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ANTIMICROBIAL AND ANTIOXIDANT ACIVITIES OF *OCIMUM BASILICUM VAR. PURPUREUM* ETHANOL EXTRACT

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Many species of *Ocimum* genus have a long history in folk medicine and food industry. The aim of this study was to evaluate the antimicrobial and antioxidant activity of ethanol extract of leaves of *Ocimum basilicum var. purpureum* cultivated in Armenian (Ararat province, village Dvin, 1000–1200 *m* a.s.l.). Antimicrobial activity of *O. basilicum* leaves' extract was determined by agar disk-diffusion method. Different Gram-positive (*Bacillus subtilis* WT-A, *Staphylococcus aureus* MDC 5233, *Enterococcus hirae* ATCC 9790) and Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* MDC1754, ampicillin resistant *E. coli* dhpα-pUC18 and kanamycin resistant *E. coli* pARG25) bacteria and yeasts (*Saccharomyces cerevisiae* ATCC 9804 and *S. cerevisiae* ATCC 13007) were used as test-microorganisms. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was applied to measure the radical scavenging capacity of *O. basilicum* ethanol extract. The total flavonoid content in *O. basilicum* extract was determined employing $AlCl_3$ colorimetric assay, and the value was 46.9±0.884 μg QE mg^{-1}. Total phenolic content of studied extract was investigated by Folin–Ciocalteu assay. The content for *O. basilicum* extract was 317.75±4.105 μg of GAE mg^{-1}. Antiradical activity of *O. basilicum* extract expressed as IC_{50} value of 19.37±0.38 $\mu g \cdot mL^{-1}$. Thus, *O. bacilicum* can be considered as potential source of biologically active substances.

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Keywords: *Ocimum basilicum,* antimicrobial activity, ampicillin and kanamycin resistant *Escherichia coli*, antiradical activity, flavonoid, phenol.

Introduction. In recent years, the herbal extracts have attracted a great deal of scientific interest due to their potential as source of natural biologically active compounds [1, 2].

Medicinal and aromatic plants constitute a large part of the natural flora and are considered an important resource in various industries (pharmaceutical, food, perfumery, cosmetics) [3]. Currently, more than 80% of the world population uses plant-based drugs in the treatment process of various kinds of health problems [4, 5]. Synthetic antioxidants and antimicrobial agents could exert numerous adverse effects, therefore the interest of food producers and consumers for natural ingredients is growing [6].

Common basil (*Ocimum basilicum* var. purpureum) a member of the Lamiaceae family, is an annual plant. It is called the "king of herbs", which contains plenty of phytochemicals with significant nutritional as well as antioxidant

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capabilities and health benefits [2]. The *Ocimum* genus comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family. Plants belonging to this family can be found in almost all continents [1, 2, 7–10].

Through the centuries basil was cultivated for culinary and medicinal purposes in many countries, which created a great diversity of species within the *Ocimum* genus. It is known, that different cultivars of basil have the genetic ability to generate and keep different chemical compounds. This ability leads to a big variety of chemotypes within the same basil species [8, 11]. Many studies of basil have been demonstrated its significant anti-inflammatory, antioxidant, antistress and anti-microbial properties. Traditionally, basil has been extensively utilized in food and perfumery as a flavoring agent, as well as in medical industry [1, 6, 7, 9, 12–22]. *O. basilicum* has a long history as culinary herb. It is used as an ingredient in various dishes and food preparation, which imparts a unique flavor to many foods, which can be attributed to its foliage [2, 9, 10, 23].

O. basilicum is commonly used as an antibacterial and antifungal agent [24–26]. In intraditional medicine *O. basilicum* is used to treat various infections, inflammatory, skin and liver disorders, stomach ache, flatulence, constipation, diarrhea colds, coughs, fever and malaria [6, 9, 10, 24].

Traditionally, the basil leaves are used in folk medicine as a remedy for a large number of diseases, including cancer, convulsion, epilepsy, gout, nausea, sore throat, toothaches, headaches, coughs, warts, worms and kidney malfunctionsand bronchitis. It is also a source of essential oil with biologically-active constituents possessing antioxidant and antimicrobial properties [6, 9, 10, 24, 27, 28]. Ocimum is also used in aromatherapy as well as in the treatment of cardiovascular diseases, diabetes and Alzheimer's disease [6]. Leaves and flowering parts of *O. basilicum* are used in folk medicine as antispasmodic, aromatic, carminative, digestive, stomachic, antispasmodic and tonic agents [1, 2, 29]. Basil is an excellent source of β-carotene, magnesium, iron and calcium [6].

