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ANTIMICROBIAL EFFECT OF SATUREJA HORTENSIS ESSENTIAL OIL GROWING IN AZERBAIJAN

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The purpose of the study was to assess the antimicrobial effect of essential oil obtained from the Satureja hortensis L plant grown in Azerbaijan. The qualitative composition and content (mg/kg) of Satureja hortensis L essential oil was determined by chromatography-mass spectrometry. Antimicrobial effect of Satureja hortensis L essential oil against microbial cultures is determined by disk-diffusion method. According to the results, the essential oil isolated from Satureja hortensis, both in native form and in dilutions, had a detrimental effect on the test cultures of S.aureus, E.coli, K.pneumoniae, H.pylori and P.aeruginosa, C.albicans, causing a complete or partial delay in their growth. The results obtained give grounds to assume that the synthesized essential oil of Satureja hortensis has a high antimicrobial effect. Therefore, this herbal preparation can be used in the future in the preparation of new drugs in the treatment of infectious diseases caused by these microorganisms.

Key words: Satureja hortensis L extract, chromato-mass spectrometry, serial dilution method, disk diffusion method, antimicrobial.

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ВИЗНАЧЕННЯ ПРОТИМІКРОБНОЇ ДІЇ ЕФІРНОЇ ОЛІЇ SATUREJA HORTENSIS, ВИРОЩЕНОЇ В АЗЕРБАЙДЖАНІ

У статті представлені результати вивчення антимікробної дії ефірної олії Satureja hortensis, яка росте в Азербайджані. Якісний склад і кількість (мг/кг) ефірної олії Satureja hortensis визначали хромато-мас-спектрометричним методом. Антимікробну дію фітопрепарату вивчали диско-дифузійним методом, а також методом серійних розведень. Згідно з результатами, ефірна олія, виділена з Satureja hortensis, як у нативному вигляді, так і в розведеннях, мала згубний ефект на використані в якості тест-культур S.aureus, E.coli, K.pneumoniae, H.pylori та P.aeruginosa, C.albicans, викликаючи повну чи часткову затримку їхнього росту. Отримані результати є основою припущення, що синтезована ефірна олія Satureja hortensis має високу антимікробну дію. Тому цей фітопрепарат у майбутньому можна використовувати для приготування нових лікарських засобів при лікуванні інфекційних захворювань, викликаних цими мікроорганізмами.

Ключові слова: ефірна олія Satureja hortensis, хромато-мас-спектрометрія, метод серійних розведень, диско-дифузійний метод, антимікробний ефект.

In the modern period, the rapid increase of antibiotic resistance, which is one of the main problems of the century, makes the treatment of many diseases difficult and complex. Therefore, this problem is discussed at conferences, and information on the use of antibiotics is constantly updated. The rise of resistant strains creates problems of microbial disruption, leading to pathological processes such as candidiasis, allergies, and dysbacteriosis, along with the weakening of the effectiveness of the treatment.

For this reason, there is an increasing amount of research on the development of approaches with alternative treatment systems to antibiotics. Since essential oils of plant origin have an antibacterial effect, research in this direction plays an important role in solving the problem. Antibacterial properties are observed with different indicators in plants growing in different places [6, 8].

Due to the increase in the problem of resistance, there is a need to increase the arsenal of antimicrobial drugs, and the search for new therapeutic sources began to be studied as one of the most important and urgent problems.

The search for new sources of plant materials and obtaining more effective medicines on their basis is one of the main tasks facing pharmacy and medical science. Currently, more than 21,000 species of medicinal plants are used for the treatment and prevention of various diseases in most countries of the world. Medicinal plants have some advantages over chemical medicinal preparations. Resistance to them is not formed, the effect is high, there are no additional effects, it is cheap, it gradually shows a therapeutic effect [2, 12, 13].

Preparations containing essential oils obtained from plants are used in medicine as cosmetology, aromatherapy, in the treatment and prevention of infectious diseases. It is also used as an antiseptic to prevent various infections or to clean wounds.

