume	
	β	p	β	p	β	p	β	p	β	p
Age	0.45	0.0041*	0.49	0.0000*	0.46	0.0000*	0.38	0.0000*	0.19	0.0929
Menopause	0.56	0.0000*	0.27	0.0054*	0.28	0.0036*	0.44	0.0000*	0.32	0.0064*
No of birth	0.10	0.1879	0.00	0.9696	0.01	0.9327	0.02	0.6795	0.04	0.5954
BMI	0.13	0.0742	0.01	0.8944	0.03	0.6686	0.04	0.5337	0.02	0.7644
	R=0.63; p<0.0000*		R=0.70; p<0.0000*		R=0.70; p<0.0000*		R=0.76; p<0.0000*		R=0.48; p<0.0000*	

The influence of predictor variables (age, menopausal status, BMI and number of child-birth) on the initial values of the incontinence parameters of the total tested population was assessed by Multiple regression analysis (Table 7).

Multiple regression analysis confirmed, good to very good (R ranged from 0.48 to 0.76), statistically significant ($p<0.05$) influence of the predictor variables (Table 7) on the initial values of ICIQ-UI-SF score, perineometry and post voiding residual urine. Based on the results of beta coefficients and their significance level it seems that among four predictor variables only menopausal status and age exhibited statistically significant contribution. In the case of residual urine content the menopausal status was the only significant predictor variable.

Those results were in line with the previous research identifying age and menopause as the significant predictor of incontinence (Luber, 2004; Danforth et al., 2006; Minassian et al., 2008; Zhu et al., 2009). Those authors also identified obesity as significant predictor, however, our results failed to show any significant contribution. The reason for that lies in the fact that the population from the current study had an ideal or slightly elevated BMI.

According to the results of multiple regression it seems that menopausal status is the variable with the highest influence on all assessed incontinence parameters. ICIQ-UI-SF score and post-voiding residual urine volume were significantly higher in menopausal & postmenopausal participants compared to child-bearing age participants.

On the contrary, all three perineometry parameters that determine pelvic floor muscle strength were significantly lower in menopausal & postmenopausal woman compared to those of child- bearing age.

3.4. Quantification of the difference in the treatment efficiency between the experimental and control group

The results t-test showed significant difference for perineometry parameters and residual urine volume at baseline. Consequently, the direct comparison of the efficiency between the treatment and training was not possible. Since direct comparison of the mean values of selected variables wouldn't be appropriate approach due to differences in the initial values in two tested groups the percentage of the changes (decrease or increase) of the mean value of each variable following the treatment was calculated for both groups and the differences between the percentages were tested by χ^2 test (Table 8).

There was no decrease of the total score of vulvo-vaginal disorders and vaginal pH in the control group. ICIQ-UI-SF score decreased 9.8%, perineometry parameters increased between 4.3 and 8.3% while residual urine volume decreased for 9.1%. Better results for perineometry parameters were obtained following six months PFMT (Oreščanin et al., 2018) with increase ranging between 7.3 and 26.4%.

The experimental group showed better results for all tested variables compared to PFMT group. The application of the ointment resulted in 100% decrease of vulvo-vaginal disorders, 14.2% decrease in vaginal pH, 76.9% decrease in residual urine volume and 54.9% decrease in the mean value of ICIQ-UI-SF score. In the same time, perineometry parameters determining pelvic muscle strength increased between 31.5 and 34.3%. According to the results of χ^2 test the differences between two groups were statistically significant for all tested parameters.

Current results for the experimental group were significantly better compared to the previous study (Oreščanin et al., 2018) lasting only two weeks which resulted in

30.7% decrease of the mean value of ICIQ-UI score, 68.2% decrease of residual urine volume, 11.3% decrease of vaginal pH and between 25.3 and 31.7% increase of perineometry parameters. However, two weeks of the treatment were long enough for complete disappearance of vulvo-vaginal complaints.

Table 8. The percentage of decrease of the Total score of vulvo-vaginal disorders, vaginal pH, residual urine volume and ICIQ-UI-SF score and increase of perineometry parameters in the control and experimental group compared to initial value and the results of χ^2 between control and experimental group. *-significantly different at p<0.05

Variable	Control	Experimental	χ2	P
Total score-vulvo-vaginal disorders	0.0	100.0	128.0	<0.0001*
Vaginal pH	0.0	14.2	8.1	0.0045*
ICIQ-UI-SF score	9.8	54.9	28.6	<0.0001*
Maximum pressure (mm Hg)	4.3	33.1	16.2	0.0001*
Average pressure (mm Hg)	6.3	31.5	12.1	0.0005*
Duration of pressure (s)	8.3	34.3	11.8	0.0006*
Residual urine (mL)	9.1	76.9	59.2	<0.0001*

3.5. Clinical efficiency and treatment rating by the patients

Following the treatment with Bioapigyn® ointment for pelvic muscle tonus 25.8% of the participant of the experimental and 3% of the control subjects were completely dry which was recorded as clinical cure. Clinical improvement was found in 74.2% participants of the experimental and 97% of the control group.

Both approaches have received good ratings by the patients. Treatment with Bioapigyn ointment showed a significantly better rating (4.9±0.3) compared to PFMT (4.4.±0.6) which was expected due to better performance of the ointment compared to PFMT.

3.6. Clinical safety

At Visit II the respondents using medical device are asked by the principal investigator if they experienced any side effects or adverse reaction after the first application of the ointment as well as throughout the course of the study. Furthermore, a complete gynaecological examination was performed to determine the possible occurrence of adverse reactions (irritation of the vulvo-vaginal area, allergic reaction), worsening of the existing or the occurrence of new symptoms. Patients were asked to describe in their own words the feeling after the application of the ointment.

Patients described a slight feeling of tightening and contraction in the vaginal area. After 20 to 30 minutes following the application of the ointment, they were able to empty the bladder completely. Patients with vaginal dryness and accompanying symptoms (itching, burning, and pain) after a week of administration experienced a comfortable feeling of vaginal moisture whiles the symptoms of itching and burning has disappeared. None of the patients reported severe burning or itching in the vulva-vaginal area, nor the appearance of an allergic reaction or the exacerbation of the existing symptoms or the appearance of new symptoms. The gynaecologic examination did not show any signs of irritation or worsening

of the vulvo-vaginal disorders. Just opposite, the examination confirmed the recovery of the vaginal mucosa in menopausal and postmenopausal women. There was no increase of vaginal pH in none of the subjects. On the contrary in majority of women vaginal pH was reduced significantly and only in few of them remained the same. All the patients confirmed that they could keep the urine significantly longer, the number of urination during the night decreased, patients could empty the bladder better compared to the baseline. The measurement of the perineometry parameters confirmed slight to significant enhancement of the pelvic floor muscle strength in all participants. In all patients who had residual urine at the first visit, its volume was reduced at the second visit. Based on the above facts obtained from the patients or by direct examination and measurement it is possible to conclude that the medical device does not cause any adverse effects and is safe for vaginal administration in the dose of 2.5 mL per day for up to 28 days.

4. Discussion and Conclusions

Conducted study confirmed the efficiency of Bioapigyn® vaginal ointment for pelvic muscle tonus in alleviating urinary incontinence in women. The ointment decreased the total value of ICIQ-UI-SF score in all 66 subjects. This significant improvement was the consequence of the strengthening of the pelvic floor muscles which was quantitatively confirmed by increasing in the values of perineometry parameters (maximum pressure, average pressure, duration of the pressure) and reducing the volume of post voiding residual urine.

Consequently, this study has confirmed that the main mode of action of the ointment is physico-mechanical by causing the contraction and relaxation of the smooth muscles of the pelvic floor similar to PFMT or electrical stimulation. However, compared to the PFMT conducted during the same time period the ointment showed significantly better results considering all measured parameters.

Although, there are no published data on human studies considering the influence of a single ingredient on the contraction of smooth muscles, the in vitro results or those obtained on the animal model have confirmed that the plants *Capsella bursa-pastoris* and *Urtica diodice* (Grosso, 2011; Al-Snafi, 2015) induce smooth muscle contraction/relaxation activity. Moreover, smooth muscle contraction could be caused by the astringent property of the plants such as *Quercus robur, Achillea millefolium, Salvia officinalis, Olea europaea, Plantago major.* Smooth muscle contraction/relaxation stimulated by the ointment ingredients resulted in the tightening and firming of the smooth muscles of the pelvic floor and consequently, reduced the symptoms of incontinence significantly especially in perimenopausal and menopausal women (Oreščanin and Findri Gustek, 2016, Oreščanin et al., 2018).

Besides, product is also indicated for alleviations of vulvo-vaginal disorders that are often associated with incontinence in perimenopausal and menopausal women. Disappearance of all vulvo-vaginal disorders could be explained physical parameters like low pH, high osmolarity, high viscosity and greasiness, emollient as well as low water activity of Bioapigyn® ointment for pelvic muscle tonus leading to eradication of the symptoms of vulvo-vaginal disorders due to: the creation of unfavourable conditions for the growth, adhesion and multiplication of the pathogens; the creation of the protective coating on the vaginal mucosa, enabling its recovery and preventing further irritation; alleviation of the vaginal dryness due to the presence of the humectants; preventing pain during sexual intercourse due to its lubricating and coating effect.

Conflict of Interest

The authors have declared that they have no conflict of interest.

References

1. Al-Snafi, A.E., 2015. The chemical constituents and pharmacological effects of Capsella bursa-pastoris-A review. International Journal of Pharmacology and toxicology, 5(2), 76-81.
2. Aoki, Y., Brown, H.W., Brubaker, L., Cornu, J.N., Daly, J.O., Cartwright, R., 2018. Urinary incontinence in women, Nature Reviews Disease Primers, 3, 17042. doi:10.1038/nrdp.2017.42.
3. Brown, J.S., Grady, D., Ouslander, J.G., Herzog, A.R., Varner, R.E., Posner, S.F., 1999. Prevalence of Urinary Incontinence and Associated Risk Factors in Postmenopausal Women. Obstetrics & Gynecology, 94(1), 66–70.
4. Buchsbaum, G.M., Chin, M., Glantz, C., Guzick, D., 2002. Prevalence of Urinary Incontinence and Associated Risk Factors in a Cohort of Nuns. Obstetrics & Gynecology, 100(2), 226–229.
5. Danforth, K.N., Townsend, M.K., Lifford, K., Curhan, G.C., Resnick, N.M., Grodstein, F., 2006. Risk factors for urinary incontinence among middle-aged women. American Journal of Obstetrics and Gynecology, 194, 339–345.
6. Grosso, C., Vinholes, J., Silva, L.R., Guedes de PinhoI, P., Gonçalves, R.F., Valentão P., JägerII A.K., Andrade P.B., 2011. Chemical composition and biological screening of Capsella bursa-pastoris. Brazilian Journal of Pharmacognosy, 21(4), 635-644.
7. Khandelwal, C., Kistler, C., 2013. Diagnosis of Urinary Incontinence. American Family Physician, 87(8),543-550.
8. Luber, K. M., 2004. The Definition, Prevalence, and Risk Factors for Stress Urinary Incontinence. Reviews in urology, 6(3), 3-9.
9. McPhil, J. H., 2004. The prevalence of stress urinary incontinence in women. Nursing time, 100(20), p.70.
10. Minassian, V.A., Drutz, H.P., Al-Badr, A., 2003. Urinary incontinence as worldwide problem. International Journal of Gynecology and Obstetrics, 82, 327–338.
11. Minassian, V.A., Stewart, W.F., Wood, G.C., 2008. Urinary Incontinence in Women: Variation in Prevalence Estimates and Risk Factors. Obstetrics & Gynecology, 111(2), 324-331.
12. Nygaard, IE., Heit, M., 2004. Stress Urinary Incontinence. Obstetrics & Gynecology, 104(3), 607-620.
13. Oreščanin, V., Findri Guštek, Š. Fistonić, I., Rodić, J., 2018. Efficacy of bioapigyn® ointment for pelvic muscle tonus in the treatment of stress urinary incontinence and vulvo-vagnal disorders. IJRDO - Journal of Health Sciences And Nursing, 3(10), 13- 25.
14. Oreščanin, V., Findri Guštek, Š., 2016. Development and Testing of New Herbal Ointment for the Treatment of Stress Urinary Incontinence-A Preliminary Study. Global Journal For Research Analysis, 5(7), 371-374.
15. Peyrat, L., Haillot, O., Bruyere, F., Boutin, J.M., Bertrand, P., Lanson, Y., 2002. Prevalence and risk factors of urinary incontinence in young and middle-aged women. BJU International, 89(1), 61–66.
16. Radzimińska, A., Strączyńska, A., Weber-Rajek, M., Styczyńska, H., Strojek, K., Piekorz. Z., 2018. The impact of pelvic floor muscle training on the quality of life of women with urinary incontinence: a systematic literature review. Clinical Interventions in Aging, 13, 957–965.
17. Rovner, S.E., Wein, J.A., 2004. Treatment Options for Stress Urinary Incontinence. Reviews in urology, 6 (Suppl 3), S29–S47.
18. Zhu, L., Lang, J., Liu, C., Han, S., Huang, J., Li, X., 2009. The epidemiological study of women with urinary incontinence and risk factors for stress urinary incontinence in China. Menopause, 16(4), 831-836.

