Physiological, antioxidant and antimicrobial properties of some herbal extracts

Mehdi Khorshidi¹ and Masoomeh Vaghef²*

1. School of Biology & Institute of Biological Science, Damghan University, Iran
2. MS Student, plant physiology, School of Biology, Damghan University, Iran

Corresponding author: Masoomeh Vaghef

ABSTRACT: The main purpose of this study is to investigate phytochemical and antibacterial effects of several species of native plants of Iran against pathogenic bacteria and introduce them as one appropriate alternative drug against these bacteria. First, the plants were collected and named scientifically and then their effects were examined on growth inhibition of Staphylococcus aureus, Bacillus cereus, Escherichia coli and Salmonella enteritidis after providing methanol extract with 500, 250, 125, 62.5, 31.25, 15.62 and 3.9 mg/ml dilutions via Macro dilution & Disk plate methods. Also, phytochemical experiments on provided extracts were conducted to examine the existence of Alkaloid, Tannin, Saponin, Anthocyanin and measuring the level of phenol and antioxidant effect (DPPH method). The results showed that the methanol extract of these 3 species have significant antimicrobial effect on standards strains. The maximum and minimum level of phenol was in Heracleum Persicum and Salvia Officinalis, respectively. The results of DPPH showed that 50% inhibitory concentration in Teucrium Polium, Heracleum Persicum and Salvia Officinalis was 5.74±0.099, 5.005±0.154 and 6.208±0.224 mg/ml, respectively. The result of this study and others show that the chemical drugs could be replaced by extract of herbs for treatment of infections.

Keywords: Teucrium Polium, Heracleum Persicum, Salvia Officinalis, antioxidant activity, phenol, antimicrobial activity

INTRODUCTION

The herbs were used for different goals from a long time ago. The evident of using these natural sources in Iran were addressed to history and given documents from Avicenna's books that is noted many medicinal properties for herbs (Akin. 2010). Plants are forming a large part of the nature, so they were used as the first choice for solving problems. Until a few decades ago somewhat that is used as drug, was provided from natural sources and specially plants. On one hand Science development and in the other hand economics are caused reducing usage of drugs and in lots of cases, herbal drugs are replaced with industrial ones (Bonjar. 2004). Increasing use of chemical drugs is going to cause more important issues include self-immune that is due to continuous and indiscriminate consumption without considering the way of usage and side effects that could be even more dangerous than the drug, itself. In recent years, due to resistance of microorganisms against chemical drugs and un-wanted side effects of them, using extracts and herbs that are showing antimicrobial activities have increased (Brain and Turner, 1975). On the other hand, bacterial resistance to antibiotics is increasing so the men are willing to replace antimicrobial agents, which have less effect and more un-wanted side effects, with effective antimicrobial agents that have minimum side effects. In order to defend against insects and microorganisms, plants synthesis some elements. Also, it might be spattered antimicrobial metabolites to affect the natural growth of microorganisms (Brand. 1995). In recent years, in order to evaluate the antimicrobial effect of extracts, extensive research are conducted which show the
capability of these components to prevent the growth of a wide variety of pathogenic microorganisms (Chhabra. 1984).

Teucrium Polium with Persian name of "Maryam-Nokhodi" is a member of mint family with more than 340 species in all over the world and including 12 plant and herbaceous species that three of them are exclusively in Iran (Eloff, 1998). In recent years, a large amount of interest has made for using extract of these plants with purpose of improving the sustainability of foods, delay fungal growth and bacteria and preventing the Mycotoxins (Heber 2004). Heracleum Persicum or Iranian Gol-par (that is from Apiaceae family) is one of the Heracleum species out of 10th in Iran. Root of Heracleum Persicum consists of different coumarins. Various investigations by different authors about Heracleum Persicum extract have proved the inhibitory effect of this plant on some of positive and negative bacteria and pathogenic fungus such as Candida albicans and Candida utilis (Kumar and Chauhan 2006; Lee, 2003; Mirjana. 2004; Miski., 1983). The Salvia Officinalis is also called Garden sage and means liberation or healer. Mint family, especially Salvia Officinalis, is under critical attentions due to different Terpenoids components, extract of phenols and flavonoids, and their antimicrobial effect. For example, extract of Salvia Palaestina has significant antimicrobial effect on Staphylococcus aureus, Staphylococcus Epidermidis and Escherichia coli (Mohamed Saleem. 2010).

