**INTRODUCTION**

Interest in the raw materials of natural herbs, already great, is increasing day by day [1]. The flora of Armenia is rich in its variety of herbal raw materials [2]. Plants of the *Lamiaceae* family are valuable medicinal, mostly aromatic plants, many of which produce essential oils, used in traditional and modern medicine, as well as in the food, cosmetics, and pharmaceutical industries [3].

*Origanum vulgare*, belonging to the Lamiaceae family, is a common and widespread plant in the Armenian highlands and has been used for medical and traditional purposes for many years [4]. The essential oil of *O. vulgare* is known for its analgesic, anti-inflammatory, and antimicrobial properties [5].

This study aims to investigate the content of the essential oil of the *O. vulgare* of the Armenian highlands (OVA) in different periods of vegetation and to investigate its antinociceptive and anti-inflammatory effects in mice (in vivo) and cytotoxic action in cultured cells (in vitro). OVA essential oil was extracted from fresh plant material by hydro-distillation.

**Background:** Essential oils are of great interest for their analgesic and anti-inflammatory properties. We aimed to study the content of the essential oil of the *Origanum vulgare* of the Armenian highlands (OVA) in different periods of vegetation and to investigate its antinociceptive and anti-inflammatory effects in mice (in vivo) and cytotoxic action in cultured cells (in vitro). OVA essential oil was extracted from fresh plant material by hydro-distillation.

**Methods:** For OVA essential oil contents determination the gas chromatography-mass spectrometry method was used. Formalin and hot plate tests and analysis of cell viability using the methyl-thiazolyl-tetrazolium (MTT) assay were used.

**Results:** The maximal content of β-caryophyllene and β-caryophyllene oxide in OVA essential oil was revealed in the period of blossoming (8.18% and 13.36%, correspondently). In the formalin test, 4% OVA essential oil solution (3.5 mg/mouse) exerts significant antinociceptive and anti-inflammatory effects (P = 0.003). MTT assay shows approximately 60% cytotoxicity in HeLa and Vero cells for 2.0 μL/mL OVA essential oil in media.

**Conclusions:** The wild oregano herb of Armenian highlands, harvested in the blossoming period, may be considered as a valuable source for developing pain-relieving preparations.

**Key Words:** Analgesia; Anti-Inflammatory Agents; Beta-Caryophyllene; Caryophyllene Oxide; Cell Survival; Gas Chromatography-Mass Spectrometry; Nociception; Oils, Volatile; Origanum; Pain; Pain Measurement.
illy, is widely spread in Armenia, where its raw material resources have been investigated [4]. Nowadays, the research on oregano is of a great scientific interest. The essential oil extracted from this plant has positive effects as antioxidant, and as an anti-inflammatory, anti-diabetic, anti-proliferative and antibacterial agent [5,6]. According to European and Russian Pharmacopeias, the essential oil of O. vulgare contains thymol and carvacrol, which serve as a basis for the classification of herbal raw material [7]. However, modern scientific perceptions about oregano are based on other criteria, taking into a consideration the chemotype of the plant. The literature data suggest four chemotypes of oregano: the first chemotype is distinguished by the high content of thymol, the second possess high content of carvacrol, the third chemotype has moderate thymol content, and the fourth is characterized by a low or complete absence of phenol in the essential oil and a high content of sesquiterpenes [8].

The composition of biologically active substances in plants are defined by growth, as well as climatic and other environment factors [9] which have great influence on the essential oil production dynamics [10]. The content of essential oil from the Origanum vulgare of the Armenian highlands (OVA) differs from the essential oils of the same plants represented in the territories of other countries of the region (Turkey, Iran, and Georgia) [11]. It is known that essential oils have painkiller and anti-inflammatory properties [12]. The rather high percentage of β-caryophyllene, β-caryophyllene epoxide, and some other physiologically active substances suggest that OVA essential oil may have a pronounced antinociceptive and anti-inflammatory effect. β-caryophyllene is a substance belonging to the class of cannabinoids and have expressed analgesic effects. Therefore, the antinociceptive effect of O. vulgare can be of particular interest.