O. basilicum extracts have been shown to contain polyphenolic compounds, vitamins and essential oils that possess insecticidal, nematicidal, antifungal, antimicrobial, antioxidant and anti-inflammatory properties [9, 10, 21, 27, 30–34].

Oxidative stress cause by reactive oxygen species (ROS), which are known to contribute pathogenesis of various chronic diseases, including heart failure, carcinomas, coronary heart disease atherosclerosis, ventricular remodeling, and many other health problems [2, 29, 35, 36].

Antioxidants have been widely used as food additives to avoid the degradation of the foods and also protect the living cells from oxidative damage that occur due to formation of free radicals and reactive oxygen species during metabolic activity. This oxidative damage of cellular constituents lead to cell injury causing to cell death [2].

Antioxidants have an important role in preventing a variety of lifestyle-related diseases and aging because these are closely related to the active oxygen and lipid peroxidation. Current research of free radicals confirmed that food rich in antioxidants play an essential role in reducing the risk of incidence of cardiovascular diseases as well as other chronic diseases and certain types of cancer. A large number of plant species have already been tested for potential antioxidant activity [10, 37].

Generally, the aromatic plants and spices of the Lamiaceae family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties. Natural antioxidants are being extensively studied for their ability to protect organisms and cells from damage caused by oxidative stress. There is an increasing demand to evaluate the antioxidant properties of the herbal extracts and in the last years, the attention has been focused on the antioxidant products from natural sources [9, 21, 30–33].

In view of its therapeutic potential and its importance as a culinary base ingredient, basil deserves further scientific attention.

The aim of this study was to investigate the phenolic and flavonoids composition as well as antioxidant capacity and antimicrobial activity of *O. basilicum* extract.

Materials and Methods.

Chemicals and Reagents. Folin–Ciocalteu (FC) reagent, ethanol, gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), kanamycin, ampicillin, fluconazole, Mueller–Hinton agar and catechin were purchased from "Sigma-Aldrich GmbH" (Taufkirchen, Germany).

Plant Material Collection, Identification and Extraction. The plant material (*O. basilicum var. purpureum*) was collected from Ararat province (Armenia, 900–1200 *m* a.s.l.) (July 2019). The collected leaves were washed, dried in the shadow at room temperature and subsequently crushed to obtain the powder, which was stored in a dry and dark place until use. Plant material was extracted using ethanol. The obtained dried extracts were stored at 4°C until further use [38].

Determination of Radical Scavenging Activity. Free radical scavenging potential was determined by DPPH assay. Catechin was applied as standard. Sample solution contained 125 μL (1 mM) DPPH, 375 μL ethanol and 500 μL of test-solution (extract and catechin with different concentrations (1000 $\mu g \cdot mL^{-1}$, 500 $\mu g \cdot mL^{-1}$, 100 $\mu g \cdot mL^{-1}$, 50 $\mu g \cdot mL^{-1}$ and 10 $\mu g \cdot mL^{-1}$, respectively). Test-solution was replaced by ethanol in the control sample. The absorbance was measured at the wavelength of 517 *nm* using spectrophotometer Genesys 10S UV-Vis (Thermo Scientific USA).

The radical scavenging activity was calculated using the following formula:

$$\text{Radical scavenging activity, \%} = (A_c - A_s) / A_c \times 100,$$

where A_c is absorbance of control (DPPH without the addition of test solution), A_s is sample absorbance. IC_{50} calculated denote the concentration of investigated samples required to decrease the DPPH absorbance at 517 *nm* by 50% [38–40].

