



The impact of hydroalcoholic extract of the upper limb of *Stachys lavandifolia* on *Streptococcus pneumoniae* and *Pseudomonas Aeruginosa*

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ABSTRACT

According to the resistance of bacteria to antibiotics, researchers have been trying to discover new antibiotics, among medical plants have played an important role. *Stachys lavandifolia* is one of these plants which has been used for treatment of pneumonia by the people of Boyerahmad in old traditional medicine. First, we provide hydroalcoholic extract of *Stachys lavandifolia* and then during several different experiments and by a plate method minimum inhibitory concentration (MIC) and *Pseudomonas Aeruginosa* growth obtained, and then diameter of this extract were compared with the diameter of the standard antibiotics. *Stachys lavandifolia* extract had a good effect on pneumococcal that concentrations mg / ml1000 formed a diameter equal to 3.5. At concentrations mg / ml 750 and mg / ml500 diameter of the respectively is mm33 and mm27 at MIC and MLC equal to mg / ml 25/31. *Stachys lavandifolia* extract had a modest effect on *Pseudomonas* concentration mg / ml1000 and mg / ml500 and mg / ml750 each zone that formed the MIC and MLC equivalent mm 15 mg / ml 500 registered. In concentrations 750mg/ml and 500mg/ml their diameters are respectively 33 mm and 27 mm that its MIC is equivalent to 31/25 mg/ml. *Stachys lavandifolia* extract had a modest effect on *Pseudomonas* which in concentration of 1000mg/ml and 500mg/ml 750mg/ml each three have conformed 15mm halo that its MIC and MLC is equivalent to 500mg/ml. The result of this research showed that hydroalcoholic extract of *stachys Salavan-difolia* in comparison with standard antibiotic has shown a positive antimicrobial effect, however, we hope with more efforts done on this plant, we can use the plant as an alternative to antibiotics, synthetic and semi-fabricated. So that, concentration of 750mg/ml its effect on *streptococc pneumoniae* is better than all compared antibiotics, but about *psedomonas aenginosa aerations* in concentration of 1000 mg/ ml which was the highest concentration of our experiment from the most compared antibiotics, had a weaker effect.

Keywords: hydroalcoholic extract of *stachyslavandifolia*, *pseudomonus aeruginosa*, *streptococci pneumoniae*

INTRODUCTION

Since the trend today is towards the use of natural ingredients tend to not only meet its desired properties, but concerns about adverse effects of chemical compounds decreases [1] the use of native plants in human life: the life of medicinal plants may be as old as human life. Throughout human history, there is no choice but to resort to herbs to treat diseases and more people have been using a variety of medical plants and they are mixed with their religious history. For example, in the Avesta the name of useful plants are frequently mentioned. What makes use of these plants is experience. Local doctors have cured different illnesses by using these plants. Infrastructure of medical science is based on these experiences. Books of qualified physicians like, Avesina is full of various utilities of plants. By entering the Renaissance era and increasing development of different sciences, usage of plants entered a new phase. The emergence of advanced laboratory equipment along with development of different sciences, the attitude of using local plants from normal status changed into experimental and laboratory. Therefore, given that *stachyslavandifolia* is one of the native plants of Kohkiluyeh Boyer Ahmad and it has been used for a long time;

This plant is one of the largest sex and gender Labyath dark stachys more than 270 species. This essential oil monoterpenes hydrocarbons composed of 14 90 to 96 percent of the oil composition which includes and among these compounds (20%) Terpinene, (3/16%) Pinene, (9/20%) Myrcene and (7/8%) Bicyclogermacrene is the main component. This plant has good antibacterial effect against Staphylococcus and Escherichia coli and also accelerates the healing of gastric ulcer [2]. This plant is one of the wild plants in the province Kohgiluyeh mint family and short-hairy-Perennial plant with numerous stems and green or gray is more or less [2]. Since the bacterium Pseudomonas aeruginosa and warm-negative, aerobic, motile and is commonly found in water, soil, plants and animals are sometimes found as normal flora in the digestive tract and skin. It is one of the most common causes of hospital infections in patients with Pseudomonas burn and aerobic bacterial cultivated environments was forced and smells like grape or tortilla some types of blood hemolysis and smooth, round colonies with a green fluorescence. Pigments this bacterium produces pigments, including: 1) non-fluorescent Pyocyanin blue pigment that is released into the cultivated environment and is produced by Pseudomonas aeruginosa 2) Pyoverdine; the green color cultivated area 3) Pyoverdine which is red. The bacteria in people with compromised immune systems, such as chemotherapy and patients with burns causing disease the bacteria can cause infections. In wounds and burns it produces a blue-green pus. It causes necrotizing pneumonia in people who are connected to the ventilator and also cause serious infections of swimmer's ear in people with diabetes[3]. Other bacteria Streptococcus pneumoniae (streptococcus pneumoniae) is gram-positive, lancet-shaped (lancet shape) with a chain arrangement which has a polysaccharide capsule that is based on the types of pneumococcal polysaccharides is a naturally in 5 to 40% of upper respiratory tract are causing the pneumonia, sinusitis, otitis, bronchitis, meningitis and bacteremia.