The exact mechanism of the essential oil’s influence on the endocannabinoid system activity is still poorly understood [13]. There are two types of cannabinoid receptors. Type 1 (CB1) cannabinoid receptors are predominantly present in the central nervous system, making them a potential target for neuropsychological disorders and neurodegenerative diseases. Therefore, CB1 activation can cause psychosis and panic in some cases. On the other hand, inhibition of CB1 can lead to depressive or anxious behavior [14]. Cannabinoid receptors of the type 2 (CB2) are widely represented in the immune system and the periphery (in leukocytes and lymphocytes, mast cells, and microglia), which determines their participation in immune modulation [15]. CB2 is a promising target for treating inflammatory diseases, neuropathic pain, and immune modulation [16]. CB2 activation can affect different signaling pathways. Cannabinoids are able to modulate the function of immune cells and thus influence the secretion of cytokines. Inflammation plays a decisive role in the defense of the immune system against damaging factors such as pathogen attack and mechanical injury. Exogenous as well as endogenous cannabinoids can modulate pain and inflammation both through a direct influence on appropriate receptors as well as by inhibiting inflammatory neuropeptide release [17].

Pain relief is a major challenge in the current health care system. Pain is probably the most common symptomatic reason to seek medical consultation. Despite improved knowledge of the underlying mechanisms and better treatments, many people who have any type of pain receive inadequate care and non-effective drugs. There is a proven causal link between pain and inflammation. Inflammation, key players are macrophages, T-lymphocytes, cytokines, and chemokines. In the spinal cord and brain, microglia and astrocytes are involved in these processes too. Many physiologically active substances reduce inflammation, resulting in pain relief [18].

The aim of this research was to reveal the chemical composition of OVA essential oil, and to study its possible analgesic, cytotoxic, and anti-inflammatory properties.

**MATERIALS AND METHODS**

1. **Experimental animals**

Male outbred albino mice (4–5 weeks old, 20 ± 2 g) were used throughout these experiments. Mice were obtained from L. A. Orbeli Institute of Physiology National Academy of Sciences of the Republic of Armenia (NAS RA). The animals were maintained in a room with a controlled temperature (22°C ± 2°C) and a 12-hour light/dark cycle, and all mice had unlimited access to food and water. Twelve hours before each experiment, the animals received only water to avoid possible interference of the food with the absorption of the drug. The study was conducted according to the “Principles of Laboratory Animal Care” and was carried out in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU) and was approved by the Institutional Review Board of the Orbeli Institute of Physiology of NAS RA (protocol code N4, date of approval: 22.07.2021).

For the formalin test, eleven groups (six mice in each group) were investigated. During the implementation of the hot plate test, there were two groups of mice (twelve in each). Totally, ninety mice were used. No animal was euthanized during experiments.
2. Plant material

The wild *O. vulgare* plant served as the investigation material, and was harvested between May and August 2018 in different phenological periods (pre-blossoming, blossoming, and fruiting, Gegharkunik region, village Chkalovka, 1930 meter above sea level). The identification of the plant was carried out at the Department of Pharmacognosy, Yerevan State Medical University, Yerevan (Armenia), and the plant samples were deposited and are available at the Herbarium of the Institute of Botany, National Academy of Sciences of Armenia, Yerevan (Armenia): voucher specimen number is ERE 191395.

3. Raw materials preparation

The primary processing of the raw materials was carried out immediately after collection: discarding organic and mineral mixtures, washing, and drying [19].

4. Essential oil extraction

OVA essential oil was extracted from fresh plant material (aerial parts only) by hydro-distillation for 3 hours, using a Clevenger-type apparatus. The distilled essential oil had been dehydrated with anhydrous sodium sulfate and stored at 4°C in dark, airtight bottles until further analysis [7].

5. Determination of essential oil chemical composition

The essential oil composition was analyzed at the FDA Analytical laboratory (Tonus-Les LLC., Yerevan, Armenia). The gas chromatography (GC) analysis was carried out using a Bruker gas chromatograph (Bruker 450-GC; Bruker Corporation, Billerica, MA), fitted with 60 m × 0.25 mm × 0.25 μm OPTIMA-FFAP column (Macherey-Nagel, Düren, Germany). The oven temperature varied from 40 to 220°C with a scanning rate of 3°C/min, evaporator temperature was 220°C. Helium (purity 5.6) was used as a carrier gas at a flow rate of 1 mL/min. The GC was equipped with a Hewlett-Packard 5972 Series mass spectrometry (MS) detector. The MS operating parameters were an ionization voltage of 70 eV and an ion source temperature of 250°C.