Determination of Total Phenolic Content. The concentration of phenolics in plant extracts was determined using Folin-Ciocalteu assay. The reaction mixture consists of 0.5 *mL* of extract (1 $mg \cdot mL^{-1}$) and 0.1 *mL* of Folin-Ciocalteu reagent. After 5 *min*, 1 *mL* of 7 % sodium carbonate (Na_2CO_3) was added. The volume was made up to 2.5 *mL* by adding distilled water. A set of standard solutions of gallic acid (5–100 $\mu g \cdot mL^{-1}$) were prepared in the same manner as described earlier. The mixtures were incubated for 90 *min* at room temperature and the absorbance for test and standard solutions was determined against the reagent blank at 765 *nm* with a Genesys 10S UV-Vis (Thermo Scientific USA) spectrophotometer. Total phenolic content was expressed as μg of GAE mg^{-1} of extract [38, 41, 42].

Determination of Total Flavonoid Content. The total flavonoid content in plant extracts was determined employing $AlCl_3$ colorimetric assay. The extract was dissolved in 80% ethanol to obtain a final concentration of 1 $mg \cdot mL^{-1}$. 0.5 *mL* of this extract solution was mixed with 0.1 *mL* of $AlCl_3$ (10%), 0.1 *mL* of sodium acetate (1 *M*) and 2.8 *mL* of distilled water. The sample was incubated for 15 *min* and the absorbance of the samples was measured at 415 *nm* against a blank consisting of distilled water utilizing a UV–Vis spectrophotometer (Genesys 10S, Thermo Scientific, USA). Total flavonoid content was calculated employing a calibration curve of quercetin (*Q*), as a reference flavonoid (0–1000 $\mu g \cdot mL^{-1}$) and results were expressed in terms of *Q* equivalents (*QE*) per *g* extract dry weight [36, 38, 43].

Investigation of Antimicrobial Activity. The antimicrobial activity of *O. basilicum* leaf ethanol extracts was determined by agar the disk-diffusion method. Mueller-Hinton agar and LB broth (pH 7.5) medium were used. Different Gram-positive (*Bacillus subtilis* WT-A, *Staphylococcus aureus* MDC 5233, *Enterococcus hirae* ATCC 9790) and Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* MDC 1754, ampicillin resistant *E. coli* dhpα-pUC18 and kanamycin resistant *E. coli* pARG25) bacteria and yeasts (*Saccharomyces cerevisiae* ATCC 9804 and *S. cerevisiae* ATCC 13007) were used as test-microorganisms. The following concentrations of plant ethanol extracts were used: 1000; 500; 250; 125 $\mu g \cdot mL^{-1}$. Microorganisms were grown on Mueller-Hinton agar for 24 *h* at 37°C temperature. Ampicillin (25 $\mu g \cdot mL^{-1}$), kanamycin (25 $\mu g \cdot mL^{-1}$) and fluconazole (25 $\mu g \cdot mL^{-1}$) were used as a positive control. Data were expressed in minimal inhibitory concentrations (MIC) values [38].

Results and Discussion. The antimicrobial activities of *O. basilicum* extract against the microorganisms examined in the present study, and their potency, were qualitatively and quantitatively assessed by the presence or absence of growth inhibition zones, and MIC values. The results are given in Fig. 1. In case of ampicillin and kanamycin resistant *E. coli* strains both investigated extracts did not shown any activity. They were not any activity also against *St. aureus* and *E. hirae* strains. The MIC value of *O. basilicum* was 250 $\mu g \cdot mL^{-1}$ against *B. subtilis*, 125 $\mu g \cdot mL^{-1}$ against *S. typhimurium* and 500 $\mu g \cdot mL^{-1}$ against *E. coli*. The investigated extracts did not shown any suppressing activity against tested yeasts.

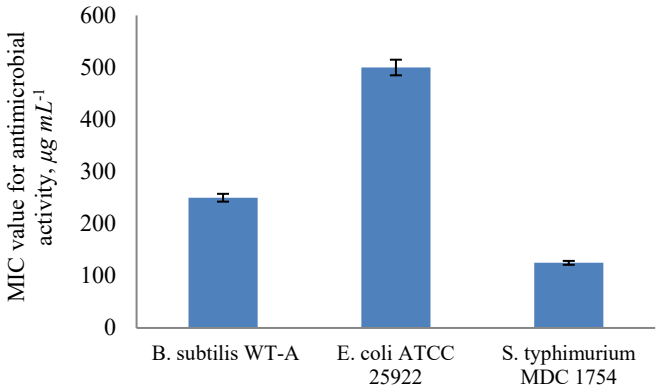

Fig. 1. The antimicrobial activity of *O. basilicum var. purpureum* ethanol extract.

