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SCREENING OF SOME PLANT MATERIALS USED IN ARMENIAN TRADITIONAL MEDICINE FOR THEIR ANTIMICROBIAL ACTIVITY

M. M. GINOVYAN [*], A. H. TRCHOUNIAN [**]

Chair of Biochemistry, Microbiology and Biotechnology YSU, Armenia

Nowadays, in the era of widespread antibiotic resistance, plants are considered as one of most prospective sources for new antimicrobial compounds. The goal of our research was to screen the antimicrobial efficiency of 48 plant parts of 28 wild plants species, which are commonly used in Armenian folk medicine. Maceration technique was used to obtain plant crude extracts. Antimicrobial activity of plant materials was evaluated by agar well diffusion assay against five bacterial and two yeast strains. *Agrimonia eupatoria*, *Hypericum alpestre*, *Lilium armenum*, *Sanguisorba officinalis* and *Rumex obtusifolius* expressed the highest and broadest antimicrobial activity against tested strains and they were selected for further comprehensive studies.

***Keywords*:** Plant material, antimicrobial activity, Armenian folk medicine, crude extract, multidrug resistance.

Introduction. Antibiotic resistance has become one of the most urgent challenges of humanity. There is increasing need for new effective antimicrobials. Plant materials are considered as one of the most promising sources. Large amount of studies are conducted all over the world in order to find antimicrobial properties in plant materials. Several antimicrobial compounds isolated from plants are already registered [1–7].

Republic of Armenia has large diversity of flora. This diversity is mainly due to the variety of climate and landscape [8]. According to literature data, antimicrobial potential of traditional herbs of almost all countries of the region were evaluated in many research works [9–11]. In contrast, there is no massive study, which tried to evaluate antimicrobial properties of plants from RA. There were only some studies, which mainly focused on particular plant species or genera. On the other hand, traditional medicine is well developed in Armenia since ancient times [12, 13]. Moreover, many plant materials were widely used to treat infections of various origin, as well as wounds, burns, inflammations, etc., which can imply the presence of antimicrobial activity. Taking into account these facts, we tried to perform antimicrobial screening of plants of Armenia in order to fill in the existing gap.

We chose prospective plants for screening based on their use in folk medicine. We also paid attention to the plants typical to Armenian region.

Our main goal was to evaluate the antimicrobial activity of 48 water, methanol, chloroform, acetone and hexane crude extracts of 28 wild herbs in order to select most prospective plants for further comprehensive studies.

[*] E-mail: mikayel.ginovyan@ysu.am [**] E-mail: Trchounian@ysu.am

Materials and Methods. The collection of plant materials was done in dry and sunny days. Leaves, flowers and whole plants were harvested during their flowering period. Fruits, seeds and roots were harvested after maturation according to recommendations [14]. Identification of plant materials was done with help of specialists from the Chair of Botany and Mycology of YSU. Harvested fresh plant materials were dried under proper conditions. Then they were fine grounded with a homogenizer and stored in hermetically sealed glass jars at room temperature till further use. Collected plant materials and their details are listed in Tab. 1.

