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Antibacterial activity screening of some endemic plants of Adjara floristic region and secondary metabolites - essential oils

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ABSTRACT

In order to find alternative remedies for replacement of so-called food antibiotics in livestock and poultry, antibacterial activity screening was carried out on the methanolic extract of some endemic plants of Adjara and Adjara-Lazeti, as well as some essential oils and compositions based on them. Based on conducted experimental researches, it was established that *Hypericum nordmanni* Khokhr., *Hypericum ptarmicifolium* var. *adzharicum* and *Linaria adzharica* Kem. methanolic extracts have pronounced and wide spectrum antibacterial action. Also, it should be noted that 0.1% aqueous solutions of *Thymus vulgaris* L., *Salvia sclarea* L. and *Monarda didyma* L. essential oils and compositions made of them was found to have high antibacterial activity. The obtained results testify to the necessity of further research of the identified antibacterial plants and essential oils for their use in medical practice.

Keywords: Endemic plants, Secondary metabolites, Essential oils, Antibacterial activity, Methanolic extracts, Spot test.

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Introduction

Modern technologies for farming animals and poultry involve wide use of antibiotics. In the 1950s, took start usage of antibiotics to eradicate the pathogenic microflora in the food, to prevent disease, as well to stimulate growth and productivity in animals and poultry. Most of the antibiotics used for these purposes are synthetic. However, the constant and unsystematic use of antibiotics in poultry has dramatically reduced their effectiveness. Inappropriate or excessive use of antibiotics causes their accumulation over than permissible in food products, which endangers human health, causes dysbiosis, allergic reactions, weakens immunity, etc. The first signal regarding antibiotics enter the human body

through the food chain appeared in scientific literature as early as the '60s of past century. Along with destroying the intestinal microflora they produce resistant forms of bacteria. Resistant strains of pathogenic microorganisms have become a huge problem worldwide. That is why in 2006 the EU banned the use of antibiotics in the territories of its countries, in stock farming and poultry as a stimulant for growth, for neutralizing food from pathogen microorganisms and for prophylactic goals [1]. The growth of antibiotic-resistant strains and the reduction of their efficacy have paved the way for research of new ways to combat pathogenic microorganisms. Recent researches have led scientists to conclude that antibiotics for the above mentioned purposes in stock rising and poultry can be replaced with herbal remedies. The abundance of infections caused by

multiresistant microbes and the complexity of combating them has brought widespread recognition to biologically active substances of plant origin. They do not cause addiction, have no side effects and most importantly, do not develop resistance in bacteria and fungus [2]. From plant origin biologically active substances of antibacterial activity, essential oils have special place with high antibacterial, antioxidant and immune modulating activity [3-5]. The use of essential oils prevents the development of various intestinal infections, which significantly affects the productivity and maintenance of stock and fowls. In addition, essential oils provide a pleasant aroma and make food attractive, also they have anti-stress activity, increase production of digestive enzymes, even improve their mood [6]. Essential oils are products of secondary metabolism that contain numerous easily evaporated substances: terpenes, terpenoids, phenolic products, aliphatic and aromatic components [7]. It is known that essential oils have a wide range of biological activity, above all with bactericidal and fungicidal properties [8]. The mechanism of their action on microorganisms is following: different organic compounds within it change speed of biochemical reactions, resulting in their destructive effects on microorganism's mesosomes and cytoplasmic membranes, thus reducing oxidative phosphorylation activity, also inhibit cellular respiration [7,9].

In recent years, in the scientific community interest in medicinal plants and herbal extracts has increased significantly in terms of antimicrobial activity. Researchers [2] found that essential oils of bergamot, carnation, cypress, big fennel, eucalyptus, lavender, rosemary, peppermint, clary, thyme, show pronounced antibacterial activity against various pathogens. That is why for today there is no doubt regarding use of essential oils as new antibacterial chemical modifiers, on the basis of which it is possible to create different compositions with therapeutic, prophylactic effects [10]. Studies in poultry, particularly in chickens, have shown that watery extract of *Salvia sclarea* L. flowers has anti-inflammatory, antiseptic properties, also has a positive effect on the musculoskeletal system [11]. Studies have also established the antioxidant and antibacterial activity of *Salvia sclarea* L. [12]. Besides, the essential oils of *Monarda didyma* L. possess interesting antibacterial action for poultry [13]. Among well-known essential oils one with high antibacterial activity is the essential oil of Thyme (*Thymus vulgaris*) containing 50% thymol [14]. Chemistry,

Antioxidant, Antibacterial and Antiviral Activity of essential oils of *Thymus Transcaucasicus* Ronniger, widespread in Georgia was studied in details [15]. We must also take into account the fact that usage of only one type of food supplement cannot completely replace synthetic food antibiotics. In this case, it is necessary to create alternatives with complex composition and action. In the modern stage One of the most effective ways to combat resistant bacteria is to use antibacterial ingredients that act with different mechanisms at the same time. In this case, it is possible to inhibit different processes of metabolism in the microbial cell at the same time, leading to its rapid death and significantly inhibits the development of resistance in microorganisms.

