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Essential Oils of *Polygonum* L. Plants Growing in Kazakhstan and Their Antibacterial and Antifungal Activity

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***Abstract*—**The article represents the results of isolation and component chromatographic analysis of essential oils of *Polygonum* L. plants growing in Kazakhstan in commercial reserves at the territory of Kazakhstan. The results of research of antibacterial and antifungal activity of isolated compounds have been represented.

***Keywords*—**Antibacterial, antifungal, bioactive substances, essential oils, isolation, *Polygonum* L.

I. Introduction

BIOACTIVE substances of plant origin can be one of the advanced means of solution to the issue of combined therapy to inflammation. Usage of different plants for treatment purposes is known when still a high antiquity. Currently, irrespective of intensive growth of synthetic chemistry of medicines, further research of natural pharmacologically active substances is still a topical. This is proved by the fact that about 40% of all medicines applied in the modern medicine are obtained from plant raw material. Along with other biologically active groups of chemical compounds, essential oils with wide range of pharmacological effects became very ingrained in medical practice [1]-[6].

The main advantages of medical plants are softness and width of their therapeutic effect on an organism, the absence of side effects and complications even if the used continuously, high tolerability by patients. Moreover, medial plants are often the only and (or) cost-effective sources of natural biologically active substances and medicines.

In this connection, *Polygonum* L. plants 44 species of which grow in Kazakhstan (8 endemic) and only 4 species pharmacopoeal one are especially noteworthy. Other species are widely used in popular medicine, the reserves of 12 species of wild plants are sufficient for commercial development, but they are not used in official medicine so far [7].

The common property of *Polygonum* L. plants is blood-stopping (vitamin K), anti-inflammatory (flavonoids), astringent (tannins), coating and tonic (polysaccharides) effect [8]-[11].

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The purpose of the research is to study composition of essential oils of *Polygonum* L. plants, establish their antibacterial, antifungal and wound healing effect.

II. Materials and Methods

A. Plant Materials

Plant raw materials (*Polygonum amphibium* L., *Polygonum minus* Huds. Fl. Angl., *Polygonum alpinum* All., *Polygonum persicaria* L.) were collected in the foothill of Zailiyskiy Alatau (the Republic of Kazakhstan) in blossoming period in July 2014. Essential oil was obtained by the method hydrodistillation air-dry aerial part of *Polygonum* L. plants using Clevenger apparatus within 3 hours [12], [13].

B. Chromatography-Mass Analysis

Qualitative composition of essential oils was analyzed by chromatography-mass-spectrometry method using Agilent 6890N apparatus. Silica column DB-XLB FSC (30m ×0.25mm) with helium as a carrier gas was used. The feed rate was 1 ml/min. Gas chromatographic column was kept at 40^0C within 10 min; with temperature programming to 240^0C, with temperature change rate equal to 2^0C/min, and then it was kept in isothermal conditions within 10 min. The sample injection mode is splitless. The sample volume is 1 ml. Evaporator temperature is 250^0C.

Mass-spectra were recorded within the range of m/z 10-425 [14], [15]. The percentage of an essential oil was calculated based on area responses without using adjusting factors. The qualitative analysis is based on the comparison of retention time and full mass-spectra with respective data on components of reference oils and pure compounds, if there were any, and with the data of libraries of mass-spectra Wiley 7th edition and NIST 02.

C. Antibacterial Examinations

Antibacterial activity was researched relating to strains of gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis, Streptococcus agalacticae*, relating to gram-negative strain *Escherichia coli* and to yeast fungus *Candida albicans* using agar diffusion method [16], [17]. The medicines of comparison were gentamicin for bacteria and nystatin for yeast fungus *Candida albicans*.

Daily broth culture was cultivated on liquid medium with pH 7.3±0.2 at the temperature from 30 to 35^0C within 18-20 hours. The cultures were diluted at ration 1:1000 in sterile 0.9% normal saline, put by 1ml to dishes with respective

selective media for researched test-strains and inoculated as per 'solid lawn' method.

After predrying on the surface of agar, wells with 6.0 mm in width were formed using puncher, and alcohol solution of essential oil of *Polygonum* L. plants (1:10) and gentamicin were put by 10 mcl, in control group 96% ethyl alcohol was used in equal-volumetric amounts. The dishes with inoculated strains were incubated at 37^0C; growing cultures were recorded in 24 hours.

