



## Poulk Plant (*Stachys schtschegleevii*) and Its Antibacterial Specifications

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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### ABSTRACT

Poulk plant (*Stachys schtschegleevii* Sosn. ex Grossh.) is one of the medicinal plants with a long history in traditional medicine and is used to remedy many diseases. Polk plant possesses some properties, including the internal anti-infectious, antibacterial, Anti-asthma, anti-sinusitis, anti-inflammatory and it is used to remedy the respiratory inflammatory diseases and has been identified as a natural penicillin. This study aims to investigate the phytochemical and antibacterial effects of ethanolic extract, 2-Propanol and n-hexane of the Poulk plant against *Escherichia coli* and *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella enterica*. In this study, extracting was done by Soxhlet extractor with ethanolic, 2-Propanol and n-hexane solvents and after evaporation of the solvent and methylation by the rotary evaporator, the obtained substance was injected into the GC-MASS and the substance was detected, as well as the Inhibition zone, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) studies were carried out. The ranges of MIC and MBC of ethanolic extract, 2-Propanol and n-hexane for MIC were 0.78-12.5%, 0.78 -6.25% and 12.5-50%, respectively. MBC for ethanolic extract, 2-Propanol and n-Hexane were 0.78-12.5%, 0.78 -6.25% and 12.5-100%, respectively.

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## 1. INTRODUCTION

Medicinal plants' consumption and the herbal medicines' prescription originated from the medical history of the human being, and nowadays it has been increasingly accepted in most communities [1,2]. Therefore, the scientific and research communities in the world have recently been considered to study their effect mechanism and proving the effectiveness of the plants [3].

Nowadays, treating the infectious diseases has been difficult due to the resistance of pathogenic bacteria against the antibiotics, and besides, because of the side effects of the chemical drugs, the use of herbal medicines increases more and more [4].

One of these herbs is the Poulk plant which is used to remedy the infectious diseases of the respiratory tract in the Iranian traditional medicine (for colds and sinusitis), as well as it is used for asthma, rheumatism and other inflammatory disorders.

The scientific name of this plant is *S. schtschegleevii*. The antimicrobial effects of the *S. schtschegleevii* species are still unknown" [5], but, there is an emphasis on the antibacterial effects of this plant in the academic literature. "The *Stachys* genus has more than 300 species, but 34 species have been identified in Iran. *Stachys* is a genus of shrubs and annual or perennial and rhizome herbs with a length of about 30 cm that is covered by white soft hairs, as well as its stem has numerous, dense and thick leaves in standing posture with abundant short branches with oval or ovate-triangular leaves with a very short point or almost without point and specific leaf vein [6].

This plant is distributed in the provinces of Mazandaran, Azerbaijan and Semnan of Iran. Antibacterial effects have been found in some species of this plant" [7-9]. Some investigations have been conducted by the researchers to identify the chemical composition of the Poulk plant, including:

Kumarasamy et al. [10], analyzed the phenolic and glycosylated compounds of Poulk plant by chromatography method (GC-MASS) [10,11], Reza Zadeh et al. [12], identified some chemical compositions of the essential oil of some types of

Poulk plant such as *S. balausaebioss* and *S. schtschegleevii*. Also Marriotti et al. [13], identified various compounds of the essential oil of a Poulk plant species by the chromatography method and isolated compounds such as Terpinene, alpha-Pinene and alpha-Terpineol. Considering the isolated compounds from the essential oil, one can be said that the extract of the Poulk plant has a lot of chemical compounds and its antimicrobial effects are related to these compounds, therefore, it is recommended for identifying compounds of the Poulk plant in the future studies.

In this research, in addition to extracting the Poulk plant in different solvents, the antimicrobial effects were investigated as well. Initially, n-hexane, ethanolic and 2-Propanol extracts were prepared and then the microbial effects of the Poulk plant were investigated and also studies on inhibition zone, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was conducted.

## 2. MATERIALS AND METHODS

### 2.1 Specifications of the GC-MASS Instrument

The BD5 column with a temperature gradient of 60°C-140°C, and finally 260°C with a split/splitless ratio of one to three, and helium as a carrier gas with the rate of 1 mL/min and 1 µL injection volume of the sample was used and the purity of helium gas was 99.999.



**Fig. 1. GC-MASS instrument**

**Table 1. GM-MASS conditions for injection of extract sample**

| Rate (C <sup>0</sup> /min) | Value (°C) | Hold time (min) |
|----------------------------|------------|-----------------|
|                            | 60         | 0               |
| 40                         | 140        | 0               |
| 20                         | 240        | 10              |

Thermal Aux: 280, Solvent delay: 3 min, Post time: 3 min

### 2.1.1 Conditions of the GC-MASS instrument

The execution terms for any sample of n-hexane, 2-Propanol and ethanol extracts from the obtained factions of the essential oil are according to samples Table 1.

## 2.2 Soxhlet Extractor

This instrument is one of the laboratory tools that is usually made by the glass and used for extracting plants.