Table 1

The list, description and traditional uses of tested 28 plant species

Plant Name[a]	Common name	Family	Part tasted [b]	Traditional uses [c]
Achillea filipendulina Lam.	yarrow	*Compositae*	A	Purulent wounds, oral, colon and other inflammations
Achillea nobilis subsp. *neilreichii* (A. Kern.) Takht.	yarrow	*Compositae*	A	Purulent wounds, oral, colon and other inflammations
Agrimonia eupatoria L.	common agrimony	*Rosaceae*	A	Hepatitis, nephritis, stomatitis, yellow fever, purulent wounds
Alchemilla sericata Rchb. ex Buser	lady's mantle	*Rosaceae*	A	Purulent wounds, eyelid inflammations
Alchemilla spp.	lady's mantle	*Rosaceae*	A	Purulent wounds, eyelid inflammations
Chelidonium majus L.	greater celandine	*Papaveraceae*	WP	Various skin conditions (wart, scabies, cutaneous tuberculosis), wounds during venereal diseases, purulent wounds, gastrointestinal inflammations, dysentery, syphilis, malaria, helminthiasis, fungal diseases *etc.*
Cichorium intybus L.	common chicory	*Compositae*	A, R	Hepatitis, nephritis, cholecystitis, mucosal inflammations, malaria, fungal infections
Cuscuta europaea L.	greater dodder	*Convolvulaceae*	WP	Helminthiasis, malaria quartana, cholecystitis, gastritis, splenomegaly, hepatitis
Gentiana cruciata L.	star gentian	*Gentianaceae*	A, R	Mucosal inflammation, hepatitis, splenomegaly
Hypericum alpestre subsp. *polygonifolium* (Rupr.) Avet. & Takht.	hypericum	*Hypericaceae*	A	Pneumonia, wounds, hepatitis, cholecystitis gastrointestinal inflammation, nephritis, skin diseases
Inula helenium L.	horse-heal	*Compositae*	L, Fl	Gastrointestinal inflammation, whooping cough, yellow fever
Leonurus cardiaca L.	Mother wort	*Lamiaceae*	A	Cardiac muscle inflammation
Lilium armenum (Miscz. ex Grossh.) Manden.	unknown	*Liliaceae*	L, St, B	Whooping cough, purulent wounds, burns, leprosy, fungal diseases, mastitis, cystitis
Origanum vulgare L.	wd. marjoram	*Lamiaceae*	A	Gastritis, whooping cough, yellow fever
Polygonatum odoratum (Mill.) Druce	angular Solomon's seal	*Asparagaceae*	Rh, A	Lymphnode inflammation, abscesses
Peganum harmala L.	wild rue	*Nitrariaceae*	S, R, St, L, F	Gastritis, intestinal inflammation, nephritis, leprosy
Rubus anatolicus Focke	holy bramble	*Rosaceae*	L, F	Leprosy, gastrointestinal, inflammation, hepatitis, nephritis, yellow fever, burns
Rumex obtusifolius L.	broad-leaved dock	*Polygonaceae*	L, R I, S	Infectious diseases, skin rash, mucosal inflammation
Sambucus ebulus L.	danewort	*Adoxaceae*	L, I, Fr	Various inflammations
Sambucus nigra L.	elderberry	*Adoxaceae*	L, I, Fr	Bronchitis, stomatitis, tonsillitis, dysentery, erysipelas, various inflammations
Sanguisorba officinalis L.	great burnet	*Rosaceae*	A, R	Tonsillitis, purulent wounds, inflammations, skin infections, dysentery, typhoid fever, trichomoniasis, stomatitis, gingivitis, flu

Stachys sylvatica L.	hedge woundwort	*Lamiaceae*	A	Wounds
Thymus kotschyanus Boiss. & Hohen.	wild thyme	*Lamiaceae*	A	Otitis, gastrointestinal inflammation, hepatitis, nephritis, yellow fever, leprosy
Tilia caucasica Rupr.	basswood	*Malvaceae*	L, I	Tonsillitis, stomatitis, red measles, mumps, flu, various inflammations, pneumonia, etc.
Veratrum album L.	European white hellebore	*Melanthiaceae*	A, R	Tuberculosis, pleurisy, purulent wounds, arthritis, hepatitis, pneumonia
Verbascum thapsus L.	common mullein	*Scrophulariaceae*	L, I, R	Whooping cough, cough, hepatitis
Veronica anagallis-aquatica L.	water speedwell	*Plantaginaceae*	A	Burns
Viscum album L.	common mistletoe	*Santalaceae*	WP	Inflammation of lymphnode and other organs, diarrhea helminthiasis, abscesses, wounds, tuberculosis

[a] The plants names has been checked with http://www.theplantlist.org/

[b] The following abbreviations are used: A – aerial part; B – bulb; F – flower; Fr – fruit; I – inflorescence; L – leaf; R – root; Rh – rhizome; S – seed; St – stalk; WP – whole plant.

[c] In this section there are presented only such traditional plants, which can imply the presence of antimicrobial compound.

Plant crude extracts were prepared using maceration technique using five different solvents: distilled water, methanol (98%), chloroform (99%), acetone (99.8%) and hexane (97%) ("Sigma-Aldrich"). Grinded plant materials were soaked with solvents at 10 : 1 solvent-to-sample ratio (v/w). The mixture was thoroughly vortexed for one minute and left in refrigerator at 5º*C* for 24 *h* according to the method developed in [15]. Then the mixtures were filtered through Whatman filter paper. The filtrates were placed in a vacuum chamber at 40º*C* temperature for drying. Further, fresh solvents were added to the residue at the same ratio and incubated at 5º*C* for next 24 *h*. This step was repeated three times in order to achieve maximal extraction of active compounds. Dried crude extracts were weighed and kept at 4–8º*C* till further use.

The samples for antimicrobial assay were prepared by dissolving crude dried extracts in pure DMSO ("Sigma-Aldrich") and then diluted in sterile distilled water in order to get 500 $\mu g/mL$ concentrations.

Following microorganisms were used as test strains: bacteria *E. coli* (EC) VKPM-M17, *P. aeruginosa* (PA) GRP3, *B. subtilis* (BS) WT-A1, *S. typhimurium* (ST) MDC 1754 and *S. aureus* (SA) MDC, yeasts *C. albicans* (CA) WT-174 and *C. guilliermondii* (CG) HP-17.