Antibacterial activity was assessed based on the diameter of zones of test strains growth inhibition (mm). The diameter of zones of growth inhibition less than 10 mm and confluent growth in a dish was assessed as the absence of antibacterial activity, 10-15 mm – slight activity, 15-20 mm as moderate activity, and over 20 mm as high activity. Every sample was tested in four simultaneous tests.

The cultures of bacteria were maintained in meat peptone agar slants at 4^0C throughout the study and used as stock cultures. For preparation of inocula, cultures were grown until logarithmic phase, and then bacterial density was adjusted to approximately 10^8 colony forming units per ml for disk diffusion method and 10^5 colony forming units per ml for microdilution method with sterile saline solution. Bacterial counts were confirmed by plating out on meat-peptone agar, plates were incubated at 37^0C for 24 h.

Disk diffusion method was used for the screening of essential oils for increase of antibacterial activity in the presence of *Polygonum* L. oil. Bacterial suspension was spread over the plates 85 mm in diameter containing Mueller-Hinton agar using a sterile cotton swab in three directions in order to get a uniform microbial growth. Under aseptic conditions, empty sterile disks were impregnated with 5 µl of essential oil. Disks were left for 5 min at room temperature for better oil absorption and were then placed on inoculated agar surface. A standard disc with ciprofloxacin (10µg/disc) was used as a reference control. The Petri dishes were left for 30 min at room temperature for better oil diffusion and were then placed to an incubator at 37^0C for 24 h. After an incubation period, diameters of inhibition zones around the disks with essential oils were measured.

We assessed diameter of inhibition zones around the disks with essential oils mixtures. For this purpose, we prepared blends of essential oils in sterile eppendorf tubes by mixing 50 µl of *Polygonum* L. oil with 50µl of correspondent second oil. Paper disks were then impregnated with 5 µl of appropriate mixture of essential oils.

Results of disk diffusion assay for study of essential oil mixture were assessed by comparing the experimental inhibition zone area of oils mixture with theoretical inhibition zone area of indifferent combinatory effect (calculated as ½ of inhibition zone area for *Polygonum* L. oil + ½ of inhibition zone area for the second oil).

We prepared serial doubling dilutions of each plant essential oil in 96-well microtiter plates in volume 50 µL of Muellor Hinton Broth to give a range of concentrations from 0.0025% to 5% (v/v). After preparations of suspension of tested cultures 50 µL were added to oil dilutions to produce total volume of 100 µL. The resulting suspensions were then mixed with a micro-pipettor. Two controls were used: positive (50 µL of medium and 50 µL of culture), and negative (100 µL of medium). All microtiter plates with microorganisms were incubated at 37^0C for 24 h. Inhibition of bacterial growth in the wells containing test oil was judged by comparison with growth in negative control well. The minimum inhibitory concentrations were determined by measuring optical density at 570 nm and defined as the concentration of oil at which there was a sharp decline in the absorbance value [18].

Minimum inhibitory concentrations determination of mixtures of essential oils, Mixture of *Polygonum* L. and different essential oils in ratios 1:1 were tested for determinations of minimum inhibitory concentrations by broth microdilution method. In order to assess results of minimum inhibitory concentrations of essential oils in mixtures we calculated fractional inhibitory concentrations with fractional inhibitory concentration indexes [19].

Because mixtures were used in ratio 1:1, individual minimum inhibitory concentration of essential oil in blend was calculated as ½ of minimum inhibitory concentration of blend.

Accordingly to this, fractional inhibitory concentration indexes were calculated as the following : fractional inhibitory concentration of *Polygonum* L. oil = (1/2 minimum inhibitory concentration of blend)/(minimum inhibitory concentration of *Polygonum* L. oil alone); fractional inhibitory concentration of second oils =(1/2 minimum inhibitory concentration of blend) / (minimum inhibitory concentration of second oil alone); fractional inhibitory concentration index = (fractional inhibitory concentration of *Polygonum* L. oil) + (fractional inhibitory concentration of second oil), where second oil is the essential oil which was tested in combination with *Polygonum* L. oil.

Fractional inhibitory concentration indexes were interpreted as following: synergy, fractional inhibitory concentration <0.5; addition, $0.5 \leq$ fractional inhibitory concentration ≤ 1; indifference, $1 <$ fractional inhibitory concentration ≤ 4; antagonism, fractional inhibitory concentration > 4 [20].

