ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF PLANTS BELONGING TO *ASTERACEAE* FAMILY ENDEMIC FOR UZBEKISTAN

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Abstract

The increase in the number of diseases that threaten human health in the world causes the need to create new pharmacological drugs to expand year by year. In this regard, the use of natural compounds of plants and their synthetic analogues in the field of pharmaceuticals opens up wide opportunities. In this study the antimicrobial activities of the extracts obtained from 16 endemic plants belonging to the *Asteraceae* against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and yeast (*Candida albicans*) were studied. According to obtained results, ethyl alcoholic extracts were more active compered low polar solvents extracts. Moreover, *Achillea filipendulina, Ligularia macrophylla, Artemisia leucodes, Artemisia annua, Erigeron canadensis, Handelia trichophylla, Lactuca sp., Onopordum acanthium and Tragopogon malicus* extracts showed different levels of activity.

Keywords: Antimicrobial, extracts, antibiotics, endemic plats.

Introduction

Antibiotic resistance is an important issue due to the frequent use of antibiotics for treatment common bacterial infections, indicating that we are running out of effective antibiotics. Enhancement of antimicrobial resistance is strengthening the pathogenicity and virulence of infectious microbes [1,2]. Antibacterial or antifungal drug resistance leads to longer treatment times, higher medical costs, and increased mortality. Data published by the Pan American Health Organization, which coordinates the collection of antibiotic resistance data in hospitals and laboratories in 21 countries, it shows that *Escherichia coli* is resistant to cephalosporins and highly resistant to third generation fluoroquionolones. Fluoroquionolones are one of the most important and widely used types of antibacterial drugs [3,4]. Blood-borne *Klebsiella pneumonia* is one of the most important causes of infectious diseases in newborns and intensive care units, and is high and widespread in all regions of the world. In many parts of the EU (60%) *Staphylococcus aureus* infections are reported to be methicillin-resistant, which means that

treatment with standard antibiotics is ineffective [5]. Therefore, research in the field of creating new, effective antibacterial drugs is a very urgent task [6].

Nowadays, medicinal plants provide an enormous bioresource of potential use in modern medicine and agriculture [7]. Although the biological activity of extracts and natural compounds obtained from approximately 20% of plants in the world has been established [8]. Medicinal plants, on the one hand, have a high biological activity, and on the other hand, the concentration of low molecular weight antioxidants in them is practically unorganized, making them special objects of research [9-12]. In recent years, it is clear that the attitude of doctors towards medicinal plants has changed dramatically and that these plants have a special importance in maintaining the health of the population. For example, 30 crude extracts of 8 plants belonging to the *Asteraceae* family from the Colombian Regional Park of Ucumari were tested for antibacterial activity. As a result, the extracts from the *Asteraceae* family were more bioactive against *Bacillus subtilis* and *Staphylococcus aureus* as well as biologically active against *Candida albicans* and *Fusarium solani* fungi. In addition, the extracts of *Asteraceae species* showed the greatest cytotoxic activity [13]. Therefore, study of antimicrobial activities of extracts of plants of *Asteraceae* family endemic for Uzbekistan is important.

Materials and methods

Collection and extraction of the plants

The studied 16 plants were collected from different regions of Uzbekistan and dried in the shade. The above ground parts of the plants used for research. Plant materials were extracted with ethyl alcohol and non-polar solvents (benzene and choroform) (Table 1).

Table 1

List of the plants used in the experiment

№	Plants	Extraction with ethyl alcohol	Extraction with non-polar solvents
1	*Achillea filipendulina*	+	benzene
2	*Achillea millefolium*	+	choroform
3	*Acroptilon repens*	+	choroform
4	*Artemisia annua*	+	benzene
5	*Artemisia leucodes*	+	benzene
6	*Centaurea ruthenica*	+	choroform

7	*Cichorium intubus*	+	benzene
8	*Cirsium sp.*	+	choroform
9	*Cousinia sp.*	+	choroform
10	*Erigeron canadensis*	+	benzene
11	*Handelia trichophylla*	+	choroform
12	*Lactuca sp.*	+	-
13	*Ligularia macrophylla*	+	choroform
14	*Onopordum acanthium*	+	-
15	*Inula sp.*	+	benzene
16	*Tragopogon m.*	+	-