The diluted samples of essential oils of 2 μL had been injected manually. To avoid overloading the GC column, the essential oils were diluted 1:50 (v/v) in methanol. The identification of peaks was carried out based on a library search using the NIST-2013 [20]. It was also determined retention time and retention index would be basic parameters for the descriptive components, which are very important for the chemometrics of essential oils [21]. The substances are presented in ascending order of retention indices (Table 1).

6. Chemicals, compounds, and drugs

As a standard analgesic sodium metamizole (Analgin®; Yerevan Chemical-Pharmaceutical Firm, Yerevan, Armenia) and as a standard anti-inflammatory agent diclofenac sodium (Diclofenac®, Hemofarm A.D., Vršac, Serbia) (positive controls) were used. The formalin (Sigma-Aldrich, St. Louis, MO) and other chemicals were analytical or sequencing grade.

7. The formalin test

The formalin test was used to evaluate the antinociceptive properties of the OVA essential oil after intraperitoneal injection in mice [22]. Counting the bites/licks of the hind paw was used for estimating nociceptive behavior. An intraplantar injection was performed on the hind paw of the mice, using 20 μL of 5% formalin. The nociceptive behavior (the biting/licking number) was recorded for 45 minutes. Sodium metamizole (Analgin®, 8.3 mg/kg) and sodium diclofenac (Diclofenac®, 10 mg/kg) were used as standard drugs. Dosage selection was based on protocols widely used in the literature [23]. The initial mixture of OVA essential oil for the experimental groups were made up of 50 μL dimethyl sulfoxide (DMSO), 100 μL Tween 80, and 50, 100, 150, 200, and 250 μL of OVA essential oil in 1 mL saline (respectively for each group). Then, the volume of the solution was brought to 5 mL using saline. Tested solutions were injected intraperitoneally (15 minutes and 30 minutes before formalin injection), with the concentrations of OVA essential oil being 2%, 3%, 4%, and 5% per mouse, respectively. The actual amount of essential oil administered to one mouse corresponded to 2.0, 3.0, 4.0, and 5.0 μL (essential oil density is equal to 0.87, the OVA essential oil mass was 1.74, 2.60, 3.48, and 4.35 mg/mouse) according to one or another group of experimental animals. The whole experiment lasted 60 or 75 minutes from the moment the essential oil was injected. The quantity of biting/licking of the hind paw was recorded for 45 minutes after intraplantar injection of formalin.

8. The hot plate test

This test was used to assess acute thermal pain [24]. The mice (20 ± 2 g) were placed into a Plexiglas cylinder, one by one, on a surface maintained at a stable 55°C. An intraperitoneal injection of 0.1 mL 4% OVA essential oil was made in the experimental group after 15 minutes. The time...
Antinociceptive properties of essential oil

The antinociceptive properties of essential oil were studied. The sensitivity of hind paw licking and/or shaking, or jumping, was recorded, and at this point the animal was immediately removed from the hot plate to avoid further damage and the next animal was placed on it.

9. The investigation of cytotoxicity and growth inhibition

The HeLa (human cervical cancer cells) and Vero cells (African green monkey’s kidney epithelial cells; American Type Culture Collection, Manassas, VA) were used. Both cell lines were cultivated in Dulbecco’s modified Eagle medium with 5% v/v fetal bovine serum (Gibco/Termo Fisher Scientific, Rochester, MN), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO₂ (Memmert ICO105). The metabolic effects of OVA essential oil were assessed by methylthiazolyl-tetrazolium (MTT) colorimetric assay [25]. In all experiments both HeLa and Vero cells were seeded at 1 × 10⁵ cells/mL (1 × 10⁴/well of 96-well plate) a day before the MTT assay. Initial studies to test the influence of different concentrations of DMSO as a solvent of essential oil in the culture media of the HeLa cells were carried out. The 0.1% concentration of DMSO proved to be comfortable for the proliferation of both HeLa and Vero. In the first series of experiments, twenty-four hours after culture seeding, the media was replaced, and 100 μL of fresh media containing essential oil were added to each well at the following v/v ratios: 0.1, 0.25, 0.5, 1.0, and 2.0 μL/mL. The data of cytotoxicity was evaluated after twenty-four hours of essential oil addition (totally after 48 hours). In the second series of experiments, culture seeding in the media containing the essential oil was carried out at the same ratios: 0.1, 0.25, 0.5, 1.0, and 2.0 μL/mL and data of growth inhibition was evaluated after twenty-four hours.