Based on experimental studies carried out by the authors of the presented article [16-19], was obtained and studied antibacterial activity of Clary sage, Eucalyptus, Perilla essential oils [20]. The antioxidant and anti-inflammatory effects of Perilla was also explored [21-23].

The aim of the study is to determine the antibacterial spectrum of some endemic plants of Adjara and Adjara-Lazeti, essential oils and bio-composition.

To achieve the goal following task is set: Research antibacterial activity of some endemic plants of Adjara and Adjara-Lazeti, essential oils and bio-composition.

Objectives and methods

Research objects are:

Endemic plant species of Adjara:

1. *Angelica adzharica* M.Pop. – Umbellifereae Juss., Apiaceae Lindl.
2. *Centaurea adzharica* Sosn. – Asteraceae Dumort. (Compositae Giseke)
3. *Erysimum contractum* Somm. et Levier. - Crucifereae Juss.(=Brassicaceae Burnett.)
4. *Ranunculus ampelophylus* var.adzharica - Ranunculaceae Juss.
5. *Rubus adzharicus* Sanadze - Rosaceae Juss.

Endemic plant species of Adjara-Lazeti:

6. *Amaracus rotundifolius*(Boiss.) Briq. (*Origanum rotundifolium*) - Lamiaceae Juss.(=Labiaceae)
7. *Astragalus sommieri* Freyn. - Fabaceae Lindl.
8. *Hypericum nordmanni* Khokhr. - Hypericaceae

Juss.

9. *Hypericum ptarmicifolium* var. *adzharicum* - *Hypericaceae* Juss.
10. *Linaria adzharica* Kem.-Nath. (= *L. syspirensis* C. Koch.) - *Scrophulariaceae* Juss.
11. *Primula megasaeifolia* boiss. Et Bal. - *Primulaceae* Vent.
12. *Quercus petra* var. *dshorochensis* c. Koch. - *Fabaceae* Lindl.
13. *Rhododendron smirnovii* Trautv.- *Ericaceae* DC.
14. *Rhododendron ungeri* Trautv. - *Ericaceae* DC.
15. *Rhynchospora caucasica* Vahl. - *Cyperaceae* Juss.

Essential oils:

16. *Thymus vulgaris* L. essential oil 0.1% aqueous solution
17. *Salvia sclarea* L. essential oil 0.1% aqueous solution
18. *Monarda didyma* L. essential oil 0.1% aqueous solution
19. *Perilla nankinensis* Decne. essential oil 0.1% aqueous solution
20. Composition of *Thymus vulgaris* L., *Salvia sclarea* L. and *Monarda didyma* L. essential oils 0.1% aqueous solutions in the ratio of 2:1:1
21. Control object

The research objects were prepared in Adjara region in the period of May-August of 2019. Preparation of methanolic extracts from dry and finely fragmented raw herbal materials was carried out by the maceration method, with the raw material and menstruum in a ratio of 1: 5 and left to stand by for 24 hours at room temperature.

Extraction of essential oils from the study objects was carried out by the method of hydrodistillation.

Experimental Section

Antibacterial activity research of study objects was conducted using Spot test (screening). 5ml of LB broth was added to each LB agar slant containing overnight bacterial culture and eluted using vortexes. Tenfold dilution (using LB broth) of eluted bacterial suspension was prepared: 0,5ml of bacterial suspension was added to reaction tubes with 4,5ml LB broth and vortexed gently on low speed;

The lawn of the diluted bacterial suspension was made on 1.5% LB agar plate. Plates were allowed to set on bench top or in biosafety cabinet for at least 10-15 min and then sequentially spotted 10μl of each research substance on the bacterial lawn. To avoid mixing of spotted drops max 4 different

