DEFINITION OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *SATUREJA HORTENSIS* AGAINST DOMINANT OPPORTUNISTIC PATHOGENIC BACTERIA ISOLATED FROM PATIENTS WITH ACUTE RESPIRATORY INFECTIONS

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Abstract

Antimicrobial Activity of *Satureja hortensis* Essential Oil Against Dominant Opportunistic Pathogenic Bacteria Isolated From Patients With Acute Respiratory Infections

The essential oil of *Satureja hortensis*, derived from wild populations in Azerbaijan, represents a significant source of antimicrobial compounds. This study evaluates its antibacterial activity against opportunistic pathogens causing acute respiratory infections (ARIs). Essential oil was extracted throug steam distillation, and its chemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS). Antimicrobial activity was assessed using the disk diffusion method against standard bacterial strains (*Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853) and clinical isolates from sputum samples of ARI patients.

The essential oil demonstrated notable antibacterial properties, with varying inhibitory effects depending on the bacterial species. The strongest inhibition was observed against clinical isolates of *S. aureus*, *E. coli*, and *K. pneumoniae*, while *P. aeruginosa* exhibited the lowest sensitivity. GC-MS analysis revealed that thymol (37.61%), γ-terpinene (21.47%), and p-cymene (12.31%) were the primary components, distinguishing the Azerbaijani variety of *Satureja hortensis* from others.

These findings show the potential of *Satureja hortensis* essential oil as a natural antimicrobial agent. Its high efficacy against opportunistic pathogens suggests its suitability for developing treatments for ARIs, offering an alternative to synthetic antibiotics in combating antibiotic resistance.

Keywords: acute respiratory infections, antimicrobial activity, *Satureja hortensis*, essential oil, disk diffusion method, thymol, GC-MS.

The essential oil of Satureja hortensis, derived from wild-growing populations of garden savory in Azerbaijan, is an important source of antimicrobial substances. The aim of the study was to assess the antibacterial activity of the essential oil of Satureja hortensis against opportunistic pathogenic bacteria – causative agents of acute respiratory infections. The essential oil was obtained by steam distillation. The component composition of the studied essential oil was analyzed by gas chromatography-mass spectrometry using an Agilent Technologies 6890 chromatograph. Antimicrobial activity was determined by the disk-diffusion method. The experimental strains used were standard cultures of *Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 35218, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC,* and 9 clinical bacterial strains: *Klebsiella pneumonia (3), Pseudomonas aeruginosa (3), Staphylococcus aureus (2), E. coli (1)* isolated from patients with acute respiratory infections (ARI). It was found that the essential oil demonstrated excellent antibacterial properties against both reference and clinical strains, with the intensity of the effect varying slightly depending on the bacterial species. The highest inhibitory effect of the essential oil of *Satureja hortensis* was observed for clinical strains *of S. aureus, E. coli, and K. pneumoniae*, while the least effect was observed for P. aeruginosa strains.

Keywords: acute respiratory infections, antimicrobial activity, essential oil of Satureja hortensis, component composition, disk-diffusion method.

In the modern world, the high frequency of acute respiratory infections (ARI) among both children and adults is due to increased susceptibility to potential respiratory pathogens, which determines the medical importance of this issue and its social significance [1,2]. Etiologically significant pathogens of ARI are bacteria and viruses that cause similar clinical symptoms, due to the tropism of the infectious agent to the epithelial cells of the respiratory tract [3]. Drug therapy for ARI includes antimicrobial agents, immunomodulatory drugs, and vaccines against the main pathogens of bronchopulmonary infections [4,5]. Most antibacterial drugs can cause various side effects with prolonged use, which limits their application in the treatment of respiratory infections. Furthermore, the increasing resistance of respiratory infection agents to synthetic chemotherapeutic drugs forces researchers to find new, more effective, and environmentally safe methods for the treatment and prevention of ARI [6,7,8].

In recent years, the rise of antibiotic resistance in microorganisms has led researchers to focus more on natural compounds with various biological effects (like antibacterial, antiseptic, and immune-boosting properties). One such group is plant-based essential oils, which show different levels of antimicrobial activity depending on their chemical makeup [9].The long history of our ancestors and recent progress in pharmacognosy create new opportunities to use essential oils in making antimicrobial drugs for preventing and treating various diseases, including infections [10,11].

Studies by many researchers, both local and international, have shown that essential oils from mint, tea tree, savory, clove, and eucalyptus have antimicrobial properties against many harmful microorganisms, including some viruses. This effect is due to compounds like carvacrol, thymol, terpinen-4-ol, and various flavonoids, making them an interesting topic for pharmacological research [12,13,14,15].

Azerbaijan has a strong tradition of folk medicine, where healers have used medicinal plants for centuries, thanks to the country's rich and diverse plant life. Special attention is given to plants that have been used in traditional medicine for many years [16]. One such plant in Azerbaijan is Satureja hortensis (garden savory), which belongs to the mint family (Lamiaceae). This plant is important in pharmacology, cosmetology, and medicine due to its high content of valuable components [17]. In medicine, it is used as an expectorant, antibacterial, diuretic, and antifungal treatment [18,19]. In Azerbaijan, Satureja hortensis, known as "ət otu," is also used as a seasoning in cooking, especially for meat.

