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Full Length Research Paper

Antimicrobe characteristics of essential oils of some species of the *Nepeta* L. genus

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Antimicrobial properties of the essential oils of the species *Nepeta trautvetteri* Boiss., *Nepeta velutina* Pojark, *Nepeta zangezura A.* Grossh. and *Nepeta grosshemi* Pojark. have been studied as well as it was ascertained that essential oils of the *N. velutina* and *N. grosshemi* can be used as antifungal means.

Key words: *Nepeta trautvetteri Boiss.*, *Nepeta velutina Pojark*, *Nepeta zangezura Grossh*, *Nepeta grosshemi Pojark.*, essential oils, antimicrobial properties.

INTRODUCTION

About 800 essential oil plants have been formed in the rich and various vegetation cover of Azerbaijan. These plants are being investigated by scientists periodically (Ibadullayeva et al., 2007). Many new fields of the essential oils use have been ascertained. As the essential oils consist of compounds, that is, set of bioactive substances, it uniquely affects to human body physiologically, psychologically, curatively etc. Essential oils do not lose their quality and they continue remaining safe and effective even if they were conserved for a long period. Majority of the species belonged to *Nepeta* genus are rich of essential oils and their several properties have been studied. Accumulation dynamics of the essential oil in these plants were defined in different stages of onthogenesis and it was realised that it occured during the florescence phase of the generative period most often. In the accumulation dynamics of the essential oil, the synthesis runs weakly at first, maximum output of the essential oil occures in the florescence; recurrent reduce is observed in the next phases (Mammedova et al., 2009). Essential oils obtained from these species are used in making various canning products of food industry, in perfumery and cosmetics, as well as in medicine. Structure of the essential oils was composed of valuable components such as citroneol, citral, geranylacetat etc (Mammedova and Abbasov, 2009). Separate obtaining from these components is also possible in the production condition by their extraction out of the essential oil. Essential oil of catmint is used in making toothpastes, antiseptic substances, as well as balsam and tinctures are used against weakness, in disease of nervous system and respiratory disease, anaemia, gastrointestinal disease, cholagogue, expectorant and other diseases. It is used as indispensable spicery in food industry. On the other hand, it is considered as valuable nectar.

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MATERIALS AND METHODS

The investigated species by us are widely spread mostly in forests, bushy, forest glades, steppes, orchards, meadows and gardens of Azerbaijan as *Nepeta trautvetteri* Bioss, *N. velutina* Pojark in the low and middle belts of the Nakhichevan Autonomous Republic, rare *N. zangezura A.* Grossh in the Ordubad region, *N. Grossheimi* Pojark around Bichanak village (Goryayev, 1952). The phenological observations were held on the base of Beydman methods during the whole season (Beydman, 1974). Output rate of the essential oil was defined by the Ginsberg method (Ginzberg, 1932). Essential oils were defined by means of disk diffusion and purification series method applied against microbes.

EXPERIMENTAL PART

As a continuation of the conducted experimental investigations, essential oils have been obtained from the various bodies of *N. trautvetteri* Boiss., *N. velutina* Pojark, *N. zangezura* A. Grossh. and *N. grosshemi* Pojark.species in different years (Ibrahimov et al., 1996; Gasymov et al., 2007). Output rate of the essential oil of the *N. trautvetteri* Boiss. and *N. velutina* Pojark which are

representatives of shizocalis section was defined during all phases of the vegetation. The output rate of the essential oil of *N. trautvetteri* is 0.15%; amount of the essential oil of the upper part of the plant in whole is 0.2% in virginile state, in the generative period is 0.25%, in the semination period is 0.15% and light refraction rate is nd^{20}-14641. Amount of the essential oil of the *N. velutina* is 1.6%, in virginile state is 1.7%, in the generative period is 1.9%, in the semination period is 1.5%, light refraction rate is nd^{20}-1.4600. Essential oil of the *N. zangezura* A. Grossh. and *N. grosshemi* species that has got more economical significance than *Camapua* section being light yellow colour differs with their private aroma, because aliphatic spirits, aldehydes, a little amount of ketons appear as main components. Amount of the essential oil in *N. zangezura* A. Grosshis 0,2% in the vegetation period, 0.25% in the floressence period, in the semination period is 0.18%, light refraction rate is nd^{20}-1.4740. Output rate of the essential oil of the *N. grosshemi* in virginile state is 0.22%, in the generative period is 0,26%, in the semination period is 0.195%, light refraction rate is nd^{20}-1.5460. The investigated species are of a big significance from the medical point of view. That is why several preparations are being made from the biologically active substances obtained from the upper part of the *N. trautvetteri* species that are used in paralysis, heart attack, insomnia, relief of blood pressure/arteriotony. The extraction obtained of the plant's green mass is widely used in peoples medicine for treatment of respiratory tracts.

