

## THE ANTIBACTERIAL ACTIVITY OF THE EXTRACTS FROM THE *STACHYS SIEBOLDII* MIQ

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### Abstract

Plants of the genus *Stachys* contain various secondary metabolites, including flavonoids, iridoids, fatty acids, phenolic acids, and essential oils, which are associated with anti-inflammatory, cytotoxic, antibacterial, and antioxidant activities. It is believed that the antibacterial effect of *Stachys sieboldii* is associated with the total content of polyphenols and flavonoids contained in the plant and which are extracted with methanol and ethanol.

Using the method of spectrophotometry, the quantitative content of tannins, total polyphenols, and flavonoids in the herb and tubers of the study plant was defined. The content of tannins in the herb of *Stachys sieboldii* was 1.67 %, in tubers – 1.35 %. The results of studies of the number of polyphenols in terms of pyrogallol showed that in the herb of *Stachys sieboldii*, their content was 7.93 %, and in the tubers – 11.08 %. The total quantitative content of the number of flavonoids in terms of rutin in the herb of the studied plant was 2.42 %, in the tubers – 0.39 %.

Antibacterial and antifungal action was studied by agar diffusion (“wells” method). Experiments showed that a dry extract of *Stachys sieboldii* herb has a more effective antimicrobial effect. All test microorganisms were sensitive to its action. The diameters of their growth retardation ranged from (19.75±2.12) mm (for *S. typhimurium*) to (24.55±1.20) mm (for *P. aeruginosa*). A homogenate from the herb of the studied plant showed the most pronounced antimicrobial effect against gram-positive cocci (19.35±1.08) mm and yeast fungi (22.35±1.15) mm. Gram-negative rods of *E. coli*, *S. typhimurium*, *P. aeruginosa*, as well as *S. aureus* and *C. albicans* were insensitive to a thick extract from the tubers of *Stachys sieboldii*. The results of the studies confirmed that the biologically active substances of *Stachys sieboldii* have antimicrobial and anticandidal effects.

**Keywords:** extract, herb, tubers, *Stachys sieboldii*, antibacterial activity, tannins, polyphenols, flavonoids

## Introduction

Extensive and often uncontrolled or insufficiently substantiated use of modern antibacterial drugs (primarily antibiotics) for the prevention and treatment of various infectious processes leads to a rapid increase in antibiotic resistance of opportunistic pathogens and the actual pathogens of infectious diseases. Therefore, it will always be relevant to find new antimicrobials, the source of which can be medicinal plants [1]. Throughout many years plants using not only as a source of meal but also in the fight against diseases [2-4]. Modern pharmacotherapy increasingly takes into account the centuries-old experience of folk medicine with the use of phytopreparations as monotherapy and in combination with synthetic drugs [5-9]. Medicinal plants contain biologically active substances that are chemically similar to a number of substances in the human body (enzymes, hormones, vitamins, etc.), so when introduced into the macroorganism, they are actively involved in biochemical processes, have a positive effect on the human body as a whole and some of them are able to show antibacterial action [1]. Phytotherapy is a justified method for the prevention and treatment because it has some advantages, such as relatively low toxicity, mild pharmacological effects, and the possibility to be used for long periods without significant side effects, and it often well combines with synthetic drugs [10-14]. Moreover, herbal drugs, as a rule, have a wide range of pharmacological properties, which are realized through different groups of phytochemicals [15-17].

Plants of the genus *Stachys* contain various secondary metabolites, including flavonoids, iridoids, fatty acids, phenolic acids and essential oils, which are associated with anti-inflammatory, cytotoxic, antibacterial and antioxidant activities [18]. There is knowledge about the study of essential oils of *Stachys sieboldii* in the sources of scientific literature. It was found that they contain caryophyllene, germacrene D, and cadinene. It is believed that  $\beta$ -caryophyllene and germacrene D are the primary components of the essential oil of *Stachys sieboldii* characterized by moderate antibacterial activity [19-21].

Mi Ra Yang et al. studied the antimicrobial activity of ethanol and methanol extracts from the herb, leaves, and tubers of *Stachys sieboldii* [18]. It was found that methanol extract from the leaves and root tubers and ethanol extract from the root tubers of *Stachys sieboldii* has an expressive antibacterial action on the culture of *Salmonella typhimurium*. In addition, methanol extract from the leaves of *Stachys sieboldii* showed a substantive antibacterial effect on the culture of *Bacillus cereus*. It is believed that the antibacterial effect of *Stachys sieboldii* is associated with the total content of polyphenols and flavonoids contained in the plant and which are extracted with methanol and ethanol [18, 22]. As active principals phenylethanoid glycosides [20, 23], triterpenoids, steroids [24, 25] and flavonoids [26] were identified in the genus *Stachys*.

