Antimicrobial Activity of Essential Oils of Aromatic Plants

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ABSTRACT: In this paper it was studied the antimicrobial activity of essential oils of the Azerbaijan aromatic plants. The antibiotic influence of essential oils can be different depending on both species of microorganisms and kind of essential oil. It was showed that, some of essential oils exert specific influence on microorganisms. So, essential oil of Faeniculum vulgare significantly inhibited only gram-positive bacteria, essential oil of Rosmarinus officinalis - only gram-negative bacteria, essential oil Thymus transcaucasicus - both gram-positive and gram-negative bacteria, essential oils of Mentapiperifae, Salvia officinalis and Saturea laxiflora - gram-negative bacteria and yeasts.

KEYWORDS: Essential oils, antimicrobial activity, gram-positive and gram-negative bacteria, yeasts

INTRODUCTION

Nowadays the study of antimicrobial properties of biologically active substances of plant origin requires special attention. First of all, it depends on the quick adaptation of pathogenic microorganisms to individual antimicrobial preparation [5,8,12].

The essential oils occupy special place among the substances with antimicrobial properties of plant origin. The plants with essential oils are spread in all climatic zones of the world. Currently, 2500 species of plants are known to form essential oils, most of them are included in the family of Labiateae, Apiaceae and Compositae [2,10,11,13]. Essential oils from various plants have antibacterial, antifungal, antivirus, antiparasite, anti-inflammatory, antiallergic, antioxidant, immunostimulating and wound recovering properties. First of all, it is related to the synergetic effect of the substances in the composition of essential oils. The composition and properties of essential oils can be various depending on climate conditions and kind of plants [1,4,9,15].

More than 800 species of essential oil plants are spread in the territory of Azerbaijan Republic, among them representatives of the family of Apiaceae occupy the first place [6].

The antibacterial and antifungal properties of essential oils of plants: Achillea millefolium L., Origanum vulgare L., Salvia officinalis L., Juniperus susbina L., Saturea hortensis L., Foeniculum vulgare Mill., Pimpinella saxifrage L., Ziziphora L. and Thymus L. have been studied [7].

The aim of the present work was to study the antimicrobial activity of essential oils of the Azerbaijan aromatic plants.

II. MATERIALS AND METHODS

Essential oils were extracted from 10 species of aromatic plants: Coriandrum sativum L.(Coriander), Foeniculum vulgare Mill.(Foenell), Mentapiperifae L.(Peppermint), Nepetacataria L.(Catmint), Ocimum basilicum L.(Sweet basil), Rosmarinus officinalis L.(Rosmary), Salvia officinalis L.(Garden sage), Saturea laxiflora C.Koch., Thymus transcaucasicus Ronn., Ziziforatenior L. by hydrodistillation method [3].
Antimicrobial activity of essential oils was determined using the dispersion-contact method. Essential oils have been used in the 6 dispersion forms. 96% ethyl alcohol diluted with distilled water in the ratio of 1:3, 1:4, 1:6, 1:7 and 1:8 for obtaining different dispersion forms (water-alcohol solution). Essential oils have been used in the form of the solution with ethyl alcohol. 1.9 ml each dispersion forms of water-alcohol solution poured in the test tube, then was added 0.1 ml solution of essential oil (2%) in the ethyl alcohol. As a result, we obtained 0.1% emulsion of essential oil in the different dispersions (1:3-1:8). Control variants (dispersion forms) which are chosen for comparison have been prepared by the same way, then 0.1 ml 96% ethyl alcohol has been added, instead of the solution of essential oil in the alcohol, into 1.9 ml of water-alcohol solution. Test tubes which were prepared by the same way (control and experiment) were added 0.1 ml drop of different culture suspension.