Apart from the essential oil hydrocarbons, organic acids and tannin agents have been ascertained in the content of the *N. velutina*. 0.9% fat oil has been found in its seed. Content of the *N. zangezura is* rich of vital vitamins and acids considered as useful for human organism. It is considered as a qualitative edable plant. Vitamins and watery carbons have been obtained from watery-spirit mixture of the *N. grosshemi* species.Taking into account all of these essential oils obtained from the abovementioned plants have been microbiologically investigated. The investigation was held in the microbiology department of the Azerbaijan Medical University (AMU) and in the Ethnobotany Laboratory of the Institute of Botany of the Azerbaijan National Academy of Sciences (IB ANAS) as a component a joint research works conducted according to the agreement on the scentific collaboration signed between AMU and IB of the ANAS. Disk diffusion and purification series method were applied. E*seherichia* coli have been taken as a test-culture. A suspension with 1 mgr microcell in 1ml has been made from a microorganism culture of a day (24 h). Then the suspension was added into Petri vessel with Sabouraud's peptone agar in it as well this was equally spread onto medium surface. The rest fluid was sucked by a pipet and this vessel has being dried in a thermostate during 10-15 min. A sterile filter paper put the disks onto that surface wetting them by preparations placed it into a thermostate; the result has been registered after its cultivasion in the thermostate for 18-24 h at 37°C temperature. A sterile space of 20 mm was obtained in the *N. velutina* and *N. grosshemi* substance; but a space of 18 mm was obtained in *N. trautvetteri;* there was no sterile space in the controle and *N. zangezura.* And in the purification series method, 5 sterile sample bottles were taken. 1 ml substance was poured into the 1st and 2nd bottles, 1 mg abacterial liquid petrolatun was added into all sample bottles beginning from the 2nd bottle. Then 1 ml mixture was taken out of the 2nd bottle and was poured into the 3rd bottle. It was passed out of the 3rd bottle into the 4th bottle, and out of the 4th into the 5th sample bottle. 1 ml mixture from the 5th bottle was poured away. So, essential oils in the sample bottles got: in the 1st bottle – pure, that is, as it was before; in the 2nd bottle – twice purified; in the 3rd bottle – 4 times purified; in the 4th bottle – 8 times purified; and in the 5th bottle – 16 times purified. After the purification, a drop of the microbe suspension was added into each sample bottle by means of a Paster piptte. Then, after exposition for 10, 20, 40, and 60 min, sowing was conducted onto the located in the Petri vessel in each sample bottle. The results were registered after keeping the sowings in a thermostat of 37°C for 24 h. *N. trautvetteri, N. velutina* and *N. grosshemi* out of the newly synthesised oils can be considered as active ones with antimicrobic property. So the 2nd and the 4th bottles in sterile state killed bacilli at once. The 2nd bottle – the form purified 4 times killed bacilli in 20 min and the 4th bottle effecting more actively killed *E-coli* in 20 min even when it was purified 8 times. *N. trautvetteri* has a weaker effect than others – as its antimicrobic effect was not observed even in 60 min when it was perified more than 4 times. Purification of *N. velutina* and *N. grosshemi* for 16 times took life activities of the bacilli in an hour. The results are shown in Table 1. In order to study antibacterial and antifungal properties of the newly synthesised essential oils *Staphylolococcus aurens* of gram positive bacteria as a test-culture, *E. coli* (bacilli) and *Pseudomosa auriginosa* (dark blue and green bacilluae pyogenic (hot) abscesses) of gram negative bacteria as well as *Candida albicans* that is leaven-shaped fungus as a representative of fungus were taken.

To study the antimicrob properties disk-diffusion and purification, series methods were used. In the disk-diffusion method a suspension (posessing 1 mGr cell in 1 ml) was prepared from microorganism's culture of a day. Then a little suspension was added into the Petri vessel with Sabouraud's peptone agar in it and it was equally spread onto the medium surface. The rest liquid was sucked by means of pipette and the vessel was dried in the thermostat for 10-15 min. Sterile filter paper disks were put on that surface wetting them by the preparations, then were placed into the thermostst; the result was registered after their cultivasion at 37°C temperature for 18-24 h. So a sterile zone of 20 mm was

Table 1. Effect of 4 species essential oils of the *Nepeta* L.onto bacilli (*E. coli*).

Exposition duration (min)	*N. trautvetteri*					*N. velutina*					*N. zangezura*					*N. grosshemi*					Vaseline(con trole) oil				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
10	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
20	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
40	-	-	-	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+
60	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+

+, Growing; **-**, absence of the growing; 1, 2, 3, 4 and 5, sequence number of the vessel.