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	K
<i>Streptococcus pyogenes</i>	R	R	R	±	R	R	R	2+	3+	±	±	R	R	R	R		1+		1+	4+	
<i>Escherichia coli</i>	R	R	R	R	2	R	R	R	±	2+	R	±	R	R	R		4+	4+		4+	
<i>Enterobacter cloacae</i>	R	±	R	R	R	R	R	4+	±	3+	R	R	R	R	±	4+				4+	
<i>Salmonella typhimurium</i>	R	R	R	±	2	R	R	2+	R	2+	R	±	R	R	R	4+	2+			4+	
<i>Klebsiella pneumoniae</i>	2+	±	R	2+	2	±	R	2+	R	2+	R	±	R	R	R	4+	2+			4+	+
<i>Proteus vulgaris</i>	2+	R	R	R	+	R	R	4+	2+	2+	±	R	R	R	2+	4+	4+			4+	
<i>Shigella flexneri</i>	R	R	R	R	+	3	R	3+	3+	±	2+	R	R	R	±	4+	4+	3+	1+	4+	
<i>Enterococcus faecalis</i>	±	2+	R	2+	2	3	R	2+	4+	3+	3+	R	R	R	±	4+	4+	4+		4+	
<i>Staphylococcus aureus</i>	±	R	R	R	+	R	R	2+	2+	R	±	R	R	R	±	4+	4+			4+	
<i>Pseudomonas aeruginosa</i>	2+	R	R	2+	+	2	R	R	2+	2+	2+	R	R	R	R	4+	4+			4+	1+
	R		R				R	R	2+	R	R	R	R	R	R	4+					

Table. Results of determination of antibacterial action of research objects

research substances were spotted on one bacterial lawn. After drying the drops, the Petri dishes were placed upside down into the incubator at appropriate temperature; Spot test results were examined 18-24 hours after incubation. The presence of clear zones in the research substance spot area indicated a positive result.

Results and analysis

The investigation antibacterial activity of methanolic extracts obtained from 15 endemic herbs, 0.1% aqueous solutions of 4 essential oils and essential oils composition took place. The results are presented on Table.

Given data shows that against listed strains no antibacterial effect was found for *Erysimum contractum* Somm. et Levier., *Astragalus sommieri* Freyn., *Rhododendron smirnovii* Trautv. and *Rhododendron ungerii* Trautv. Weak antibacterial effect was determined for *Angelica adzharica* M.Pop., *Centaurea adzharica* Sosn., *Ranunculus ampelophylus* var. *adzharica*, *Rubus adzharicus* Sanadze, *Quercus petra* var. *dshorochensis* c. Koch. and *Rhynchospora caucasica* Vahl. With pronounced antibacterial effect and narrow spectrum of action is characterized *Amaracus rotundifolius* (Boiss.) Briq. and *Primula megasaefolia* boiss. Et Bal., while *Hypericum nordmanni* Khokhr., *Hypericum ptarmicifolium* var. *adzharicum* and *Linaria adzharica* Kem. Methanolic extracts along with pronounced antibacterial effect have comparably wide spectrum of action.

From secondary metabolites, aqueous solution of thyme and clary essential oils is standing out with pronounced and wide antibacterial activity. *Monarda didyma* L. essential oil 0.1% aqueous solution has pronounced antibacterial activity but with narrow spectrum. *Perilla nankinensis* Decne essential oil 0.1% aqueous solution shown weak antibacterial activity. Essential oils composition, with constitution of thyme, clary and *monarda* in the ration of 2:1:1, had effect of each bacterial strain on each bacterial strain.

Conclusion

Based on conducted experimental researches, it was established that *Hypericum nordmanni* Khokhr., *Hypericum ptarmicifolium* var. *adzharicum* and *Linaria adzharica* Kem. methanolic extracts have pronounced and wide spectrum antibacterial action. 0.1% aqueous solutions of *Thymus vulgaris* L., *Salvia sclarea* L. and *Monarda didyma* L. essential oils and of their compositions with 2:1:1 ratio possess high antibacterial activity.