**MATERIALS AND METHODS**

1. **Consumables:**
   antibiotic disks from "Padtan Teb" Inc., medium and methanol from MERK Company, lyophilized bacteria from Pasteur institute of Iran were purchased.

2. **Bacterial strains:**
   in this study, it was used 4 pathogenic bacteria that was hold in microbial bank of plant physiology laboratories which are inclusive: Staphylococcus aureus and Bacillus cereus as gram-positive bacteria; Escherichia coli and Salmonella enteritidis as gram-negative bacteria.

3. **Required plants:**
   the herbs like Teucrium Polium, Heracleum Persicum and Salvia Officinalis were chose from traditional species. These plants were named scientifically in herbarium of biology faculty. These plants were ground with an electric mill and hold within a sealed container without any light and heat till the experiment.

4. **Providing extract and required dilutions:**
   300 gr of the plants noted above are weighted and extraction with methanol by maceration method was done. The resulted extract after concentration with vacuum distillation unit dried in oven and kept in refrigerator until experiment. The dilutions were varied from 3.9 to 500 mg/ml.

5. **Phytochemical investigation:**
   in order to do this experiment, the certain amount of dried plants was studied for analyzing the existence of alkaloid, tannin, saponnin and anthocyanin.

   A. The experiment of alkaloid was in this way that 0.25gr methanol extract of 5ml hydrochloric acid (1%) was added and boiled for 5 minutes; then the volume is reached to primal value and the resulted acidic solution was filtered by filter paper. The resulted solution was extracted with appropriate amount of 10% alkaline ammonia and ethyl ether. The ether solution was evaporated and 5ml hydrochloric acid 5% was added to it. Then the solution was divided into 3 parts. On part as control and two other were received Bosharda and Mayer reagents. The brown sediment by adding Bosharda reagent and the yellowish white sediment by adding Mayer reagent show the alkaloid (Motamedi. 2010; Naserian, 1997).

   B. The diagnostic experiment of tannin was in a way that 0.25gr extract was dissolved in 10ml distilled water and 3-4ml sodium chloride 10% was added. 2 drops of ferric chloride was added to 5ml distilled water in a test tube. Then a few drops of extract solutions were added to test tube. The bluish green color shows tannin (Motamedi. 2010; Naserian, 1997).

   C. The diagnostic experiment of saponnin was determined through the height of sustainable flooring due to extreme movement of extract near the water (Motamedi. 2010; Naserian, 1997).

   D. The diagnostic experiment of anthocyanin was in a way that 0.25gr extract was dissolved in 10ml distilled water and the resulted solution was acidic by chloric acid 1%. The red color at PH 3-4 and changing colors with PH alteration shows anthocyanin (Motamedi. 2010; Naserian, 1997).
6. Analyzing the phenol content of extracts:
The amount of phenol compounds via folin ciocalteu method was measured. The normal folin ciocalteu 0.2 was added to 0.5ml concentrations of 500, 250, 125, 62.5, 31.25, 15.6 mg/ml; after five minutes, 2ml of sodium carbonate 75gr/l was added to it. Mixture absorption was seen at 760nm wavelength via spectrophotometer after 2 hours. Gallic acid was applied as a standard for drawing calibration curve. The amount of phenolic was reported according to equivalent rate of “milligram Gallic acid on extract gram”. The experiments were repeated 3 times and the average was reported.

7. Analyzing the antioxidant activity (The ability to capture DPPH)
In this experiment, the stable radicals of DPPH were used. 1ml DPPH solution 0.1 molar was added to following extract concentrations (500, 250, 125, 62.5, 31.25, 15.6 and 7.8 mg/ml) and the resulted mixture was shaken and incubated in a dark room for 15 minutes; then the absorption was read at 517nm wavelength through spectrophotometer by blanch methanol. Ascorbic acid was used as standard. Finally the capturing percentage of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was calculated with this equation: I% = A_S -A_B / A_S.
Then, in order to compare the activity of the extracts IC_{50} parameter was applied. IC_{50} is an extract concentration which restrains 50% of radicals.