Soxhlet extractor

## 2.3 Rotary Evaporator

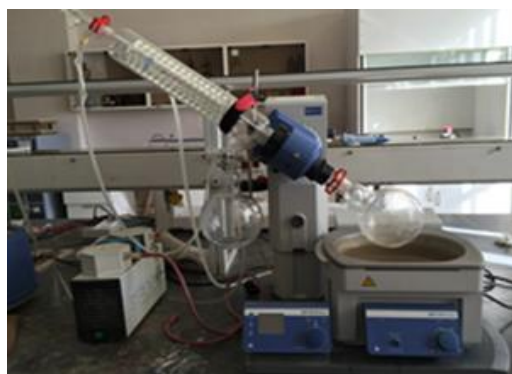
This apparatus conducts the operation of the solvent's removal in chemical material by evaporation method.

## 2.4 Microbial Strains

### 2.4.1 Preparation of bacterial strains

The used microorganisms, including *S. aureus*, *E. coli*, *B. subtilis* and *S. enterica* were prepared

as lyophilized form from the Biotechnology Research Institute of Tehran University (Table 2). The microbial samples were revived according to standard methods. Since the number of inseedinated bacteria is one of the most important variables that affect the outcome of this study, the concentration of inseedinated microbial suspension should be standard. For preparing the microbial suspension from a fresh and young growth, the multi-colony bacteria was transferred to Multan Hinton broth growth medium. It was then incubated for 2 hours at 37°C to obtain turbidity similar to 0.5 McFarland turbidity standard (turbidity =  $10^8 \times 1.5$  per ml). Then, to achieve a concentration of  $10^6 \times 1.5$  per ml, the bacterial suspension was diluted with turbidity equal to 0.5 McFarland tube to a ratio of 0.01.



Rotary evaporator

**Table 2. Used microbial strains of the study**

|   |   |
|---|---|
| 1 | <i>Staphylococcus aureus</i> PTCC: 1112 |
| 2 | <i>Escherichia coli</i> PTCC: 1270      |
| 3 | <i>Bacillus subtilis</i> PTCC: 1254     |
| 4 | <i>Salmonella enterica</i> PTCC: 1709   |

## 2.5 Methods

Extraction was done using a Soxhlet extractor in various solvents such as n-hexane, ethanol, isopropanol, hydrocarbons, etc. Then, the disk diffusion procedure in the Muller Hinton Agar growth medium was used to determine the inhibition zone by a 12 cm diameter large plate in containing Muller Hinton Agar medium, and the prepared concentrations were 0.39% -10 mg/ml. To determine the minimum inhibitory concentration (MIC) of the target complex on the bacteria studied, the Micro Dilution method was utilized using 96-well Microplates by successive two-fold dilutions of concentrations. After reading the MIC result (by the Micro Dilution method),

MBC step was carried out {with growth medium (BHI, Brain Heart Infusion) Agar} was performed. The used bacteria in this study are *S. aureus*, *B. subtilis*, *E. coli* and *S. enterica*.

### 2.5.1 Soxhlet method

The classical methods of extracting are based on plant placement in a proper solvent and to increase the speed of the process, mixing or heating is also used and in this regard, one can be referred to classical methods such as Soxhlet extraction, distillation, maceration and percolation methods. Soxhlet extraction is a standard method that is used as the main reference for the assessment of other methods. The Soxhlet extraction is a general method and is mainly utilized to extract compounds with low or moderate volatility, which are stable against heating.

### 2.5.2 Preparation of the poulk plant and the method of extracting

The Poulk plant was purchased from Ahar's market and was completely milled by a small electric mill, and packed completely in a paper bag without sunshine and moisture. The milled plant was used in an interval of 12-hour and the extract of the Poulk plant was prepared by the following steps:

- 1) 500 grams of the Poulk plant are pulverized.
- 2) A closed-bottom tube should be formed by a filter paper. The paper tube must be able to enter easily in the Soxhlet pipe.
- 3) The milled Poulk plant (step 1) must be poured into the paper tube and the tube should be slowly placed into the Soxhlet (upside and downside of the filter paper must be fastened).
- 4) Soxhlet and the refrigerant must be installed on the balloon and attached to the clamp. However, before the installation of the system, the polished fittings should be lubricated a little using silica gel for disassembling the fittings at the end of the work and 7 boiling stones should be placed in the balloon (Note: the refrigerant must be tested before the installation for leaking, and the water inlet and outlet hoses must be connected to it).
- 5) The n-hexane is added from the top of the Soxhlet to fill the tube of the Soxhlet. Then it is added one more time until the place of the bubble (about 1000 mL).

- 6) The chiller is switched on to allow water to flow into the refrigerant.
- 7) At this stage, the balloon is placed over the heating mantle.
- 8) The time is recorded when the first drop of the solvent is distilling and dripping from the refrigerant, (note: Installed device must be completely vertical to put the solvent droplets exactly on the materials in the paper).
- 9) Extracting must be continued for 24 hours.
- 10) After 24 hours, the heat must be cut off and let the system get cold a little, and all the vapours should get cold in the refrigerant and entered into the liquid phase.
- 11) Before reaching the bubble place of the machine, the machine automatically pours the solution into the balloon and we turn off the machine.
- 12) Firstly, the refrigerant should be removed, then the Soxhlet must be separated, and at the last step, the balloon should be removed from the clamp (Note: if some solution is left in the Soxhlet, it should be poured into the balloon slowly and carefully, so that the Poulk plant does not enter into it).
- 13) Under a vacuum, the solvent must be evaporated by a rotary apparatus.
- 14) The prepared extract should be kept in the refrigerator.