To the best of our knowledge, reports on the chemical composition of the polyphenols and flavonoids content and antimicrobial profiles of *Stachys sieboldii* are scant in the literature. The present study was done to determine the inhibitory concentrations of *Stachys sieboldii* extracted against pathogenic bacteria.

## Methods

### Plant Materials

The herb and tubers of *Stachys sieboldii* was collected on research grounds of Educational and Scientific Centre "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv in 2017 [27]. A voucher specimen was deposited in the laboratory herbarium of the Department of Pharmacognosy and Medical Botany (TSMU, Ternopil, Ukraine) [28-31]. The herb and tubers were authenticated by professor Svitlana Marchyshyn (TNMU, Ternopil, Ukraine) [32, 33]. The study plant materials were dried using the conventional method and stored in paper bags in a dry, protected from direct sunlight place [34-37].

### Preparation of extract

About 500 g of dried *Stachys sieboldii* herb and tubers were powdered with the help of a suitable crusher [38]. It was taken in an extractor and extracted using 70 % ethanol for herb and 40 %

ethanol for the tubers of the *Stachys sieboldii* as a solvent [39]. The extract was concentrated under vacuum to half under volume and dried at a temperature of  $50 \pm 2^\circ \text{C}$ .

#### *Chemicals reagents*

Chloroform, sodium molybdate dehydrate, phosphotungstic acid hydrate, sodium carbonate, pyrogallol, methanol, sodium hydroxide, potassium hydroxide, aluminium chloride, were purchased from Ltd. Sfera Sim (Lviv, Ukraine), were of the highest purity available. Water used in the studies was produced by MilliQ Gradient water deionization system (USA) [40, 41].

#### *Microorganisms*

In research used a standardized diurnal suspension of testing strains American Type Culture Collection (ATCC) of the microorganisms, namely: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Staphylococcus typhimurium* ATCC 55, and *Candida albicans* ATCC 885-653. Cell concentration was 0.5 McFarland [42].

#### *Determination of tannins and polyphenols*

The quantitative content of biologically active substances was determined out by the spectrophotometric method according to the European Pharmacopeia and State Pharmacopoeia of Ukraine. The investigation of the content of polyphenols and tannins was carried out in a fivefold repetition.

#### *Determination of quantitative content of tannins*

0.50 g of powdered raw material was placed in a 250 ml round flask, 150 ml of water R was added to the flask; heated for 30 minutes in a water bath, cooled under running water R, and quantitatively transferred to a 250 ml graduated flask. The roundbottomed flask was rinsed with water, the flushing waters were transferred to a measuring flask and the solution volume was diluted with water R to 250 ml. The precipitate was allowed to settle and the liquid was filtered through filter paper with a diameter of 125 mm. The first 50 ml of the filtrate was discarded.

#### *The amount of polyphenols*

5 ml of the filtrate was diluted with water R to 25 ml. A mixture of 2 ml of the obtained solution 1 ml of phosphomolybdotungstic reagent R and 10 ml of water R was brought to a solution of 290 g/l sodium carbonate R to a volume of 25 ml. After 30 min, the optical density of the solution was measured at a wavelength of 760 nm, using water R as a compensatory solution.

#### *Polyphenols not adsorbed on the hide powder*

0.10 g of the pharmacopoeial reference standard of cutaneous powder was added to 10 ml of the filtrate and shaken vigorously for 60 min. The mixtures were filtered and dilute 5 ml of filtrate with water R to a volume of 25 ml. A mixture of 2 ml of the obtained solution, 1 ml of phosphomolybdotungstic reagent R, and 10 ml of water was diluted with a solution of 290 g/l sodium carbonate R to a volume of 25 ml. After 30 min, the optical density of the solution was measured at a wavelength of 760 nm, using water as a compensatory solution.

#### *Standard solution*

Immediately before trial, 50.0 mg of pyrogallol R was dissolved in water R and diluted the volume of the solution with the same solvent to 100 ml. 5 ml of the resulting solution were diluted to 100 ml with water R. A mixture of 2 ml of the obtained solution, 1 ml of phosphomolybdotungstic reagent R, and 10 ml of water R was diluted to a solution of 290 g/l sodium carbonate R to a volume of 25 ml. After 30 min, the optical density of the solution was measured at a wavelength of 760 nm, using water R as a compensatory solution [43-45].