There is a strong need for effective antioxidants from natural sources as alternatives to the synthetic antioxidants.

The DPPH radical is one of the most commonly used substrates for fast evaluation of antioxidant activity because of its stability in radical form and the simplicity of the assay. In the DPPH assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was investigated [44].

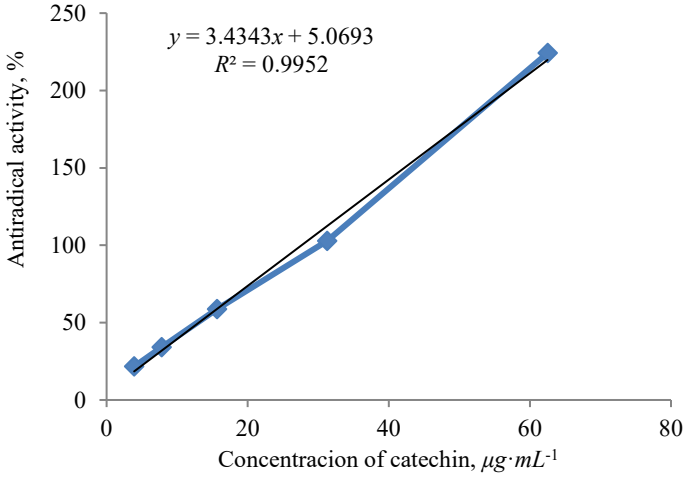

Fig. 2. Antiradical activity (%) of catechin.

The antiradical activity of *O. basilicum* extract was expressed as IC_{50} value. IC_{50} calculated denote the concentration of investigated sample required to decrease the DPPH absorbance at 517 *nm* by 50%. So, according to obtained results the IC_{50} value for the positive control (catechin) was determined to be 13.08±0.035 $\mu g \cdot mL^{-1}$ ($y = 3.4343x + 5.0693$, $R^2 = 0.99$) (Fig. 2).

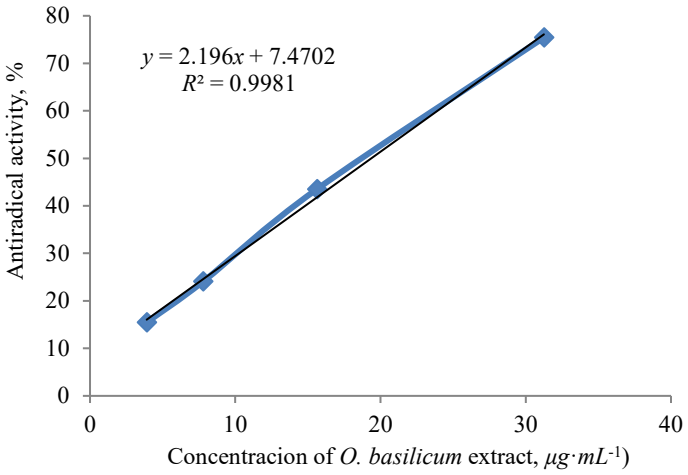

Fig. 3. Antiradical activity (%) of *O. basilicum* ethanol extract.

In case of *O. basilicum* this parameter had the following value: 19.37 ± 0.38 $\mu g \cdot mL^{-1}$ ($y = 2.196x + 7.4702$, $R^2 = 0.99$) (Figs. 3, 4).

The antioxidant activity value obtained showed that the extract of *O. basilicum* has almost the same antioxidant potential as catechin. These data indirectly support the known facts that antioxidant potential is important in the manifestation of antimicrobial activity of plant metabolites [38].

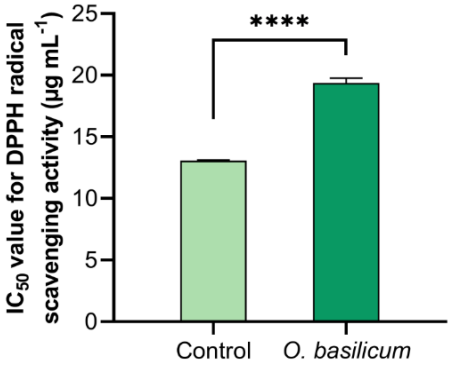

Fig. 4. The radical scavenging activity of *O. basilicum* ethanol extract ($^{****}p < 0.0001$).

