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Screening of Microbicidal Activity of Some Plants of the Azerbaijan flora in Relation to Antibiotic-Resistant Microorganisms

E. M. Agayeva[1], S. J. Ibadullaeva[2], N. V. Movsumova[2], R. E. Mammadova[1], V. N. Abbasova[3] and I. D. Ganbarly[1]

[1]Azerbaijan Medical University, Baku, Azerbaijan
[2]Institute of Botany of the Azerbaijan National Academy of Sciences, Baku AZ1004, Badamdarshosse 40
[3]Azerbaijan State Agrarian University, Ganja, Azerbaijan

**Corresponding author*

Abstract

Results of study of microbicidal activity and comparative assessment of 9 preparations - water, ethanol extracts, juice and essential oilfrom *Geranium collinum, Menthalongifolia, Rosacanina, R. nisami* species in relation to *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*, MRSA and the antibiotic-resistant of *E.coli* 0127K99 strain have been shown in manuscript. 1, 3, and 4 samples possessed the best microbicidal property in relation to *S. aureus, E. coli, P. aeruginosa*and *C. albicans*. The antimicrobial activity of the 1st sample in relation to the standard and epidemic strains of *S.aureus, E.coli, P.aeruginosa, C.albicans*, as well as strains with antibiotic resistance (MRSA and E.coli strains) have been studied. Thus, geranium ether oil in the indicated concentrations can be used in complex therapy against purulent-inflammatory diseasesof skinand diarrheal diseases. The possibility of replacing chemotherapeutic agents byphytopreparations to prevent the spread of antibiotic resistance of the main pathogens of purulent-inflammatory and diarrheal infections have been established.

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Microbicidal activity, Medicinal plant, Antibiotic-resistant

Introduction

The antibiotic resistance of microorganisms tends to progress due to the widespread use of antibiotics in the last decade [Agaeva *et al*., 2016; Gordinskaya 2013; Sablin 2012; Sidorenko 2002; Warren *et al.*, 2001].

The rapid increase in antibiotic resistance depends both from antibiotic type (its frequent use, prolonged exposure to microorganisms in low concentrations, violation of rational antibiotic therapy schemes, etc.), and from the microorganism type (genetic, biochemical mechanisms) and the state of the macroorganism (decreased of immunobiological reactivity, change of normal microflora, colonization by hospital strains, etc.) [Andreeva *et al.*, 2002; Berezyakov, 2001; Glushenko, 2010; Sidorenko, 2010; Williame, 2001].

Extremely difficult to stop or prevent the formation of resistance of microorganisms, since the basis of this phenomenon is the ability of microorganisms to adapt to changing of environmental conditions. This is the natural

biological response of microorganisms which is formed as a result of natural selection and the ability to survive and reproduce the species [Bogun 2007; Sidorenko 2003].

Thus, resistant strains arise as a result of adaptation, mutation and selection of microorganisms. However, this process by applying the principles of rational antibiotic therapy is possible to slow down.

Significant amount of work is devoted to the development of promising approaches to overcoming the resistance of microorganisms by selecting new treatment methods without the use of antibiotics as shown references review [Ibadullayeva, 2013; Alexandrova, 2007].

The growing interest in herbal medicines is associated with the absence of a side effect, low cost, milder effect, low toxicity, high bioavailability and especially important the absence of development of resistance to the microorganism.

All of the above prompted us to monitor and check the effectiveness of herbal preparations for bactericidal activity against antibiotic-resistant strains of pathogens of purulent-inflammatory and diarrheal diseases.

Thus, the search for new domestic medicinal plants with microbicidal properties to antibiotic-resistant strains is an urgent goal.

The aim of our research is to screen the antimicrobial activity of medicinal plants in Azerbaijan and to select the most effective drugs with antimicrobial activity against antibiotic-resistant strains.

Materials and Methods

The standard cultures and clinical isolates of *S.aureus, E.coli, P.aeruginosa, K.pneumonie, P.vulgaris, C.albicans* isolated from various organs and tissues from 58 patients and 10 *E.coli* strains isolated under diarrheal diseases. Clinical isolates previously isolated from purulent-inflammatory diseases (PID), MRSA, *E. coli, P. aeruginosa, K. pneumonia* and *P. vulgaris* also have been used.