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References

- [1] Srivastava, M. K., Antibiotic growth-promoters in food animals. *Pharma Times*, 42 (2010)17-21.
- [2] Zhuchenko E.V., Influence of essential oils on microorganisms of different taxonomic affiliation in comparison with modern antibiotics. *News III. Effects of essential oils of lavender, Rosewood three, Eucalyptus, Fir on Some Gram-Negative Bacteria* / Zhuchenko E.V., Semenova E.F., Markelova et al., *Volga Region/*, *Natural sciences*, № 1 (9) (2015), 30–41 (in Russian).
- [3] Guo Qunqun et al., Antibacterial activity of *Perilla Frutescens* leaf essential oil, *Science and technology of Food Industry*. 2003-09 International J. of Innovative Research in Science, Engineering and Technology (An ISO 3297: 2007 Certified Organization). Vol. 5, Issue 11 (2016).
- [4] Minarchenko V.M., Effect of silver nanoparticles on the physical and chemical properties of plant oils and their antimicrobial activity / V.M. Minarchenko, R.V. Kutsyk, N.P. Kovalska / *Biotechnologia Acta*. Vol. 10, No 6 (2017) 35-44.
- [5] Sandy van Vuuren, Alvaro Viljoen, Plant-Based Antimicrobial Studies – Methods and Approaches to Study the Interaction between Natural Products *Planta Med.* 78(03) (2012) 302-302.
- [6] Saini R., Davis S. and W. Dudley-Cash., Oregon essential oil reduces necrotic enteritis in broilers, *Fifty-Second Western Poultry Disease Conference*, 2003, pp. 95–98.
- [7] Atajanova G.A., Terpenoids of Plant Essential oils. Distribution, chemical modification and biological activity. Moscow, 2008 (in Russian).
- [8] Bakkalia F., Biological effects of essential oils – a review / F. Bakkalia, S. Averbecka, D.Averbecka et al. / *Food and Chemical Toxicology*, Vol. 46 (2008) 446–475.
- [9] Nazzaro F., Effect of essential oils on pathogenic bacteria / F. Nazzaro, F. Frattani, L. De Martino et al. / *Pharmaceuticals (Basel)*, Vol. 6 (2013) 1451–1474.
- [10] Reichling J., Essential oils of aromatic plants with antibacterial, antifungal, antiviral and cytotoxic properties an overview / J. Reichling, P. Schnitzler, U. Suschke et al. / *Research in Complementary Medicine*, № 16 (2009) 79–90.
- [11] Khromenko A.V. Analysis of the precedent use of clary sage extracts. XXIII International Scientific and Production Conference, Innovative solutions in agricultural science – a look into the future”. May, 28-29, 2019, pp. 156-159 (in Russian).
- [12] Gulchin I., Uguz M., Oktay M. Evolution of antioxidant and antimicrobial activities of clary sage (*Salvia sclarea* L.). *Turk. Jour. Agric. For.*, Vol.28 (2004) 25-33.
- [13] Paola Mattarelli, Francesco Epifano, Paola Minardi, Maura Di Vito, Monica Modesto, Lorenzo Barbanti Chemical Composition and Antimicrobial Activity of Essential Oils from Aerial Parts of *Monarda didyma* and *Monarda fistulosa* Cultivated in Italy (2017) 76-86.
- [14] https://lekostyle.com/files/pdf/konservir_de_istvie_1.pdf
- [15] Ersan Bektas, Gönül Serdar, Münevver Sokmen, Atalay Sokmen. Biological Activities of Extracts and Essential Oil of Thymus transcaucasicus Ronniger. Published online: 30 March (2016) <https://doi.org/10.1080/0972060X.2014.895208>
- [16] Berashvili D., Bakuridze A., Alania M., Gvazava L., Balansard G., Elias R. Apigenin glucuronide from *Perilla nankinensis* leaves. *Chemistry of Natural Compounds* №1 (2005), 78-79 (in Russian).
- [17] Berashvili D., Bakuridze A., Alania M., Kuchukhidze D., Gvazava L., Balansard G., Elias R. Luteolin diglucuronide from *Perilla nankinensis*. *Chemistry of Natural Compounds*, №1 (2006), 97-98 97-98 (in Russian).
- [18] Shashiashvili N., Jokhadze M., Tushurashvili P., Bakuridze A., Berashvili D., Analysis of *Perilla nankinensis* Decyne essential oil using Gas Chromatography coupled with Time-of-flight Mass Spectrometry. *Georgian Med News*, № 4 (229) (2014) 92-96.
- [19] Anti-Inflammatory, Antioxidant, Antibiotic, and Cytotoxic Activities of *Tanacetum vulgare* L Essential Oil and Its Constituents Héloïse Coté, Marie-Anne Boucher, André Pichette and Jean Legault, *Medicines* 4, 34 (2017) 3-9.

- [20] Akvlediani L.T., Koiava T.N., Lomtadze L.B., Djoxadze M.C., Mschiladze L.V., Berashvili D.T., Bakuridze A.D. Comparative analysis of anti-bacterial effect of phytoantibiotics and antibiotics”. Georgian Med News, № 11 (260) (2016) 79-86.
- [21] Gapdiremen A., Berashvili D., Oktay M., Halici Z., Shengelia D., Bakuridze A., Karanadze N. Invitroantioxidant and acute antiinflammatory profiles of *Perilla nankinensis* Decne. and *Aloe arborescens* Mill. Extracts. Allergology and Immunology, vol. 5, 3, Tbilisi (2004) 447-480.
- [22] Gulcin I., Berashvili D., Gepdiremen A., Antiradical and antioxidant activity of total anthocyanins from *Perilla nankinensis* Decne. Ethnopharmacology (2005).
- [23] Mehmet Emin Buyukokuroglu, Dali berashvili, Akcahan Gepdiremen, Mustafa Altinkeser., Antiinflammatory and Antinociceptive properties of Luteolin Diglucuronide and Apigenin Diglucuronide from *Perilla nankinensis*. Asian Journal of Chemistry, vol. 20, N3 (2008) 1900-1906.