### 2.5.3 Test method

Initially, the herbal material was extracted with a non-polar solvent such as n-hexane. Then the de-solvation of the obtained extract was done. In the next step, the retained material from the previous step was again extracted by using the 2-Propanol polar solvent. The obtained extracts from 5% dimethyl sulfoxide (DMSO) solvent was used for agar well diffusion test and determination of MIC and MBC with concentrations (0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100 mg/mL). In this study, the antimicrobial effect of the extracts was amplified uniformly by 500  $\mu$ L agar well diffusion and microbial suspension methods with a concentration of  $10^8 \times 1.5$  cfu/mL methods on the Mueller Hinton Agar culture surface. Then, some wells with 6 mm diameter were created on a plate with a distance of 2.5 cm apart. Afterwards, 100  $\mu$ L of each of the obtained concentrations from the extract of the Poulk plant were transferred to the wells. DMSO as a negative control and antibiotic chloramphenicol is

considered as a positive control. At the end of the work, all growth medium were placed in an incubator for 24 hours at 37°C. The microbial growth was evaluated after a specified period for the formation or non-formation of the inhibition zone around the wells and the diameter of the inhibition zone was measured in millimeter. The diameter of the zones is a response to the concentration of the tested extract. This phenomenon was a linear relationship between the zone and the concentration logarithm of the extract, and the antimicrobial activity of the extract was determined by measuring the diameter of the inhibition zone and comparing it with the specified standard. Determination MIC and MBC was done by Resazurin micro-titer assay plate testing. In this method, the wells 1-9 are related to dilutions of extract (39-100 mg) in a 96-well sterile U-bottom Microplate. The well-10 was bacteria control, the well-11 relates to medium control and the well-12 was extracted control.

- First Step: 100 µL of the culture medium of the Mueller Hinton Broth was poured in each well, except the first well.
- Second Step: In the first and second wells, 100 µL of the prepared extract was poured (because the concentration of extract in the first well is 100%), then 100 µL of the extract was taken from the second well, afterwards this act continued from third well to fourth one and up to ninth well and finally 100 µL of the extract was taken from the ninth well.
- Third step: Dilution of 1 to 100 was prepared from 0.5 McFarland turbidity standard (turbidity =  $10^8 \times 1.5$  cfu/mL) after 24-hour culture of the desired bacteria and a dose of 100 mL was poured in all wells.
- Fourth step: A dose of 10 µL of Resazurin agent was poured in all wells.
- The above method was used for extracting.
- After incubation time, the wells were examined in terms of color change of Resazurin agent from blue dye to pink due to the growth of the inoculated bacteria. The minimum dilution of the extract was considered as MIC, because the colour change was not observed (lack of turbidity). To determine the extract of MBC, all non-growth tubes were cultured in

Muller Hinton Agar growth medium surface and inoculated growth medium was incubated 24-hour at 37°C. The growth of the Bacterium was not observed in the plate related to the tube containing the lowest concentration of extract and this plate was considered as the MBC concentration of the extract.

### 3. RESULTS

#### 3.1 Determination of Inhibition Zone

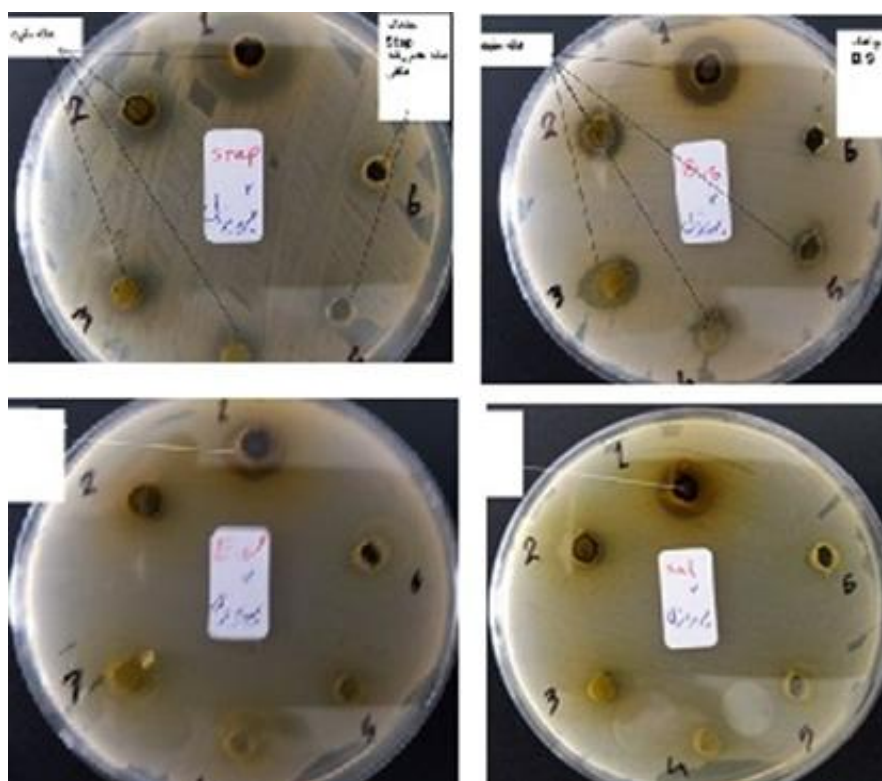
To determine the lack of inhibition zone, a 12 cm diameter large plate of containing the Muller Hinton Agar medium was used for this experiment and the obtained concentrations were 0.039 to 10 mg/mL. After 24 hours of placement of the plates at 37°C, the diameter of the inhibition zone was examined. The results are given in Table 3.