8. Microbial suspension:
one day before the experiment, part of mother culture was added to Mueller Hinton Broth medium, and after 24 hours incubation in logarithmic phase of bacteria development (by spectrophotometer), bacteria concentration was equaled with standard McFarland tube No.0.5.

9. Microbiologic evaluation:
this process was done by Agar diffusion method.
A. Disc diffusion: the concentrations 1000, 750, 500, 250 and 125 mg/ml were used. 100Ml bacterial suspension was drop on Agar medium and distributed with a sterile cotton swap. Raw sterile discs with 6mm diameter were put on the medium and 20ml Heracleum Persicum solution was drop on discs with distinct concentrations. Antibiotic discs were used as standard discs. The plates were incubated 24 hours at 37°C and the aura of inhibitory bacteria growth was measured and recorded by caliper. The experiments were repeated three times.
B. The minimum inhibitory growth and bactericidal concentration: it was determined by macro dilution method. For each bacteria, it was used a series of 9 test tubes. 7 tubes for different dilutions, one tube as positive control, one tube as negative control and one tube as positive-positive control were used. 1ml Mueller Hinton Broth sterile solution was added to each tube and 1ml stoke extract was added to first tube and after homogenizing, 1ml of homogenized liquid was added to second tube. This process went on and 1ml solution was thrown out from the seventh tube. Therefore, the concentrations were 500mg/ml to 3.9mg/ml, respectively. 50Ml microbial suspension was added to all tubes except negative control. All tubes were incubated 24 hours at 37°C. For MIC reporting, 50Ml Color reagents nitro blue tetrazolium chloride was used and incubated 3 hours at 37°C. However, a higher concentration that shows the red tetrazolium is considered as extract MIC for microbe (Nazeml., 2005). In order to determine the minimum bactericidal concentration (MBC), certain volume of no turbidity tubes was cultured on Muller Hinton Agar medium. The last dilution of the extract that was able to kill 99.9% of primary bacteria is considered as minimum bactericidal concentration (Oganesian. 1991). These processes were repeated for the extracts and every four strains bacteria three times. The controls were as follows: the medium and the extract without bacteria: no growth (negative control), the medium and DMSO with bacteria: growth (positive-positive control), medium and bacteria: growth (positive control).

10. Statistical analysis:
statistical analysis was done based on factorial and a completely random pattern three times. It was done by SPSS software version 16. In order to determine the average of data, the Duncan test with a 95% confidence level was used. The data were presented by ± average of standard deviation.

RESULTS AND DISCUSSION
Phytochemical analysis of studied plants in table 1 shows that the available alkaloid in Teucrium Polium from mint family is considerable, while Salvia Officinalis has no alkaloid. Tannin exists in every methanol extracts. The existence of Saponnin in Salvia officinalis is significantly high. Methanol extracts of Teucrium polium and Heracleum persicum have very low saponnins.

The studies have shown that the extracts with phenol contents cause protective effects for cells by decreasing the oxidative stress. Phenols and polyphenols are widely found in many foods with vegetable origin and have significant antioxidant effects. The phenol content was calculated in terms of milligram Gallic acid on gram extract based on standard curve equation by folin ciocalteu method. The phenol contents of Teucrium Polium, Heracleum Persicum and Salvia Officinalis have shown on figure 1 on given concentrations. Phenol content of the samples in the highest concentration (from the highest to the lowest) was:
Salvia Officinalis < Teucrium Polium < Heracleum < Persicum

DPPH radical is a free stable radical with nitrogen as central atom which will change its color from purple to yellow by rehabilitation and producing stable molecule DPPH-H. The inhibitory concentration IC$_{50}$ of Teucrium Polium, Heracleum Persicum and Salvia Officinalis are 5.74±0.099, 5.005±0.154 and 6.208±0.224, respectively. The capturing activity of 2, 2-diphenyl-1-picrylhydrazyl has the most and the less value in Heracleum Persicum and Salvia Officinalis, respectively. Also, variance analysis of data shows significant difference between DPPH tests in the samples (P<0.05) (figure2).
The results concluded from disc plate method are an acceptable quantitative method and showed the inhibitory effects of extracts on bacteria, such that in the next repetition was significantly effective. Examining the results of screening of antibacterial effect by disc plate method showed that the most appropriate dilutions were 1000, 500 and 250 mg/ml, respectively. As it could be seen at comparing the studied plants in highest dilution (1000 mg/ml), Heracleum Persicum showed significant effect on growth inhibitory of microorganisms. Teucrium Polium showed the lowest antimicrobial effect on given bacteria.