| Compound                  | Pre-blossoming |              | Blossoming |              | Fruiting |              |
|---------------------------|----------------|-------------|------------|-------------|----------|-------------|
|                           | Concentration (%) | RT/RI      | Concentration (%) | RT/RI      | Concentration (%) | RT/RI      |
| (+)-Sabinene              | 2.42           | 13.43/971  | 3.1        | 3.36/897    | 3.29     | 13.36/897   |
| β-Pinene                  | 2.06           | 15/983     | -          | -           | -        | -           |
| trans-β-Ocimene           | 6.13           | 18.37/1,041| 3.81       | 19.35/978   | 2.61     | 18.4/1,042  |
| β-Pinene epoxide          | -              | -          | 2.37       | 26.69/979   | -        | -           |
| 2-Hexenal diethyl acetal, trans-β-Ocimene | 4.04               | 19.19/1,043| 3.27       | 21.22/993   | -        | -           |
| α-Cymene                  | 2.22           | 20.32/1,045| 5.22       | 20.43/1,045| 9.41     | 20.33/1,045|
| Eucalyptol                | -              | -          | 1.95       | 17.7/1,059  | 4.18     | 17.68/1,037|
| α-Terpineol               | -              | -          | -          | -           | 1.0      | 20.69/1,089 |
| β-Linalool                | -              | -          | 2.90       | 34.26/1,083 | 1.18     | 34.21/1,100 |
| trans-β-Terpineol         | 1.01           | 21.79/1,125| -          | -           | -        | -           |
| Neo-allo-ocimene          | 1.65           | 25.35/1,131| -          | -           | -        | -           |
| p-Menthyl-3-one           | 1.18           | 30.43/1,275| -          | -           | -        | -           |
| L4-terpineol              | 2.19           | 37.14/1,335| 2.34       | 37.19/1,137 | 2.18     | 37.12/1,137|
| cis-β-Terpinol            | -              | -          | 2.57       | 30.42/1,158 | -        | -           |
| Carvacrol/Isothymol       | -              | -          | 2.38       | 62.23/1,262 | -        | -           |
| α-Terpineol               | -              | -          | -          | -           | 1.74     | 41.53/1,181 |
| DihydrocedulenII          | -              | -          | 1.93       | 31.68/1,342 | -        | -           |
| Elicene                    | 1.27           | 30.68/1,375| -          | -           | 1.75     | 42.89/1,375 |
| β-Caryophyllene           | 7.0            | 36.7/1,417 | 8.18       | 36.9/1,494  | 6.41     | 36.62/1,410|
| γ-Elemene                 | 1.18           | 42.89/1,433| -          | -           | -        | -           |
| α-Humulene                | 1.68           | 39.97/1,456| 2.68       | 40.06/1,456 | 1.53     | 39.93/1,455|
| l-β-Bisabolene            | -              | -          | 3.24       | 42.47/1,501 | 2.18     | 42.31/1,561|
| Germacrene D              | 3.80           | 41.83/1,482| 3.80       | 41.92/1,915 | 9.22     | 41.8/1,915  |
| β-Caryophyllene epoxide   | 5.6            | 53.91/1,517| 13.36      | 54.18/1,517 | 11.2     | 53.91/1,517|
| cis-Z-α-Bisabolene epoxide| 1.97           | 81.02/1,536| -          | -           | -        | -           |
| Isoromadenrene epoxide    | 3.72           | 82.25/1,579| -          | -           | -        | -           |
| Ent-Spathulenol           | 6.75           | 59.2/1,590 | 3.77       | 59.26/1,536 | 2.51     | 59.15/1,590|
| tau-Cadinol               | 1.48           | 60.76/1,628| -          | -           | 1.53     | 60.75/1,628|
| α-Cadinol                 | 6.9            | 61.41/1,652| -          | -           | 6.93     | 63.09/1,653 |
| α-Humulene epoxide II     | -              | -          | 2.38       | 56.17/1,579 | -        | -           |
| Palmitic acid             | -              | -          | 2.52       | 84.11/1,968 | -        | -           |

RT: retention times, RI: retention indexes, OVA: Origanum vulgare of the Armenian highlands.
10. Statistical analysis

Data analysis was performed by Graph Pad Prism 8 software (Graph Pad Software Inc., San Diego, CA). The results of the formalin test observations at each minute were averaged both for the entire experimental period (45 minutes) and in 5 minutes intervals (for the analysis of dynamic changes in nociceptive behavior). One-way analysis of variance followed by the Bonferroni multiple comparison test was used for statistical analysis. Values of $P < 0.05$ were considered as significant. Results are given as mean ± standard error of mean (SEM).