To determine the minimum inhibitory concentration (MIC) of the desired extract against bacteria studied, concentrations of 0.039 to 10 mg/mL was used, using 96-well Microplates based successive two-fold dilutions. All microbes were prepared by 0.5 McFarland turbidity ( $1.5 \times 1.8$  bacteria per ml). Antibacterial activity of ethanolic, 2-Propanol and n-hexane extracts against four bacteria are assessed, including gram-negative bacteria *E. coli* (PTCC: 1270) and gram-negative bacteria *S. enterica* (PTCC: 1709) as well as gram-positive bacteria *S. aureus* (PTCC: 1112) and gram-positive bacteria *B. subtilis* (PTCC: 1254). N-hexane extract is inactive against the *B. subtilis* and also ethanol extract is inactive against the *E. coli*, while these extracts are active against other tested bacteria.

For all bacteria, the largest inhibition zone is the concentration of 100%. The n-hexane extract with a concentration of 100% indicates the diameter of inhibition zone for *S. aureus*, *S. enterica* and *B. subtilis*, 0, 10 and 10 mm, respectively. 2-Propanol extract with a concentration of 100% exhibits the diameter of inhibition zone for *S. aureus*, *S. enterica*, *E. coli* and *B. subtilis*, 13, 10, 10 and 12 mm, respectively. Ethanol extract with a concentration of 100% indicates the diameter of the inhibition zone for *S. aureus*, *S. enterica* and *B. subtilis*, 12, 10 and 13 mm.

**Table 3. Diameter of inhibition zone of gram-positive and gram-negative bacteria of complexes at different concentrations**

| Compound/Concentration (%) | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.87 | 0.39 |
|----------------------------|-----|----|----|------|------|------|------|------|------|
| <b><i>S. aureus</i></b>    |     |    |    |      |      |      |      |      |      |
| Ethanol                    | 12  | 10 | 7  | 0    | 0    | 0    | 0    | 0    | 0    |
| 2-Propanol                 | 13  | 10 | 7  | 0    | 0    | 0    | 0    | 0    | 0    |
| n- Hexane                  | 10  | 7  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| <b><i>B. subtilis</i></b>  |     |    |    |      |      |      |      |      |      |
| Ethanol                    | 13  | 9  | 7  | 6    | 0    | 0    | 0    | 0    | 0    |
| 2-Propanol                 | 12  | 9  | 7  | 0    | 0    | 0    | 0    | 0    | 0    |
| n- Hexane                  | 0   | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| <b><i>E. coli</i></b>      |     |    |    |      |      |      |      |      |      |
| Ethanol                    | 0   | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| 2-Propanol                 | 10  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| n- Hexane                  | 8   | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| <b><i>S. enterica</i></b>  |     |    |    |      |      |      |      |      |      |
| Ethanol                    | 10  | 0  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| 2-Propanol                 | 10  | 7  | 0  | 0    | 0    | 0    | 0    | 0    | 0    |
| n- Hexane                  | 10  | 8  | 6  | 0    | 0    | 0    | 0    | 0    | 0    |

**Fig. 2. Inhibition zone of 2- propanol extract against the studied bacteria**

The values of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of the ethanolic extract of the Poulk plant has been shown in Table 4. The MIC of ethanolic extract possesses the highest activity against *S. aureus* and *B. subtilis* with a concentration of

0.78% and the lowest activity against *E. coli* at a concentration of 12.5%. MBC of ethanolic extract possesses the highest activity against *S. aureus* and *B. subtilis* with a concentration of 0.78% and the lowest activity against *E. coli* at a concentration of 12.5%.

**Table 4. Values of Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC) of ethanol extract**

| Name of bacterium  | percentage | 1    | 2   | 3   | 4     | 5     | 6     | 7     | 8     | 9     | 10                   | 11                | 12                   |
|--------------------|------------|------|-----|-----|-------|-------|-------|-------|-------|-------|----------------------|-------------------|----------------------|
|                    |            | 100% | 50% | 25% | 12.5% | 6.25% | 3.12% | 1.56% | 0.78% | 0.39% | Control of bacterium | Control of medium | Control of substance |
| <i>E. coli</i>     | MIC        | -    | -   | -   | -     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1270         | MBC        | -    | -   | -   | -     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>S. enterica</i> | MIC        | -    | -   | -   | -     | -     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1709         | MBC        | -    | -   | -   | -     | -     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>B. subtilis</i> | MIC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |
| PTCC: 1254         | MBC        | -    | -   | -   | -     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>S. aureus</i>   | MIC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |
| PTCC: 1112         | MBC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |

**Table 5. Values of Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC) of 2-propanol extract**

| Name of bacterium  | Percentage | 1    | 2   | 3   | 4     | 5     | 6     | 7     | 8     | 9     | 10                   | 11                | 12                   |
|--------------------|------------|------|-----|-----|-------|-------|-------|-------|-------|-------|----------------------|-------------------|----------------------|
|                    |            | 100% | 50% | 25% | 12.5% | 6.25% | 3.12% | 1.56% | 0.78% | 0.39% | Control of bacterium | Control of medium | Control of substance |
| <i>E. coli</i>     | MIC        | -    | -   | -   | -     | -     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1270         | MBC        | -    | -   | -   | -     | -     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>S. enterica</i> | MIC        | -    | -   | -   | -     | -     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1709         | MBC        | -    | -   | -   | -     | -     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>B. subtilis</i> | MIC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |
| PTCC: 1254         | MBC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |
| <i>S. aureus</i>   | MIC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |
| PTCC: 1112         | MBC        | -    | -   | -   | -     | -     | -     | -     | -     | +     | +                    | -                 | -                    |