From each species of bacteria and yeast separately, physiological solution (0.9% NaCl solution) has been prepared for studying the influence of essential oils on the culture test. The number of cells in the suspension was determined by the number of generated colonies on the solid medium and expressed in the colony forming unit (CFU) [14]. The quantity of cells in the suspension which was prepared from bacterial culture test was $10^3$ CFU/ml, while in the suspension which prepared from yeast culture test was $10^4$ CFU/ml. The number of cells in the culture test was determined by the number of generated (forming) colonies on the solid medium which had been survived after direct contact with essential oil.

Cultures of gram-positive (Bacillus anthracoides TU-1, Bacillus subtilis BDU-43; Staphylococcus aureus TU-4), and gram-negative (Escherichia coli TU-2, Pseudomonas aeruginosa TU-3) bacteria and yeast (Candida albicans BDU-47, Candida tropicalis BDU-30) were used as a culture as a culture test in order to determine antimicrobial activity of essential oils. These cultures were taken from the culture collection of the department of Microbiology of Baku State University.

III. RESULTS AND DISCUSSION

The amount of dead cells which died 5 minutes later by the influence of 0.1% dispersion emulsion of essential oils on gram-positive bacteria Bacillus anthracoides TU-1, Bacillus subtilis BDU-43 and Staphylococcus aureus TU-4 was comparatively analyzed. The high antibiotic effect belongs to the essential oils of Thymus transcaucasicus, Foeniculum vulgare and Salvia officinalis and very weak antibiotic effect belongs to the essential oils of Coriandrum sativum, Rozmarinus officinalis and Nepeta cataria. 27, 37 and 51% cells, correspondingly, of Bacillus anthracoides TU-1, B. subtilis BDU-43 and Staphylococcus aureus TU-4 died 5 minutes later by the influence of essential oil of Thymus transcaucasicus; 23; 31 and 49%-died by the influence of the essential oil of Foeniculum vulgare; 15; 20 and 42%-died by the influence of the essential oil of Salvia officinalis; 7; 7 and 9%-died by the influence of the essential oil of Coriandrum sativum; 8.6 and 7.7 and 9%-died by the influence of the essential oil of Zizifora tenior; 4.4 and 10%-died by the influence of the essential oil of Rozmarinus officinalis (fig.1).

The species of genus Bacillus have the same sensitivity against antibiotic impact of essential oil of Coriandrum sativum, Rozmarinus officinalis and Zizifora tenior. However, the sensitivity can change depending on the kind of essential oils. The sensitivity of bacteria against essential oil of Zizifora tenior was 1.6 and 2.8 times more than essential oils of Coriandrum sativum and Rozmarinus officinalis. On the other hand, bacteria have the same sensitivity to different kind of essential oils. For example, the bacterium Bacillus subtilis BU-43 indicates the same sensitivity to essential oils of Mentha piperita, Foeniculum vulgare, Coriandrum sativum, Satureja halleri, Zizifora tenior and Nepeta cataria; the bacterium Bacillus subtilis BU-43 indicates the same sensitivity to essential oils of Mentha piperita, Coriandrum sativum and Nepeta cataria; the bacterium Staphylococcus aureus TU-4 shows the same sensitivity to essential oils of Mentha piperita, Foeniculum vulgare, Coriandrum sativum, Satureja halleri,
The antibiotic influence of essential oils on gram-positive bacteria. A – the amount of killed bacterial cells, in 5 minutes impact, as compared with control, in %: a) Bacillus anthracoides TU-1; b) Bacillus subtilis BDU-43; c) Staphylococcus aureus TU-4; B – essential oils: 1-menta piperitate; 2-foeniculum vulgare; 3-ocimnum basilicum; 4-coriandrum sativum; 5-saturea laxiflora; 6-rosmarinus officinalis; 7-salvia officinalis; 8-zizifor tenior; 9-nepeta cataria; 10-thymus transcaucasicus; 11-composition.