Gentamycin 10 $\mu g/mL$ (for bacteria) and nystatin 20 $\mu g/mL$ (for yeasts) were used as positive control agents; 5% DMSO was used as negative control agent.

Mueller-Hinton broth medium (MHB) and Mueller-Hinton agar medium (MHA) ("Liofilchem", Italy) were used for cultivation of bacteria. Both NHB and MHA supplemented with 2% Glucose and 0.5 $\mu g/mL$ Methylene Blue Dye were used for cultivation of yeasts [16].

Antimicrobial Assay. Antibacterial and anti-yeast activities of selected plants materials were evaluated by modified agar well diffusion method [17]. Sterile MHA (25 *mL*) was poured into Petri dishes at 50–70°C and left to solidify under ultraviolet light for 15 *min*. Subsequently, a sterile cotton swab was dipped into overnight bacterial or yeast suspensions of indicator strains (adjusted to turbidity of 0.5 McFarland Standard). An agar plate was inoculated by evenly streaking cotton swab over the agar medium. Then wells with a diameter of 8 *mm* were cut in the medium with a sterile cork-borer. The tested samples and controls (100 μL) were added into the

wells. The plates were incubated at 37°*C* for 24 *h*. Then the diameters of growth inhibition zones around the wells were measured.

Antimicrobial tests were independently repeated 3 times. Standard deviations were calculated using GraphPad Prism 5.03 (GraphPad Software, Inc.; USA) software.

Results and Discussion. *In vitro* antimicrobial activity of 48 water, methanol, chloroform, acetone and hexane crude extracts of 28 wild plants collected from Armenia were studied against five bacterial and two yeast strains. The obtained results (except of those with absence of any activity vs all tested microbes) are presented in Tab. 2.

Table 2

Antibacterial and anti-yeast activity of crude extracts of tested 28 wild plant species determined by agar well diffusion assay

Plant spaces, Part tasted	Extract [a]	Diameter of growth inhibition zone with standard deviation, *mm*						
		SA[b]	BS	PA	EC	ST	CG	CA
Achillea filipendulina, L	Met	–	–	–	–	9±0.6	–	–
Achillea nobilis, A	Met	–	–	9±0.6	–	–	–	–
	Chlo	–	–	9±0.6	–	–	–	–
	Acet	9±0.6	–	11±0.6	–	–	–	–
	Hex	9±0.6	–	10±0.6	–	–	–	–
	Wat	–	–	–	9±0.6	–	–	–
Agrimonia eupatoria, WP	Met	10±0.6	11±0.6	11±0.6	11±0.6	–	11±0.6	–
	Chlo	9	11±0.6	10±0.6	10±0.6	11±0.6	12±0.6	9±0.6
	Acet	12±0.6	10±0.6	11±0.6	10±0.6	10±0.6	12±1	9±0.6
	Hex	13±0.6	10±0.6	10±0.6	–	9±0.6	9±0.6	–
	Wat	–	–	–	9±0.6	–	–	–
Alchemilla sericata, A	Met	10±0.6	9±0.6	10±0.6	9±0.6	–	11±0.6	–
	Chlo	–	–	9±0.6	9±0.6	–	10±0.6	–
	Acet	–	–	10±0.6	–	–	–	–
	Hex	–	–	9±0.6	–	–	–	–
	Wat	–	–	10±0.6	–	–	9±0.6	9±0.6
Alchemilla spp., WP	Met	–	9±0.6	9±0.6	9±0.6	–	9±0.6	–
	Chlo	–	–	9±0.6	9±0.6	–	10±0.6	–
	Acet	–	10±0.6	–	11±0.6	–	–	10±0.6
	Hex	–	9±0.6	–	9±0.6	–	–	–
Chelidonium majus, A	Met	–	9±0.6	10±0.6	–	–	–	–
	Chlo	–	–	10±0.6	–	–	–	–
	Acet	–	–	9±0.6	–	–	9±0.6	–
	Hex	–	–	9±0.6	–	–	–	–
Cichorium intybus, A	Wat	9±0.6	–	–	–	9±0.6	9±0.6	–
	Met	10±0.6	–	–	–	10±0.6	–	–
	Chlo	11±0.6	–	–	9±0.6	11±0.6	10±1	–
	Acet	10±0.6	–	–	10±0.6	10±0.6	10±0.6	–
	Hex	10±0.6	–	–	–	–	–	–
Cichorium intybus, R	Wat	9±0.6	9±0.6	–	–	–	–	–
	Met	10±0.6	9±0.6	–	–	–	–	–
	Chlo	–	–	10±0.6	–	–	–	–
	Acet	10±0.6	10±0.6	10±0.6	–	–	–	–
Cuscuta europaea, WP	Chlo	–	–	10±0.6	–	–	–	–
	Acet	10±0.6	10±0.6	10±0.6	–	–	–	–
	Met	–	–	11±0.6	9±0.6	–	–	–
	Chlo	–	–	10±0.6	9±0.6	–	9±0.6	–
	Acet	–	–	12±0.6	–	–	9±0.6	–
	Hex	9±0.6	–	14±0.6	9±0.6	9±0.6	9±0.6	–
Gentiana crusiata, R	Wat	–	–	12±0.6	–	–	–	–
	Met	–	–	12±0.6	–	–	–	–
	Chlo	–	–	9±0.6	–	–	–	–
	Acet	–	–	10±0.6	–	–	–	–
Gentiana crusiata, A	Wat	–	–	12±0.6	10±0.6	–	–	11±0.6
	Met	–	–	11±0.6	–	–	–	9±0.6