Azerbaijan, rich in its fauna and flora, is a source of raw materials for the production of many medicinal substances. Given the presence of various valuable plants growing here with useful properties, it is necessary to study their antimicrobial properties for use in medicine [3, 5, 11].

The expansion of the range of various infectious agents and the growth of diseases caused by antibiotic-resistant pathogens make it relevant to study alternative methods of treatment that affect pathogenic or opportunistic microflora with a wide spectrum of action. According to the literature, essential oils have a destructive effect on the cytoplasmic membrane of microorganisms, weaken its permeability, reduce the activity of aerobic respiration, and have a destructive effect on non-chromosomal genetic elements of bacteria – plasmids [5, 11, 15].

Satureja hortensis L seed essential oil and various amounts of extracts have been found to be effective (antimicrobial effect) against microorganisms such as many Gram-positive and Gram-negative, spore-forming bacteria and fungi that cause many diseases. In addition to this, many experiments have proven that it has properties that kill cancer cells and strengthen the immune system in the body. Resistance of microorganisms to antibiotics, toxic effects on the human body, dysbacteriosis, etc. due to its side effects, searches are underway for harmless alternative drugs for treatment. As can be seen from the literature, the antimicrobial activity of the same plant growing in different places, or essential oils derived from it, is not the same [3, 7, 13]. It is for this reason that the purpose of the research was to study the antimicrobial effect of the plant Satureja hortensis L, which grows in Azerbaijan, on some microorganisms, to study the effectiveness of medicinal plants for treatment and prevention.

The purpose of the study was to evaluate the effect of essential oil obtained from the Satureja hortensis L plant grown in Azerbaijan on the microbial cultures.

Materials and methods. In the laboratory of the Department of Pharmacognosy of the Azerbaijan Medical University (AMU), the essential oil of the newly synthesized plant Satureja hortensis L in Azerbaijan, its antimicrobial properties were studied in the Department of Medical Microbiology and Immunology of the AMU, the Reference Clinical Laboratory Center and the Analytical Expert Center.

Antimicrobial effect of Satureja hortensis L essential oil against microbial cultures (S.aureus, E.coli, K.pneumoniae, H.pylori и P.aeruginosa, C.albicans) is determined by disk-diffusion method. A suspension is prepared from a daily culture of a microorganism with a concentration of 1 ml of microbial cells per 1 ml, that is, from a daily culture of microbes on the agar surface with a bacteriological loop adjusted to the McFarland standard. Then the microbial suspension is poured separately into a Petri dish containing meat-peptone, Sabouraud and Campilo agar and cultivated in special conditions.

Additionally, antimicrobial effect of Satureja hortensis L essential oil against microbial cultures is determined by dilution method. In the serial dilution method, 6 sterile test tubes are taken, then 1 ml of the test substance is poured into the 1st and 2 nd, and 1 ml of sterile vaseline oil is added to all test tubes, starting from the 2 nd. Then 1 ml of the mixture was taken from the 2 nd and added to the 3 rd, from the 3 rd to the 4 th, from the 4 th to the 5 th, and finally, 1 ml of the mixture from the 5 th was discarded. So, in the experimental vials, the essential oil was stored undiluted (pure) for control in the 1st, diluted 2 times in the 2 nd, 4 times in the 3 rd, 8 times in the 4 th and 16 times in the 5 th. After washing, one drop of a microbial suspension containing 500 million microbial particles per 1 ml was added to each tube with a sterile pipette. Then, from the 10 min exposure, 20 min, 40 min and 60 min exposures, each test bottle was inoculated onto the surface of the culture media in a petri dish. The results were recorded after keeping the seedlings in a thermostat at 37°C for 24 hours (and the fungus was kept at 28°C for 2 days, then the results were recorded).