So far, a lot of research has been done on the active compounds, like terpenoids, found in the essential oil of Satureja hortensis. These compounds play an important role in the plant's life and are also a valuable source of natural antioxidants and biologically active substances [20,21,22]. Satureja hortensis (L.) is a small shrub that grows up to 50 cm tall, with small, leathery, lance-shaped leaves that have tiny glands on the upper side. It grows naturally in the Shakhbuz district of the Nakhchivan Autonomous Republic, on dry, rocky slopes (39°21'44.27" E; 45°40'18.07" N, altitude 1592 m). The soil there is loamy, well-drained, and rich in potassium and phosphorus, with a pH of 6.5 to 7.0. The unique climate and geography of Azerbaijan help create a special chemical composition in the essential oil, which has high levels of thymol and carvacrol, known for their antibacterial effects [23,24]. This makes studying the antimicrobial properties of Satureja hortensis essential oil especially important.

The aim of this study was to test the antibacterial activity of Satureja hortensis essential oil against common pathogenic bacteria found in sputum samples from patients with respiratory infections. The objectives included extracting and analyzing the chemical composition of the essential oil, and isolating bacterial strains from clinical sputum samples to test the oil's antimicrobial effects on both reference and clinical bacterial strains.

Materials and Methods

For this study, the leaves of Satureja hortensis were used. The harvest was collected in July-August 2023 during the mass flowering of Satureja hortensis to minimize the loss of essential oil. The plant material was dried by air drying to avoid the destruction of biologically active substances and to remove excess moisture. The drying process was carried out in a shaded area at a temperature of 25-30°C for 5-7 days. The dried plant material was then ground in a laboratory mill to particles passing Through a sieve with 2 mm holes. The essential oil was extracted using the steam distillation method. The ground plant material was placed in a 1000 ml round-bottom flask, 300 ml of purified water was added, and the flask was sealed with a rubber stopper. The flask with the contents was heated on a flask heater and boiled for two hours. After distillation (6–8 hours) and cooling to room temperature, the volume of the essential oil was measured and collected in a sterile test tube. The samples of essential oil were dissolved in hexane at a 1:300 ratio.

The composition and individual compounds of the essential oil were analyzed using gas chromatography-mass spectrometry (GC-MS) on an Agilent Technologies 6890 chromatograph with a mass spectrometer. The analysis was done using a DB-5ms capillary column (0.25 mm diameter, 0.25 μm stationary phase thickness, 30 m length) under the following conditions: the temperature started at 60°C for 3 minutes, then increased to 250°C at a rate of 15°C per minute, and was held at 250°C for 3 minutes. Helium gas was used at a flow rate of 1 ml/min, with a 1:100 separation factor. The mass spectrometry analysis was done with a 3-minute retention time, 50 mA emission, and a scan range of 30–350 amu, scanning at 1600 amu/s. The ion source temperature was set to 230°C, the transfer temperature to 280°C, and the analysis lasted 21 minutes. Compounds were identified by comparing their mass spectra with reference libraries (NIST 2011 and Wiley 2010), and a compound was considered identified if the match was over 85%.

Clinical bacterial strains were collected from sputum samples of 302 patients with acute respiratory infections (ARI) at the "Referans" Clinical Laboratory in Baku. The samples were cultured on solid nutrient media like blood agar, Endo medium, egg-salt agar, and meat-peptone agar. Bacterial identification was done using the automated VITEK 2 system. Among the pathogens isolated, the most common were Klebsiella pneumonia (53 strains), Pseudomonas aeruginosa (38 strains), Staphylococcus aureus (20 strains), and Escherichia coli (9 strains).

The antibacterial activity of the essential oil was determined by the disk-diffusion method, as described in guidelines for determining the sensitivity of microorganisms to antimicrobial agents [25]. The method is based on the visual detection of inhibition zones. The test cultures used were reference strains *(Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 35218, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853)* from the bacterial culture collection of the Department of Medical Microbiology and Immunology, Azerbaijan Medical University. Nine clinical isolates were also used, corresponding to *Klebsiella pneumonia (3), Pseudomonas aeruginosa (3), Staphylococcus aureus (2), and E. coli (*1), isolated from sputum samples of patients with various respiratory tract pathologies. Suspensions of the bacterial cultures were prepared in sterile physiological saline, achieving a final concentration of 1.5 × 10^8 CFU/ml (0.5 on the McFarland standard). For microbial load control, a measured inoculum was plated onto Mueller-Hinton agar. A bacterial lawn was obtained by inoculating 1 ml of the inoculum onto a Petri dish, where the suspension was spread with a sterile swab over the Mueller-Hinton agar surface. After 20 minutes of absorption, a paper disk (6 mm in diameter) adsorbed with *Satureja hortensis* essential oil was placed on the surface. The results were evaluated after 18-24 hours of incubation at 37°C. The antibacterial activity was assessed by measuring the diameter of the growth inhibition zone: very high sensitivity (22 mm and above), moderate (15–21 mm), and weak (14 mm and below).

Results and Discussion

The content of essential oil in the plant material was 2.6%. The isolated essential oil was an oily liquid of light-yellow color with a pungent, spicy taste and a characteristic smell.

Gas chromatography-mass spectrometry analysis of the essential oil of Satureja hortensis obtained from air-dried plant material identified at least 32 compounds, with the largest mass fractions being thymol (37.61%), γ-terpinene (21.47

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