	Wat	–	–	12±0.6	–	–	–	–
Gentiana crusiata, R	Met	–	–	12±0.6	–	–	–	–
	Chlo	–	–	9±0.6	–	–	–	–
	Acet	–	–	10±0.6	–	–	–	–
	Wat	–	–	11±0.6	–	–	–	–
Hypericum alpestre, A	Met	10±0.6	11±0.6	18±0.6	10±0.6	–	–	–
	Chlo	13±0.6	12±0.6	23±1	–	–	–	–
	Acet	15±0.6	13±0.6	21±0.6	–	10±0.6	9±0.6	–
	Hex	17±0.6	16±0.6	21±0.6	–	–	–	–
Inula helenium, L	Met	–	–	9±0.6	–	–	–	–
	Chlo	–	–	9±0.6	–	–	–	–
	Acet	–	–	9±0.6	–	–	–	–
	Acet	10±0.6	10±0.6	10±0.6	–	–	–	–
Inula helenium, Fl	Chlo	–	–	10±0.6	–	–	–	–
	Hex	–	–	–	–	9±0.6	–	–
	Met	–	–	11±0.6	9±0.6	–	–	–
	Chlo	–	–	10±0.6	9±0.6	–	9±0.6	–
Leonurus cardiaca, A	Acet	–	–	12±0.6	–	–	9±0.6	–
	Hex	9±0.6	–	14±0.6	9±0.6	9±0.6	9±0.6	–
	Wat	–	–	12±0.6	–	–	–	–
	Met	–	–	12±0.6	–	–	–	–
Lilium armenum, A	Chlo	–	–	9±0.6	–	–	–	–
	Acet	–	–	10±0.6	–	–	–	–
	Wat	–	–	12±0.6	10±0.6	–	–	11±0.6
Lilium armenum, B	Met	–	–	11±0.6	–	–	–	9±0.6
	Wat	–	–	12±0.6	–	–	–	–
	Met	–	–	12±0.6	–	–	–	–
Origanum vulgare, A	Chlo	–	–	9±0.6	–	–	–	–
	Acet	–	–	10±0.6	–	–	–	–
	Wat	–	–	11±0.6	–	–	–	–
	Met	10±0.6	11±0.6	18±0.6	10±0.6	–	–	–
Polygonatum odoratum, Rh	Chlo	13±0.6	12±0.6	23±1	–	–	–	–
	Acet	15±0.6	13±0.6	21±0.6	–	10±0.6	9±0.6	–
	Hex	17±0.6	16±0.6	21±0.6	–	–	–	–
	Met	–	–	9±0.6	–	–	–	–
Polygonatum odoratum, A	Chlo	–	–	9±0.6	–	–	–	–
	Acet	–	–	9±0.6	–	–	–	–
	Met	–	–	10±0.6	–	–	–	–
Peganum harmala, S	Acet	–	–	–	–	9±0.6	–	–
	Chlo	–	–	–	9±0.6	–	–	–
Peganum harmala, R	Acet	–	–	9±0.6	9±0.6	–	–	–
	Hex	–	–	9±0.6	10±0.6	–	–	–
	Wat	–	–	10±0.6	–	–	–	–
	Met	–	–	9±0.6	–	–	–	–
Peganum harmala, BL	Chlo	–	–	–	9±0.6	–	–	–
	Acet	–	–	–	11±0.6	10±0.6	–	–
	Hex	–	–	–	–	9±0.6	–	–
	Met	–	–	–	9±0.6	–	–	–
	Chlo	–	–	–	9±0.6	–	–	–
Peganum harmala, Fl	Acet	–	9±0.6	9±0.6	9±0.6	9±0.6	–	–
	Hex	–	9±0.6	13±0.6	–	–	–	–
	Wat	–	–	9±0.6	–	–	–	–
	Met	–	–	12±0.6	–	9±0.6	–	9±0.6
Rubus anatolicus, LFl	Chlo	–	–	12±0.6	–	9±0.6	14±0.6	–
	Acet	–	–	11±0.6	–	9±0.6	10±0.6	9±0.6
	Hex	10±0.6	–	–	–	–	9±0.6	9±0.6