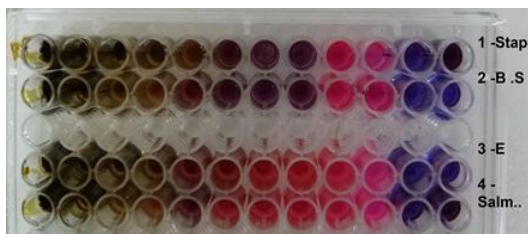
**Table 6. Values of Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC) of n-hexane extract**

| Name of bacterium  | Percentage | 1    | 2   | 3   | 4     | 5     | 6     | 7     | 8     | 9     | 10                   | 11                | 12                   |
|--------------------|------------|------|-----|-----|-------|-------|-------|-------|-------|-------|----------------------|-------------------|----------------------|
|                    |            | 100% | 50% | 25% | 12.5% | 6.25% | 3.12% | 1.56% | 0.78% | 0.39% | Control of bacterium | Control of medium | Control of substance |
| <i>E. coli</i>     | MIC        | -    | -   | +   | +     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1270         | MBC        | -    | -   | +   | +     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>S. enterica</i> | MIC        | -    | -   | +   | +     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1709         | MBC        | -    | +   | +   | +     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>B. subtilis</i> | MIC        | -    | -   | -   | +     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1254         | MBC        | -    | +   | +   | +     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| <i>S. aureus</i>   | MIC        | -    | -   | -   | -     | +     | +     | +     | +     | +     | +                    | -                 | -                    |
| PTCC: 1112         | MBC        | -    | -   | -   | -     | +     | +     | +     | +     | +     | +                    | -                 | -                    |



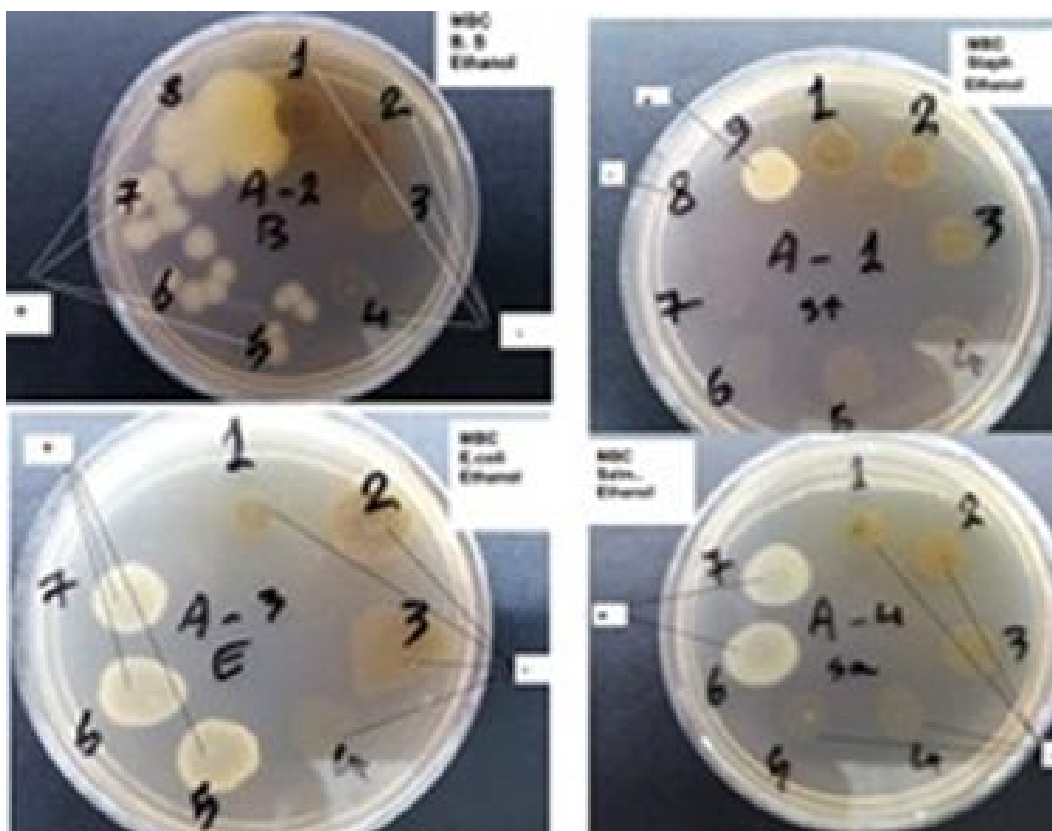
Ethanol extract for *S. aureus* has the highest MBC of 78%. The lowest MBC is in the concentration of 12.5% by *B. subtilis* and *E. coli*.

The results of the MIC test of the Poulk plant in an ethanol solvent against the tested bacteria are shown in Fig. 3.



**Fig. 3. Results of MIC test of ethanol extracts compared to tested bacteria**

The results of the MBC test of the Poulk plant in the ethanol solvent compared to the tested bacteria in the Fig. 4.