Identification of microorganisms was carried out taking into account morphological, cultural, biochemical and antigenic properties.

Antimicrobial activity was screened by the diffusion method, as well as by VITEK and MariPok.

The minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) of the preparations was determined by the method of serial dilutions.

Suspension of microorganisms was sown with a lawn on the surface of meat peptone agar (MPA) in Petri dishes. Sterile filter paper disks onto which 0.04 ml of herbal preparation was applied (ethanol or aqueous extracts). Crops were incubated for 18-24 hours at 37°C. The results were taken into account by the diameter of the growth inhibition of microorganisms around the disk.

Daily broth culture of bacteria *S.aureus* PM, *E.coli* K12, *E.coli* I.G. and, as well as daily broth cultures of bacteria of museum strains used to assess the spectrum of action of medicinal plants.

Antibiotic-resistant strains *S.aureus, K.pneumoniae, P.aeruginosa, E.coli* and others were also used.

The microbial load was 1.0 million cells/ml, the concentration of microorganisms was determined using a DEN-1B densitometer.

The object of the study was 5 medicinal plants species (Table 1). Plant materials for research were collected in natural meadow and steppe phytocenoses on the territory of the Azerbaijan Republic in 2018-2019 years, during flowering, fruiting and vegetation periods. The collected raw materials were air-dried to an air-dry state and stored in paper bags.

Water and alcohol-water extracts were obtained by the methodology of the State Pharmacology under aseptic conditions. Plant extract and ether oil are prepared in accordance with the requirements of the State Pharmacology (1989).

Ethanol extracts were obtained by percolation in 1:5 ratio using 40% and 70% ethyl alcohol. The obtained ethanol extracts were standardized by the yield of extractives. For this purpose, a suspension by 5 ml weighing was dried at 30-35^{0}C temperature in porcelain evaporation cups to dryness. Then it was cooled for 30 minutes in a desiccator with anhydrous calcium chloride and weighed. The yield of extractives was calculated [g 100 ml (xISx, n = 6)]. Dried ethanol extracts were suspended in water before use.

Screening of antimicrobial activity against 5 mm in the control was observed to *C.albicans*. Antimicrobial activity of aqueous and ethanol extracts from the above herbal preparations have been studied (Table 4).

Water and ethane extracts of all the studied species of the samples had antimicrobial activity to varying degrees have been established in result of the studies.

Results and Discussions

Monitoring of the antimicrobial activity of medicinal plants against pathogens of purulent-inflammatory diseases and diarrheal diseases indicates an increasing of number of antibiotic-resistant strains.

The increasing number of antibiotic-resistant microorganisms in Azerbaijan has prompted us to continue the search for medicinal plants with high microbicidal properties.4 plants used in researches (Table 1).

3%, 5%, 10% solutions prepared and checked their bactericidal and bacteriostatic effects on reference cultures. All studied medicinal plants with the exception of rosehip juice were active against test cultures (*S. aureus, E. coli, P. aeruginosa, C. albicans, K. pneumoniae, P. vulgaris*) and clinical isolates isolated during purulent-inflammatory diseases and diarrheal diseases (Table 3).

Geranium ether oil was possessed by the greatest microbicidal activity.

So, the growth inhibition zone is 15 mm against 8 mm in the control to *S.aureus*, 16 mm against 8 mm in the control to *E. coli*, 10 mm against 7 mm to *P.aeroginosa*, and the highest fungicidal effect - 18 mm.

Alcoholic extract of geranium (home pelargonium) had the highest microbicidal activity for *S.aureus* as can be seen from the table. The growth inhibition zone was 19 mm, while the aqueous solution was 16 mm.

We recommend using alcohol solutions of medicinal plants in future work.

Thus, geranium essential oil was selected as the most active in relation to all cultures, from which various concentrations were prepared (3%, 5% and 10% solutions) and its microbicidal properties were studied.