According to some literature data, the IC_{50} value of the ethanol extract of *O. basilicum* from Vietnam was lower than the extracts of *O. basilicum* from Romania, Algeria, different areas of Egypt (91.31 ± 4.28 $\mu g \cdot mL^{-1}$, 124.95 ± 4.46 $\mu g \cdot mL^{-1}$, 0.679 ± 0.0383 $mg \cdot mL^{-1}$, 1.29–2.98 $mg \cdot mL^{-1}$, respectively) [9, 10, 45, 46]. So, the ethanol extract of *O. basilicum* from Armenia showed more powerful antioxidant activity. The radical scavenging activity value of the investigated extract was in strong correlation with the total phenolic content.

Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense. Polyphenols interact with pathogens, herbivores, and other plants; they protect from ultraviolet radiation and oxidants, repel or poison predators and attract beneficial insects or microbes [34].

Phenolic compounds are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action. Many studies of the antioxidant activity of plant extracts have confirmed a correlation between total phenolic content and antioxidant activity [10].

Phenolic compounds, often characterized by the phenolic rings and the structural elements which combine these rings to different classes, may include phenolic acids, flavonoids, stilbenes, tannins and lignans. Flavonoids are the most prominent group of antioxidant compounds among plant phenolic compounds [46].

The results of total phenolic content of *O. basilicum* extract expressed as μg of GAE mg^{-1} (see Table) (the standard curve equation: $y = 0.0047x - 0.0201$, $R^2 = 0.99$) (data not shown). The results of total flavonoids content of *O. basilicum* extract expressed as μg QE mg^{-1} (the standard curve equation: $y = 0.003x+0.0322$, $R^2=0.99$) (data not shown). The extract of *O. basilicum* contained the high amount

of polyphenolic and flavonoid compounds (317.75 ± 4.105 μg of GAE mg^{-1}, 46.9 ± 0.884 μg QE mg^{-1}, respectively).

Total phenolic (expressed in terms of gallic acid equivalent) and the total flavonoid (expressed in terms of quercetin equivalent) contents of *O. basilicum* extract

Extract	Total phenolic content, μg of GAE mg^{-1}	Total flavonoid content, μg QE mg^{-1}
O. basilicum	317.75 ± 4.105	46.9 ± 0.884

Concerning the content of polyphenols, the extract of *O. basilicum* species from Algeria was richer than the extracts of *O. basilicum* from Pacistan, Romania, Vietnam and different areas of Iran (226, 191.2, 175.57 ± 2.43, 29.6 ± 1.64 and 22.9–65.5 μg of GAE mg^{-1}, respectively) [9, 44–47]. Thus, the ethanol extract of *O. basilicum* from Armenia is richer in polyphenols. The rather high content of total phenols and flavonoids content in the investigated extract explains the high antiradical and antioxidant activity.

Comparing the flavonoid content, the ethanol extract of *O. basilicum* from Vietnam was richer than the ethanol extract from Pakistan (19.58 ± 0.93 and 13.3 μg QE mg^{-1}, respectively) [44, 45]. The result obtained for our sample exceeded these limits.

In conclusion, we can suggest the *O. basilicum* leaves as potential source for biologically active substances.

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OCIMUM BASILICUM VAR. PURPUREUM –Ի ԷԹԱՆՈԼԱՅԻՆ ԼՈՒԾԱՄՋՎԱԾՔԻ ՀԱԿԱՄԱՆՐԷԱՅԻՆ ԵՎ ՀԱԿԱՌԱԴԻԿԱԼԱՅԻՆ ԱԿՏԻՎՈՒԹՅՈՒՆԸ