	Met	9±0.6	–	–	–	10±0.6	14±0.6	–
Rumex obtusifolius, L	Chlo	11±0.6	–	10±0.6	–	10±0.6	9±0.6	–
	Acet	11±0.6	–	12±0.6	–	10±0.6	12±0.6	–
	Hex	9±0.6	–	11±0.6	9±0.6	–	–	–
	Wat	–	–	11±0.6	–	–	–	–
	Met	10±0.6	–	10±0.6	–	–	–	9±0.6
Rumex obtusifolius, R	Chlo	11±0.6	–	10±0.6	–	9±0.6	12±0.6	9±0.6
	Acet	10±0.6	9±0.6	12±0.6	–	–	13±0.6	9±0.6
	Hex	9±0.6	–	–	–	–	12±0.6	–
	Met	–	9±0.6	9±0.6	10±0.6	–	–	–
Rumex obtusifolius, I	Chlo	–	9±0.6	9±0.6	9±0.6	–	–	–
	Acet	11±0.6	11±0.6	9±0.6	10±0.6	9±0.6	9±0.6	–
	Hex	9±0.6	9±0.6	10±0.6	–	–	–	–
	Wat	9±0.6	–	10±0.6	–	–	–	–
	Met	12±0.6	11±0.6	10±0.6	11±0.6	12±0.6	10±0.6	–
Rumex obtusifolius, S	Chlo	–	9±0.6	9±0.6	–	–	–	–
	Acet	12±0.6	12±0.6	10±0.6	10±0.6	12±0.6	10±0.6	–
	Hex	9±0.6	9±0.6	10±0.6	–	–	–	–
	Met	–	–	–	9±0.6	–	–	–
Sambucus ebulus, L	Chlo	–	–	–	9±0.6	–	–	–
	Acet	–	–	–	9±0.6	–	–	–
	Wat	9±0.6	–	11±0.6	–	10±0.6	–	
	Met	9±0.6	–	12±0.6	9±0.6	–	11±0.6	–
Sambucus ebulus, I	Chlo	–	9±0.6	12±0.6	–	–	–	–
	Acet	–	–	12±0.6	–	–	–	–
	Hex	–	–	–	–	12±0.6	9±0.6	–
	Wat	–	–	–	–	9±0.6	–	–
	Met	–	–	–	–	9±0.6	–	–
Sambucus ebulus, Fr	Chlo	–	9±0.6	–	–	–	–	–
	Acet	–	11±0.6	9±0.6	–	–	–	–
	Hex	–	9±0.6	9±0.6	–	–	–	–
	Wat	9±0.6	–	–	11±0.6	9±0.6	10±1	–
	Met	11±0.6	9±0.6	–	11±0.6	–	11±0.6	–
Sambucus nigra, L	Chlo	10±0.6	–	–	10±0.6	–	10±0.6	–
	Acet	–	–	–	–	–	9±0.6	–
	Hex	9±0.6	–	–	–	9±0.6	–	–
	Met	–	–	9±0.6	–	–	–	–
Sambucus nigra, I	Chlo	–	–	11±0.6	–	–	–	–
	Acet	–	9±0.6	11±0.6	–	–	9±0.6	–
	Hex	–	–	9±0.6	–	–	9±0.6	–
	Wat	9±0.6	–	9±0.6	–	–	–	–
	Met	9±0.6	–	10±0.6	–	–	–	–
Sambucus nigra, Fr	Chlo	10±0.6	–	10±0.6	–	–	–	–
	Acet	11±0.6	9±0.6	10±0.6	–	–	–	–
	Hex	9±0.6	9±0.6	9±0.6	–	–	–	–
	Wat	–	–	10±0.6	–	9±0.6	–	–
	Met	12±0.6	11±0.6	10±0.6	10±0.6	12±0.6	9±0.6	10±0.6
Sanguisorba officinalis, A	Chlo	10±0.6	9±0.6	10±0.6	–	–	12±0.6	11±0.6
	Acet	13±0.6	13±0.6	12±1	12±0.6	11±0.6	10±0.6	10±0.6
	Hex	11±0.6	10±0.6	10±0.6	9±0.6	–	10±0.6	9±0.6
	Met	10±0.6	–	10±0.6	10±0.6	–	10±0.6	10±0.6
Sanguisorba officinalis, R	Chlo	–	–	–	–	–	10±0.6	11±0.6
	Acet	10±0.6	10±0.6	10±0.6	10±0.6	–	10±0.6	10±0.6
	Hex	–	–	–	–	–	–	10±0.6
	Wat	–	–	10±0.6	–	–	–	–
	Met	–	–	12±0.6	–	–	–	–
Stachys sylvatica, A	Chlo	–	10±0.6	11±0.6	–	–	–	–
	Acet	–	9±0.6	11±0.6	–	–	–	–
	Hex	–	–	10±0.6	–	–	–	–