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The Effect of *Corchorus olitorius* L. Extract on Viability of Breast Cancer Cells: A friend or a foe?

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Abstract

This *Corchorus olitorius* L. is known to be a medicinal plant widely consumed in the Eastern Mediterranean region and has anti-bacterial, anti-inflammatory and anti-cancer properties. Breast cancer has one of the highest mortality rates among all cancers. Therefore, the main aim of the study is to evaluate the effectiveness of *C. olitorius* plant on viability of estrogen receptor (ER) positive breast cancer cell line, MCF-7. *C. olitorius* leaves first extracted with ethanol, then LC-MS/MS analysis was done for identification of phytochemicals. MTT assay was used for assessment of cell viability of MCF-7 cells. Cells were treated with *C. olitorius* extracts at five concentrations (5, 10, 20, 50, 100 µg/ml) and four different incubation periods (24, 48, 72, 96 h). LC-MS/MS analysis identified seven phytochemicals in the extract, mainly quercetin and caffeoylquinic acid derivatives. MTT results showed that the extract was only slightly effective in terms of reduction of cell viability at 50 and 100 µg/ml doses which were incubated for 24 and 48 h. Lower concentration doses did not show any effect in cell viability of MCF-7 cells. Longer incubation periods tend to increase cell viability of breast cancer cells. Quercetin identified within the extract might interfere with ER and promote MCF-7 cell proliferation. Therefore, in ER positive breast adenocarcinoma, quercetin intake and doses should carefully be monitored. More studies regarding the relationship between *C. olitorius*, quercetin and breast cancer should be done for further clarification of the topic.

Key Words: *Corchorus olitorius* L., MCF-7, Breast Cancer, Cell Viability

1. Introduction

Corchorus olitorius L. or molohiya in its traditional name belongs to Tiliaceae family in botany and is known to be a medicinal plant widely consumed in the Eastern Mediterranean area and the Middle East (İşeri et al. 2013). According to earlier studies *C. olitorius* was known with its diuretic, sedative and laxative properties (Duke, 1986). With enlightenment of recent literature, it has been shown that *C. olitorius* indeed has anti-bacterial, anti-cancer and anti-inflammatory effects (Pal et al. 2006;

Adegoke et al. 2009; Li et al. 2012; Handoussa et al. 2013; Soykut et al. 2018). *C. olitorius* contains phytochemicals such as quercetin, chlorogenic acid as well as antioxidant vitamins and minerals, which are thought to aid the exertion of its medicinal properties (Azuma et al. 1999; Handoussa et al. 2013).

Breast cancer is known to be the most common cancer in women and has a high mortality rate especially in the developing world (WHO, 2019). There are several factors which contribute to the development of breast cancer, including sex, genetics, breastfeeding and lifestyle. Hormones actively play part in breast cancer progression and so; cancer cells can be classified according to the presence of estrogen receptor (ER) (ER positive and ER negative cells) (WCRF, 2019). Use of medicinal plants with potential anti-cancer properties to either directly slow progression of cancer or to increase effectiveness of chemotherapeutic drugs/agents with dual action is regarded as a new approach for cancer treatment (Kasiri et al. 2019). Therefore the main aim of the study is to evaluate the effectiveness of phytochemical rich *C. olitorius* on the viability of breast cancer cell line, MCF-7.

2. Material and Method

2.1. Extraction

C. olitorius leaves were collected during the summer months and dried. The plant sample was registered with Near East Herbarium at Near East University under the Herbarium number 6904. The dried leaves of *C. olitorius* (100 g) were powdered (Waring Commercial Blender, United States of America, USA) and extracted with 80% ethanol while incubated overnight at room temperature with frequent stirring.

The extract was vacuum filtered and concentrated by rotary evaporator (BUCHI Rotavapor R-210). The extract was evaporated and lyophilized (Christ Alpha 1-4 LD Plus, Germany) and the final yield of crude extract was found to be 14.8 grams. Extraction composition, LC-MS/MS analysis was done by Becer et al. (2020).

2.2. Cell line and cell culture

MCF-7 cell line which is breast adenocarcinoma was purchased from ATCC: HTB-22. MCF-7 cells are also known for being estrogen and progesterone receptor positive, and HER2 negative. Cells were maintained in their usual medium; RPMI-1640 (Biochrom, FG 1215). In total, 10% heat inactivated fetal bovine serum (Capricorn Scientific, FBS-11B), 1% penicillin-streptomycin (Biochrom, A2213) and 1% glutamine amino acid (EMD Millipore, K0282) were also added to the culture medium for sustaining optimal medium. Cells were cultured in a humidified atmosphere at 37°C in 5% CO_2 until 80% confluency.

2.3. Cell viability – MTT assay

The extract was first dissolved with dimethylsulfoxide DMSO, (Sigma-Aldrich). The final concentration of DMSO in cell lines was less than 0.05% to prevent any possible effect on the cytotoxicity levels. Then extract was further diluted in culture medium with five concentrations; 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL and 100 µg/mL.

MCF-7 cells were seeded in 96-well culture dishes at a density of 5×10^4 cells in each well. The cells were treated with each extract dilutions and were incubated for 24, 48, 72 and 96 h. The cell viability was estimated by MTT assay. MTT solution (Biotium, #30006) was heated to 37°C, after the addition of 10 µl solution to each seeded well. The cells were then incubated for 4 h at 37°C in 5% CO_2. After this, 200 µl DMSO was added to each well to prevent crystallinization formazan salts. The absorbance was measured at 570 nm by a spectrophotometer (Versa Max, Molecular Device, Sunnyvale, USA).

3. Results and Discussion

3.1. Identification of Corchorus olitorius L. content

Identification of polyphenol content of *C. olitorius* extract was done by liquid chromatography high performance coupled with mass spectrometry (LC-MS/MS). In total, seven main polyphenolic compounds were identified. The results showed that the extract contains mainly derivatives of caffeoylquinic acid and quercetin such as 3-caffeoylquinic acid, quercetin glucoside, quercetin acetylglucoside, 3,5-dicaffeoylquinic acid (Table 1). Our results are coherent with the scientific literature. According to other studies *C. olitorius* was found containing nearly same content including caffeoylquinic acid and quercetin derivatives as well as antioxidant vitamins; ascorbic acid (Vitamin C) and *alpha*-tocopherol (Vitamin E) (Azuma et al. 1999; Handoussa et al. 2013). *C. olitorius* had shown to contain more Vitamin C and quercetin when compared with the richest sources for the reference component (Azuma et al. 1999; Bhagwat et al. 2011; Handoussa et al. 2013; Türkomp 2018). The identified compounds in the plant gives its strong antioxidant properties with the aid of free radical scavenging ability. Studies showed that *C. olitorius* was significantly able to reduce oxidative stress by increasing endogenous antioxidant enzymes; superoxide dismutase and catalase activities (Dawanjee et al. 2013; Boye et al. 2014,). Besides, *C. olitorius* due to its rich polyphenolic content showed reduction in Thiobarbituric acid reactive substances (TBARS) level which is known to increase oxidative stress due to lipid peroxidation (Dawanjee et al. 2013b).

Table 1. Identification of *Corchorus olitorius* L. extract content.

Rt	$[M-H]^-$	MS^2	Identified compounds
4.1	341	179, 161	Caffeoyl glucose
4.7	353	191, 179	3-Caffeoylquinic acid
9.9	463	299, 271, 255	Quercetin glucoside
10.9	505	299, 271, 255	Quercetin acetylglucoside
11.5	515	353, 191, 179, 173	3,5-Dicaffeoylquinic acid
12.1	515	353, 191, 179, 135	1,3-Dicaffeoylquinic acid
12.6	489	284, 255, 227	Luteolin / kaempferol acetylglucoside

**Adapted from Becer et al., 2020 (article ahead of publication)*

3.2. Cell viability and toxicity

MCF-7, breast adenocarcinoma cells were treated with five concentrations of *C. olitorius* EtOH extract at four different incubation period. Our results showed that the extract was only slightly effective in terms of reduction of cell viability at 50 and 100 µg/mL doses, incubated for 24 and 48 h. The maximum decline in cell viability of MCF-7 cells was observed at 100 µg/mL dose at 24 h (Figure 1). The decline was only about 10% which is not regarded as an efficient treatment dose. On the other hand, lesser concentrations and longer incubation periods not only cause any decrease in cell viability but increase breast cancer cell viability. Therefore, overall results suggest that the EtOH extract prepared from *C. olitorius* extract leaves is ineffective for reduction of cell viability and does not show cytotoxic effects in breast cancer cells *in vitro* condition.

There is a plausible mechanism behind the possibility of *C. olitorius* extract not showing sufficient effect in decreasing cell viability in breast cancer cells. It was stated earlier in the

article that the plant is rich in terms of phytochemical specifically quercetin and caffeoylquinic acid. Quercetin can be regarded as a phytoestrogen as it is an uncompetitive ligand for oestrogen receptors (Miodini et al. 1999). It is reported that at lower doses that can stimulate transcriptional activity, quercetin might promote cellular proliferation in MCF-7 cells through ERα domain (Maggiolini et al. 2001).

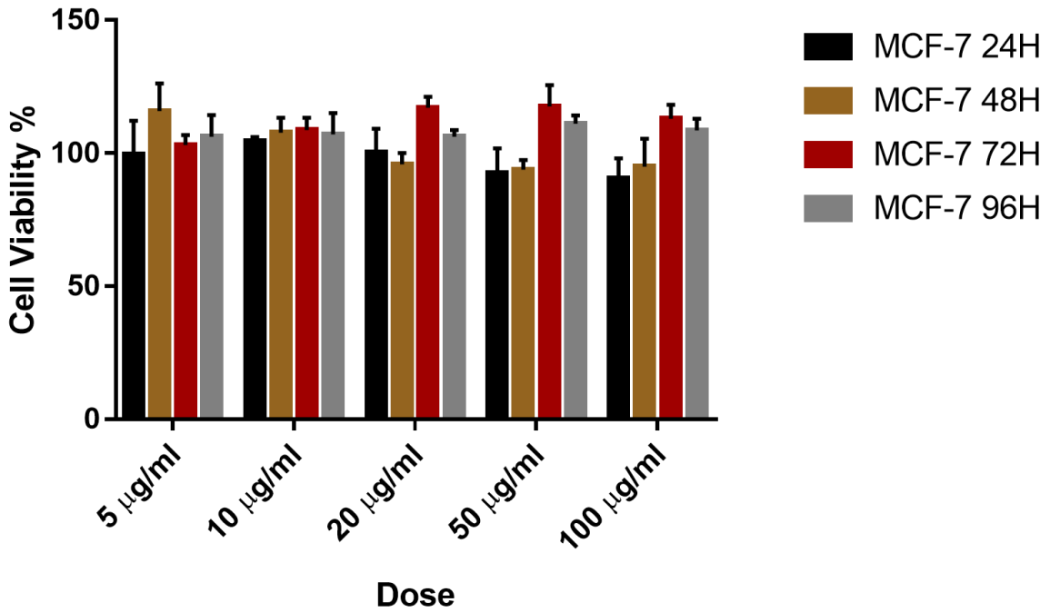

Figure 1. The effect of different concentrations of *C. olitorius* extract in MCF-7 breast adenocarcinoma cells at four incubation period (data are expressed as means ± SEM).