Pneumococcal infection causes fluid secretion in alveolar fibrin, which is followed by releasing the red and white blood cells, resulting in the formation of lung congestion. *Stachys lavandifolia* pneumonia, fever, - chills and severe pain in the next screen appears. Induced sputum or blood-colored brick color (rust) can be seen. In 10-20% of cases the cause of bacteremia. The purpose of this research was to evaluate the antimicrobial properties of the hydroalcoholic extract *Stachys lavandifolia* on pneumococcal bacteria and Pseudomonas aeruginosa is [2]. The purpose of this research was to evaluate the effect of plant extract *Stachys lavandifolia* upper limb (*Stachys lavandifolia*) on Streptococcus pneumoniae is *Pseudomonas aeruginosa*. This plant can be used as an alternative to antibiotics which nowadays are used against infections from these bacteria and they may have adverse effects on human beings.

MATERIALS AND METHODS

First we provide *Stachys lavandifolia* from Yasouj pharmacy and dry it at room temperature away from direct sunlight. Then it ground into powder by an electric mill and then it is closed tightly and stored at room temperature until it was extracted. Then 200 grams *Stachys lavandifolia* with 1000 cc solution of ethyl alcohol and distilled water in the flask with a 50/50 2000 cc mixed and it is put on a shaker for 24 hours then we have crossed the solution through Whatman filter paper No. 1 (Whatman's filter paper NO.1) and the filtrate solution collected by the Rotary-evaporator was isolated from extract hydroalcoholic *Stachys lavandifolia* this extract kept in the refrigerator and we used it to do the project, totally from every 200 grams of *Stachys lavandifolia* The new bacterial suspensions of 5.0 McFarland tube which is equivalent to 10⁶ bacteria, produced by swap Hinton agar medium and incubated for 10 minutes at ambient temperature laboratory. After this period by Borr, Meanwhile, on the other stopped producing antibiotics diameter were measured (using antibiotic discs in culture any bacteria) and then compared the extract in different concentrations[4]. MIC and MBC of this extract on Streptococcus pneumoniae tube standard method specified amount of each heating plant extract which was prepared *Stachys lavandifolia*, calculated in terms of cost-effectiveness compared to chemical drugs and side effects they were reported, 500 mg extract *Stachys lavandifolia* in 1 ml of alcohol Distilled we solve the microtubes cc5 / 1 and 7 micro-tubes we send it to domestic numbered 7 0.5cc water pipes we send then to extract *Stachys lavandifolia* mg500 [1]. We send the tube and mix well then we Shaker devices to concentration mg / ml500 to fixed Then cc5 / 0 solution No. 1 pick and the tube number 2 added, as well as to tube No. 7 continued after cc5 / 0 from tube 7 taken away We send to all the tubes cc5 / 0 to *Stachys lavandifolia* extract is created equal. Then, on 8 to medium pneumococcal 8 wells produce brought in from each of the 7 tubes 50 microliter 7 to plate We send into the well number 8, 50 micro-liters of distilled water and alcohol We send (For example, the control group) and 24 hours put into the incubator for 24 hours to measure the size of the halo according to the good results and the concentration of 750 mg / ml 1000 also provided on the plate and we tested it on sinks and cultivated, areas of Streptococcus pneumoniae. These tests for each concentration of 5 times and then average them were obtained. It should be noted that the tests in 5 times and was calculated using SPSS statistical work.