Researches of wound healing effectiveness were conducted on 28 white pubertal male rats with 200-250 g in average body weight. The animals were fed in standard vivarium conditions.

To assess the influence of essential oil on healing processes the model of flat dermal wound. To reproduce the model of flat dermal wound the wool and underwool in the area of the middle back was trimmed and skin flap of round shape with 2x2 cm in size was cut off (as well as cutting off subcutaneous fat) under ether narcosis and as per a standard template. Skin defects were left uncovered during the entire period of observations. The speed of wound healing on rats of different groups was judged based on assessment the area of a wound from time to time.

D.Statistical Analysis

The results were statistically processed using 'Statistica 6.0' software package. The obtained results are represented as 'average value ± standard error of an average value'. Inter-group differences were assessed using non-parametric test

Mann-Whitney U-test. The reliable values were those at achieved significance point $p<0.05$.

III. Results and Discussion

The main components of essential oil are for: *Polygonum amphibium* L. - γ-terpinene, borneol, piperitol, 1,8-cyneole, α-pinene, linalool, terpinolene and sabinene; *Polygonum minus* Huds. Fl. Angl. – linalool, terpinolene, camphene, borneol, 1,8-cyneole, α-pinene, 4-terpineol and 1-octen-3-ol; *Polygonum alpinum* All. – camphene, sabinene, 1-octen-3-ol, 4-carene, p- and o-cymol, γ-terpinene, borneol, α-terpineol; *Polygonum persicaria* L. - α-pinene, sabinene, α-terpinene, 4-carene, 1,8-cyneole, borneol, 4-terpineol. Comparison composition of the researched samples of essential oils has been represented in Tables I and II.

TABLE I
Composition of Essential Oils of *Polygonum Amphibium* L. and *Polygonum Minus* Huds. Fl. Angl.

Component	Quantity, %	
	Polygonum amphibium L.	*Polygonum minus* Huds. Fl. Angl.
α-thujene	0.6	0.8
α-pinene	1.6	1.1
Camphene		1.6
β-pinene	0.5	
Sabinene	1.2	0.7
1-octen-3-ol	0.4	1.0
β-myrcene	0.9	
3-octanon		0.2
3-octanol		0.4
α-terpinene	0.5	0.7
2-carene	0.6	0.9
4-carene	0.9	
Terpinolene	1.3	1.8
p-cymol	0.3	
o-cymol	0.2	0.3
Dipentene		0.9
D-limonene	0.4	0.2
1,8-cyneole	1.8	1.3
γ-terpinene	2.1	0.9
cis-β-terpineol	0.3	0.5
Terpinolene	0.8	0.2
Linalool	1.6	1.9
1-terpineol	0.2	0.4
Borneol	2.1	1.6
α-terpineol		0.3
4-terpineol	0.6	1.1
Piperitol	1.8	0.9
Geraniol	0.6	0.8
Bornylacetate	0.1	

Note. The components with the content of not less than 0.1% have been represented in increasing retention time.

The essential oils of *Polygonum* L. plants growing in Kazakhstan were researched for antibacterial and antifungal activity, the results are shown in Table III.

TABLE II
Composition of Essential Oils of *Polygonum Alpinum* All. and *Polygonum Persicaria* L.

Component	Quantity, %	
	Polygonum alpinum All.	*Polygonum persicaria* L.
α-thujene	0.3	0.6
α-pinene	0.8	1.5
Camphene	1.7	0.7
β-pinene	0.9	0.4
Sabinene	2.4	1.5
1-octen-3-ol	1.5	
β-myrcene	0.3	0.6
3-octanon		0.5
3-octanol		0.2
α-terpinene		1.1
2-carene	0.3	0.4
4-carene	1.7	1.6
Terpinolene		0.2
p-cymol	2.2	
o-cymol	1.4	
Dipentene		
D-limonene	0.2	0.8
1,8-cyneole		1.4
γ-terpinene	1.3	0.4
cis-β-terpineol		0.3
Terpinolene	0.6	
Linalool	0.5	0.7
1-terpineol	0.4	
Borneol	1.8	1.1
α-terpineol	1.1	0.9
4-terpineol	0.3	1.8
Piperitol		0.2
Geraniol	0.7	0.3
Bornylacetate	0.5	0.4