Antimicrobial activity of plant extracts

The extracts obtained by extraction of plants belonging to *Asteraceae* family growing in Uzbekistan in different organic solvents were tested for antimicrobial activity by the agar disk-diffusion method [14,15]. The antimicrobial activity was evaluated using the following five species of microorganism: Gram-positive bacteria *Bacillus subtilis* RKMUz - 5 and *Staphylococcus aureus* ATCC 25923; Gram-negative bacteria *Escherichia coli* RKMUz - 221 and *Pseudomonas aeruginosa* ATCC 27879; the yeast *Candida albicans* RKMUz - 247. The RKMUz microorganism cultures were obtained from the strain collection of the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan. Sterile nutrient agar (28 g agar/l distilled water) was inoculated with bacterial cells (200 µl of bacterial cell in 2 ml 0.9% NaCl suspension and 25 ml medium) and poured into Petri dishes to give a solid medium. *Candida albicans* (1×10^6 colony forming units per ml) was inoculated into sterile Mueller-Hinton-agar. 2 mg/per disc of test material (the extracts) was applied on sterile paper discs (Whatman No.1, 6 mm diameter). Ampicillin, ceftriaxone and fluconazole (20 µg/disc) were used as positive controls and the solvents as negative controls. The solvents were allowed to evaporate in a stream of air. The discs were deposited on the surface of inoculated agar plates. Plates were kept for 3 h in refrigerator to enable the diffusion of the substances into the agar. Plates with bacteria were incubated for 24 h at 37°C and plates with *Candida albicans* for 48 h at 28 °C. The inhibition zone diameter (including the disc diameter) was measured and recorded

after the incubation time. An average zone of inhibition was calculated for the three replicates in independent assays.

Results and discussion

The plants selected in this work have important medicinal properties and are used in folk medicine. In addition, the biologically active substances in the plant have different natures and are extracted in different organic solvents. Therefore, in our study, we extracted plant materials in ethyl alcohol and other less polar solvents. This helps us identify plants with antimicrobial activity [16,17].

The benzene extract of *Achillea filipendulina* showed the appropriate activity in 8.12±0,13 mm and 11.08±0,12 mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively. These were the highest antibacterial activities of extracts obtained using organic less polar solvents. It was observed that the activities of other plants are relatively lower or absent (Table 2). Ampicillin was used as a control for Gramm-positive bacteria and showed 26.04± 0.10 mm and 27.08±0.12 mm activities against *Staphylococcus aureus* and *Bacillus subtilis* accordingly. None of the plants extracted with non-polar solvents showed activity against Gramm-negative bacteria and yeast *Candida albicans*. Ceftriaxone was used as a control for Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* and exhibited 25.04 ± 0.10 mm and 26.04 ± 0.10 mm activities respectively. Fluconazole was used as a control for *C. albicans* and its inhibition zone diameter was 28.12± 0.13 mm (Table 2).

Table 2

In vitro antimicrobial activities of non-polar solvent extracts isolated from plants belonging to the *Asteraceae* family, n=3

№	Samples	Inhibition zone (mm, ± SD, P≤0.05)				
		Gramm-positive bacteria		Gramm-negative bacteria		Yeast
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. albicans*
1	*Achillea filipendulina*	8.12± 0.13	11.08± 0.12	N.a.	N.a.	N.a.
2	*Achillea millefolium*	7.04± 0.10	6.04± 0.10	N.a.	N.a.	N.a.
3	*Acroptilon repens*	6.04± 0.10	6.04± 0.10	N.a.	N.a.	N.a.

4	*Artemisia annua*	6.08± 0.12	8.04± 0.10	N.a.	N.a.	N.a.
5	*Artemisia leucodes*	6.04± 0.10	7.12± 0.13	N.a.	N.a.	N.a.
6	*Centaurea ruthenica*	6.04± 0.10	6.04± 0.10	N.a.	N.a.	N.a.
7	*Cichorium intubus*	N.a.	N.a.	N.a.	N.a.	N.a.
8	*Cirsium sp.*	N.a.	N.a.	N.a.	N.a.	N.a.
9	*Cousinia sp.*	N.a.	N.a.	N.a.	N.a.	N.a.
10	*Erigeron canadensis*	6.08± 0.12	7.04± 0.10	N.a.	N.a.	N.a.
11	*Handelia trichophylla*	7.04± 0.10	6.04± 0.12	N.a.	N.a.	N.a.
12	*Inula sp.*	7.04± 0.10	7.08± 0.12	N.a.	N.a.	N.a.
13	*Ligularia macrophylla*	8.08± 0.12	7.04± 0.10	N.a.	N.a.	N.a.
Ampicillin		26.04± 0.10	27.08± 0.12	N.t.	N.t.	N.t.
Ceftriaxone		N.t.	N.t.	25.04± 0.10	26.04± 0.10	N.t.
Fluconazole		N.t.	N.t.	N.t.	N.t.	28.12± 0.13

N.a.*-Not active; N.t.*- Not tested.