**Fig. 4. MBC test of ethanol extract compared to tested bacteria**

The values of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of the 2-Propanol extract of the Poulk plant against the four tested bacteria has been shown in Table 5. The results showed that the 2-Propanol extract possesses the lowest activity against *E. coli* and *S. enterica* with a concentration of 6.25% and the highest effect against *S. aureus* and *B. subtilis* with a concentration of 0.78%.

MBC of the 2-Propanol extract is 6.25% for *E. coli* and *S. enterica* at a concentration of 12.5%.

Ethanol extract for *S. aureus* has the highest MBC of 78%. The lowest MBC is in the concentration of 12.5% by *B. subtilis* and *E. coli*. While the highest MBC is 0.78% by *S. aureus* and *B. subtilis*.

The results of the MIC test of the Poulk plant in the 2-Propanol solvent compared to the tested bacteria.



The MIC of n-hexane extract possesses the lowest activity against *E. coli* and *S. enterica* with The MIC of 50% and the highest activity against *S. aureus* with the MIC of 12.5% (Table 6).

*S. aureus* and *B. subtilis* with a concentration of 0.78% and the lowest activity against MBC of n-hexane extract possesses the lowest activity against *S. enterica* and *B. subtilis* with MBC of 100% and the highest effect against *S. aureus* with MBC of 12.5%.

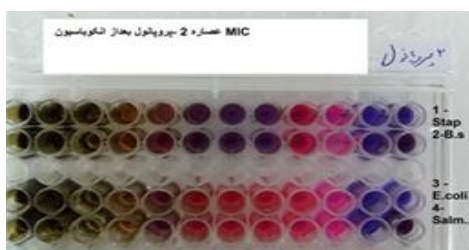


Fig. 5. Results of MIC test of the 2-propanol extract compared to tested bacteria

#### 4. DISCUSSION

Plants are still considered as a potential source for pharmaceutical compounds. Across the world, plants are traditionally used to treat many diseases, especially infectious diseases such as diarrhea, fever, colds, and also to control births and oral hygiene [14-16].

With the increasing number of antibiotic-resistant bacterial strains, many attempts have been made to use the potential antimicrobial properties of plants. On the other hand, the emergence of resistant strains among Gram-negative Bacilli and Gram-negative Coccus such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Staphylococcus*, and *Enterococcus*, have led to creating some problems in treating infections caused by these bacteria [17].

*Staphylococcus aureus* is one of the main causes of hospital-acquired infections, and the prevalence of it is increasing nowadays. This

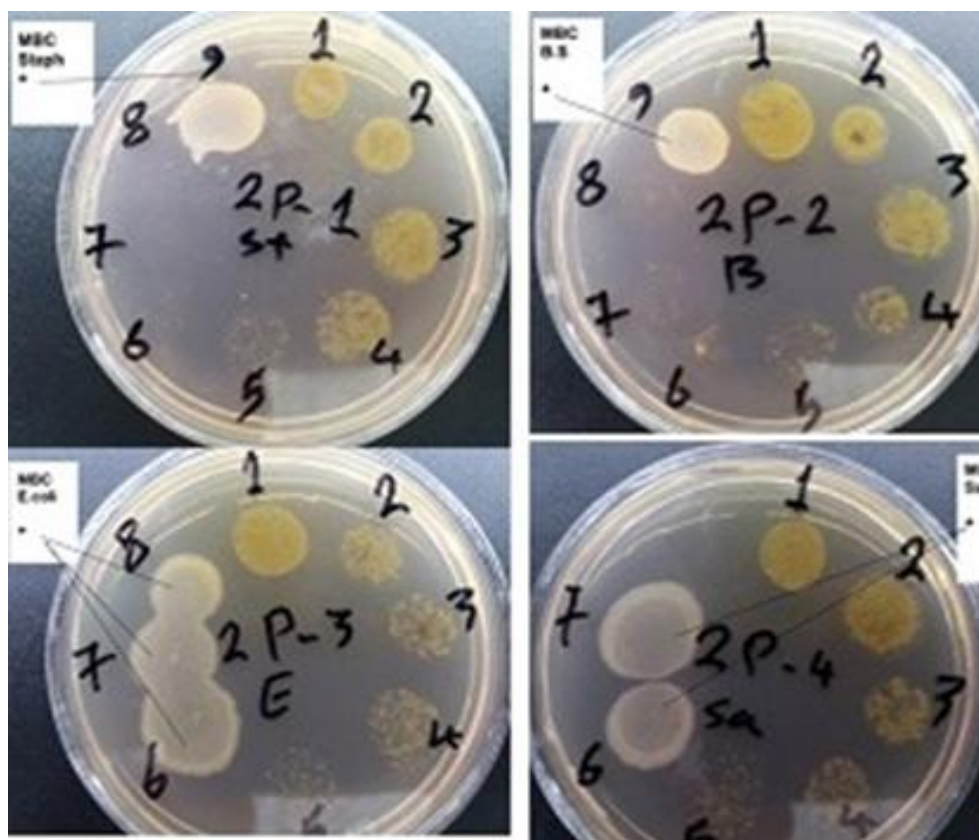


Fig. 6. The results of the MBC test of the poulk plant in the 2-propanol solvent compared to the tested bacteria

bacterium causes a wide range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome, boil, etc. [18].