The qualitative composition and amount (mg/kg) of volatile compounds in the composition of the newly synthesized Satureja hortensis L in Azerbaijan were determined by chromato-mass spectrometric method on an Agilent Technologies 6890 chromatograph with a 5973 mass spectrometric detector [1]. To a sample of air-dried plant material (1000 mg) in a flask with a capacity of 200 ml, an internal standard (tridecan) is added (at the rate of 50 μg per sample) and, based on the concentration obtained, a further internal standard is calculated.

Chromatographic column HP-5ms capillary with an inner diameter of 0.25 mm and a length of 30 m. The velocity of the carrier gas (helium) was 1.0 ml/min. The temperature of the incoming sample heater is 250°C. Sample heater temperature is programmable from 60°C to 320°C at a rate of 7 deg/min.

In order to identify the components, the obtained spectra are based on the general patterns of decomposition of molecules of organic compounds (phytosterol compounds under study) due to the electronic effect, as well as by comparing the results obtained with the data of the NIST05 Mass Spectra Library and WILEY 2007, if the total number of spectra exceeds 470,000 AMDIS for identification, and the NIST programs were considered jointly.

Quantitative content (X, mg/kg) was determined by the internal standard method according to the following formula:

$$X = \frac{P_1 \times 50}{P_2 \times m}$$

Here P1 is the peak area of the test substance; 50 is the mass of the internal standard included in the sample, μg; P2 is the peak area of the standard; m–a sample of raw materials, g.

Identification and quantification of steroid compounds was carried out by gas chromatography/mass spectrometry (QX/MS). 0.05 g of crushed raw material was placed in a 20 ml vial and tridecane was added to it as an internal standard and 6 ml of methylene chloride as a solvent. Raw materials in vials were extracted for 1 day at room temperature with ultrasound for 3 hours. Then the extract was transferred into vials of 2 ml and concentrated with a stream of special pure nitrogen (flow rate 100 ml/min) to a residual volume of 10 μl.

The experiment was carried out on an Agilent Technologies 6890 chromatograph with a 5973 mass spectrometric detector with a HP-5ms capillary column (diameter 0.25 mm, length 30 m). Carrier gas velocity (helium) 1.0 ml/min. Sample heater temperature 350°C, oven temperature programmed from 150°C to 300°C at 7 degrees/min.

The components were identified using the AMDIS and NIST identification programs using the library and mass spectra together.

The bowls are carefully moved so that the suspension is evenly distributed in all directions. After that, the remaining suspension is sucked off through a pipette and thrown into a disinfectant solution. The dishes are kept at 37°C for 10 minutes so that the solution dries out a little. After that, the bowls are removed from the thermostat and each bowl is divided into 2 parts (for experiment and control). Sterile filter paper discs are soaked in essential oil and petroleum jelly, and a control disc and a disc impregnated with essential oil are placed in each bowl and pressed gently with tweezers. After that, Petri dishes with meat peptone and campylo agar are placed in a thermostat at 37 °C and an anaerostat for cultivating H. pylori under microaerophilic conditions.

For the cultivation of Candida, Petri dishes planted on a Sabouraud nutrient medium are incubated in a thermostat at a temperature of 28 °C. The essential oil diffuses into the agar and attacks the microorganisms. After 24–48 hours, the bowls are removed from the thermostat. The presence of a sterile zone (no growth) around the filter paper impregnated with essential oil leads to the effect of killing microorganisms and a weak effect if there is growth around the filter paper. Statistical processing of the results was carried out using the Statistica 6 computer program. Arithmetic means of each indicator were calculated using the Student's T–test, also the standard deviation, the mean error of the arithmetic mean, and the estimation of the distribution of values were determined.

Results of the study and their discussion. The data obtained were presented in the tables below. Table 1 demonstrated the results of antimicrobial effect of Satureja hortensis L essential oil on microbial cultures obtained by disk diffusion method.