Conversely, at higher doses, quercetin can act as a cytotoxic agent that can kill MCF-7 cells in an ER independent way. Therefore, the dietary intake of quercetin should be carefully monitored in patients with breast cancer (Maggiolini et al. 2001). The quercetin derivatives may have different cytotoxic effects on MCF-7 cells. Therefore, the nature of the quercetin found in the nutrient content of foods should be established carefully (Kasiri et al. 2019). Additional to cytotoxic effects, quercetin may have apoptotic effects at 100µM concentration. This might be due to the generation of cellular reactive oxygen species (ROS) and increase in pro-oxidative state in MCF-7 cells. Quercetin may show phytotherapeutic effects through activating ROS dependent intrinsic apoptotic pathway (Wu et al. 2018). However, in our results, the EtOH extract of *C. olitorius* leaves did not exert an effective cytotoxic effect which further proves that its rich quercetin content inhibited MCF-7 cytotoxicity probably through the interaction of ER. Moreover, the extract increased cancer cell proliferation at longer incubation periods more specifically at 72 and 96 h. This might be due to quercetin increasing its activity during longer periods. One study discussed that quercetin might have the capacity to increase breast tissue carcinogenesis due to aiding the estrogen transformation of normal cells to malignant cells (Singh et al. 2010).

Another possible, suggested mechanism is quercetin in its glucoside form which was identified in our analysis, had shown lower affinity to estrogen receptors (Sørensen, 2018). Therefore, this can also reduce the potency of the extract for lowering the cell viability of MCF-7 cells. Contrary to other studies, which showed *C. olitorius* anti-cancer effects in different cell lines, our results stated increased cancer cell proliferation in

MCF-7 cells (Li et al. 2012; İşeri et al. 2013; Soykut et al. 2018).

4. Conclusion

Our results stated that *C. olitorius* plant contains strong phytochemicals which exert antioxidant properties such as quercetin and caffeoylquinic acid. These phytochemicals are usually effective at reduction in cancer cell proliferation. However, cell viability results stated that the EtOH extract of the leaves only showed a slight decrease in cell proliferation at 100 µg/ml dose, incubated for 24 h. Other doses were not effective at reducing cell proliferation and even caused increased in cancer cell proliferation. This effect might be due to quercetin found in plant extract interacting with ER positive MCF-7 cells by triggering transcriptional activity and promoting malignant cell proliferation. Therefore, people with ER positive breast carcinoadenoma should carefully monitor their quercetin intake as it might be a double-edged sword and might deteriorate the treatment process. However, it should be stated that more studies should be done for further clarification of the relationship between *C. olitorius*, quercetin and breast cancer.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Adegoke, A.A., Adeboye-Tayo, B.C., 2009. Phytochemical composition and antimicrobial effects of Corchorous olitorius leaf extracts on four bacterial isolates. Journal of Medicinal Plants Research, 3(3), 155-159.
2. Azuma, K., Nakayama, M., Koshioka, M., Ippoushi, K., Yamaguchi, Y., Kohata, K. et al., 1999. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. Journal of Agricultural and Food Chemistry, 47, 10, 3963-3966. DOI 10.1021/jf990347p
3. Becer, E., Soykut, G., Kabadayı, H., Mammadov, E., Çalış, İ., Vatansever, S. 2020. Obtaining stem cell spheroids from foreskin tissue and the effect of *Corchorus olitorius* L. on spheroid proliferation. Turkish Journal of Phamaceutical Sciences. DOI: 10.4274/tjps.galenos.2019.05658 [article ahead of printing]
4. Bhagwat, S., Haytowitz, D.B., Holden J.M. 2011. USDA Database for the flavonoid content of selected foods (Report No: 3). Beltsville Human Nutrition Research Center: United States Department of Agriculture.
5. Boye, A., Barku, V.Y.A., Amoateng, P., Mbroh, L.A. 2014. Anti-nociceptive and antioxidant activities of an aqueous leaf extract of *Corchorus olitorius* L. (Tiliaceae). International Journal of Biological and Chemical Sciences, 8(6), 2395-2406. DOI 10.4314/ijbcs.v8i6.3
6. Dewanjee, S., Sahu, R., Karmakar, S. Gangopadhyay, M., 2013. Toxic effects of lead exposure in Wistar rats: Involvement of oxidative stress and the beneficial role of edible jute (*Corchorus olitorius*) leaves. Food Chemical Toxicology, 55, 78-91. DOI 10.1016/j.fct.2012.12.040
7. Duke, J.A. 1983 Handbook of Energy Crops; *Corchorus olitorus* L. Accessed online at 23.11.2015. https://hort.purdue.edu/newcrop/duke_energy/Corchorus_olitorius.html
8. Handoussa, H., Hanafi, R., Eddiasty, I., El-Gendy, M., El-Khatip, A., Linscheid, M. et al. 2013. Anti-inflammatory and cytotoxic activities of dietary phenolics isolated from *Corchorus olitorius* and *Vitis vinifera*. Journal of Functional Foods, 5, 1204-1216. DOI 10.1016/j.jff.2013.04.003
9. İşeri, Ö.D., Yurtcu, E., Sahin, F.İ., Mehmet, H., 2013. *Corchorus olitorius* (jute) extract induced cytotoxicity and genotoxicity on human multiple myeloma cells (ARH-77). Pharmaceutical Biology, 51(6), 766-770. DOI 10.3109/13880209.2013.765897
10. Kasiri, N., Rahmati, M., Ahmedi, L., Eskandari, N., Motedayyen, H. 2019. Therapeutic potential of quercetin on human breast cancer in different dimensions. Inflammopharmacology. DOI 10.1007/s10787-019-00660-y. [Epub ahead of print]
11. Li, G.J., Huang, S.Y., Wu, M.Y., Chen, Y.C., Tsang, S.F., Chyuan, J.H., et al. 2012. Induction of apoptosis by ethanolic extract of *Corchorus olitorius* leaf in human hepatocellular carcinoma (HepG2) cells

via a mitochondria-dependent pathway. Molecules, 17, 9348-9360. DOI 10.3390/molecules17089348.
12. Maggiolini, M., Bonofiglio, D., Marsico, S., Panno, M.L., Bruno Cenni, B., Picard, D., And`O, S., 2001. Estrogen receptor α mediates the proliferative but not the cytotoxic dose-dependent effects of two major phytoestrogens on human breast cancer cells. Molecular Pharmacology, 60(3), 595-602.
13. Miodini, P., Fioravanti L., Di Fronzo G., Cappelletti V., 1999. The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. British Journal of Cancer, 80(8), 1150-1155. DOI 10.1038/sj.bjc.6690479
14. Pal, D.K., Mandal, M., Senthilkumar, G.P., Padhiari, A, 2006. Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. Fitoterapia, 77, 589-591. DOI 10.1016/j.fitote.2006.06.015
15. Singh, B., Mense, S.M., Bhat, N.K., Putty, S., Guthiel, W.A., Remotti, F., Bhat, H.K., 2010. Dietary quercetin exacerbates the development of estrogen-induced breast tumors in female ACI rats. Toxicology and Applied Pharmacology, 247(2), 83–90. DOI 10.1016/j.taap.2010.06.011
16. Soykut, G., Becer, E., Calis, I., Yucecan, S., Vatansever, S. 2018. Apoptotic effects of Corchorus olitorius L. leaf extracts in colon adenocarcinoma cell lines. Progress in Nutrition, 20, 4, 689-698. DOI 0.23751/pn.v20i4.6892
17. Sørensen, B.G., 2018. Quercetin - creating an estrogenic response. University of Copenhagen. Accessed online at 27.11.2019 https://natoxaq.ku.dk/toxin-of-the-week/quercetin/
18. Türkomp. 2018. Ulusal Gıda Kompozisyon Veri Tabanı, C Vitamini. Accesed online at 17.06.2018. http://www.turkomp.gov.tr/component_result-c-vitamini-32
19. WCRF, World Cancer Research Fund/American Institute for Cancer Research (2018). Diet, Nutrition, Physical Activity and Breast Cancer: A Global Perspective (Report No: 2018 revised). Dietandcancerreport.org: World Cancer Research Fund/American Institute for Cancer Research.
20. WHO World Health Organization. 2019. Breast Globacan 2018. Accessed online at 27.11.2019 https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf
21. Wu, Q., Needs, P.W., Lu, Y., Kroon, P.A., Daoyuan Rena, D., Yang, X., 2018. Different antitumor effects of quercetin, quercetin-3'-sulfate and quercetin-3-glucuronide in human breast cancer MCF-7 cells. Food & Function, 9, 1736–1746. DOI 10.1039/c7fo01964e

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Do Microbial Fertilizer Applications Affect the Yield and Essential Oil Ratio in Mint (*Mentha piperita* L.) Cultivars?

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Abstract

Recently, excessive and unconscious uses of chemical fertilizer cause significant hazards on natural resources and subsequently cause serious environmental problems. As an alternative instead of chemical fertilizer, microbial fertilizers are used for healthier soil and plant. The aim of this study was to determine the effect of different doses of microbial fertilizers on yield and essential oil ratio in Mint (*Mentha piperita* L.) cultivars. The experiment was carried out in a pot according to the randomized parcel design. The study was carried out with three cultivars namely, *Mentha piperita* swiss, M. *piperita* chocolate, M. *piperita* multimentha, four doses (control, 1, 1.5 and 2 doses) and three replicates. In the study; plant height (cm), fresh herb yield (g/m^2), drog herb yield (g/m^2), drog leaf yield (g/m^2), essential oil ratio (%) and essential oil yield (L/m^2) were examined. Along with the study, Microbial fertilizers exhibited significant effects on the parameters examined herein. The yield of fresh herb yield and drog leaf yield were found between 1342.5-2001.1 g/m^2, 160.9-228.0 g/m^2 respectively and the essential oil ratio varied between 1.55-1.93% according to microbial fertilizer doses.

Key Words: *Mentha piperita,* Microbial Fertilizer, Yield, Essential Oil Ratio

1. Introduction

From prehistoric times to present time, plants met many demands of human beings for their many uses. Of the plant groups, medicinal and aromatic plants have gained great interest among people due to side effects of synthetic and semi-synthetic pharmaceutical agents (Gezgin 2006). Various and numerous functions and uses including food, medicine, cosmetics and spices have been attributed to those plants. The mentioned uses have also been common since the beginning of human history. Those plants are gathered either from the wild or cultivated and produced (Demirezer, 2010).

Of the significant medicinal and aromatic plants, mint (*Mentha* sp.) belonging to Lamiaceae (Labiatae) widely spreads over the world but mainly concentrated in Central Europe and Asia. In Turkey, it can be grown everywhere (Demirez, 2013). Of the *Mentha* spp., peppermint (*Mentha piperita*), Japanese peppermint (*Mentha arvensis*) and spearmint (*Mentha spicata*) are the most important species cultivated in the world (Baydar, 2016).

Mentha species possess essential oils varying between 1 and 4%. Regarding major essential oil components, *M. piperita* and *M. arvensis* species are characterized with menthol and mentone while carvone is of the major components of *M. spicata* (Baydar, 2016; Karakaplan, 2017).

Fresh shoots and leaves of mint are used as a seasoning for meals and added to salads in Mediterranean countries. It is used as a fresh vegetable in traditional Turkish cuisine and takes its place in salads. A significant portion of the mint is used as powder by being dried and packaged by various companies for sale or used by the producers (Demirez, 2013).

Peppermint largely requires nitrogen and potassium fertilizers. It has been found that although potassium fertilizer decreases the essential oil ratio, nitrogen increases yield and essential oil ratio. Both potassium and phosphorus fertilizers should be given according to the results of soil analysis. Mint demands nitrogen fertilizer and requires plenty of organic fertilizer. Fertilizer is most needed between the buds and flowering cycles (Megep, 2016).

In addition to the chemical fertilizers, biofertilizers exhibit significant effects on biomass of the plants, suppressing the soil harmful pathogens and increasing the effectiveness of other beneficial bacteria. Consequently, biofertilizers are of the alternative methods used to establish the balance between plant and soil microorganisms. With the present study, different doses of microbial fertilizers on yield and quality of different mint cultivars were examined.

2. Materials and Method

The study was conducted as a pot experiment in the randomized parcel experiment design in Medical and Aromatic Plants greenhouse of Field Crops Department. Commercially available Microbial fertilizer was used in the experiment.