Table 2. Antimicrobic effect of newly synthesised essential oils.

Test culture	Exposition duration (min)	*N. trautvetteri*					*N. velutina*					*N. zangezura*					*N. grosshemi*					Vaseline oil (control)				
		1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
E. coli	10	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
	20	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
	40	-	-	-	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+
	60	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
S. aurens	10	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
	20	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+
	40	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	+	+	+	+
	60	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+
P..auraginoza	10	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
	20	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
	40	-	-	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+
	60	-	-	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+	+	+	+	+	+
C. alhicas	10	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+
	20	-	-	-	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+
	40	-	-	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+
	60	-	-	-	-	+	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+

+, Complete growing; **-**, absence of growing; 1,2,3,4 and 5, maintenance number of the vessel.

origioned in the *E. coli* and dark blue and green bacilluae pyogenic (hot) abscesses around the disks wetted by *N. velutina* and *N. grosshemi.* A zone of 18 mm was registered at *Candida* 22 and *Staphylococcus*. Zone of 18 mm was registered at *Candida* 22 and *Staphylococcus*. *N. trautvetteri, N. velutina* and *N. grosshemi E. coli* (bacilli) and *P. auriginosa* (dark blue and green bacilluae pyogenic (hot) abscesses). A zone of 18 mm for *N. trautvetteri E. coli* (bacilli) and *P. auriginosa* as well as a zone of 20 mm for *Candida* and *Staphylococcus* were appared. A zone of 16 mm for *Candida* only has been ascertained around the *N. zangezura* disks; also a sterile zone (vaseline saturated) was not met in control. And 5 sterile vessels were taken from the purification series methods, 1mgr of the examined substance was flowed into the 1st and the 2nd bottles. 1 ml of sterile vazeline was added into each vessel beginning from the 2nd bottle.

Consequently, getting 1 ml of mixture out of the 2nd bottle was poured into the 3rd bottle, getting 1 ml of mixture out of the 3rd into the 4th bottle, getting 1 ml of mixture out of the 4th into the 5th bottle; 1 ml of mixture was thrown away out of the 5th bottle. So essential oils in the sample bottles got: in the 1st bottle – pure, that is, as it was before; in the 2nd bottle – twice purified; in the 3rd one – 4 times purified; in the 4th one – 8 times purified; and in the 5th bottle – 16 times purified. So, after the purification a drop of the microbe suspension posessing 500 mln microe particls in 1 ml solid was added into each sample bottle by means of a sterile paster pipette. Then, after the expositions for 10, 20, 40, and 60 min, sowing was conducted onto the nourishing medium surface located in the Petri vessel in each sample bottle. The results were registered after keeping the sowings in a thermostat under 37°C for 24 h (concerning to fungi they were registered after keeping them under temerature of 28°C for 48 h). The obtained results were shown in Table 2. As it is indicated in Table 2, this characteristics seems enough at all essential oils which antimicrobial effect were investigated. *N. velutina* and *N. grosshemi* have rendered more effective anticrobial effect among them. Thereby in the case of purification of *N. velutina* by means of vaseline oil for 4 times *E. coli* (bacilli) and *P. auriginosa* were killed in 20 min, but in the case of its purification for 8 times life activity of those bacteria was stopped in 40 min. *N. grosshemi* in the case of purification for 4 times killed *Staphylococcus*, dark blue and green bacilluae pyogenic (hot) abscesses in 20 min. Only after its purification for 16 times, *N. velutina* and *N. grosshemi* effected to the bacilli in an hour. *C. albicance* effected onto *N. velutina* most actively. Its purificated form for 8 times could stop development of leaven-shaped fungus in 10 min.

N. trautvetteri and *N. zangezura* are falling behind the others according to their antimicrobic characteristics. So, *N. trautvetteri* in the state of purification for 4 times is able to kill *S. aurens* and *P. auriginosa* in 40 min, but it stopped life activity of *C. albicance* in 40 min. *N. zangezura* is considered as the most passive one among the 4 substances. In other words, it could stop development of *E. coli* (bacilli) and green bacilluae pyogenic (hot) abscesses even in pure state (not perificated) only for 40 min. In the state of its twice purification, it could kill *Candida*, green bacilluae pyogenic (hot) abscesses for 40 min and it could kill *S. aurens* and *E. coli* (bacilli) only for 60 min. At all experiments, full termination (growing) happened. It is realised from the obtained results according to antimicrobial that effect of *N. velutina* and *N. grosshemi* are considered as the most active preparations (*N. trautvetteri* is considered the weaker one). That is why they can be recommended as antimicrobial substance.

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