Nowadays, spreading antibiotic resistance to species of *S. aureus* is one of the main problems for physicians and the emergence of resistant strains to the antibiotic for *S. aureus* have led to reducing the number of available antibiotics to treat these infections [19].

*Pseudomonas aeruginosa* is a common cause of hospital-acquired infections and also an important factor of the death of patients that are suffering from fibrosis, neoplasm disease and severe burns. It also causes serious infections such as septicemia, pneumonia, endocarditis, otitis and keratitis. The intrinsic resistance of *P. aeruginosa* is the result of the presence of specific proteins of the outer membrane related to lipoprotein I, and the external membrane lipoprotein related to Peptidoglycan, which is involved in transfusion systems or permeability of the bacterial cell [20].

Antimicrobial compounds obtained from plants eliminate bacteria with different mechanisms of antibiotics, and this issue is important in the treatment of infections caused by resistant microbial strains. Regarding use of the herbal medicine and products, studying the medicinal properties of plants is so important. In this study, the anti-bacterial effect of n-hexane, 2-Propanol and ethanol extracts of the Poulk plants against *S. aureus*, *B. subtilis*, *E. coli* and *S. enterica* bacteria were investigated.

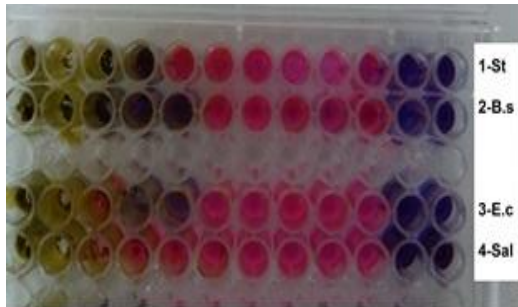


Fig. 7. Results of MIC test of n-hexane extract compared to tested bacteria

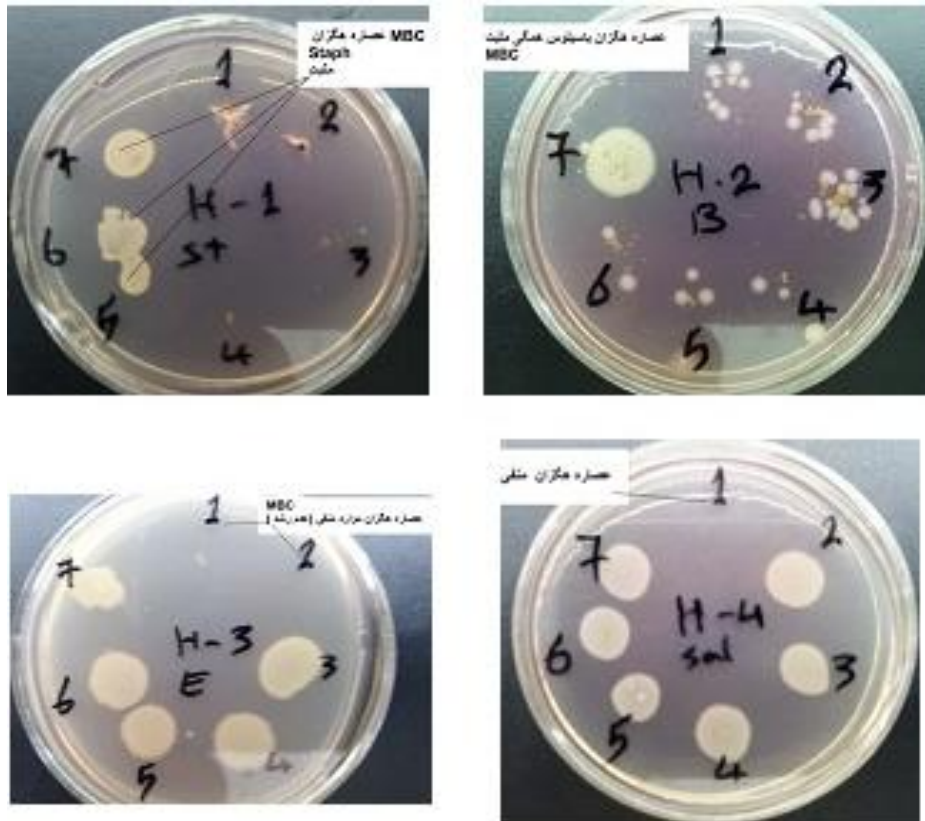


Fig. 8. The results of the MBC test of the poulk plant in the n-hexane solvent compared to the tested bacteria

The species of *Stachys* (*Lamiaceae*) include a variety of secondary metabolites of the plant, including phenylethanoid glycosides, terpenoids, steroids and flavonoids.

*S. schtschegleevii* are commonly known as Poulk and spike of Arasbarani. Spike of Arasbarani is widely used in medicine. These species are cultivated in the Arasbaran area, at the northwest of Iran. The appearance of this plant is similar to *S. inflata* and *S. schtschegleevii*, and sometimes these plants are sold by non-professional persons in the local market instead of *S. schtschegleevii*.

The blue extracts from non-flowering aerial parts of this plant are traditionally used in the northwest of Iran for the treatment of infection, asthma, rheumatism and other inflammatory disorders. One has been reported that the methanolic extract of flowering aerial parts of this plant has anti-inflammatory properties as well [21].