The antimicrobial activities of alcoholic extracts of above-mentioned plants were carried out. As a result, *Ligularia macrophylla* extract showed the highest activity with 15.08±0,12 mm against *Staphylococcus aureus*. *Achillea filipendulina* extract showed the highest activity that 13.08±0,12 mm against *Bacillus subtilis*. Ampicillin was used as a positive control against Gram-positive bacteria, and it showed 27.08±0,12 mm and 28.04±0,10 mm inhibition zone diameter against *Staphylococcus aureus* and *Bacillus subtilis* respectively. However, there were no samples showing strong activity against Gram-negative bacteria among the extracts. Ceftriaxone showed 26.12± 0.13 mm and 27.12± 0.13 mm activities against *Pseudomonas aeruginosa* and *Escherichia coli* respectively. Only *Erigeron canadensis* extract exhibited 10.04± 0.10 mm inhibition zone diameter against *Candida albicans*. The inhibition zone diameter of fluconazole was 28.04± 0.10 mm (Table 3).

Table 3

In vitro antimicrobial activities of alcohol extracts isolated from plants belonging to the *Asteraceae* family, n=3

№	Samples	**Inhibition zone (mm, ± SD, P≤0.05)**				
		Gramm-positive bacteria		**Gramm-negative bacteria**		**Yeast**
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C.albicans*
1	*Achillea filipendulina*	12.04± 0.10	13.08± 0.12	6.08± 0.12	7.08± 0.12	N.a.
2	*Achillea millefolium*	10.04± 0.10	8.04± 0.10	N.a.	6.08± 0.12	N.a.
3	*Acroptilon repens*	8.04± 0.10	6.04± 0.10	N.a.	N.a.	N.a.
4	*Artemisia annua*	9.08± 0.12	11.04± 0.10	6.08± 0.12	6.04± 0.10	N.a.
5	*Artemisia leucodes*	12.08± 0.12	10.16± 0.20	6.08± 0.12	6.04± 0.10	N.a.
6	*Centaurea ruthenica*	6.04± 0.10	6.04± 0.10	N.a.	N.a.	N.a.
7	*Cichorium intubus*	6.04± 0.10	8.04± 0.10	N.a.	N.a.	N.a.
8	*Cirsium sp.*	N.a.	N.a.	N.a.	N.a.	N.a.
9	*Cousinia sp.*	6.04± 0.10	6.08± 0.12	N.a.	N.a.	N.a.
10	*Erigeron canadensis*	10.08± 0.12	11.04± 0.10	N.a.	7.12± 0.13	10.04± 0.10
11	*Handelia trichophylla*	10.12± 0.10	8.04± 0.12	N.a.	N.a.	N.a.
12	*Lactuca sp.*	10.08± 0.12	8.04± 0.10	N.a.	N.a.	N.a.
13	*Ligularia macrophylla*	15.08± 0.12	10.04± 0.10	N.a.	N.a.	N.a.
14	*Onopordum acanthium*	10.08± 0.12	12.04± 0.10	N.a.	N.a.	N.a.
15	*Inula sp.*	8.04± 0.10	8.04± 0.10	N.a.	N.a.	N.a.
16	*Tragopogon malicus*	10.08± 0.12	8.08± 0.12	N.a.	N.a.	N.a.
17	Ampicillin	27.08± 0.12	28.04± 0.10	N.t.	N.t.	N.t.
18	Ceftriaxone	N.t.	N.t.	26.12± 0.13	27.12± 0.13	N.t.
19	Fluconazole	N.t.	N.t.	N.t.	N.t.	28.04± 0.10

N.a.*-Not active; N.t.*- Not tested.

In this work, the antimicrobial activities of extracts of 16 plants belonging to the *Asteraceae* family in low polar solvents and ethyl alcohol were studied. According to obtained results, ethyl alcoholic extracts were more active compered low polar solvents extracts.