RESULTS

1. Chemical composition of the OVA essential oil in different periods of vegetation: GC-MS analysis

Since most plants at different stages of vegetation differ in the quality and quantity of the chemical composition of essential oils, their determination in experimental mixtures is necessary. Depending on when the plant is collected, the essential oil in one case can demonstrate pronounced antibacterial properties, in another case analgesic properties, and the third case anti-cancer effects, etc.

2. Pre-blossoming

The results of the quantitative and qualitative analysis of essential oil obtained in the pre-blossoming period have shown that OVA essential oil contains 20 components with concentrations of more than 1.0% (total amount 74.99%). Another 125 components were found with concentrations lower than 1.0% (total amount 25.0%) (Fig. 1).

3. Blossoming

The results of the quantitative and qualitative analysis of essential oil obtained in the blossoming period have shown that OVA essential oil contains 20 components with concentrations of more than 1.0% (total amount 80.89%). Another 120 components were found with concentrations lower than 1.0% (total amount 19.0%) (Fig. 2).
4. Fruiting

The results of the quantitative and qualitative analysis of essential oil obtained in the fruiting period have shown that OVA essential oil contains 20 components with concentrations of more than 1.0% (total amount 84.38%). Another 101 components were found with concentrations lower than 1.0% (total amount 15.6%) (Fig. 3). The GC-MS analysis of OVA essential oil additionally showed that it contains acyclic sesquiterpenes, acyclic sesquiterpene alcohols, bicyclic sesquiterpenoids, tricyclic sesquiterpenoids, tricyclic sesquiterpene alcohols, monocyclic terpenoids, bicyclic monoterpenoids, acyclic monoterpene alcohols, monocyclic monoterpene ketones, monoterpene acids, and aromatic monoterpenoids. The dominant components of OVA essential oil during different vegetation periods are shown in Table 1.

The results of the GC-MS analysis of OVA essential oil showed that it contains acyclic sesquiterpenes, acyclic sesquiterpene alcohols, bicyclic sesquiterpenoids, tricyclic sesquiterpenoids, tricyclic sesquiterpene alcohols, monocyclic terpenoids, bicyclic monoterpenoids, acyclic monoterpene alcohols, monocyclic monoterpene ketones, monoterpene acids, and aromatic monoterpenoids. As can be seen, the content of essential oil components is quite variable during different stages of growth. For the major components (with contents more than 5% in the essential oil at different periods), the data is summarized in Fig. 4. The maximal amounts of β-caryophyllene and β-caryophyllene epoxide are present in the OVA essential oil in the blossoming period. Therefore, the essential oil of O. vulgare distilled in this period was chosen for further investigations.

5. Analgesic and anti-inflammatory properties of OVA essential oil: the formalin test

First, the formalin test for the intact mice was carried out. The analgesics were injected intraperitoneally (15 minutes or 30 minutes before the formalin intraplantar injection). The analgesic effects of Analgin and Diclofenac pretreatment 15 minutes before the formalin intraplantar injection are shown in Fig. 5. It is known that only local anesthetics affect the acute pain phase [26]. Both Diclofenac and Analgin showed a significant pain relief effect in the second phase (16–25 minutes, P = 0.028). Then the analgesic effects of different doses of OVA essential oil were tested (Fig. 6). As can be seen from the data, the 4% OVA essential oil has the most effective analgesic effect, but there is a delay in the second phase, so it was decided to conduct a study of OVA essential oil antinociceptive action 30 minutes before the