The study was carried out with three cultivars, four doses (control, 1, 1.5 and 2 doses) and three replications. Recommended microbial fertilizer dose by company is 100cc to 1000 lt water. Recommended dose used as 1 dose in study and other doses were adjusted according to it. Prior to planting, 0.5 liters of water was put into 12 buckets and then the microbial fertilizer applied in these buckets and mixed. Only water was put in to buckets for control application. In the meantime, 10 stolon were cut from each three mint cultivars with the help of hand pruners. These stolons were put into buckets for each dose application and kept for 1 hour. The pots 72 cm long, 13 cm wide and 13 cm high were used in the experiment and filled with soil up to 8 cm high. These pots were planted with 10 pieces of 10 cm stolon after 1 hour. Extra soil was added to the top of the planted stolon and the soil was compacted and watered. Each pot was considered as a parcel. The total experiment consisted with 36 pots. Mint stolons planted to pots in 18 February 2019 and harvest was made after 75 days later in 3 May 2019.

The statistical analysis of the data was performed according to the SAS package program and the differences between the averages were grouped according to the significance level (5% or 1%) in LSD test.

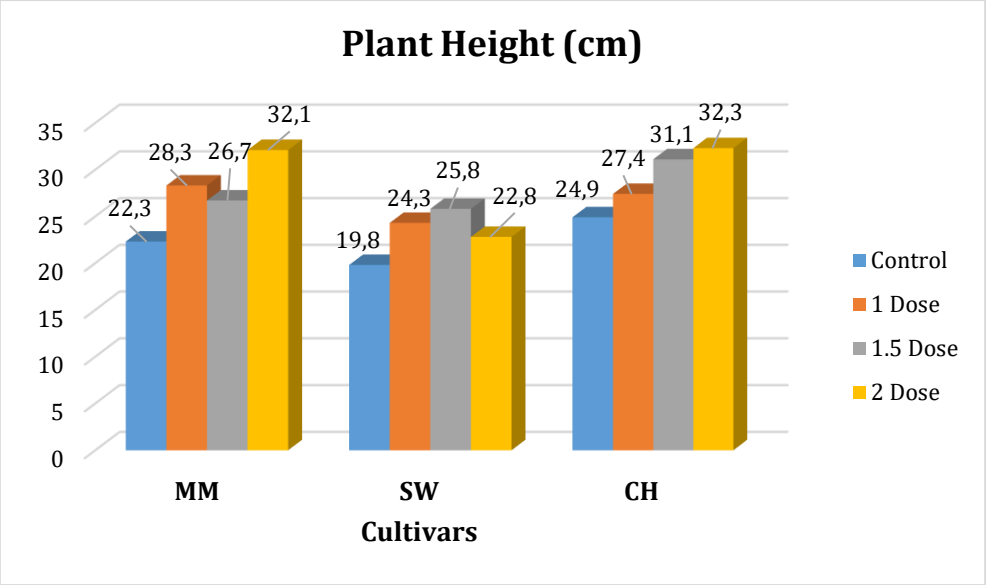
3. Results and Discussion

In order to determine the effects of microbial fertilizer applications on *Mentha piperita* cultivars in Aydın greenhouse conditions, F values and significance levels were determined by variance analysis and significant features were grouped.

3.1. Plant height (cm)

Cultivar x dose interaction was found significant. The highest plant height belongs to chocolate cultivar 2 doses (32.3 cm) and the shortest plant height was found in the swiss cultivar control application (19.8 cm) (Figure 1). Mint species can grow up to 100-

150 cm (Kokkini, 1983). First cultivation year of mint (2016), plant height (cm) was found between 45.4-75.8 cm in first harvest, 32.5-47.4 in second harvest and in second year (2017) first harvest 39.5-73.2 cm, second harvest 20.8-58.2 cm (Yılmaz, 2018). It can be said that the differences between this study and other studies are due to the early harvesting (before flowering) and the doses of microbial fertilizer.

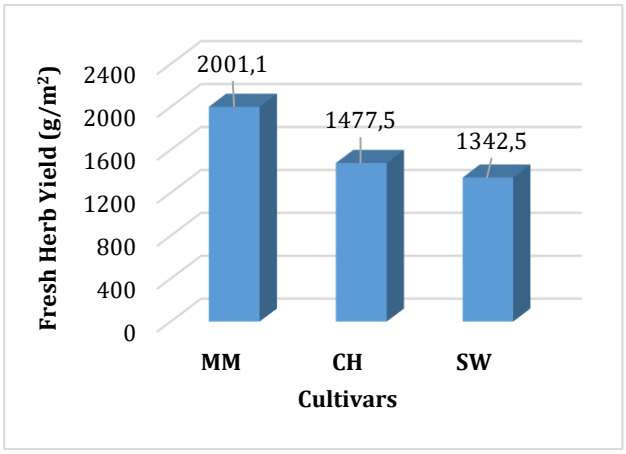


MM: Multimentha, SW: Swiss and CH: Chocolate

Figure 1. Effect of cultivars and doses on plant height (cm)

3.2. Fresh Herb Yield (g/m²)

The highest fresh herb yield value was obtained as 2001.1 g/m² in multimentha cultivar (Figure 2). There is no significant effect of microbial fertilizer on fresh herb yield. Yılmaz (2018) has found that fresh herb yield of different mint cultivars vary between 387.3-2493 kg/da and multimentha cultivar has the highest fresh herb yield than other mint cultivars (Swiss, Chocolate, Citaro and Piperita T.). In our study, It is clear that our results similiar with Yılmaz (2018) findings. However, our fresh herb yield is 2001.1 (kg/da) lower that researcher due to first year harvest. It can be said that if there is harvests in second year, our finding might be higher than researcher.



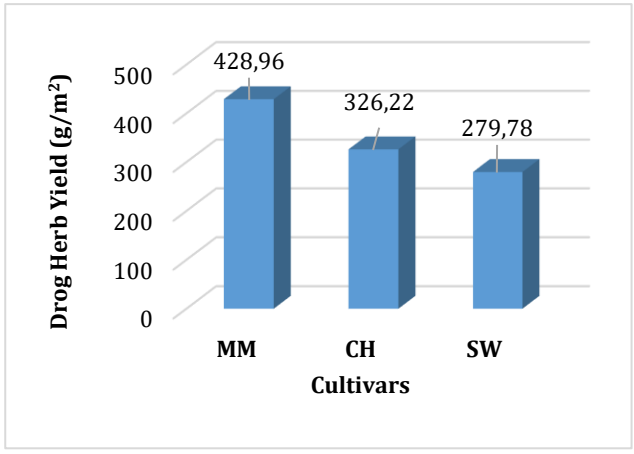


Figure 2. Effect of cultivars on fresh herb yield and drog herb yield (g/m²)

3.3. Drog Herb Yield (g/m²)

The highest drog herb yield was obtained as 428.96 g/m² from multimentha cultivars. The lowest yield value was found 279.78 g/m² from the swiss cultivar and in the same statistical group with Chocolate cultivar (Figure 2).

According to Yılmaz (2018) results drog herb yield vary between 263.6–457 kg/da and multimentha has the highest drug herb yield with 457 kg/da in first harvest of first year.

Our study results were found similiar with researcher. Drog herb yields in mint vary according to the climatic conditions of the plant (Piccaglia and Marotti, 1993; Özgüven and Kırıcı, 1999) and growing conditions (Munsi, 1990; Court et al., 1993; Alkire and Simon, 1996). These low yield cultivars awaking late in the spring and show better development in heat. Drog herb yields in mint vary according to the genetic structure of the plant (Ceylan, 1987; Özgüven and Kırıcı, 1999; Tuğay et al., 2000).

3.4. Drog Leaf Yield (g/m²)

Dried leaves of mint are used as spices so drog leaf yield is an important yield element in mint. In the study, effect of the cultivars on the drog leaf yield was found significant (Figure 3). However, the doses were not statistically significant but it can be said that one dose has highest value (Figure 3).

The highest value for drog yield was obtained from multimentha cultivars (227.99 g/m²), while the lowest value was obtained from swiss cultivars (160.88 g/m²). Yılmaz (2018) found that drog leaf yield changed between 173.5-183.9 first harvest of first year and the highest drog leaf yield belongs to multimentha cultivar.

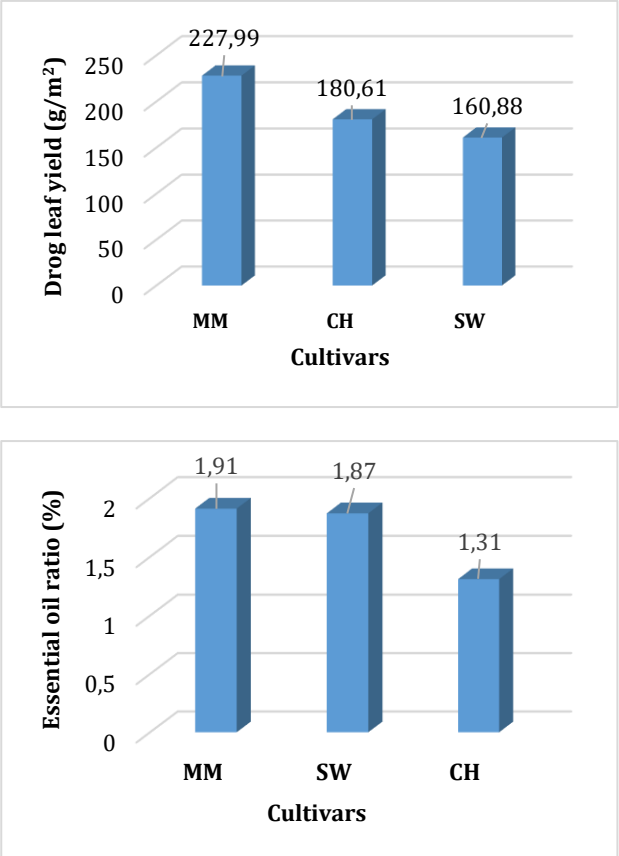


Figure 3. Effect of cultivars and doses on drog leaf yield (g/m²)

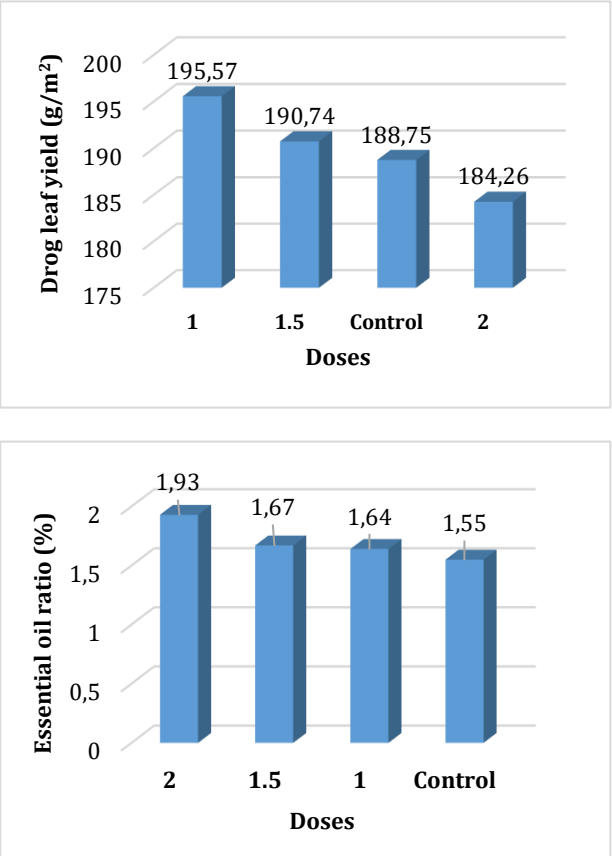


Figure 4. Effect of cultivars and doses on essential oil ratio (%).

3.5. Essential Oil Ratio (%)

The highest value of essential oil ratio in multimentha cultivar was found 1.91%, followed by swiss cultivar with 1.87% and chocolate with 1.31%, respectively (Figure 4). The highest value of essential oil ratio was found in 2 time doses (1.93%) of microbial fertilizer and the lowest essential oil ratio was found in control with 1.55% (Figure 4).

When the previous studies were examined, Yılmaz (2018) essential oil ratio values ranged between 1.3-2.1% in first year (2016). In the second year (2017), the essential oil ratios ranged between 2.2-3.2%. In previous studies on mint; it was determined that essential oil ratio vary according the plant's genetic structure, climate condition and different application in cultivation (Ceylan, 1987; Munsi, 1990; Piccaglia and Marotti, 1993; Court et. al., 1993; Alkire and Simon, 1996; Ozguven and Kırıcı, 1999; Tugay et al., 2000).