Moreover, we can invite for search antimicrobial active compounds from following plants: *Achillea filipendulina, Ligularia macrophylla, Artemisia leucodes, Artemisia annua, Erigeron canadensis, Handelia trichophylla, Lactuca sp., Onopordum acanthium and Tragopogon malicus.* Because, the plant extracts have shown antimicrobial activity, and their antimicrobial activity can be even higher if the active substances are isolated from them.

References

1. Deshmukh Gao, Y.H.; Zheng, R.; Li, J.; Kong, W.S.; Liu, X.; Ye, L.; Mi, Q.L.; Kong, W.S.; Zhou, M.; Yang, G.Y.; et al. Three new diphenyl ether derivatives from the fermentation products of an endophytic fungus Phomopsis fukushii. J. Asian Nat. Prod. Res. 2019, 21, 316–322.
2. Habboush Y, Guzman N. Antibiotic Resistance. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023 Jan.
3. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, Shah T, Chaudhry TH, Rasool MH, Shahid A, Xueshan X, Baloch Z. Antibiotic Resistance: One Health One World Outlook. Front Cell Infect Microbiol. 2021 Nov 25;11:771510. doi: 10.3389/fcimb.2021.771510.
4. Nowbuth AA, Asombang AW, Tazinkeng NN, Makinde OY, Sheets LR. Antimicrobial resistance from a One Health perspective in Zambia: a systematic review. Antimicrob Resist Infect Control. 2023 Mar 3;12(1):15. doi: 10.1186/s13756-023-01224-0. PMID: 36869351; PMCID: PMC9982795.
5. Kot B. Antibiotic Resistance Among Uropathogenic *Escherichia coli*. Pol J Microbiol. 2019 Dec;68(4):403-415. doi: 10.33073/pjm-2019-048.
6. Yakovlev, S.A. Infectious diseases as a global problem of our time/ S.A. Yakovlev // Territory of Science. 2017. S. 98-113
7. Qi, X., Wang, E, Xing, M., Zhao, W., and Chen, X. (2012). Rhizosphere and non-rhizosphere bacterial community composition of the wild medicinal plant *Rumex patientia*. World J. Microbiol. Biotechnol. 28, 2257–2265. doi:10.1007/s11274-012-1033-2.
8. Barba-Ostria C, Carrera-Pacheco SE, Gonzalez-Pastor R, Heredia-Moya J, Mayorga-Ramos A, Rodríguez-Pólit C, Zúñiga-Miranda J, Arias-Almeida B, Guamán LP. Evaluation of Biological Activity of Natural Compounds: Current Trends and Methods. Molecules. 2022 Jul 13;27(14):4490. doi: 10.3390/molecules27144490.
9. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges

and Future Perspectives. Microorganisms. 2021 Sep 27;9(10):2041. doi: 10.3390/microorganisms9102041.

10. Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, Marquez L, Salam AM, Shabih S, Farrokhi DR and Quave CL (2021) A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. Front. Pharmacol. 11:586548. doi: 10.3389/fphar.2020.586548
11. Bakhora Turaeva, Azam Soliev, Farkhod Eshboev, Lukhmon Kamolov, Nodira Azimova, Husniddin Karimov,Nigora Zukhritdinova and Khurshida Khamidova. The use of three fungal strains in producing of Indole-3-acetic acid and Gibberelllic acid. Plant Cell Biotechnology and Molecular Biology 21(35&36):32-43; 2020
12. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Public Health. 2020 May 12;17(10):3376. doi: 10.3390/ijerph17103376.
13. Antibacterial, antifungal and cytotoxic activities of eight *Asteraceae* and two *Rubiaceae* plants from Columbian biodiversity: Jaim N, Diana M Narvaez, Oscar M.Mosquera. 2007
14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI document M02. 13th Edition. PA, USA. (2018).
15. Askarova, O.K., Bobakulov, K.M., Muradov, M.T. *et al.* Constituent Composition and Antimicrobial Activity of Essential Oil from *Scutellaria oxystegia*. *Chem Nat Compd* 58, 355–357 (2022). https://doi.org/10.1007/s10600-022-03679-w
16. Aldughaylibi FS, Raza MA, Naeem S, Rafi H, Alam MW, Souayeh B, Farhan M, Aamir M, Zaidi N, Mir TA. Extraction of Bioactive Compounds for Antioxidant, Antimicrobial, and Antidiabetic Applications. Molecules. 2022 Sep 13;27(18):5935. doi: 10.3390/molecules27185935
17. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1-10. Epub 2010 Oct 2.