**Fig. 2.** GC-MS chromatograph of OVA essential oil in the period of blossoming. 1: β-Thujene, 2: (+)-Sabinene, 3: β-Pinene, 4: α-Terpinolene, 5: D-Limonene, 6: Eucalyptol, 7: γ-Terpinene, 8: Ethyl amyl ketone, 9: α-Cymene, 10: 4-methyl-3-(1-methylethylidene)-1-cyclohexene, 11: 2-Hexenal diethyl acetal, trans, 12: 3-Octanol, 13: cis-β-Terpinol, 14: α-Copaene, 15: Dihydroedulan II, 16: (+)-β-Bourbonene, 17: β-Linalool, 18: β-Caryophyllene, 19: L-4-terpineol, 20: Alloaromadendrene, 21: Humulen, 22: Germacrene D, 23: L-β-Bisabolene, 24: Elixene, 25: α-trans-Famesene, 26: β-Caryophyllene epoxide, 27: α-Humulene epoxide II, 28: Ent-Spathulenol, 29: Isaromadendrene epoxide, 30: Carvacrol/Isothymol, 31: α-Cadinol, 32: Ledene oxide-(II), 33: Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-, 34: Dibutyl phthalate, 35: Palmitic acid. GC-MS: gas chromatography–mass spectrometry, OVA: *Origanum vulgare* of the Armenian highlands.
formalin intra plantar injection. The results are shown in Fig. 7. We also performed a study of the analgesic effect of 5% OVA essential oil to determine the optimal dose, but after intra peritoneal injection of 5% OVA essential oil, the animals’ condition had deteriorated markedly, which suggested that this dose probably had a toxic effect and was not suitable for provided study. Thus, according to our results, the 4% OVA essential oil (30 minutes before the formalin intraplantar injection) has the highest analgesic therapeutic potential, which may be compared with standard analgesics (Fig. 8). The significance of this data is given in Fig. 9.

6. Analgesic and anti-inflammatory properties of OVA essential oil: hot plate test

According to obtained data, there was no significant difference between the intact and experimental groups. So, the antinociceptive effect of OVA essential oil is not related to pain and heat sensitive TRPV1 receptors. The results are shown in Fig. 10.
7. The cytotoxic properties of OVA essential oil: MTT assay

The essential oil of *O. vulgare* from the Armenian highlands may be a candidate for being a pain-killer drug, so its action on the survival and proliferation of cells is of great interest. The growth-inhibitory and cytotoxic properties of OVA essential oil diluted in cultural media by the MTT assay were investigated. Data are shown in Fig. 11 and Fig. 12. According to our results, the 1.0–2.0 µL content of essential oil in each well (100 µL) showed a certain influence on both cancer and non-cancer cells. When cells were already attached, all doses showed about 30% of the cytotoxic effect of OVA essential oil both for cancer (HeLa) and non-cancer (Vero) cells. When cells were seeded in media with preliminarily added OVA essential oil, cancer...
cells (HeLa) showed significantly higher viability (about 60%, $P = 0.041$) than non-cancer (Vero) cells (about 35%), when growing in media with OVA essential oil (Fig. 13). So, OVA essential oil shows growth-inhibitory effect on both HeLa and Vero cells.

**DISCUSSION**

The results of the present investigation directly confirm that the essential oil is exposed to quantitative and qualitative changes depending on vegetation period of plant (Fig. 4). The amount of the essential oil of investigated aerial parts of the oregano of the Armenian highlands changes depending on differing hours of daylight, air temperature, amount of light received, moisture, and the intensity of solar radiation. The variability of scientific data is often defined by climatic features [27].

The results of our investigations showed that the wild *O.
*vulgare* of Armenia belongs to the fourth chemotype (with the prevailing content of sesquiterpenoids and the very low quantity of phenolic compounds). Carvacrol, which has been discovered only in the blossoming period, was quantitatively equal to 2.38% and thymol was absent. The latter has been discovered in the fruiting period, but only in small amounts (0.01%), and in this case carvacrol was absent. It has been confirmed once more that the qualitative and quantitative composition of the essential oil of *O. vulgare* depends most on the geographical location of the plant’s growing area. The yield and the quality of the essential oil of *O. vulgare* is defined by ontogenetic factors, external environmental conditions, individual development periods, the geographical position of the growing location, as is also described in the data in the literature.

This certain correlation of the main components of the essential oil of Armenian *O. vulgare* with the prevailing content of sesquiterpenoids, such as β-caryophyllene and β-caryophyllene epoxide, gives accented possibilities for investigating it as an analgesic and anti-inflammatory agent in mice.

It was shown that essential oil expresses significant antinociceptive effects for all concentrations of OVA essential oil (2%, 3%, and 4%). As was mentioned above, a 5% solution of OVA essential oil had manifested slight signs of behavioral discomfort in tested mice and we decided do not to exceed the concentration of 4%, despite the fact that it is known that, for example, β-caryophyllene is not toxic even in much higher doses [28]. In our case, the injected effective dose of whole OVA essential oil was equal to 3.5 mg/mouse. This means approximately 286 μg/mouse in terms of β-caryophyllene (8.18%) and 467.6 μg/mouse in terms of β-caryophyllene oxide (13.36%). Based on scientific data it is known that β-caryophyllene’s effect on pain-like behavior and its anti-inflammatory action is mediated by interaction with CB2 receptors and then β-endorphin release, which lead to activation of the opioid receptors. In contrast, β-caryophyllene oxide expresses its antinociceptive and anti-inflammatory effect through interaction with central pain receptors. In addition, it is known, that both β-caryophyllene and β-caryophyllene oxide decrease the release of inflammatory mediators of pain [15].