3.6. Essential Oil Yield (L/m²)

In the study, the highest essential oil yield values were obtained from multimentha cultivar and the essential oil yield was determined as 4.31 L/da. The lowest essential oil yield (L/da) was found as 2.37 L/da in chocolate cultivar (Figure 5).

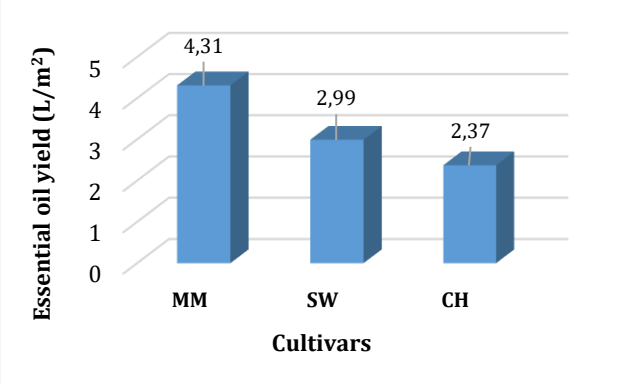


Figure 5. Effect of cultivars on essential oil yield (L/m²).

When the previous studies were examined; Yılmaz (2018), was found the essential oil yields in the first year of the study (2016) between 4.0-7.2 L/da in the first harvest and 1.2-5.5 L/da in the second harvest.

4. Conclusions

Fresh herb yield, drog herb yield and drog leaf yield although there was no effect of the doses, the multimentha cultivar has the highest yield values. Technological properties (essential oil ratio and essential oil yield) were economically valuable features and values of these features reached the highest values with 2 time dose and multimentha cultivars.

If it is desired to produce fresh herb yield, drog herb yield and drog leaf yield, it is recommended to use multimentha cultivar. Or if it is desired to produce essential oil ratio, it is recommended to use 2 times microbial dose and multimentha cultivar.

Our yield results found to be higher than other researchers except essential oil ratio and essential oil yield due to early (before flowering) harvest. However, our study was conducted Piccaglia in greenhouse condition in pots so results might be different in field condition. It is useful to enrich this study with another study in field conditions.

Conflict of Interest

The authors declare that they have no conflict of interet.

References

1. Alkire, B. H., and Simon, J. E. 1995. Response of Midwestern peppermint (Mentha x piperita L.) and native spearmint (M. spicata L.) to rate and form of nitrogen fertilizer. In International Symposium on Medicinal and Aromatic Plants 426 (pp. 537-550).
2. Baydar, H., 2016. Tıbbi Aromatik Bitkiler Bilimi ve Teknolojisi (Genişletilmiş 5. Baskı), SDÜ Yayın No: 51, p. 264. Isparta.
3. Ceylan, A. 1983. Tıbbi Bitkiler II, Ege Üniversitesi Ziraat Fakültesi Yayınları, Yayın No: 481, İzmir.
4. Court, W. A., Roy, R. C., Pocs, R., More, A. F., and White, P. H. 1993. Optimum Nitrogen Fertilizer Rate for Peppermint (Mentha piperita L.) in

Ontario, Canada. Journal of Essential Oil Research, 5(6), 663-666.
5. Demirezer, L.Ö, 2010. Bitkilerin Tıpta Kullanılması Konusundaki Sorumluluklarımız. Bitkilerle Tedavi Sempozyumu 5-6 Haziran 2010 Zeytinburnu/İstanbul Bildiri Kitabı, s: 87- 88.
6. Demirez M, 2013. Nane‟nin Fitoterapi Açısından Değerlendirilmesi. Gazi Üniversitesi Sağlık Bilimleri Enstitüsü Farmakognozi Anabilim Dalı Fitoterapi Programı. Ankara
7. Gezgin, D. 2006. Bitki Mitosları. Sel Yayıncılık, İstanbul İlisulu, 1992. Aromatik Bitki Nane. http://www.cine-tarim.com.tr/dergi/arsiv52/sektorel07.htm
8. Karakaplan, N., 2017. Nane (Mentha Spicata) Bitkisinden Uçucu Yağ Eldesi için Optimum Koşulların Araştırılması. İnönü Üniversitesi Fen Bilimlkeri Enstitüsü. Malatya.
9. Kokkini, S., 1983. Taxonomic studies in the genus Mentha in Greece. PhD Thesis. University of Thessalonniki, Thessaloniki., pp:171.
10. Megep, 2016. Tohumundan Ve Meyvelerinden Yararlanılan Bitkiler. Millî Eğitim Bakanlığı. Ankara
11. Munsi, P. S., 1990. Nitrogen and phosphorus nutrition response in Japanese mint cultivation. In International Symposium on Medicinal and Aromatic Plants, XXIII IHC 306 (pp. 436-443).
12. Özgüven M. and Kirici S., 1999. Farklı Ekolojilerde Nane (Mentha) Türlerinin Verim ile Uçucu Yağ Oran ve Bileşenlerinin Araştırılması. Çukurova Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Anabilim Dalı, 01330 Adana-TÜRKİYE
13. Piccaglia, R., and Marotti, M. 1993. Characterization of several aromatic plants grown in northern Italy. Flavour and Fragrance Journal, 8(2), 115-122.
14. Tuğay, M. E., Kaya, N., Yılmaz, G., Telci, I., and Dönmez, E. 2000. Tokat ve çevresinde yaygın olarak bulunan bazı aromatik bitkilerin bitkisel ve teknolojik özellikleri. TÜBİTAK, Tarım ve Ormancılık Araştırma Grubu, Proje Kesin Sonuç Raporu (TOGTAG-1690).
15. Yılmaz, K., 2018. Isparta koşullarında yetiştirilen Mentha piperita L. türüne ait klon ve çeşitlerin verim ve kalite özelliklerinin belirlenmesi. Doktora Tezi. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı, Isparta.

Research article
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Determination of Essential Oil Content and Composition, Total Phenolic Content and Antioxidant Activities of *Pastinaca sativa* L. subsp *urens* (Req. Ex Gordon)

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Abstract

This study was carried out in 2017 in order to determine the essential oil composition, total phenolic contents and antioxidant activity of fruits and above-ground parts of *Pastinaca sativa* L. subsp *urens* (Req. Ex Gordon) Celak, which grow naturally in the flora of Göller Region, Turkey. Plant samples were collected at full flowering and fruit samples were collected at theyellow maturing period. The essential oils were obtained by hydro-distillation and components of the oils were identified by gas chromatography/mass spectrometry. Methanol extracts of fruit and plant samples were used for total phenolic content and antioxidant activity. Total phenolic contents were 50.40 ± 1.40 mg/gr and 67.86 ± 1.02 mg/gr in the fruit and herb samples, respectively. Antioxidant activity of the herb extract was higher than the fruits. Essential oil contents of fruit and herb samples were 3.20% and 0.33% and the numbers of components forming essential oils were 28 and 29, respectively. There were 47 different components identified in total for fruit and herb samples. The main component of the fruit oil was octyl butyrate (90.4%), while cis-ocimen (38.2%), Furanone (14.1%), octyl Butyrate (13.2%) and Butanoic acid (11.1%) were the major components of the herba essential oil.

Key Words: *Pastinaca sativa* L. subsp*. urens,* Phenolic Content, Antioxidant Activity, Essential Oil Content and Component

1. Introduction

The genus *Pastinaca* (Apiaceae) is represented in Turkey by five species and altogether seven taxa: *Pastinaca sativa* L. subsp. *urens* (Req. ex Godron) Celak, *Pastinaca armena* Fisch. & Mey, *Pastinaca armena* Fisch. & Mey. subsp. *armena*, *Pastinaca armena* Fisch. & Mey. subsp. *dentata* (Freyn & Sint.) Chamberlain, *Pastinaca pimpinellifolia* Bieb., *Pastinaca zozimioides* Fenzl (endemic), *Pastinaca glandulosa* Boiss. & Hausskn. (Davis, 1972). The Pastinaca genus plants are perennial plants with small flowers and yellow fruits typical reprehensive of the Mediterranean flora (Davis, 1972). Different parts of *Pastinaca sativa* L. (Parsnip in English, Karakok, Kelemşir and Yabani havuc in Turkish) used for different purposes and, therefore, is widely cultivated and commercially traded in Europe (Baytop, 1999; Doğan, 2014). A whole plant is used for

strengthening the appetite, better digestion, and as a diuretic. The fruit is bitter aromatics that increases milk yield (Tucakov, 1986). The root is rich in starch and sugar and is used as food (parsnip), animal fodder, and for wine making. The sap is liable to cause skin irritation by sensitizing skin to UV radiation (Zehui and Watson, 2005). The seed is used as a condiment (Launert, 1981). It is similar in taste to dill (Facciola, 1990). Native parts of plants have been applied in feeding as a spice, pharmaceutical industry and folk medicine (Nikolić, 1973).

Above-ground parts, fruits and roots of *P. sativa* species contain essential oils of different properties. In the oil obtained from crushed seeds of *P. sativa* subsp. *urens* from Turkey, 18 components were characterized representing 95% of the oil with octyl butyrate (79.5%) and octyl hexanoate (5.3%) as the major constituents (Kurkcuoglu et al., 2006). The dominant constituents in fruit oil of *P. sativa* from Serbia were reported as myristicin (64.20%), *β*-ocimene (29.10%) and β-farnesene (24.50%) (Janciec et al., 1995). In the above-ground parts of *P sativa*, E-*β*-ocimene, Z-*β*-ocimene, *α*-terpinolene and E-*β*- farnesene has been identified as major compounds (Kubeczka and Stahl, 1977). The root essential oil has previously been reported to contain terpinolene (40–70%) and myristicin (17–40%) as the main constituents in the essential oil from fresh *P. sativa* roots (Stahl, 1975; Kubeczka and Stahl, 1975; Lawrence, 1979).

The occurrences of hydrocarbons and coumarins in the fruits were also reported (Brown et al., 1975; Stein and Posocco, 1984). Furanocoumarin compounds reported from the roots, stems, leaves, buds, flowers and fruits of cultivated and wild *P. sativa* included; angelicin, apterin, bergapten, imperatorin, isobergapten, isoimperatorin, isopimpinellin, pimpinellin, psoralen, sphondin, xanthotoxin and xanthotoxol (Pathak et al., 1962; Berenbaum, 1985; Berenbaum and Zangerl, 1986; Berenbaum et al., 1991). There is considerable variation in the toxicity and photoactivity of these different furanocoumarin compounds (Berenbaum et al., 1991). Considering the potential of essential oils and extracts for substituting synthetic antioxidants and preservers, the study of potential of *P. sativa* L. subsp *urens* (Req. Ex Gordon) can be useful for industrial application as a natural antioxidant sources.

To the best of our knowledge, there is no previous study on the antioxidant activity and phenolic content of fruits and above-ground parts of *P. sativa* species. The aim of this study was to evaluate and compare the essential oil content and constituents, total phenolic matter and antioxidant activities of methanol extracts from the fruits and aerial parts of *P. sativa* L. subsp *urens* (Req. Ex Gordon) from Turkey.

2. Materials And Methods

2.1. Plant Material

Fruits and above-ground parts of *P. sativa* L. subsp *urens* were collected by the authors from their natural habitats in Eğirdir, Isparta, Turkey in 2017 by random sampling from a single established population. All samples were collected at full flowering and fruit maturing stages for species identification and essential oil, antioxidant activity and total phenolic analyses. Plant materials were identified by Prof. Dr. Hasan ÖZÇELİK according to “Flora of Turkey” (Davis et al., 1988) and voucher specimens (63.73.1.1) were deposited in the Herbarium GUL, Suleyman Demirel University.

2.2. Essential oil analysis

Air-dried fruits (50 g) and fresh above-ground parts (500 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (1980). The obtained oils were dried over anhydrous sodium sulfate

and stored in sealed vials at +4°C in the dark until chemical analyses.