Thymus transcaucasicus, Rozmarinus officinalis and Nepetacataria (fig.1). The essential oils can be divided into 3 groups according to the influence on Staphylococcus aureus TU-4. The essential oils of Foeniculum vulgare and Thymus transcaucasicus are concerned into the first group which has a high impact. To the second group is concerned the essential oils of Mentapiperitae, Osimumbasicumand Saturejalanxiflora which have average degree of effect. Essential oils Coriandrumsativum, Rozmarinus officinalis and Nepetacataria are the third group which has a very weak influence. Bactericidal influence of the first group was 1,8-2,1 and 4,9-5,7 times is more than, corresponding, second and third groups (fig.1).

Thus, it was determined that essential oils of Foeniculum vulgare, Rozmarinus officinalis and Thymus transcaucasicus, have high antibiotic influence on gram positive bacteria. Maximal antibacterial activity was observed in the essential oil of Thymus transcaucasicus. Gram-positive nonsphere-forming bacterium Staphylococcus aureus TU-4 shows high sensitivity to essential oil more than sphere-forming Bacillus anthracoides TU-1 and Bacillus subtilis BDU-43. Staphylococcus aureus TU-4 has 2 times more sensitivity to antibacterial activity of essential oils composition than individual essential oils of Rozmarinus officinalis and Thymus transcaucasicus.

The number of dead cells gram-negative bacteria Escherichia coli TU-2 and Pseudomonas aeruginosa TU-3 in 5 minutes influence of 0,1 % 1:3 dispersion emulsion of essential oils was comparatively studied (fig.2). It was determined that the bacteria Escherichia coli TU-2 and Pseudomonas aeruginosa TU-3 were killed in full by the essential oils of Salvia officinalis and Thymus transcaucasicus and the composition of essential oils. Essential oils of Mentapiperitae and Saturejalanxiflorakill 100% of Escherichia coli TU-2 cells, but kill 88 and 61% of Pseudomonas aeruginosa TU-3 cells. Essential oil of Foeniculum vulgare could kill 46% of Escherichia coli TU-2 and Pseudomonas aeruginosa TU-3 cells, butessential oil of Nepetacataria - 9% cells. 20% cells of Escherichia coli TU-2 and 24% of Pseudomonas aeruginosa TU-3 were killed by the essential oil of Ocimum basilicum. Essential oil of Coriandrum sativum killed 17% cells of Escherichia coli TU-2 and 12% cells of Pseudomonas aeruginosa TU-3. Essential oil of Rozmarinus officinalis could kill 39% cells of Escherichia coli TU-2, 34% cells of Pseudomonas aeruginosa TU-3 and essential oil of Ziziforatenior could kill 92% cells of Escherichia coli TU-2 and 15% cells of Pseudomonas aeruginosa TU-3 (fig.2).

The antibiotic influence of essential oils on gram-negative bacteria can be different or similar depending on both species of bacteria and kind of essential oil. Essential oil of Ocimum basilicum, Salvia officinalis, Nepetacataria and Thymus transcaucasicus show the same influence on both species of bacteria. However, the antibiotic influence may be
very strong or weak depending on the types of essential oils. For example, this influence is observed 100% in the essential oils *Salvia officinalis* and *Thymus transcaucasicus*, 46%-in the *Foeniculum vulgare*.

Fig. 2. Antibiotic effect of 1:3 dispersive emulsion of essential oils against gram-negative bacteria. A – the amount of killed bacterial cells, in 5 minutes impact, as compared with control, in %: a) *Escherichia coli* TU-2; b) *Pseudo monas aeruginosa* TU-3. B – essential oils: 1 – mentapiperiate; 2 – foeniculumvulgare; 3 – ocimumbasiculum; 4 – coriandrumstivium; 5 – saturejalaxiflora; 6 – rosmarinusofficinalis; 7 – saliva officinalis; 8 – ziziforatenior; 9 – nepetacataria; 10 – thymus transcaucasicus; 11 – composition.

and 90%-in the *Nepetacataria* (fig.2).