Figure 3. Comparison of growth inhibitory of studied microorganisms by the methanol extract of 3 herbs (with dilution of 1000mg/ml) via disk plate method

The results of antimicrobial effects of extracts with macro dilution method are presented by the average of minimum inhibitory and bactericidal concentrations in tables 2 and 3.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>The studied methanol extracts</th>
<th>The studied methanol extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Teucrium Polium extract</td>
<td>Heracleum Persicum extract</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>31.25</td>
<td>62.5</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>7.8</td>
<td>62.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>15.62</td>
<td>31.25</td>
</tr>
<tr>
<td>Salmonella entritidis</td>
<td>62.5</td>
<td>125</td>
</tr>
</tbody>
</table>

Table 3. The average of minimum inhibitory concentration (MIC)(mg/ml) of methanol extracts

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Teucrium Polium</th>
<th>The studied methanol extracts</th>
<th>Salvia Officinalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>250</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>250</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Salmonella entritidis</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
</tbody>
</table>

Conclusion

Microorganisms play an important role in human disease. The death caused by this case have made human to prevent such microorganisms. Since the resistance of bacteria have been increased against common antibiotics, so the medical experts are going to replace antibiotics with new antimicrobial agents. The products with herbal antibiotics properties have gained more attention due to their availability and low side effects. In this study, antibacterial and phytochemical effects of 3 species on 4 strains pathological microorganisms were investigated with disc plate and macro dilution methods. As the phytochemical results showed, it seems that antimicrobial effects of studied herbs are related to material such as alkaloid, saponin and tannin which are investigated in methanol extracts.

According to results, methanol extract of Heracleum Persicum has more phenol rather than other species, and it could be say that the antioxidant effect of this herb is do phenol amounts. Phenols and polyphenol components, like flavonoids, have found in food and drug products, and indicated that they have significant antioxidant activity (Rakic. 2007). The compounds that change the purple color of free radical hydrogen or electron to yellow have
antioxidant capabilities (Sayyah, 2005). Based on this, for evaluating the ability of DPPH on free radical capturing, it was used stable radical trapping model (Shahrani, 2006). Measuring the inhibition rate with valid, accurate and DPPH free radicals is easy, affordable and reproducible which were used in experimental condition for investigating the antioxidant activity of the extracts (Singh and Singh, 2008). It was determined, based on the inhibition rate of free radical, that the methanol extract of Heracleum Persicum has more activity. DPPH results showed that the ability of Heracleum Persicum, Teucrium Polium and Salvia Officinalis extracts in free radicals inhibition is depended to their concentration, so the anti-radical activity increases with concentration increase. Two kinds of oaks, Quercus chris and quercus robur, were evaluate and investigated in different concentrations for inhibition rate of DPPH free radicals, thus increasing the methanol extract from 12.5-100 µg/ml would significantly increase the inhibition rate of free radicals (Van Acker. 1996). The antimicrobial results show that the studied methanol extracts have significant antimicrobial effects on microorganisms and Heracleum Persicum extract plays an important role in growth inhibition of microorganisms with providing inhibition zone. Teucrium Polium and Salvia Officinalis extracts have less effect on standard strains. The mentioned extracts showed various levels of antioxidant activity and they are also useful sources for providing natural antioxidants.

REFERENCES


Kumar VP and Chauhan NS. 2006. Search for antibacterial and antifungal agent from India medicinalplants. J Ethnopharmacol. 2: 107


Shahrani M. 2006. Effect of Heracleum Persicum extract on acid and pepsin secretion level in both basic and stimulated conditions with Pentagastin in rat. Shahrekord University of Medical Sciences Journal 4:41-35.