Thymus kotschyanus, A	Met	9±0.6	–	–	–	–	–	–
	Chlo	–	–	9±0.6	10±0.6	–	–	–
	Acet	–	–	–	9±0.6	–	–	–
	Hex	10±0.6	–	–	9±0.6	–	–	–
Tilia caucasica, L	Met	–	–	9±0.6	–	–	–	–
	Chlo	–	–	–	9±0.6	9±0.6	9±0.6	–
	Acet	–	–	–	10±0.6	9±0.6	9±0.6	–
	Hex	–	–	–	9±0.6	–	–	–
Tilia caucasica, Fl	Met	–	–	10±0.6	–	9±0.6	–	–
	Chlo	–	–	–	–	9±0.6	–	–
	Acet	9±0.6	9±0.6	10±0.6	–	9±0.6	9±0.6	–
	Hex	–	–	9±0.6	–	–	9±0.6	–
	Wat	–	–	13±0.6	–	–	12±0.6	–
Veratrum album, A	Met	–	–	12±0.6	–	–	–	–
	Chlo	–	–	11±0.6	9±0.6	–	–	–
	Acet	9±0.6	–	11±0.6	9±0.6	–	10±0.6	–
	Hex	9±0.6	–	10±0.6	–	–	–	–
	Wat	10±0.6	–	–	–	–	12±0.6	–
Veratrum album, R	Met	–	–	–	10±0.6	10±0.6	–	–
	Acet	10±0.6	–	–	–	10±0.6	10±0.6	–
	Hex	–	–	–	–	–	11±0.6	–
Verbascum thapsus, L	Wat	–	9±0.6	–	–	–	–	–
	Chlo	–	9±0.6	–	–	–	–	–
Verbascum thapsus, I	Met	–	–	9±0.6	9±0.6	–	–	–
	Chlo	9±0.6	–	–	10±0.6	–	–	–
	Acet	9±0.6	9±0.6	10±0.6	10±0.6	10±0.6	–	–
Verbascum thapsus, R	Met	–	–	9±0.6	–	–	–	–
	Chlo	–	–	10±0.6	–	–	–	–
	Acet	–	–	10±0.6	–	–	–	–
Veronica anagallis, A	Met	10±0.6	–	–	–	–	10±1	–
	Hex	9	–	9±0.6	–	–	–	–
	Wat	–	–	10±0.6	–	–	–	–
Viscum album, WP	Met	–	9±0.6	–	–	–	–	–
	Chlo	10±0.6	–	–	9±0.6	–	–	–
	Acet	9±0.6	–	9±0.6	9±0.6	–	–	–
PC [c]		20±0.6	30±1	28±1	19±0.6	23±0.6	24±0.6	23±0.6

[a] Extracts used–Wat – water, Met – Methanol, Chlo – chloroform, Acet – acetone, Hex – hexane.

[b] Used test strains: *Escherichia coli* EC, *Pseudomonas aeruginosa* PA, *Bacillus subtilis* BS, *Salmonella typhimurium* ST, *Staphylococcus aureus* SA, *Candida albicans* CA, *Candida guilliermondii* CG.

[c] PC – positive control (gentamicin, 10 *μg/mL* (for bacteria), nystatin, 20 *μg/mL* (for yeasts)).

During evaluation of plants antimicrobial activity we used 500 *μg/mL* concentration of their crude extracts (50 *μg* dry material for each well), which is quite low and in accordance with recommendations [5, 18]. Using low concentration of crude extracts allowed as to avoid false positive results and to choose plants with only high activity for further studies.

According to obtained data, all tested plant materials possessed antimicrobial activity against at least one tested strain (Tab. 2). The results showed that crude extracts of 29 tested plant materials of 21 plant species were active against *S. aureus*, *B. subtilis* was susceptible to 24 plant materials of 16 plant species, whereas 40 extracts of 25 plant species were active against *P. aeruginosa* making it the most sensitive strain toward tested extracts. 26 extracts of 18 plant species were active against *E. coli*, 25 extracts of 18 plant species were active against *S. typhimurium*, only 8 plant materials of 7 plant species were active against *C. albicans*, while 25 plant materials of 18 plant species were active against another tested yeast strain *C. guilliermondii*.