The quantitative content of tannins and polyphenols was determined on a spectrophotometer Lambda 25 UV Perkin Elmer (USA).

#### *Total flavonoids content*

#### *Stock solution*

To 0.6 g (exact weight) of the powdered raw material was added 90 ml of methanol R, heat in a water bath under a reflux condenser until the extraction liquid is colourless, cooled. Transfer the methanolic solution to a 100 ml volumetric flask.

Wash out the extraction flask with little milliliters of methanol R. Union the methanolic solutions in a 100 ml volumetric flask and dilute methanol R to the mark. Dilute 10 ml the obtained solution to 100 ml with water R and stir [46].

#### Test solution

Place 10 ml of the stock solution in a 100 ml volumetric flask, added a 20 g/l solution of aluminium chloride R in methanol R to the mark.

#### Compensation solution

Place 10 ml of the stock solution in a 100 ml volumetric flask and added methanol R to the mark. The optical density of the test solution and the compensation solution is measured 15 min after preparation to the compensatory solutions for each one respectively. The quantitative content of flavonoids in the raw materials were determined on a spectrophotometer Lambda 25 UV (Perkin Elmer, USA) at a wavelength of 415 nm [47-49]. The results were expressed as g. Used a specific absorption of rutin equal to 370.

#### Antibacterial and antifungal test

The antimicrobial action of the obtained dried extracts of *Stachys sieboldii* herb and tubers were studied in vitro according to the State Pharmacopoeia of Ukraine [50].

*Antibacterial and antifungal action was studied by agar diffusion ("wells" method).*

Two layers of dense nutrient medium poured into Petri dishes were used in the study. In the lower layer, "hungry" media were used, which were not sown. Thin-walled cylinders made of stainless steel were installed on the lower layer. Standardization of "well" studies of the agar diffusion was secured by 6 mm "well" diameter and 10 mm medium thickness. The top layer was filled around the cylinders, namely a sterile agar medium, melted and cooled to 40 °C, in which the corresponding standard of the daily test culture of the microorganism (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus typhimurium* ATCC 55, *Staphylococcus aureus* ATCC 6538, *Candida*

*albicans* ATCC 885-653). After solidification of the upper layer, the cylinders were removed with sterile tweezers, and 0.06 ml of the substance was added to the formed "wells". The plates were dried for 30-40 minutes at room temperature and placed in a thermostat to incubate the seed at 37 °C for 24 hours. The diameter of the growth retardation zone of the test cultures was measured in millimeters [51]. The antibacterial activity was determined by measuring of inhibition zone. Results were evaluated according to the parameters suggested by Alves et al. (2000) [52].

#### Statistical analysis

All the assays were carried out five times. Obtained results were expressed as mean value  $\pm$  SEM [53-55]. Statistical processing and data analysis were performed using program package Statistica v 7.0 (Statsoft, USA) for Microsoft Office for Windows –MS Exel 2007 [56]. Statistical significance of differences between mean values was assessed by the Student's t-test [57-59]. The level of significance was set at \* $p < 0.05$  for all statistical analyses [60-64].

#### Results and Discussion

In recent years, the information about bioactive compounds, especially polyphenolic compounds, has widely increased. Numerous polyphenols, especially tannins and flavonols, extracted from different plants have shown antibacterial, antiviral and antifungal activity [65]. These compounds could intervene with the physiology of bacteria by various mechanisms of action. Normally, they interfere with membrane functions or suppress some virulence factors, including toxins, enzymes, signal receptors and the formation of bacterial biofilm. Some polyphenols also have a synergistic effect with antibiotics, i.e. catechins which modulate  $\beta$ -lactams resistance in multiresistant strains like *S. aureus* and ESBL producing *E. coli* [66, 67].

Tannins are polymeric phenolic substances found in almost every plant part [68]. The biological properties of tannins depend on the oxygenation pattern and the polymerization degree [69]. The antimicrobial character of tannins present in variety plant foods have been well documented [70]. In



general, tannins seem to affect bacterial growth and virulence in several mechanisms, like inhibition of extracellular enzymes and oxidative phosphorylation [69].

*Quantitative content of tannins and polyphenols in the raw material of Stachys sieboldii was determined by spectrophotometric method [44].*

The research results are shown in Table 1.