2.3. Gas chromatography–mass spectrometry

GC–FID-MS analysis was performed on QP5050 GC-MS equipped with FID detector. The GC was equipped with CP-Wax 52 CB capillary column (50 m x 0.32 mm; film thickness = 0.25 µm) and helium was used carrier gas with flow rate of 1 mL/min. The GC oven was heated from 60°C to 230°C at a rate of 3°C/min, the final temperature was then maintained during for 20 min. The injector was maintained at a temperature of 250°C. Injection volume 0.1 mL of 1% solution prepared in n-hexane; split ratio 20:1. The mass spectrometer was operating in EI mode at 70 eV with mass scan range of 40–450 amu. Identification of constituents was done on the basis of RI (determined with reference to homologous series of n-alkanes C8–C25, under identical experimental condition), MS library search (NIST 08MS Library (Version 2.0 f) and Wiley MS 9th edition), and by comparison with MS literature data (Adams, 1995). The relative amounts of individual components were calculated based on GC peak area (FID response) without using the correction factor.

2.4. Determination of total phenolic content

Ground-dried above-ground parts and fruits (0.2 g) were extracted with 10 mL of 80% methanol at room temperature, using a magnetic stirrer for 15 min. After centrifugation for 10 min, the supernatant solution was filtered under vacuum into a volumetric flask. The residue was re-extracted in the same way and the final volume of the solution was set at 25 mL.The phenolic content of the fruit and above-ground parts of the species were made according to Singleton and Rossi (1965) using Folin-Ciocalte colorimetric method. The obtained results were read in a spectrophotometer at 765 nm wavelength and total phenolic contents were calculated as gallic acid equivalent in mg/g with the following equation.

Total Phenolic Content (mg/g)= [(reading * final volume) / volume in the pit) * (1 / weighing)]

2.5. Determination of antioxidant activity by DPPH method

The free radical trapping activity of fruits and above-ground parts were compared with synthetic antioxidants such as BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole) and Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane -2-carboxylic acid] by DPPH (1,1-diphenyl-2-picryl-hydrazyl) method. (Shimada et al. 1992). Samples were prepared at 50, 100 and 250 ppm in 1 ml of methanol and 1 ml of 0.2 mM DPPH was added. The vortexed samples were incubated for 30 minutes at darkroom temperature and then measured on a spectrophotometer at a wavelength of 517 nm (PG Instruments T70 Plus Dual Beam Spectrophotometer, Arlington, MA, USA). The free radical trapping activity of the fruits and above-ground parts used in the study was determined by the following formula:

Antioxidant activity (%) = [(control abs - sample abs) / control abs] x 100

3. Results and Discussion

Fruits and above-ground parts of *P. sativa* L. subsp *urens* afforded yellowish oils, with yields (mean of three replicates) 3.20% and 0.11% (v/w), respectively. The GC-MS results of the essential oils are given in Table 1. A total of 39 compounds were identified which was 27 for fruits and 29 for above-ground parts representing more than 99% of the volatile fraction. Considering the different groups of compounds, a large part of the fruit oil was composed of esters (93.96%), whereas above-ground parts oils was formed monoterpene (49.55%) and esters (40.08%).

The total sesquiterpene content of the above-ground parts essential oils (4.74%) were higher than the fruits (0.37%) (Table 1). The components of both oils were substantially similar, but the proportions of the components were different. As shown in Table 1, the main compound in the essential oil from the fruits were octyl butyrate (90.43%), while these compound were found by 13.23% in above-ground parts oil. Hexyl butyrate (2.27%) and octyl hexanoate (2.87%) were the other important compounds found in fruit essential oils. On the other hand, Octyl Hexanoate was only found in the fruit essential oils. *Cis*-Ocimene (38.23%) predominates in the essential oil of above-ground parts and this compound present in very low concentrations in fruits essential oil (0.16%). Essential oils from the above-ground parts have a high level of octyl butyrate (13.23%) and butanoic acid (11.10%) which are found very low content in the oil from the fruits. Some components with content greater than 1% such as (X) *β*-ocimine, *α*-terpinolene and neo allo acimene were only found in the essential oil of above-ground parts.

There are a few pieces of researches have been reported on essential oil compounds of *P. sativa* in the literature (Kubeczka and Stahl, 1977; Kurkcuoğlu et al., 2006; Matejic et al., 2014). Fruit essential oil compositions *P. sativa* L. subsp *urens* from Turkey have been reported for the first time by Kurkcuoğlu et al., (2006). These researches reported that the main constituents of fruit essential oil of *P. sativa* L. subsp *urens* were octyl butyrate (79.5%) and octyl hexanoate (5.3%). In Kubeczka and Stahl (1977) study, herb oils from the wild *P. sativa* were characterized by the presence of octyl acetate. The other important constituents of the herb oils of wild *P. sativa* reported as cis *β*-ocimene, *trans*-*β*-ocimene, terpinolene and *trans*-P-farnesene (Kubeczka and Stahl, 1977). Similar to our findings, Matejic et al., (2014) reported that the essential oil content of aerial parts of *P. sativa* from the Belgrade was 1.00%. (Z)-*β*-ocimene (10.8%), hexyl butanoate (10.4%), (E)-*β*-farnesene (6.1%) and lavandulyl acetate (5.2%) were found as the main components in the same research. The major components of the composition of the essential oil German *P. sativa* were reported octyl butyrate (40.9%), octyl acetate (32.4%), hexyl butanoate (4.6%), Z-*β*-ocimene (4.3%), E-*β*-farnesene (3.4%) and *γ*-stearolactone (3.4%). According to the present results and previous reports the principal component is octyl butyrate. Octyl butyrate was also as the major constituents of the essential oils of Malabaila aurea (Vuckovic et al., 2014) and Heracleum sphondylium (Maggi et al., 2014) from Apiaceae. The genera *Pastinaca*, *Heracleum*, *Zosima* and *Tordylium* are characterized by the presence of octylesters and octanol in their essential oils (Özek et al., 2006; Figueiredo et al., 2008). These compounds, such as octyl acetate, octyl butyrate, octyl hexanoate, octyl octanoate and octyl isobutanoate, often constitute nearly the entire essential oil, with other compounds occurring only as minor ones (Chizzola, 2010).

Total phenolic analyses showed that the amount of extractable phenolic compounds in above ground part extract (67.86 ± 1.02 mg/g galic acid) is higher than that detected in fruit extracts (50.40 ± 1.40 mg/g galic acid). Numerous studies have been conducted on the phenolic contents of Apiaceae species, but no similar studies were found in *Pastinaca* species. Bagdassarian et al., (2013) reported that the phenolic contents of some Apiaceae species were highly variable and they found the phenolic contents of *Foeniculum vulgare*, *Anethum graveolens*, *Pimpinella anisum*, *Carum carvi* and *Coriandrum sativum* were 116, 70, 46, 26 and 17 mg GAE /100g, respectively. The methanol extract of *P. ferulacea,* a species of Apiaceae, herb' antioxidant activities was found to be 152 and the total phenolic

content 65.1 in the study carried out by Çoruh et al. (2007). Pandey et al. (2012) reported that phenolic content for methanol extracts of seven spices of Apiaceae ranged from 12.81 in *Carum carvi* to 45.26 mg RE/g extract in *C. sativum*.

Table 1. Percentage composition of the essential oil of the fruits and of above-ground parts Pastinaca *sativa* L. subsp *urens* (Req. Ex Gordon) Celak

RI	Components	Fruit	Above ground parts
1064	Sabinene	-	0.14
1069	*β*-Pinene	0.12	0.91
1074	Butyl Butanoic Acid	0.10	-
1079	*β*-Myrcene	0.47	1.09
1118	l-Limonene	0.02	0.16
1122	***Cis*-Ocimene**	**0.16**	**38.23**
1133	**(X) *β*-Ocimine**	**-**	**5.75**
1137	2-Methyl Butyl Butyrate	0.03	-
1149	Octilin	0.73	-
1176	*α*-Terpinolene	-	1.26
1180	Linalool	-	0.39
1215	Neo Allo Ocimene	-	1.62
1266	Hexenyl Butyrate	0.02	0.20
1274	**Hexyl Butyrate**	**2.27**	**1.01**
1284	*n*-Decanal	0.23	0.23
1293	Caprylyl acetate	0.06	0.20
1330	RT:16.792	-	0.10
1347	RT:17.325	-	0.14
1364	Anethole	0.06	-
1378	Heptyl Butyrate	0.02	-
1422	Benzyl Butyrate	0.08	0.77
1470	Octanol	0.45	-
1490	**Octyl Butyrate**	**90.43**	**13.23**
1496	*α*-Duprezianene	-	0.28
1515	*α*-Longipinene	-	0.21
1525	Benzyl Carbyl Butyrate	0.49	3.17
1531	*Trans*-Caryophyllene	0.13	2.62
1564	**Butanoic Acid**	**0.19**	**11.10**
1579	RT:24.317	-	0.67
1588	*α*-Curcumene	0.20	1.26
1590	*α*-Bergamotene	-	0.36
1606	Myristicin	0.06	-
1627	Isopulegyl Acetate	0.03	0.26
1637	*β*-Sesquiphellandrene	0.04	-
1645	Aromadendrene	-	0.29
1687	Dodecenylacetate	0.02	0.24
1693	**Octyl Hexanoate**	**2.87**	-
1699	Decyl Butyrate	0.62	-
2229	**Octadecanoic Acid**	**0.07**	**14.11**
Monoterpene		0.86	49.55
Ester		93.96	40.08
Sesquiterpene		0.37	4.74
Other		4.81	5.63
Total		100.0	100.0

DPPH is a stable free radical, widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu et al 2004). The methanol extract of *P. sativa* fruits and above-ground parts and standard antioxidants (BHA, BHT and Trolox) DPPH radical scavenging activity shown in Table 2. According to the results, DPPH radical scavenging activity of fruit and above-ground parts extracts and BHT change depending on the using concentration. However, Trolox and BHA showed high levels of radical scavenging activity at a concentration of 50 ppm such as more than 94.5% and 86.6%, respectively. Antioxidant activity of plant extracts increased as the used concentration increased (Table 2). DPPH radical scavenging activity of the methanol extracts of above-ground parts were higher (more than two fold) than the fruit extracts. In addition, the above-ground parts extracts showed similar antioxidant activity to the BHA (Table 2).

Table 2. Antioxidant activity of fruits and above-ground parts of *Pastinaca sativa* L. subsp *urens* (Req. Ex Gordon) Celak

		Fruit	**Above ground parts**
P. sativa L. subsp *urens*	250 ppm	24.71±0.46	55.54±2.6
	100 ppm	8.26±0.56	33.81±1.9
	50 ppm	3.28±0.18	23.1±2.3
Trolox	250 ppm		95.1±0.9
	100 ppm		94.7±0.9
	50 ppm		94.5±1.3
BHT	250 ppm		52.6±0.7
	100 ppm		34.9±1.5
	50 ppm		26.0±1.6
BHA	250 ppm		88.2±1.3
	100 ppm		88.0±0.2
	50 ppm		86.4±0.6

The natural antioxidants in plants have a great interest in natural product science, and many herbs have significant antioxidant potency (Ngi et al., 2000). Antioxidants decrease oxidative stress in cells and are therefore very useful in the treatment of many diseases and protect to plant many stress factors (Krishnaiah et al., 2011). The physiological role of antioxidant compounds is to scavenge for free radicals (Muraina et al., 2009; Halliwell and Gutterigde, 1989) in case of the overproduction of these reactive species (Wong et al., 2006). Phenolic compounds inhibited MDA concentration during lipid peroxidation; thus, they exhibited antioxidant activity. Methanol extracts contain both the nonpolar and polar compounds (aglycones and glycosides) in the plant. High correlation was reported between the antioxidant capacity and total phenol and flavonoids contents of plants (Wong et al., 2006; Leong and Shui, 2002). Besides antioxidant capacity, phenolic compounds exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, anti-allergenic, anti-microbial and anti-stress (Hu et al., 2004). It is well known that plant phenolics, in general, are the highly effective free radical scavenging and antioxidants.

4. Conclusion

This study provides useful information on the fruit essentail oil quality and quantity as well as the fenolic content and antioxidant activity of the *P. sativa* subsp. *urens*. The majority of

the essential oil components were esters for furits and monoternes and esters for above-ground parts. Fruits of the specie may be valuable for pharmacological applications due to high essential oil and ester content and above-ground parts of the specie can be evaluated for exploitation in pharmaceutical and food industries due to its moderate antioxidant activity.

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Conflict of Interest

The authors decleared that no conflict of interest.