TABLE III
Antibacterial and Antifungal Activity *Polygonum* L. Essential Oils

Name of substances	The diameter of growth inhibition zone, mm				
	Sau	Bs	Sag	Ec	Ca
Essential oil *P.amphibium*	18±0.1	20±0.1	16±0.1	15±0.2	16±0.2
Essential oil *P.minus*	14±0.1	17±0.1	20±0.1	18±0.2	12±0.2
Essential oil *P.alpinum*	21±0.1	18±0.1	17±0.1	19±0.2	18±0.2
Essential oil *P. persicaria*	19±0.1	22±0.1	18±0.1	20±0.2	13±0.2
Gentamicin	26±0.1	24±0.1	23±0.1	23±0.2	-
Nystatin	-	-	-	-	22±0.1

Note. Sau - *Staphylococcus aureus,* Bs - *Bacillus subtilis,* Sag - *Streptococcus agalacticae*, Ec - *Escherichia coli*, Ca - *Candida albicans.*

It has been shown that *Polygonum* L. essential oils have moderate antibacterial effect to gram-positive microorganisms and weak antifungal activity to *Candida albicans* yeast fungus.

At the second stage of our researches wound healing properties of ointment form of 3% essential oil was researched on the model of flat dermal wounds.

The animals were allocated into the following groups: control group, where poly ethylene oxide basis was used – 7 rats, experimental group, where Levomekol ointment

reference compounds was used for treatment – 7 rats, experimental group, where Solkoseril ointment reference compounds was used for treatment – 7 rats, experimental group, where 3% ointment based on *Polygonum* L. essential oils on polyethyleneoxide basis was used – 7 rats. The ointments were applied using spatula in the form of applications without using bandages and sponges until a wound is completely healed. During the experiment the general condition of the animals, the intensity of inflammatory reaction and wound epithelialization was assessed.

During research of wound healing properties disturbance of integral in neither group: general condition and behavior of animals, food intake, excretion.

On the second day after operation on control rats the signs of inflammation were florid, wound edges were distinguished with severe oedema. In comparison and experimental group the signs of inflammation were less florid, that gives the evidence of florid anti-exudative action of tested substances.

Healing periods of a cut dermal wound on animals of researched group are shown in Table IV:

TABLE IV
THE PERIODS OF HEALING OF FLAT DERMAL WOUNDS IF OINTMENT FORM OF *POLYGONUM* L. ESSENTIAL OIL ON POLYETHYLENE OXIDE BASIS IS USED

Group of animals	Healing duration, days
Control (polyethylene glycol basis), n=7	18.2±0.3
Levomekol ointment, n=7	16.9±0.2
Solkoseril ointment, n=7	15.7±0.3
3% ointment on the basis of *Polygonum amphibium* L. essential oil, n=7	17.1±0.3
3% ointment on the basis of *Polygonum minus* Huds. Fl. Angl. essential oil, n=7	17.9±0.3
3% ointment on the basis of *Polygonum alpinum* All. essential oil, n=7	16.7±0.3
3% ointment on the basis of *Polygonum persicaria* L. essential oil, n=7	16.3±0.3

On animals treated with polyethyleneglycol basis, the duration of complete wound healing was 18.2±0.3 days. The average period of wound heling if 3% ointment on the basis of *Polygonum amphibium* L. essential oil is used was 17.1±0.3 days, 3% ointment on the basis of *Polygonum minus* Huds. Fl. Angl. essential oil is used was 17.9±0.3 days; 3% ointment on the basis of *Polygonum alpinum* All. essential oil is used was 16.7±0.3 days and 3% ointment on the basis of *Polygonum persicaria* L. essential oil is used was 16.3±0.3 days. Average durations of wound healing using Levomekol and Solkoseril ointments were 16.9±0.2 and 15.7±0.3 days, respectively.

Thus, wound healing action of 3% ointment on the basis of *Polygonum* L. essential oil and polyethyleneglycol is comparable with the action of reference substances. As more favorable healing dynamics was observed in the experimental group than in control group, the tested ointment can be deemed more promising for further detailed study as wound healing means.

IV. CONCLUSION

The method of chromatography-mass-spectrometry was the first that characterized chemical composition of essential oils of four species of *Polygonum* L. plant growing in Kazakhstan.

It has been established that essential oil of *Polygonum* L. plants growing in Kazakhstan has moderate antibacterial action relating to gram-positive microorganisms and antifungal action relating to *Candida albicans* yeast fungus.

Wound healing action of 3% ointment of essential oil of *Polygonum* L. plants on the polyethylene glycol basis was comparable with the action of Levomekol medicine.

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