According to the studies, the content of tannins in the herb of *Stachys sieboldii* was (1.67±0.03) %, in tubers – (1.35±0.05) % (Table 1).

The results of studies of the amount of polyphenols in terms of pyrogallol showed that in the herb of *Stachys sieboldii*, their content was (7.93±0.03) %, and in the tubers – (11.08±0.05) % (Table 1).

Flavonoids are a group made up of some thousand compounds found in a wide diversity of plant-based foods. Flavonoids are characterized by both anti-inflammatory and anti-oxidant properties as well as antibacterial activity. The antibacterial activity of flavonoids has been increasingly documented and many research groups have identified the chemical structures endowed with antibacterial activity. Flavonoids can inhibit bacterial growth using different mechanisms including the inhibition of nucleic acid synthesis, particularly flavonoids with B-ring hydroxylation [71], and inhibition of cytoplasmic membrane functions [72-75].

*The quantitative content of the sum of flavonoids in the herb and tubers of Stachys sieboldii was determined by spectrophotometric method [76].*

The total quantitative content of the amount of flavonoids in terms of rutin in the herb of the studied plant was (2.42±0.02) %, in the tubers – (0.39±0.01) % (Table 2).

We studied the antimicrobial activity of extracts from tubers and herbs of *Stachys sieboldii*. Antimicrobial activity was studied in 5 museum strains: *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *S. typhimurium* ATCC 55, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 885-653 using the method of “wells”. Each experiment was repeated ten times. The results were processed by the method of variation

statistics using the value of the median (Me). The research results are shown in Table 3.

Experiments showed that a dry extract of *Stachys sieboldii* herb has a more effective antimicrobial effect. All test microorganisms were sensitive to its action. The diameters of their growth retardation ranged from (19.75±2.12) mm (for *S. typhimurium*) to (24.55±1.20) mm (for *P. aeruginosa*).

A homogenate from the herb of the studied plant was the second in terms of growth retardation of test crops. It showed the most pronounced antimicrobial effect against gram-positive cocci (19.35±1.08) mm and yeast fungi (22.35±1.15) mm. These species of microorganisms were sensitive to this homogenate of herb. Gram-negative rods of *E. coli*, *S. typhimurium*, *P. aeruginosa*, as well as *S. aureus* and *C. albicans* were insensitive to a thick extract from the tubers of *Stachys sieboldii*.

The results of the studies confirmed that the biologically active substances of *Stachys sieboldii* have antimicrobial and anticandidal effects. The extract from the herb of the studied plant is more active.

## Conclusions

It was experimentally found out that both studied extracts of *Stachys sieboldii* showed antibacterial activity. Dry extract of *Stachys sieboldii* herb showed more pronounced antimicrobial activity against gram-positive microflora, so it is promising for the creation of a drug with antimicrobial properties. The antibacterial properties of extracts of *Stachys sieboldii* herb and tubers are the result of tannins, polyphenols and flavonoids in the plant. The content of these biologically active substances was determined by the spectrophotometric method.

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**Table 1.** The quantitative content of tannins and polyphenols in the *Stachys sieboldii* herb and tubers  
(M ± SEM, n = 5)

Raw material	Content of biologically active compounds, %	
	tannins	polyphenols
Herb	1.67±0.03	7.93±0.03
Tubers	1.35±0.05	11.08±0.05

**Table 2.** The quantitative content of flavonoids in the *Stachys sieboldii* herb and tubers (M ± SEM, n = 5)

Raw material	Content of flavonoids, %
Herb	2.42±0.02
Tubers	0.39±0.01

**Table 3.** Antimicrobial activity of the extract from the root tubers and herb of *Stachys sieboldii* (zones of growth retardation of test microorganisms (mm))

Test microorganisms	Example		
	herb homogenate	thick extract of the tubers	dry extract of the herb
<i>S. aureus</i> ATCC 6538	19,35±1,08	14,15±2,08	20,35±1,18
<i>E. coli</i> ATCC 25922	19,25±1,07	13,15±1,41	20,25±1,05
<i>S. typhimurium</i> ATCC 55	17,15±1,12	15,15±1,19	19,75±2,12
<i>P. aeruginosa</i> ATCC 9027	14,35±1,25	14,20±1,16	24,55±1,20
<i>C. albicans</i> ATCC 885-653	22,35±1,15	14,35±2,03	23,25±1,64