Table 1

Antimicrobial effect of Satureja hortensis L essential oil against microbial cultures (determination by disk diffusion method)

Solutions	S.aureus (mm)	E.coli (mm)	P.aeruginosa (mm)	H.pylori (mm)	K.pneumoniae (mm)	B. anthracoides (mm)	C.albicans (mm)
Essential oil	32	31	31	30	30	30	30
Vaseline (control)	-	-	-	-	-	-	-

Satureja hortensis L oil formed a sterile zone 30-32 mm in diameter in bowls planted with S.aureus, E.coli, K.pneumoniae, H.pylori and P.aeruginosa, C.albicans. In the control (impregnated with Vaseline), no sterile zone was found around the filter paper. The results obtained by dilution method are shown in Table 2.

According to our results, the effect of the essential oil obtained from the Satureja hortensis L plant growing in Azerbaijan on the suspensions prepared from the 1-day culture of microorganisms by the disc-diffusion method was found to be noticeably strong.

So, at this time, filter paper impregnated with Satureja hortensis oil in pots planted with gram-positive bacteria – S.aureus, B. anthracoides, gram-negative bacteria – E.coli, K.pneumoniae, H.pylori and P.aeruginosa, and fungi – C.albicans. It showed a strong effect on them by forming a sterile zone with a diameter of 30-32 mm around it.

The absence of a sterile zone around the oil-impregnated filter paper of petroleum jelly taken for the control group indicated its lack of anti-microbial effect.

The obtained results indicate that the newly synthesized essential oil of the Satureja hortensis L plant growing in Azerbaijan can be considered an antimicrobial effective substance. The essential oil of Satureja hortensis L is suitable for use in the preparation of new drugs or an alternative system for the treatment of diseases caused by these microorganisms.

Table 2

Antimicrobial effect of Satureja hortensis L essential oil against microbial cultures (determination by dilution method)

Exposure time	Test cultures																							
	S.aureus						E.coli						P.aeruginosa						H.pylori					
	1	2	3	4	5	K	1	2	3	4	5	K	1	2	3	4	5	K	1	2	3	4	5	K
10	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+
20	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+
40	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+
60	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+

Exposure time	Test cultures																	
	K.pneumoniae						B. anthracoides						C.albicans					
	1	2	3	4	5	K	1	2	3	4	5	K	1	2	3	4	5	K
10	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+
20	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+
40	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+
60	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+

Notes: Symbols: "+" indicates complete completion; "-" indicates no growing; 1(1:1), 2(1:2), 3(1:4), 4(1:8),5(1:16) dilution in proportions.

The newly synthesized essential oil of Satureja hortensis L has a high antimicrobial activity. Not only in its native state, but also in a diluted state, it had a killing effect on all microorganisms taken for research within 10 minutes. The essential oil of Satureja hortensis L has a strong effect on all tested bacteria and fungi. Thus, as in Table 2 more percent of bacteria and fungi were killed in 10 minutes at 4, 8, 16 times dilution, and 4, 8 and 16 times dilution killed B.anthracoides and P.aeruginosa at 16 times dilution in 60 minutes. Complete extinction was noted for all controls at all exposures.

The cross-sectional nature of this study did not allow establishing a causal relationship between Satureja hortensis L and the pathogenicity of the mentioned pathogens. In addition, we did not assess the antimicrobial properties of Satureja hortensis L when this plant was harvested and obtained. In this study, the anti-bacterial and anti-fungal effect of the essential oil obtained from the Satureja hortensis L plant, which grows only in Azerbaijan, was studied, and the evaluation of the results may vary depending on the collection time and place.

At the same time, it can change depending on the season, so the effects of sunlight, humidity, etc. affecting the plant can change its characteristics and affect it, so it is difficult to eliminate all influencing factors.