RESULTS

Measure the diameter of the growth inhibition of Streptococcus pneumoniae by plant *Stachys lavandifolia* plate method. In different studied concentrations, *Stachys lavandifolia* able to establish growth inhibition as follows:

- 1) The concentration mg / ml1000 mm35 average size of the halo.
- 2) The concentration mg / ml750 mean diameter was equal mm33
- 3) The concentration mg / ml500 mean size diameter was equal mm27.
- 4) The concentration mg / ml250 average size mm 22 times the diameter of the
- 5) The concentration mg / ml125 mean size diameter was equal mm17.
- 6) At a concentration of mg / ml5 / 62 times the average size of the diameter of the mm11.
- 7) At a concentration of mg / ml25 / 31 and lower concentrations had an aura.

After performing these steps to determine the MIC and MBC of the tube so that its concentration include mg / ml 1000, mg / ml 750, mg / ml500, mg / ml250, mg / ml 125, mg / ml 5/62, mg / ml25 / 31, mg / ml 75/15 and mg / ml7.87 a total of 7 pipes then add half McFarland bacterial solution to keep pipes and tubes, the tubes were incubated for 24 hours. After performing these steps to determine the MIC and MBC of the tube so that its concentration include mg / ml 1000, mg / ml 750, mg / ml500, mg / ml250, mg / ml 125, mg / ml 5/62, mg / ml25 / 31, mg / ml 75/15 and mg / ml7.87 a total of 7 The tube then add half McFarland bacterial solution to keep pipes and tubes, the tubes were incubated for 24 hours.

Table 1: Antibiotics provided as standard

Antibiotics	Diameter	Discontent
Ampicillin	30-36mm	10mg
Azithromycin	19-25mm	15mg
Cefepime	28-35mm	30mg
Cefixime	16-23mm	30mg
Cefotaxime	31-39mm	30mg
Ceftriaxone	30-35mm	30mg
Clarithromycin	25-31mm	15mg
Clindamycin	19-25mm	2mg
Doxycycline	25-34mm	30mg
Erythromycin	25-30mm	15mg
Gemifloxacin	28-34mm	5mg
Linezolid	25-34mm	30mg
Meropenem	28-35mm	10mg
Moxifloxacin	25-31mm	5mg
Ofloxacin	16-21mm	5mg
Rifampin	25-30mm	5mg
Vancomycin	20-27mm	30mg

Measure the diameter of the growth inhibition of *Pseudomonas aeruginosa* by plant *Stachys lavandifolia* plate method:

As shown in the results *Stachys lavandifolia* plant has been able to:

- 1) At Concentration mg / ml1000 and mg / ml750 and mg / ml500 mm15 size of the halo was equal 10mm.
- 2) At concentration mg / ml250 size diameter was equal mm14.
- 3) At concentration mg / ml120 size diameter was equal 12mm.
- 4) At concentration mg / ml5 / 62 the size of the diameter was equal mm9.
- 5) At low concentrations does not constitute any diameter.

After performing these steps to determine the MIC and MLC tube method described previously was used in the concentrations of these include mg / ml 1000-mg / ml750 until mg / ml 7.87 in total, including 7 tubes after adding a solution of bacteria half McFarland tube and keep the tube in Ankobatorbeh 24h tubes examined the concentration mg / ml500 pipes clear which of the MIC,. Then Muller-Hinton agar medium on which the bacteria did not grow. MIC MBC in these experiments was the equivalent mg / ml500 was.The following table is then compared to standard antibiotics provided that this table is as follows:

Table 2: Compared to the standard antibiotic prepared

Antibiotics	Diameter	Discontent
Amikacin	26mm	30mg
Aztreonam	29mm	30mg
Cefepime	30mm	30mg
Cefotaxime	22mm	30mg
Ceftazidime	29mm	30mg
Ceftizoxime	17mm	30mg
Ciprofloxacin	33mm	5mg
Gemifloxacin	25mm	5mg
Gentamycin	23mm	10mg
Imipenem	28mm	10mg
Levofloxacin	26mm	5mg
Meropenem	33mm	10mg
Moxifloxacin	25mm	5mg
Norfloxacin	29mm	10mg
Ofloxacin	21mm	5mg
Pipracillin-tazobactom	33mm	mg100/10
Polymyxin-B	18mm	units300
Ticarcillin-clavulanaic acid	28mm	75.10mg