References

1. Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy; Allured Publications: Carol Stream, IL, USA.
2. Baytop, T., 1999. Turkiyeçde Bitkiler ile Tedavi, Nobel Tip Kitabevleri, Istanbul.
3. Berenbaum, M.R. 1985. Brementown revisited: interactions among allelochemicals in plants. Recent Adv Phytochem 19,139–169.
4. Berenbaum, M.R., Nitao, J.K., Zangerl, A.R., 1991. Adaptive signifi cance of furanocoumarin diversity in *Pastinaca sativa* (Apiaceae). J Chem Ecol 17(1), 207–215.
5. Berenbaum, M.R., Nitao, J.K., Zangerl, A.R., 1991. Adaptive signifi cance of uranocoumarin diversity in *Pastinaca sativa* (Apiaceae). J Chem Ecol 17(1), 207–215.
6. Berenbaum, M.R., Zangerl, A.R., 1986. Variation in seed furanocoumarin content within the wild parsnip *Pastinaca sativa* . Phytochemistry 25, 659–661.
7. Berenbaum, M.R., Zangerl, A.R., Nitao, J.K., 1984. Furanocoumarins in seeds of wild and cultivated parsnip. Phytochemistry 23(8),1809–1810.
8. Brown, S. O., Hamilton, R. J., Shaw, S., 1975. Phytochemistry, 14, 2726.
9. Chizzola, R., 2010. Essential Oil Composition of Wild Growing Apiaceae from Europe and the Mediterranean. Natural Product Communications 5 (9), 1477-1492.
10. Christova-Bagdassarian, V., Bagdassarian, S., K., Atanassova, M., 2010. Phenolic Profile, Antioxidant and Antibacterial Activities from the Apiaceae Family (Dry Seeds). Mintage journal of Pharmaceutical & Medical Sciences. 2, 4.
11. Çoruh, N., Sağdıçoğlu Celep, A.G., Özgökçe, F., 2007. Antioxidant properties of *Prangos ferulacea* L., *Chaerophyllum macropodum* Boiss. and Heracleum persicum Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory eff ects on glutathione-S-transferase. Food Chem 100, 1237-1242.
12. Davis, P., H., (ED.) 1972. Flora Of Turkey And The East Aegean Islands, Vol.4, Edinburgh University Press, Edinburgh.
13. Davis, P., H., Mill, R., R., Kit, T., (EDS.) 1988. Flora Of Turkey And The East Aegean Island Vol.10 Edinburgh University Press, Edinburgh.
14. Doğan, A., Bulut, G., Tuzlacı, E., Şenkardeş, İ., 2014. A review of Edible Plants on the Turkish Apiaceae Species. İstanbul Ecz. Fak. Derg. J. Fac. Pharm. Istanbul 44(2), 251-262.
15. Facciola, S., 1990. Cornucopia. A source book of edible plants. Kampong Publications, Vista, 677 pp
16. Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C., 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance Journal, 23, 213-226.
17. Foster, S., Duke, J.A., 1998. A fi eld guide to medicinal plants in Eastern and Central N. America. Houghton Miffl in, Boston, 366 pp.
18. Grieve, M., 1971. A modern herbal, Penguin. Dover, New York, 2 Vols, 919 pp.
19. Halliwell, B., Gutterigde, J.M.C. 1989. Free radicals in Biology and Medicine. Clarendon Press; Oxford.
20. Hu, F.L., Lu, R.L., Huang, B., Ming, L., 2004. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. Fitoterapia 75,14–23.
21. Jančić, R., Stošić, D., Mimica-Dukić, N., Lakušić, B., 1995. Aromatične biljke Srbije. NIP Dečije novine, Beograd-Gornji Milanovac.
22. Krishnaiah, D., Sarbatly, R., Nithyanandam, R. A., 2011. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 89,217–33.

23. Kubeczka K. H., Stahl, E., 1975. Planta Med., 27, 235 .
24. Kubeczka, K.H., Stahl, E., 1977. On the essential oils from the Apiaceae (Umbelliferae) II. The essential oils from the above ground parts of *Pastinaca sativa.* [Über ätherische Öle der Apiaceae (Umbelliferae) II. Das ätherische Öl der oberirdischen Teile von *Pastinaca sativa*]. Planta Medica, 31, 173-184.
25. Kurkcuoglu, M., Baser, K. H. C., Vural, M., 2006. Composition of The Essential Oil of *Pastinaca sativa* L. subsp. *urens* (Req. Ex Godron) Celak. Chemistry of Natural Compounds, 42(1), 114-115.
26. Launert, E., 1981. Edible and medicinal plants, Hamlyn, London Lawrence BM.
27. Lawrence, B. M., 1979. Essential Oils, 1976–1978, Allured Publishing Corparation, USA .
28. Leong, L.P., Shui, G. 2002.An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem 76, 69–75.
29. Maggi, F., Quassinti, L., Bramucci, M., Lupidi, G., Petrelli, D., Vitali, L.A., Papa, F., Vittori, S., 2014. Composition and biological activities of hogweed (*Heracleum sphondylium* L. subsp. *ternatum* (Velen.) Brummitt) essential oil and its main components octyl acetate and octyl butyrate. Natural Product Research 28, 1354-1363.
30. Matekic, S.J., Džamić, A.M., Mihajilov-Krstev, T., Ranđelović, N.V., Krivošej, D.Z., Marin, D.P., 2014. Antimicrobial potential of essential oil from *Pastinaca sativa* L.. Biologica Nyssana. 5(1), 31-35.
31. Muraina, I.A., Suliman, M.M., Eloff, J.N., 2009. Can MTT be used to quantify the antioxidant activity of plant extracts? Phytomedicine 16, 665-668.
32. Ngi, T.B., Liu' F., Wang, Z.T., 2000. Antioxidative Activity of Natural Products From Plants. Life Sciences, 66(8), 709-723.
33. Nikolić, V., 1973. Fam. Apiaceae. In: Josifivić M. (ed.), Flora of SR Serbia 5, Beograd, Serbia: SANU, 183-348.
34. Özek, T., Özek, G., Baser, K.H.C., Duran, A., 2005. Comparison of the essential oils of three endemic Turkish *Heracleum* species obtained by different isolation techniques. Journal of Essential Oil Research, 17, 605-610.
35. Pandey, M.M., Vijayakumar, M., Rastogi, S., Rawat, A.K.S., 2012. Phenolic content and antioxidant properties of selected Indian spices of Apiaceae. J Herb, Spice Med Plant 18, 246-56.
36. Pathak, M.A., Daniels, F., Fitzpatrick, T.B., 1962. The presently known distribution of furocoumarins (psoralens) in plants. J Investig Dermatol 39, 225–239.
37. Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybeanoil in cyclodextrin emulsion. Journal of Agricultural and FoodChemistry, 40, 945–948.
38. Singleton, V. L., Joseph, Rossi, A., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. January 16, 144-158.
39. Stahl, E., 1975. Untersuchungen uber das Aetherischen Oel von *Pastinaca sativa* L. (Apiaceae), Ph. D. Thesis, Univ. Hamburg .
40. Stahl-Biskup, E., Wichtmann, E.M., 1991. Composition of the essential oils from roots of some Apiaceae in relation to the development of their oil duct systems. Flav Fragr J 6,249–256
41. Stein M. L., Posocco, E., 1984. Fitoterapia, 55, 119.
42. Tucakov, J., 1986. Lečenje biljem, fitoterapija. Izdavačka radna organizacija "Rad", Beograd.
43. U.S., 2014. Department of Agriculture, Agricultural Research Service (USDA, ARS) (2014) USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory HomePage,http://www.ars.usda.gov/ba/bhnrc/ndl.
44. Vuckovic, I., Vujisic, L., Todosijevic, M., Stesevic, D., Milosavljevic, S., Trifunovic, S., 2014. Volatile constituents of different plant parts and populations of *Malabaila aurea* Boiss. from Montenegro. Records of Natural Products 8, 148-155.
45. Wong, C.C., Li, H.B., Chen, K.W., Chen, F. A., 2006. systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem 97:705–11.
46. Wong, S.P., Leong, L.P., Koh, J.H.W., 2006. Antioxidant activities f aqueous extracts of selected plants. Food Chem 9, 775-783.
47. Zehui, P., Watson, M.F., 2005. 94. *Pastinaca* Linnaeus, Sp. Pl. 1: 262. 1753. Flora of China, 14, 193.

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The Efficiency of Bioapifit® Wound Care Ointment in the Treatment of Diabetic Foot Ulcers

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Abstract

Objective/Purpose: The objective of this study was efficacy and safety assessment of Bioapifit® wound care ointment consisted of honey, *Cera flava,* glycerin, the oil macerates of astringent and soothing herbs combined with essential oils for the treatment of diabetic neuropathic foot ulcers of grade II. **Materials and methods:** 50 patients with Wagner grade II diabetic foot ulcers were randomized into experimental and control group (25 patients each). The experimental group was treated 28 days with Bioapifit® wound care ointment applied on the wound after cleaning with povidone iodine once a day and covered with sterile gauze and bandage during the whole course of the study. The control group wounds were cleaned with povidone iodine once a day and covered with sterile gauze and bandage during the whole course of the study without further treatment. The ulcers surface area was measured at baseline and after 14 and 28 days of the treatment. **Results:** In the end of the treatment the mean values and standard deviation of the surface area in the experimental group dropped from 8.27±6.1 cm^2 to 0.74±0.21 cm^2 for males and from 9.01±5.9 cm^2 to 0.81±0.11 cm^2 for females. Slight, insignificant decrease was also observed in the control population (up to 25%). No side-effects were observed during the course of the study. **Conclusion/Discussion:** Four weeks treatment with Bioapifit® wound care ointment resulted in complete wound closure in 84% of the patients and 91% reduction in the wound surface area in the rest of them.

Key Words: Diabetic Foot Ulcers, Honey, Beeswax, Herbal Macerate, Essential Oils

1. Introduction

Diabetic foot is defined as a foot affected by ulceration associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with diabetes. It is common complication of diabetes with prevalence between 4 and 10% of diabetic patients (Alexiadou and Doupis, 2012). Based on etiology they are divided into neuropathic and neuroishemic. The prevalence of both types of ulcers increase with age and duration of diabetes. Although, up to 80% of ulcers will heal following the standard topical treatment up to 15% will showed no closure and between 5 and 24% of ulcers will lead to limb amputation (Alexiadou and Doupis, 2012). Neuropathic ulcers were more likely to heal compared to neuroishemic ulcers.

The treatment of diabetic foot ulcers involves debridement of the wound (surgical,

enzymatic, biological and autolytic), treatment of the infection with oral antibiotics, revascularization procedures if needed, and surgical methods. Hyperbaric oxygen therapy and negative pressure wound therapy are also used (Doupis and Veves, 2008; Alexiadou and Doupis, 2012).

Recently, topical treatment with honey or honey based dressings showed very promising results in the treatment of neuropathic foot ulcers for the management of infection, autolytic debridement, exudates removal that all together leading to the wound closure (Eddy et al., 2008; Kamaratos et al., 2014; Alam, 2017, Mohamed et al., 2015; Tsang et al., 2017; Cooper, 2017). Moreover, the product based on honey, macerates of astringent plants, glycerin and beeswax was proven highly effective in the treatment of chronic wounds (Oreščanin, 2016). Consequently, the purpose of this paper was assessment of the performance and safety of multi-component Bioapifit® wound care ointment in the treatment of Wagner grade II diabetic foot ulcers (DFU) during 28 days of topical application.

2. Patients and Methods

2.1. Study design

The study was conducted at the following locations: FINDRI GUŠTEK HEALTHCARE INSTITUTION, Ninska 5a, Sesvete, Croatia and FAMILY MEDICINE CLINIC, Vilima Korajca 19 Zagreb, Croatia. The investigator recruited the patients based on their medical history, following the predefined inclusion and exclusion criteria. The study protocol was approved by the Ethics Committee of Findri Gustek Health Care Center with EudraCT number 2019- 001379-35.

The study was designed as single blind, randomized control trial. 50 patients that met inclusion/exclusion criteria with Wagner II grade neuropathic foot ulcers were randomly selected according to the randomization code into experimental and control group. Exclusion criteria were: ulcer grade higher then 2, patients with ankle-brachial index (ABI) lover than 0.9 or higher then 1.3, immunodeficient patients, cancer patients, diabetes mellitus type I, pregnancy or breastfeeding, ongoing oral antibiotic therapy, less than 18 years of age. All the participants signed informed consent and completed the demographic questioner. The experimental group was treated 28 days with Bioapifit wound care ointment applied once a day on the wound previously cleaned with 7.5% solution of povidone iodine solution. The ointment was applied onto cleaned wound in a thick layer, covered with sterile gauze and bandage. The participants of the control group were subjected to wound cleaning with 7.5% solution of povidone iodine and cleaned wound was covered sterile gauze and bandage. Wound cleaning and bandage changing was performed each day (once a day) for 28 consecutive days. The ulcer surface area was assessed at baseline and after 14 and 28 days of the treatment.