The sensitivity of bacterial species against essential oils of *Mentapiperitae, Ocimum basilicum, Coriandrum sativum, Satureja laxiflora, Rosmarinus officinalis* and *Ziziforatenior* was different. Sometimes, *Escherichia coli* TU-2 shows more sensitivity to essential oils than *Pseudomonas aeruginosa* TU-3. So, the sensitivity of *Escherichia coli* TU-2 in the essential oil of *Mentapiperitae* was 1,14 times, in the essential oil of *Coriandrum sativum* was 1,42 in the essential oil of *Saturejala laxiflora* was1,64, in the essential oil of *Rosmarinus officinalis* was1,15, in the essential oil of *Ziziforatenior* was 6,13 times more than *Pseudomonas aeruginosa* TU-3 (fig.2).

The antibiotic influence of essential oils on the gram-negative bacteria can be different depending on both species of bacteria and kind of essential oil. The bacteria species have the same sensitivity against antibiotic influence of essential oils of *Foeniculum vulgare, Salvia officinalis, Nepetacataria* and *Thymus transcaucasicus*.

*Escherichia coli* TU-2 has showed very much sensitivity to essential oils of *Mentapiperitae, Coriandrum sativum, Satureja laxiflora, Rosmarinus officinalis, Ziziforatenior*, but *Pseudomonas aeruginosa* TU-3 has showed the high sensitivity in the essential oil of *Coriandrum sativum* (fig.2).

The dead cells of yeast in 5 minutes influence of dispersion emulsion of essential oils were comparatively analyzed. So, 68 and 85%, correspondingly, of *Candida albicans* BDU-47 and *Candida tropicalis* BDU-30 cells were died by the influence of the essential oil of *Mentapiperitae*, 65 and 60%-died by the influence of the essential oil of *Satureja laxiflora*, 73 and 76%-died by the influence of the essential oil of *Salvia officinalis* (fig.3).

The influence of essential oils of *Foeniculum vulgare, Ziziforatenior, Nepetacataria* and *Thymus transcaucasicus* on yeast was weak, but the influence of essential oils of *Ocimum basilicum, Rosmarinus officinalis* and *Coriandrum sativum*. 

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was very weak. 5 and 6% of Candida albicans BDU-47 and Candida tropicalis BDU-30 cells were died by the influence of essential oil of Osimumbasilicum, 8 and 9% were died by the influence of essential oil of Coriandrum sativum, 5 and 6% were died by the influence of Rozmarinus officinalis (fig.3). It should be noted that antifungal influence of essential oils of Mentapiperitae, Saturejalaxiflora and Salvia officinalis on yeast was 8,1-15 times more than essential oils of Osimumbasilicum, Rosmarinus officinalis and Coriandrum sativum. 100% of Candida albicans BDU-47 cells and 80% of Candida tropicalis BDU-30 cells were died by the influence of essential oils composition. Consequently, yeast species significantly differ from each other the sensitivity to essential oils (fig.3). So, it was determined that Candida albicans BDU-47 and Candida tropicalis BDU-30 showed very high sensitivity against antifungal influence of essential oils of Mentapiperitae, Salvia officinalis and Saturejalaxiflora and after 10 minutes alive cells died. The essential oil composition has very high antifugal property. In 5 minutes 100% of Candidodaalbicans BDU-47 cells and 80% of Candida tropicalis BDU-30 cells were killed by this composition.

IV. CONCLUSION

It was showed that, some of essential oils exert specific influence on microorganisms. So, essential oil of Foeniculum vulgare significantly inhibited only gram-positive bacteria, essential oil of Rosmarinus officinalis-only gram-negative bacteria, essential oil of Thymus transcaucasicus-both gram-positive and gram-negative bacteria, essential oils of Mentapiperitae, Salvia officinalis and Saturejalaxiflora-gram-negative bacteria and yeasts.

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