The formalin test (consists of Phase 1 [0–5 minutes] and Phase 2 [15–45 minutes]) produces painful behavior in animals. These phases are separated by a quiet phase called the interphase, in which nociceptive reactions decrease or disappear completely [29]. The formalin test allows investigation of nociceptive behavior in the time period (second phase of formalin action) when inflammatory processes

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**Fig. 12.** The cytotoxic effect of OVA essential oil on HeLa and Vero cells viability (media without OVA essential oil addition). OVA: *Origanum vulgare* of the Armenian highlands. The data represent mean ± standard error of mean.

**Fig. 13.** Significance of cytotoxic effect of OVA essential oil. (A) The essential oil added after cell attachment. (B) The essential oil added before cell attachment (*P* < 0.05). The data represent mean ± standard error of mean. OVA: *Origanum vulgare* of the Armenian highlands, ns: not significant. ***P* < 0.0005.
and inflammation-induced pain are developing. On the basis of these data, it was chosen as the preferable test for our investigations. The results obtained in the second phase of the formalin test suggested that the antinociceptive effect of OVA essential oil is related to the inhibition of the biosynthesis of pain mediators, such as prostaglandins and prostacyclins. In the hot plate test, it seems that OVA essential oil components did not interact with heat-sensitive TRPV1 receptors, and in this case we received a null result. Presumably, OVA essential oil exerts its analgesic effect by another route, probably the CB2 receptor-activating mechanism.

There are certain articles and reviews describing the anticancer properties of $\beta$-caryophyllene and $\beta$-caryophyllene oxide [15,30]. This study has shown the cytotoxic effects of OVA essential oil on a cancer cell line in vitro. The maximal tested dose was equal to 2.0 µL of OVA essential oil per well (100 µL), which was a 2% solution in cell media. When OVA essential oil solution was added to the well after 24 hours of cultivation (with already attached cells), all investigated doses show the cytotoxic effect for cancer and non-cancer cells. When cells were seeded in media with preliminarily added OVA essential oil in the same concentration (2%), cancer cells showed a significantly higher viability (about 60%, $P = 0.030$) than non-cancer cells (about 35%). By contrast, OVA essential oil exerts a highly expressed and significant antinociceptive effect in vivo. In conclusion, OVA essential oil exerts a highly expressed and significant antinociceptive effect in vivo. So, the wild oregano herb of the Armenian highlands, harvested in the blossoming period, may be considered as a valuable source for developing pain-relieving preparations.

**CONFLICT OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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**REFERENCES**

1. Alwafa RA, Mudalal S, Mauriello G. *Origanum syriacum* L. (Za’atar), from raw to go: a review. Plants (Basel) 2021; 10: 1001.
2. Sahakyan N, Petrosyan M, Koss-Mikolajczyk I, Bartoszek A, Sad TG, Nasim MJ, et al. The Caucasian flora: a still-to-be-discovered rich source of antioxidants. Free Radic Res 2019; 53(supl): 1153-62.
3. Kimera F, Sewilam H, Fouad WM, Suloma A. Efficient utilization of aquaculture effluents to maximize plant growth, yield, and essential oils composition of *Origanum majorana* cultivation. Ann Agric Sci 2021; 66: 1-7.
4. Abrahamyan A, Barsesvaks A, Crockett S, Melikyan A. Distribution of Oreganum vulgare L. and population dynamics during the last decade in Armenia. J Life Sci (Libertyville) 2014; 8: 690-8.
5. Moghrovyan A, Sahakyan N, Babayan A, Chicchayan O, Petrosyan M, Trchounian A. Essential oil and ethanol extract of oregano (*Origanum vulgare* L.) from Armenian flora as a natural source of terpenes, flavonoids and other phytoc hemicals with antiradical, antioxidant, metal chelating, tyrosinase inhibitory and antibacterial activity. Curr Pharm Des 2019; 25: 1809-16.
6. Castronovo LM, Calonico C, Ascrizzi R, Del Duca S, Delfino V, Chioccioli S, et al. The cultivable bacterial microbiota associated to the medicinal plant *Origanum vulgare* L.: from antibiotic resistance to growth-inhibitory properties. Front Microbiol 2020; 11: 862.
7. Council of Europe. European pharmacopoeia. 5th ed. Strasbourg, Council of Europe. 2005.
8. Werker E, Putievsky E, Ravid U. The essential oils and glandular hairs in different chemotypes of *Origanum vulgare* L. Ann Bot 1985; 55: 793-801.
9. Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, He redia JB. Essential oils of oregano: biological activity beyond their antimicrobial properties. Molecules 2017; 22: 989.
10. Moghrovyan A. Qualitative analysis of Oregano ordinary herb (Herba Origani vulgaris) essential oil in various periods of vegetation. Paper presented at: Annual reporting science conference; 2014 Sep 15; Yerevan, Armenia. Yerevan: YSMU, 2014. p. 67-71.
11. Dadalioglu I, Evrendilek GA. Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.), and fennel (*Foenicu-
Antinociceptive properties of essential oil