2.2. Description of Investigational Product

Bioapifit® wound care ointment is homogeneous, greasy, viscous mass of characteristic herbal odor and olive green color with pH of 4.43±0.13. It consists of the following ingredients: honey (certified organic), beeswax (*Cera flava*), glycerol, the macerates of the plant species: *Plantago major* L., *Achilea millefolium* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Symphytum officinale* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils: Australian tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris* ct. thymol), oregano (*Origanum vulgare*).

2.3. Statistical analysis

For statistical evaluation Statistica 11.0 software package was employed. The description of the treated population was done by basic statistics and frequency tables. Statistical significance was set to $p<0.05$ in all

the tests performed. The differences in the mean values of each parameter prior and after the therapy as well as different treatment periods were assessed by Newman-Keuls test (Oreščanin et al., 2016).

3. Results

3.1. Description of the Population

The experimental group (Table 1) consisted of 15 males ranging from 40 to 61 (51.2±9.1) years and 10 females with age range between 39 and 63 years (52.4±8.7). Male population suffers from diabetes mellitus type II from 7 to 15 years (11.7±3.4) and females from 9 to 16 years (14.1±4.2). Presence of foot ulcers ranged between 12 and 20 months in male population (16.2±4.1) and from 16 to 23 months (18.3±7.1) in female participants. T-test showed no significant difference in demographic variables between males and females.

Control population showed very similar values regarding age (53.7±9.4 and 51.3±8.1 years for males and females, respectively), duration of DM type II (12.4±2.8 and 10.1±3.2 years for males and females, respectively) or duration of diabetic foot ulcers (15.8±3.7 and 16.4±3.6 months for males and females, respectively). The results of t-test showed no significant difference between males and females for neither of the mentioned variables. There was no significant difference between experimental and control group for the same gender.

Table 1. The mean values and standard deviations for demographic variables separately for males and females for experimental and control group.

Variable	Experimental group		Control group	
	Males (N=15)	Females (N=10)	Males (N=13)	Females (N=12)
Age (yrs)	51.2±9.1	52.4±8.7	53.7±9.4	51.3±8.1
Duration of DM type II (yrs)	11.7±3.4	14.1±4.2	12.4±2.8	10.1±3.2
Duration of DFU (months)	16.2±4.1	18.3±7.1	15.8±3.7	16.4±3.6

DM-diabetes mellitus; DFU-diabetic foot ulcers

3.2. Treatment efficiency

At baseline (Figure 1) both groups showed similar surface area of the ulcers regardless of group and gender with no statistically significant difference between gender and groups. Prior to the therapy the mean values and standard deviation of the surface area in the experimental group was 8.27±6.1 cm2 and 9.01±5.9 cm^2 for males and females, respectively. After 14 days of the treatment those values dropped significantly both for males (5.72±1.70 cm^2, p=0.0479) and females (5.31±1.74 cm^2, p=0.0054). There was no slough present and pink granulation tissue occurred on the edge of the wounds in both genders. Following 28 days of the treatment in 86.7% of the male participants wound were completely closed while in other two participants wound surface area was decreased to 0.74±0.21 cm^2. In the end of the treatment in 80% of the females wound were completely closed and in the rest of them the ulcer area was 0.81±0.11 cm^2. The percentage of the ulcer size reduction following the treatment was app. 91%.

In the control group at baseline the mean values and standard deviation of the ulcers surface area was 8.34±5.40 cm^2 and 8.49±5.20 cm^2 for males and females, respectively. After 14 days of the treatment there was no significant reduction in the ulcers surface area (8.07±5.43 cm^2 and 8.11±5.02 cm^2 for males and females, respectively). In the end of the treatment the ulcers surface area were reduced in both genders. However, this reduction was not statistically significant. The surface area dropped for 24.5% and 25% for male and

female population, respectively. None of the ulcers closed completely. However in 9 of 25 ulcers pink granulation tissue occurred in the edge of the wounds.

From the presented results it was obvious that experimental group performed better in the healing of diabetic foot ulcers at both follow up periods compared to the control group. T-test showed significantly higher reduction in ulcers size in male ($p=0.0443$) and female ($p=0.0111$) population, respectively following 14 days of the treatment as well as in the end of the treatment ($p<0.0001$ for both genders) in the experimental compared to the control group.

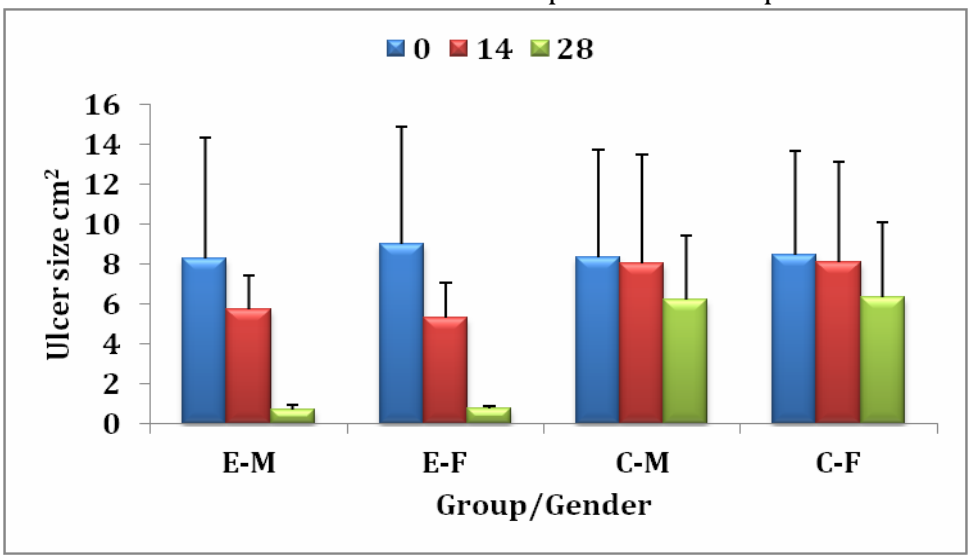

Figure 1. Mean vales and standard deviations for the mean values and standard deviation of diabetic foot ulcer area *(M) and female (F) population in the experimental (E) and control (C) group at baseline (0) and 14 and 28 days of follow up period*

4. Discussion and Conclusion

Bioapifit® wound care ointment showed very promising results in the treatment of chronic wounds like diabetic foot ulcers. Obtained results showed complete wound closure in 84% of the patients and app. 91% reduction in the wound surface area in the rest of the patients. Slough and unpleasant odor disappeared at first follow up period (after 14 days of the treatment) while in the same time pink granulation tissue occurred on the edge of all wounds. Moreover, at first follow up there were no signs of inflammation in any of the participants.

Such beneficial effect of the ointment could be linked with the presence of 30% of honey in the product which was well known ingredient since ancient time both in folk and CAM for the treatment of chronic, hard to heal wounds. Honey, due to its low pH value (4.16) forms an acidic wound micro-environment necessary for healing process. Absorption of wound exudates due to high osmotic effect/high sugar content and debridement of slough and necrotic tissue through autolytic debridement could be also linked with high content of honey in the product (Gethin et al., 2008; Alam et al., 2014) which was confirmed in numerous studies.

Mohamed et al. (2015) reported complete wound closure of foot ulcers no contractures or scars treated three weeks (once a day) with natural honey. The treatment of the patients with neuropathic diabetic foot ulcers with manuka honey impregnated dressings (Kamaratos et al., 2014) resulted in complete healing after 31±4 days. Tsang et al. (2017) reported complete closure of 50% of diabetic foot ulcers and 86% and reduction in ulcers size following 12 weeks of application of Manuka honey based dressing. Application of

beri honey impregnated dressing on 179 Wagner grade I DFU resulted in 76% of closure with median wound healing time of 18 days (Imran et al., 2015).

Researchers confirmed beneficial effect of the astringent plants rich in soluble tannins in the treatment of open wounds (Odukoya et al., 2007; Abascal and Yarnell, 2005) which could be explained by surface coagulation of the proteins resulting in the shrinking of the wound as well as by forming the protective coating over damaged tissue. For that purpose oil macerates of the plants with strong astringent properties *Plantago major* L., *Achilea millefolium* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Symphytum officinale* L., were included in the product formulation. Moreover, the macerates of marigold flowers (*Calendula officinalis* L.) and chamomile flowers (*Matricaria chamomilla* L.) were used due to its soothing and calming effect to the wounded skin (Oreščanin et al, 2016).

Additionally, herbal macerate was used in the formulation due to its low pH and coating effect. Moreover, the macerate created the environment with no water activity which was unsupportive for pathogens growth and replication. Glycerol was used in the formulation in order to provide enough moisture content of the wound necessary for the healing process. Beeswax was used in the formulation not only because of its emulsifying and thickening effect but also for wound isolation and protection from the microbial infection due to its excellent coating effect (Oreščanin et al., 2016). Essential oils served as natural preservatives and wound malodor correctors.

In conclusion, Bioapifit® wound care ointment with its low pH, high osmolarity, zero water activity, high astringency, excellent coating and moisturizing effect was promising alternative for the topical treatment of chronic wounds including neuropathic diabetic foot ulcers.

Conflict of Interest

The authors have declared that they have no conflict of interest.

References

1. Abascal, K., Yarnell, E., 2005. Botanical Treatments for Hemorrhoids. Alternative and Complementary Therapies, 11(6), 285-289.
2. Alam, F., Islam, M.A., Gan, S.H., Khalil, M.I., 2014. Honey: a potential therapeutic agent for managing diabetic wounds. Evidence-Based Complementary Alternative Medicine, Article ID 169130. doi:10.1155/2014/169130.
3. Cooper, R., 2017. The use of honey in diabetic foot ulcers. The Diabetic Foot Journal 20 (3), 154 – 159.
4. Doupis, J., Veves, A., 2008, Classification, diagnosis, and treatment of diabetic foot ulcers. Wounds, 20, 117–126.
5. Eddy, J.J., Gideonsen, M.D., Mack, G.P., 2008. Practical considerations of using topical honey for neuropathic diabetic foot ulcers: a review. Wisconsin medical journal, 107(4),187-190.
6. Gethin, G.T., Cowman, S., Conroy, R.M., 2008. The impact of Manuka honey dressings on the surface pH of chronic wounds. International Wound Journal, 5(2), 185-194.
7. Imran, M., Hussain, M.B., Baig, M., 2015. A randomized, controlled clinical trial of honey-impregnated dressing for treating diabetic foot ulcer. Journal of College of Physicians and Surgeons Pakistan, 25(10), 721–725.
8. Kamaratos, A.V., Tzirogiannis, K.N., Iraklianou, S.A., Panoutsopoulos, G.I., Kanellos, I.E., Melidonis, A.I., 2014. Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers. International Wound Journal, 11(3), 259-263.
9. Mohamed, H., Abu Salma, M., Al Lenjawi, B., Abdi, S., Gouda, Z., Barakat, N., Elmahdi, H., Abraham, S., Hamza, A.H., Al Khozaei, D., Al Majid, S., Al Majid, H., Abdini, J., Al Jaber, M., Al Masseh, F., Al Ali, A.A., 2015. The Efficacy and Safety of Natural Honey on the Healing of Foot Ulcers: A Case Series. Wounds, 27(4), 103-114.
10. Odukoya, O.A., Ilori, O.O., Sofidiya, M.O., 2007. Astringent herbs as vasoconstrictors in haemorrhoid therapy. Planta Medica, 73, 231-231.
11. Oreščanin, V., 2016. Treatment of pressure ulcers with Bioapifit® wound healing herbal ointment - a preliminary study. IJRDO-Journal of biological science, 2(10), 1- 15.
12. Tsang, K-K., Wai-Yung Kwong, E., Shing-Shun To, T., Wai-Yee Chung, J., Kwok-Shing Wong, T., 2017. A Pilot Randomized, Controlled Study of Nanocrystalline Silver, Manuka Honey, and Conventional Dressing in Healing Diabetic Foot Ulcer. Evidence- Based Complementary and Alternative Medicine, Article ID 5294890


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