It was interesting to determine whether plant crude extracts were active against Gram-negative, Gram-positive bacteria, or both of them, as it could allow us to make some assumptions about mechanisms of their activity (Tab. 2). Crude extracts of *Cuscuta europaea, Gentania crusiata* (A), *Polyganum odoratum* (Rh)*, Tilia caucasica* (L) and *Peganum harmala* (WP) were more active against tested Gram-negative bacteria compared to tested Gram-positive bacteria. On the other hand, crude extracts of *Sambucus nigra* (I, Fr), *Cichorium intybus* (R), *Hypericum alpestre* were more active against Gram-positive bacteria. In case of *Rumex obtusifolius* (S, I)*, Lilium armenum* (B)*, Agrimonia eupatoria, Alchemilla sericata* and *Sanguisorba officinalis* (A) similar activity was observed against both Gram-negative and Gram-positive bacteria.

Only several plant materials showed considerable activity against both tested yeast strains. These were *Sanguisorba officinalis* (A, R), *Rubus anatolicus*, *Alchemilla* spp., *Agrimonia eupatoria*, *Rumex obtusifolius* (R) and *Origanum vulgare*.

The obtained data (Tab. 2) show, that there were differences in antimicrobial activity of different plant parts. For instance, crude extracts of leaves of *Tilia caucasica* inhibited only growth of Gram-negative bacteria and yeast *C. guilliermondii*, whereas its flower extracts possessed inhibitory effect against Gram-positive bacteria as well. The aerial part of *Cichorium intybus* showed higher and broader activity against tested strains compared to its root extracts. In case of *Lilium armenum* bulb had higher antimicrobial effect than stalk with leaves. Inflorescence of *Verbascum thapsus* expressed better antimicrobial properties than other tested parts. All tested parts of *Rumex obtusifolius* demonstrated high antimicrobial activity with some differences. For example, only root extracts exhibited antimicrobial activity against *C. albicans.* In turn they did not inhibit the growth of *E. coli*, whereas other parts did. *B. subtilis* was more sensitive to crude extracts of inflorescence and seeds of *R. obtusifolius*. In contrast, tested yeast strains were more sensitive to leaf and root extracts of this plant.

The data collected during screening was processed and most active plant materials were selected based on diameter of growth inhibition zones and spectrum of their action. According to received data following plants were chosen for further detailed investigation: *Sanguisorba officinalis*, *Rumex obtusifolius*, *Hypericum alpestre*, *Lilium armenum* and *Agrimonia eupatoria*.

Hypericum alpestre's crude extracts induced the largest zones of inhibition among all the tested plant materials. They exhibited activity against almost all tested strains with the exception of *C. albicans* and *S. typhimurium*. We did not found any data in literature about antimicrobial activity of this species although high antimicrobial activities of many other species of the genera have already been shown [19, 20].

There were several reasons to choose *Lilium armenum* for further studies. First of all, the bulb extract of *Lilium armenum* inhibited the growth of all tested bacterial strains. Secondly, this plant is native for Armenia and there were no studies about its antimicrobial properties. And third, it has been widely used in Armenian folk medicine to treat various medical conditions, which can indicate about its antimicrobial properties [12, 13].

Acetone extract of *Rumex obtusifolius* seeds had high antimicrobial activity against all tested strains except *C. albicans*. Other parts of *Rumex obtusifolius* also demonstrated considerable activity, but with less efficiency compared to seeds. Root crude extracts had lower activity than seed extracts. However, they were also interesting due to their activity against *C. albicans.*

Crude extracts of *Agrimonia eupatoria* and *Sanguisorba officinalis* (aerial part) inhibited the growth of all tested bacterial and yeast strains. Although these plants were

widely investigated in many research works, and their high antimicrobial activity was shown [21–23], it could be interesting to study these plants in order to find out any possible differences between the activities of same plant species from various geographical areas.

Since we used five solvents in our screening protocol, the collected data allowed us to evaluate solvents for their efficiency to solubilize antimicrobial compounds from plant materials as well as their other properties. According to obtained data, acetone received best rates followed by methanol.

Thus, screening of 28 plant species used in Armenian folk medicine for antimicrobial properties allowed us to evaluate their antimicrobial properties at 500 $\mu g/mL$ concentration and choose the most active five plant species for further studies. Many of tested plants species had weak antimicrobial activity and thus had no prospective for practical use. On the other hand, the obtained results enabled the evaluation of comparative effectiveness of five deferent solvents for their ability to solubilize antimicrobial compounds from plant materials.

Conclusion. This research showed high antimicrobial properties of some herbs used in Armenian traditional medicine. According to the obtained data, five plants which possessed the highest and broadest antimicrobial properties were selected for further comprehensive studies. We also showed that acetone was the most effective solvent among the tested five solvents for solubilizing antimicrobial compounds from the tested plant materials. Therefore, we propose using acetone in antimicrobial screening protocols as well.