lum vulgare) on common foodborne pathogens. J Agric Food Chem 2004; 52: 8255-60.
12. Sarmento-Neto JF, do Nascimento LG, Felipe CF, de Sousa DP. Analgesic potential of essential oils. Molecules 2015; 21: E20.
13. Johnson SA, Rodriguez D, Allred K. A systematic review of essential oils and the endocannabinoid system: a connection worthy of further exploration. Evid Based Complement Alternat Med 2020; 2020: 8035301.
14. Rubino T, Zamberletti E, Parolaro D. Endocannabinoids and mental disorders. Handb Exp Pharmacol 2015; 231: 261-83.
15. Fidyt K, Fiedorowicz A, Strzdała L, Szummy A. β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. Cancer Med 2016; 5: 3007-17.
16. Turcotte C, Blanchet MR, Laviolette M, Flamand N. The CB2 receptor and its role as a regulator of inflammation. Cell Mol Life Sci 2016; 73: 4449-70.
17. McKenna M, McDougall JJ. Cannabinoid control of neurogenic inflammation. Br J Pharmacol 2020; 177: 4386-99.
18. DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. J Neurochem 2016; 139(Suppl 2): 136-53.
19. World Health Organization. Quality control methods for herbal materials. Updated ed. Geneva, World Health Organization. 2011, p 173.
20. National Institute of Standards and Technology. NIST Standard Reference Database 1A [computer program]. Version 2.4 Gaithersburg (MD).
21. Kováts E. Characterization of organic compounds by gas chromatography. Part 1. Retention indices of aliphatic halides, alcohols, aldehydes and ketones. Helv Chim Acta 1958; 41: 1915-32. German.
22. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977; 4: 161-74.
23. Santos LH, Feres CA, Melo FH, Coelho MM, Nothenberg MS, Oga S, et al. Anti-inflammatory, antinociceptive and ulcerogenic activity of a zinc-diclofenac complex in rats. Braz J Med Biol Res 2004; 37: 1205-13.
24. Espejo EF, Mir D. Structure of the rat’s behaviour in the hot plate test. Behav Brain Res 1993; 56: 171-6.
25. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. Cold Spring Harb Protoc 2018. doi: 10.1101/pdb.prot095505.
26. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992; 51: 5-17.
27. Granados-Chinchilla F, Villegas E, Molina A, Arias C. Composition, chemical fingerprinting and antimicrobial assessment of Costa Rican Cultivated guavas (Psidium friedrichsthalianum (O. Berg) Nied. and Psidium guajava L.) essential oils from leaves and fruits. Nat Prod Chem Res 2016; 4: 236.
28. Oliveira GLDS, Machado KC, Machado KC, da Silva APDSCL, Feitosa CM, de Castro Almeida FR. Non-clinical toxicity of β-caryophyllene, a dietary cannabinoid: absence of adverse effects in female Swiss mice. Regul Toxicol Pharmacol 2018; 92: 338-46.
29. Henry JL, Yashpal K, Pitcher GM, Coderre TJ. Physiological evidence that the ‘interphase’ in the formalin test is due to active inhibition. Pain 1999; 82: 57-63.
30. Dahham SS, Tabana YM, Iqbal MA, Ahamed MB, Ezzat MO, Majid AS, et al. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. Molecules 2015; 20: 11808-29.