Oily essential ingredients of some Iranian *Stachys* species such as *S. ixodes*, *S. pilifera* and *S. acerosa*, *S. lavandulifolia*, *S. byzantina* and *S. setifera* have been reported previously in Iran. Also, the ingredients of essential oil and anti-bacterial activity of 6 species of Serbian *Stachys* have recently been published. Distilling water of *S. schtschegleevii* leaves produce yellow oil with a yield of 2% (w/w) that is based on the dry weight of the plant. 45 compounds have been identified in this oily extract, which corresponds to 98.7% of the total oil.

Oily section of *S. schtschegleevii* possesses a significant amount of  $\alpha$ -pinene (36.4%), germacrene (18.6%), limonene (8.2%), and piperitone (6.2%) and significantly  $\beta$ -pinene (7.4%), Bicyclogermacrene (3.7%) and Valencene (2.5%). Monoterpenic hydrocarbons with two main components of  $\alpha$ -pinene and limonene is 55.6% of the total oil and therefore it is the most important oil ratio. Oxygenated monoterpene (7.9%) and piperitone of oil are the main component. Among the Sesquiterpene hydrocarbons that make up 29.9% of germacryn D of the oil is the main compound. Oxygenated Sesquiterpene consists of 5.3% of the total oil, and the rest of it includes elmol (1.4%),  $\alpha$ -cadinol (1.4%) and spatulenol (1.2%) are the significant percentage of the oil [22].

The main ingredient of the oil has been obtained by distillation with *S. schtschegleevii* water.

*Lamiaceae* that are wild plants and grow in Iran were studied by GC and GC-MASS. 42 compounds have been identified and the major components include  $\alpha$ -pinene (27.4%),  $\beta$ -Phellandrene (14.7%), germacrene (14.1%),  $\beta$ -pinene (10.5%) and  $\alpha$ -Phellandrene (7.4%).

Phytochemical study of *Stachys* species shows the existence of ethanoid glycosides, terpenoids, steroids and flavonoids. Oil extracts from some species have also been reported. Pharmacological studies have shown that the extract of some *Stachys* species possesses some properties such as anti-inflammatory, anti-inflammatory, anti-hepatitis and anti-anoxic [23].

Anti-inflammatory and painkiller activities of methanolic extracts in the aerial parts of *Schrophularia striata* and *S. schtschegleevii* show a high of bactericidal and bacteriostatic activity and the MIC and MBC values of the extract are between 12-39 mg/ml and 25-78 mg/ml, respectively. MBC and MIC of *S. schtschegleevii* extract is effective in the range of 1.56-12.5 and 3.12-50 mg/mL. Compared to the effect of two extracts, *S. schtschegleevii* shows a high activity against gram-negative bacteria. While *S. striata* extract inhibits *Streptococcus agalactiae* and *Staphylococcus spp* growth [24].

The values of MIC and MBC of the 2-Proponol extract of the Poulk plant against the gram-negative bacteria of *S. aureus* and *B. subtilis* tested were 0.78%, and 0.78% mcg/mL, respectively.

The values of MIC and MBC of the 2-Proponol extract of Poulk plant against the gram-negative bacteria of *S. enterica* and *E. coli* were obtained 6.25%, 6.25% and 6.25%, 6.25% mcg/mL, respectively.

The values of MIC and MBC of n-hexane extract of the Poulk plant against the gram-positive bacteria of *S. aureus* and *B. subtilis* were 12.5-25% and 12.5-25% mcg /mL, respectively.

The values of MIC and MBC of n-hexane extract of the Poulk plant against the gram-negative bacteria of *S. enterica* and *E. coli* were obtained 50% and 50% mcg /mL, respectively.

The values of MIC and MBC of the n-hexane extract of the Poulk plant against the gram-positive bacteria of *S. aureus* and *B. subtilis* were 12.5%, 0.78% and 12.5%, 0.78% mcg/mL, respectively.

The values of MIC and MBC of the n-hexane extract of Poulk plant against the gram-negative bacteria of *S. enterica* and *E. coli* were obtained 6.25%, 6.25% and 12.5%, 12.5% mcg/mL, respectively.

(It should be noted that all experiments were analyzed using the GC-MASS device and after analyzing the information, the identified compounds from the above experiment and their main components with the common compounds from the comparison of the experiments have been collected in the results section).

## 5. CONCLUSION

It is concluded that the 2-propanol extract of the Poulk plant possesses a high bacteriostatic and bacteriocidal effect on two gram-positive bacteria of *S. aureus* and *B. subtilis* tested and a moderate bacteriostatic and bacteriocidal effects on the gram-negative bacteria of *E. coli* and *S. enterica*. The ethanolic extract of the Poulk plant possesses a high bacteriostatic effect on two gram-positive bacteria of *S. aureus* and *B. subtilis* tested and a moderate bacteriostatic effect on the gram-negative bacteria of *E. coli* and *S. enterica*. The ethanolic extract of the Poulk plant possesses a high bacteriocidal effect on *S. aureus* and a moderate bacteriocidal effect on *B. subtilis*, *E. coli* and *S. enterica* bacteria. The n-hexane extract possesses a high bacteriocidal effect on gram-positive bacteria of *S. aureus* and *B. subtilis*. The results of the GC-MASS device show that in the n-hexane extract of the Poulk plant, nine known compounds were identified and these compounds are 98.111% of the extract.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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