Table.1 List of studied plant species

№	Plant species	Thestudiedplantcomplex
1	*Geranium collinum* Steph.	Essensialoil, distantwater
2	*Menthalongifolia* (L.) Huds	Extract (waterandethanol)
3	*Rosacanina*L. (seed)	Extract (waterandethanol)
4	*Rosacanina*L.	Juice
5	*R. nisami*Sosn.	Extract (waterandethanol)

Table.2 Screening of antimicrobial activity

Object of research	Study material	Defined indicators
*Geranium collinum, Menthalongifolia, Rosacanina, R. nisami*species (9 preparations - water, ethanol extracts, juice and essential oil)	Bacteria culture *S.aureus P.aeruginosa E.coli C.albicans*	The diameter of the bactericidal and bacteriostatic zones, the minimum inhibitory concentration, the minimum bactericidal concentration

Table.3 The results of the antimicrobial activity of the studied medicinal plants

Studied testcultures	Test samples of herbal preparations (growth inhibition zone, mm)					Control-alcohol				
	1	2	3	4	5	1	2	3	4	5
S.aureus	15	8	12	11	–	8	8	8	7	7
E.coli	16	6	7	10	–	8	8	8	8	7
P.aeruginosa	10	7	7	8	–	7	7	–	–	–
C.albicans	18	5	10	6	–	5	5	7	4	4

Table.4 Microbicidal activity of the studied extracts to *S.aureus*

Type of samples	Microbicidal activity, mm	
	Water extracts	Alcohol extracts
Geranium collinum Steph.	16	19
Menthalongifolia (L.) Huds	8	10
*Rosacanina*L. (Семена)	11	12
*R. nisami*Sosn.	8	11

Table.5 Microbicidal activity of geranium ether oil

Test culture	3% solution (mm)	Control-alcohol	5% solution (mm)	Control-alcohol	10% solution(mm)	Control-alcohol
S.aureus	5	6	8	5	11	5
E.coli	10	5	9	5	8	5
P.aeruginosa	5	5	7	6	6	5
C.albicans	7	5	7	6	8	6
MRSA	4	6	6	5	5	5
E.coli 0127K99	9	5	7	6	6	4

Antimicrobial effect of geranium ether oil in various concentrations (3%, 5% and 10% solutions) was used by the disk diffusion method.

The data are presented in Table 5.

The best bactericidal effect was possessed by 10% solution of geranium essential oil for *S.aureus* as can be seen from the table. Thus, the growth retardation zone of *S.aureus* was 11 mm against 5 mm in the control, and in the antibiotic-resistant strain MRSA (*S.aureus* E, P15) 9 mm against 5 mm.

The growth retardation zone of *E. coli* was 8 mm against 5 mm in a 10% solution. However, the 3% solution was the most effective (the growth inhibition zone was 10 mm against 5 mm), and for the antibiotic-resistant epizootic strain *E.coli* 0127K99 - 9 mm against 5 mm in the control.

In conclusion, microbicidal activity was studied and a comparative assessment of 9 preparations - water, ethanol extracts, juice and essential oil from *Geranium collinum, Menthalongifolia, Rosacanina, R. nisami*species in relation to *S.aureus, E.coli, P.aeruginosa, C.albicans* MRSA and the antibiotic-resistant *E.coli* 0127K99 strain was carried out. 1, 3, and 4 samples possessed the best microbicidal property in relation to *S.aureus, E.coli, P.aeruginosa* and *C.albicans*.

Also, the antimicrobial activity of the 1st sample in relation to the standard and epidemic strains of *S.aureus, E.coli, P.aeruginosa, C.albicans*, as well as strains with antibiotic resistance (MRSA and *E.coli* strains) have been studied.

Thus, geranium ether oil in the above concentrations can be used in complex therapy against purulent-inflammatory diseases and diarrheal diseases.

The possibility of replacing chemotherapeutic agents by phytopreparations was established for prevent the spread of antibiotic resistance of the main pathogens of purulent-inflammatory and diarrheal infections.

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