DISCUSSION

Century 21st which is named the century of returning to nature and the use of herbs to treat? We have witnessed the development of research in the field of medicinal plants and we see that every day supply of new medical Plant And we see that every day supply of new medical Plants become broader. Mico-organisms play an important role t in human diseases. Large number of deaths due to these factors, the human always has to think of ways to deal with microorganisms. As bacterial resistance to antibiotics is becoming more common, so experts are trying to find new antimicrobial as an alternative to antibiotics. Many plants have medicinal properties due to their low frequency of side effects of compounds that are effective in large studies have been conducted to identify medicinal plants. One of these plants is, of course few studies have been done on the plant. This plant grows widely in Kokiloyeh and Boyerahmad province ; it belongs to mint family, it is a steady-short-hairy plant with many stems and green or is more or less gray [3] In a research by Amiri and his colleagues in 2008 in Lorestan University, effect of hydro-alcoholic extract *Stachys lavandifolia* on the number of bacteria tested ,the bacteria Salmonella typhi and S. epidermidis and E. coli, respectively, with 29, 31 and 25 mm in diameter inhibited, but it has minimal effect on the Haemophilus influenzae and B. cereus [5] A rerearch on promastigotes Leishmania conducted by Asadi and his colleagues in laboratory situations, the research showed that it has an anti- Leishmania effect [6].In another study conducted by J. M., et al. its subject was the effect of *Stachys lavandifolia* on abortion in mice this research was carried out since pregnant women used some of it to feel relax. The study showed that low-dose effects not abortion [7]. In another study conducted by Nabavizadeh and his colleagues, *Stachys lavandifolia* has proven its effect on gastric ulcer healing [8].But our research on two bacteria Pseudomonas aeruginosa and S. pneumoniae were obtained the following results: This plant in the case of Streptococcus pneumoniae bacteria, left a very good effect of itself. For example with the concentration mg / ml500 an average diameter equal to the diameter mm27, which is equivalent to many standard antibiotics such as clarithromycin and Doxycycline and Erythromycin and Linezolid and Moxifloxacin and Rifampin and Vancomycin. The number of antibiotics its effect on the diameter of Ampicillin and Cefepime and Cefotaxime and Ceftriaxone is less and less Gemifloxacin and Meropenem. It is also due to a number of antibiotics such as Clindamycin and Ofloxacin Azithromycin and Cefixime and more. The higher concentration of 1000 mg / ml750 effect has been said about the effects of antibiotics in the table according to the diameter is greater. But the effect of alcoholic extract of plant *Stachys lavandifolia* modest effect on Pseudomonas Aeruginosa has been in place so that the diameter of those in high concentrations (750 and 1000 mg / ml500) said almost all of the standard antibiotics Polymyxin-B in the table below, except that the effect of alcoholic extract of the plant is *Stachys lavandifolia* . It should be noted that the disc contains micrograms is based on a comparable measure is only based on the diameter. According to the survey Amiri has done this plant has a maximum effect on Salmonella typhi mm31, but in this study it is shown that at high concentrations as mg / ml1000 the diameter of the inhibitory than its effect on Salmonella typhimurium is (regardless of concentration) But in the case of Pseudomonas aeruginosa showed a weak effect due to the study and research on mice and humans to treat pneumonia, strep Pnomonyay *Stachys lavandifolia* plant can be used. But weaker effects than standard antibiotics effective against Pseudomonas aeruginosa can be used as adjunctive therapy Krd. amydvarym due to the low cost and easy access to antibiotics and fewer side effects compared with *Stachys lavandifolia* plant The study in the near future of this plant as treatment of choice for streptococcal pneumoniae and Pseudomonas aeruginosa adjunctive therapy to be used. Considering that the indiscriminate use of antibiotics to treat bacterial infections Azanty (especially bacteria Pseudomonas aeruginosa and Streptococcus pneumoniae) causes increased resistance to antibiotics is increasing and

therefore requires that a drug that could the creation of effective resistance And the direction is both available and cheaper and have fewer side effects, requires society to the use of herbal medicines, (which is all the benefits above) lead. The study of the properties of herbs *Stachys lavandifolia* important than the bacteria *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were used And excellent results of the current drugs used to treat the bacteria, Respectively, and the preparation of this herbal medicine is affordable and little side effects have been reported in studies previously conducted, Can be used as a drug for the treatment of diseases caused by this bacteria used in the community. It should be noted that the need for more laboratory studies to ensure the feasibility of the plan. According to previous studies, this study can be used as a drug plant Pshmvk broad spectrum used alone or with other medicines.

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