Ocimum ցեղի շատ տեսակներ ժողովրդական բժշկության և սննդի արդյունաբերության մեջ օգտագործման երկար պատմություն ունեն: Այս ուսումնասիրության նպատակն էր գնահատել Հայաստանում աճող *Ocimum basilicum var. purpureum*-ի տերևների էթանոլային լուծամզվածքի հակամանրէային և հակաօքսիդանտային ակտիվությունը (Արարատի մարզ, գ. Դվին, 1000–1200 *մ* բ.ծ.մ.): *O. basilicum*-ի տերևներից ստացված լուծամզվածքների հակամանրէային ակտիվությունը որոշվել է սկավառակ-դիֆուզիոն մեթոդով: Որպես փորձնական միկրոօրգանիզմներ օգտագործվել են տարբեր գրամ-դրական (*Bacillus subtilis* WT-A, *Staphylococcus aureus* MDC 5233, *Enterococcus hirae* ATCC 9790), գրամ-բացասական (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* MDC1754, ամպիցիլին կայուն *E. coli* dhpα-pUC18 և կանամիցին կայուն *E. coli* pARG25) բակտերիաներ և խմորասնկեր (*Saccharomyces cerevisiae* ATCC 9804 և *S. cerevisiae* ATCC 13007): *O. basilicum*-ի էթանոլային լուծամզվածքի հակառադիկալային ակտիվությունը գնահատելու համար կիրառվել է 1,1-դիֆենիլ-2-պիկրիլհիդրազիլը՝ ԴՖՊՀ (DPPH): *O. basilicum*-ի լուծամզվածքում ֆլավոնոիդների ընդհանուր պարունակությունը որոշվել է $AlCl_3$ գունաչափական վերլուծության միջոցով, և արժեքը կազմել է 46,9 ± 0,884 *մկգ* ԿՀ *մգ*$^{-1}$: Ուսումնասիրված լուծամզվածքի ընդհանուր ֆենոլային միացությունների պարունակությունը որոշելու համար կիրառվել է Ֆոլին-Չեոկալտեուի մեթոդը: Ընդհանուր ֆենոլային միացությունների պարունակությունը *O. basilicum*-ի լուծամզվածքում կազմել է 317,75±4,105 *մկգ* ԳԹՀ *մգ*$^{-1}$: *O. basilicum*-ի լուծամզվածքի հակառադիկալային ակտիվությունն արտահայտվել է IC_{50}-ի արժեքով և կազմել 19.37±0.38 *մկգ մլ*$^{-1}$: Այսպիսով, *O. basilicum*-ը կարելի է համարել կենսաբանական ակտիվ նյութերի պոտենցիալ աղբյուր:

А. М. БАБАЯН

АНТИМИКРОБНАЯ И АНТИОКСИДАНТНАЯ АКТИВНОСТЬ ЭТАНОЛОВЫХ ЭКСТРАКТОВ *OCIMUM BASILICUM VAR. PURPUREUM*

Многие виды *Ocimum* имеют долгую историю использования в народной лекарственной и пищевой промышленности. Целью данного исследования было оценить антимикробную и антиоксидантную активность этанольного экстракта листьев *Ocimum basilicum var. purpureum*, представленного в армянской флоре (Араратская область, село Двин, 1000–1200 *м* н.у.м.). Антимикробную активность экстракта листьев *O. basilicum* определяли диск-диффузионным методом. Различные грамположительные (*Bacillus subtilis* WT-A, *Staphylococcus aureus* MDC 5233, *Enterococcus hirae* ATCC 9790) и грамотрицательные (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* MDC1754, ампициллин резистентная *E. coli* dhpα-pUC18 и канамицин резистентная *E. coli* pARG25) бактерии и дрожжи (*Saccharomyces cerevisiae* ATCC 9804 и *S. cerevisiae* ATCC 13007) использовались в качестве тест-микроорганизмов. 1,1-Дифенил-2-пикрилгидразил (DPPH) использовали для определения антирадикальной активности этанольного экстракта *O. basilicum*. Общее содержание флавоноидов в экстракте *O. basilicum* определяли с помощью колориметрического анализа $AlCl_3$, и его значение составило 46,9 ± 0,884 *мкг* КЭ $мг^{-1}$. Общее количество фенольных соединений в исследуемых экстрактах определяли методом Фолина–Чеокальтеу. Их содержание в экстракта *O. basilicum* составило 317,75±4,105 *мкг* ГАЕ $мг^{-1}$. Антирадикальная активность экстракта *O. basilicum* выражалась значением IC_{50} =19,37±0,38 $мкг \cdot мл^{-1}$. Таким образом, *O. bacilicum* можно рассматривать как потенциальный источник биологически активных веществ.