Hagh LG, et al also used Satureja hortensis extract and essential oil prepared using double dilution method against oral bacteria. The authors revealed that the inhibition effects of all concentrations of essential oil were higher for S. sanguis. S. salivarius and S. sanguis. They noted strong antibacterial effect of S. hortensis essential oil on the oral bacteria growth, that is why it can be served as herbal mouth rinse, while to confirm this antibacterial effect, further clinical studies are necessary [10].

In some works the different aspects of summer savory (Satureja hortensis L) including biological activity, medicinal properties, nutritional value, food application, prospective health benefits, and its use as an additive in broiler feed were measured. Summer savory leaves are abundant in total phenolic compounds (rosmarinic acid and flavonoids) that have a powerful antioxidant impact. Summer savory extract shows considerable biological potential in antioxidant, cytotoxic, and antibacterial assays [14]. This is similar with our results about effectiveness of Satureja hortensis L oil against several microbial agents (S.aureus, B. anthracoides, E. coli, K.pneumoniae, H.pylori P.aeruginosa, and fungi – C.albicans).

One of the review reported that Summer savory also has Fe (III) reductive and free radical scavenging properties and contains minerals and vitamins. Summer savory has important biological properties, including protective effects against Alzheimer's disease, cancer, cardiovascular diseases, diabetes, and cholesterol. [7]. Several plants of Satureja genus have shown anti-tumor activity. Asadipour M, et al investigated the antileukemia effects of different fractions of Satureja hortensis (Summer savory) and found out positive results (growth inhibitory effect of S. hortensis fractions on K562 and Jurkat leukemia cells) They revealed that hexane and dichloromethane fractions of S. hortensis had the capacity to induce death and change the cell cycle distribution in leukemia cells; therefore these fractions might be good candidates for more studies in regard to their possible therapeutic usefulness in leukemia [4]. In our study we did not assess the role of Satureja hortensis L in diseases mentioned above, but it may be the subject of further experiments.

Variuos studies showed the additional effect of polyphenols and flavonoids, such as antiparasitic, pesticidal, analgesic, hepatoprotective impact. Some authors presented the progress made in the last decade

regarding the potential applications of summer savory, suggesting future research opportunities, as they appear from the properties of other Satureja species. We confirmed antimicrobial features of this plant oil. Other researchers noted several properties of summer savory which represent a scientific support for application in industry, for developing "clean label" food products [9].

Our colleguae from Turkey, Emre İ, et al, conducted the similar study to determine some biological compounds, radical scavenging activity and antimicrobial capacity in seeds of Satureja hortensis L. and Mentha spicata L. subsp. spicata. Their results suggested that methanol extracts of Satureja hortensis L. and Mentha spicata L. subsp. spicata have significant free radical scavenging activity. According to results obtained The present results revealed that Satureja hortensis L. and Mentha spicata L. subsp. spicata showed major activity against gram-positive and gram-negative microorganisms, fungi and yeast [8]. We also have got positive data with bacteria and fungi.

Conclusively, summer savory is widely considered beneficial for human health due to its versatile properties and medicinal use. Furthermore, toxicity related to this also should be take into account.

Conclusion

Thus, filter paper impregnated with Satureja hortensis oil in pots planted with gram-positive bacteria – S.aureus, B. anthracoides, gram-negative bacteria – E.coli, K.pneumoniae, H.pylori and P.aeruginosa, and fungi – C.albicans showed a strong effect on them by forming a sterile zone with a diameter of 30-32 mm around it.

This study is limited to Satureja hortensis L, and our findings cannot be generalized to other members of this genus. Our data related to The anti-bacterial and anti-fungal effect of the essential oil obtained from the Satureja hortensis L plant, which grows only in Azerbaijan, and the evaluation of the results may vary depending on the collection time and place.

The obtained results indicate that the newly synthesized essential oil of the Satureja hortensis plant growing in Azerbaijan can be considered as an antimicrobial effective substance. The essential oil of Satureja hortensis L is suitable for use in the preparation of new drugs or an alternative system for the treatment of diseases caused by these microorganisms.

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