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REFERENCES

1. World Health Organization. Antimicrobial Resistance. Antimicrob. Resist. Glob. Rep. Surveillance. Geneva, Switzerland: WHO Press; 2014.
2. **Abreu A.C., McBain A.J., Simões M.** Plants as Sources of New Antimicrobials and Resistance-Modifying Agents. // Nat. Prod. Rep., 2012, v. 29, p. 1007–1021.
3. **Cowan M.M.** Plant Products as Antimicrobial Agents. // Clin. Microbiol. Rev. 1999, v. 12, p. 564–582.
4. **Savoia D.** Plant-Derived Antimicrobial Compounds: Alternatives to Antibiotics. // Future Microbiol., 2012, v. 7, № 8, p. 979–90.
5. **Ríos J.L., Recio M.C.** Medicinal Plants and Antimicrobial Activity. // J. Ethnopharmacol., 2005, v. 100, p. 80–84.
6. **Upadhyay A., Upadhyaya I., Kollanoor-Johny A., Venkitanarayanan K.** Combating Pathogenic Microorganisms Using Plant-Derived Antimicrobials: A Mini Review of the Mechanistic Basis. Biomed Res. Int., 2014.
7. **Iwu M.M., Duncan A.R., Okunji C.O.** New Antimicrobials of Plant Origin. Perspect. New Crop. New Uses. Alexandria: ASHS Press, 1999, p. 457–462.
8. **Tamanyan K., Fayvush G., Nanagyulyan S., Danielyan T.** Red Book of Plants of the Republic of Armenia. 2010.
9. **Pirbalouti A., Malekpoor F., Enteshari S., Yousefi M., Momtaz H., Hamedi B.** Antibacterial Activity of Some Folklore Medicinal Plants Used by Bakhtiari Tribal in Southwest Iran Abdollah. // Int. J. Biol., 2010, v. 2, p. 55–63.
10. **Uzun E., Sariyar G., Adsersen A., Karakoc B., Ötük G., Oktayoglu E.** et al. Traditional Medicine in Sakarya Province (Turkey) and Antimicrobial Activities of Selected Species. // J. Ethnopharmacol., 2004, v. 95, p. 287–296.

11. **Bazzaz B.S., Haririzadeh G.** Screening of Iranian Plants for Antimicrobial Activity. // Pharm. Biol., 2003, v. 41, p. 573–583.
12. **Torosyan A.** Armenian Herbs. Yer.: Hayastan, 1983.
13. **Harutyunyan H.** Herbs from Armenian Medieval Medical Guides. Yer.: Luys, 1990.
14. **Sarker S.D., Zahid L., Gray A.I.** Natural Products Isolation. V. 20. Springer Science & Business Media, 2005.
15. **Rojas J.J., Ochoa V.J., Ocampo S.A., Muñoz J.F.** Screening for Antimicrobial Activity of Ten Medicinal Plants Used in Colombian Folkloric Medicine: a Possible Alternative in the Treatment of Non-Nosocomial Infections. // BMC Complement. Altern. Med., 2006, v. 6, p. 1–6.
16. **Sheehan D.J., Brown S.D., Pfaller M.A., Warnock D.W., Rex J.H., Chaturvedi V.** et al. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline. NCCLS, 2004.
17. **Ginovyan M., Keryan A., Bazukyan I., Ghazaryan P., Trchounian A.** The Large Scale Antibacterial, Antifungal and Anti-Phage Efficiency of Petamcin-A: New Multicomponent Preparation for Skin Diseases Treatment. // Ann. Clin. Microbiol. Antimicrob., 2015, v. 14, № 28.
18. **Cos P., Vlietinck A.J., Berghe D.V., Maes L.** Anti-Infective Potential of Natural Products: How to Develop a Stronger *in vitro* "Proof-of-Concept." // J. Ethnopharmacol., 2006, v. 106, p. 290–302.
19. **Dall'Agnol R., Ferraz A., Bernardi A.P., Albring D., Nör C., Sarmento L.** et al. Antimicrobial Activity of Some Hypericum Species. // Phytomedicine., 2003, v. 10, p. 511–516.
20. **Saddiqe Z., Naeem I., Maimoona A.** A Review of the Antibacterial Activity of *Hypericum Perforatum* L. // J. Ethnopharmacol., 2010, v. 131, p. 511–521.
21. **Dulger B., Gonuz A.** Antimicrobial Activity of Some Turkish Medicinal Plants. // Pak. J. Biol. Sci., 2004, v. 7, p. 1559–1562.
22. **Kokoska L., Polesny Z., Rada V., Nepovim A., Vanek T.** Screening of Some Siberian Medicinal Plants for Antimicrobial Activity. // J. Ethnopharmacol., 2002, v. 82, p. 51–53.
23. **Shan B., Cai Y.Z., Brooks J.D., Corke H.** The *in vitro* Antibacterial Activity of Dietary Spice and Medicinal Herb Extracts. // Int. J. Food Microbiol., 2007, v. 117, p. 112–119.