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ANTIMICROBIAL POTENTIAL OF THE PLANT *ANCHUSA OFFICINALIS* L.

Bošković Ivana[1], Dragutin Đukić[2], Pavle Mašković[2], Leka Mandić[2]
[1]University of East Sarajevo, Faculty of Agriculture, Vuka Karadžića 30, East Sarajevo
[2]University of Kragujevac, Faculty of Agronomy Čačak, Cara Dušana 34, Čačak

ABSTRACT

The use of plants in the prevention and treatment of various diseases dates back to ancient civilizations. Knowledge about the medicinal properties of certain plant species has been passed down from generation to generation, and numerous literary sources have confirmed the historical use of plants in healing practices, both locally and globally. Due to the increasingly prevalent occurrence of bacterial resistance to a large number of antibiotics and the ability of plants to synthesize biologically active substances, the use of natural antimicrobial preparations of plant origin in the biological control of pathogenic bacteria is gaining significant importance. Numerous literary sources mention the use of plants from the Boraginaceae family in the treatment of various diseases. Therefore, the aim of this research was to investigate a series of extracts from the plant Anchusa officinalis L. in terms of assessing its antimicrobial potential. The results of our study confirmed that the tested extracts of Anchusa officinalis L. exhibited good antimicrobial potential compared to standard antibiotics such as amracin (for bacteria) and ketoconazole (for fungi).

Key words: Anchusa officinalis, plant, antimicrobial potential

INTRODUCTION

The use of plants in the prevention and treatment of various diseases dates back to ancient civilizations. Knowledge about the medicinal properties of specific plant species has been passed down from generation to generation, and numerous literary sources have confirmed the use of plants in healing practices both locally and globally.

During the process of photosynthesis, plants synthesize a wide range of organic compounds, known as secondary metabolites, which play an essential role in plant development and particularly in their ability to survive unfavorable conditions (Kliebenstein, Osbourn, 2012). In recent decades, certain secondary metabolites, especially phenolic compounds, have garnered significant interest from scientists, biologists, chemists, and microbiologists due to their proven antimicrobial, anticarcinogenic and antioxidant properties. According to Álvarez-Martínez et al. (2021), the extensive chemical diversity of plant secondary metabolites, coupled with their long history of traditional use, makes plants highly attractive as natural reservoirs for discovering new antimicrobial compounds.

Due to the increasing occurrence of bacterial resistance to a large number of antibiotics and the ability of plants to synthesize biologically active substances, the use of natural antimicrobial preparations of plant origin in the biological control of pathogenic bacteria is gaining significant importance (Đukić, Vesković, 2015).

Rapid technological advancement and the application of new, increasingly efficient methodologies have led to the identification and characterization of numerous antibacterial agents in recent years (Katz and Baltz, 2016).
Numerous literary sources mention the use of plants from the Boraginaceae family in the treatment of various diseases. *Pulmonaria officinalis* L. has long been used as a remedy for cough, while *Symphytum officinale* L. is used for throat inflammation, arthritis, joint swelling, sprains, and bone fractures (Bošković, 2017). In Turkish folk medicine, the roots of *Echium italicum* and *Echium vulgare* are used externally for wound healing (Yesilada et al., 1995; Sezik et al., 1997; Altundag et al., 2011; Fujita et al., 1995). Al-Snafi (2014) mentions the use of plants from the Boraginaceae family in traditional medicine for treating fever, asthma, kidney stones, and wound healing.
Anchusa belongs to the Boraginaceae family, and there are 30 species in the *Anchusa* genus found in Europe and Asia, with several species growing in open fields and waste places (Su et al., 1994). According to Launert's research (1981), all parts of *Anchusa officinalis* L. are traditionally used in medicine for expectorant effects, treating cuts and bruises, varicose veins, and stomach ailments. Additionally, Khare (2007) and Amin (2005) mention the use of the *Anchusa* genus in folk medicine.
Therefore, the aim of this research was to investigate the antimicrobial potential of *Anchusa officinalis* L.

MATERIALS AND METHODS

Preparation of plant extracts

The plant under investigation was crushed using a cylindrical crusher, and then extraction was performed using a series of solvents in a Soxhlet apparatus (ethanol, acetone, chloroform, ethyl acetate, petroleum ether).

Determination of antimicrobial potential of plant extracts using the microdilution method

The determination of antimicrobial potential of plant extracts was carried out using the microdilution method (CLSI, 2012) on the following bacterial cultures: *Listeria innocu* ATCC 33090, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19112, *Bacillus spieizenii* ATCC 6633, *Enterococcus faecium* ATCC 6057, *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophiticus* ATCC 15035, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 14153, *Citrobacter freundii* ATCC 43864, *Pseudomonas aeruginosa* ATCC 27853, and the fungi *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404.

RESULTS AND DISCUSSION

Plant tissues contain secondary metabolites with antibacterial and antifungal properties, making them a source of natural bioactive molecules for controlling pathogens, the main causes of diseases in plants, animals, and humans (Bošković, 2017). As a result, there has been increased interest in the antimicrobial activity of traditional medicinal plants in recent decades. The antimicrobial activity of plant extracts was investigated using the microdilution method against bacteria and fungi, determining the minimum inhibitory concentrations

(MIC) that prevent the growth of tested microorganisms. Table 1. presents the results of the MIC of the tested plant. All tested extracts exhibited good antimicrobial potential with MIC values ranging from 3.94 µg/ml to 125 µg/ml, indicating strong antimicrobial activity compared to standard antibiotics. The ethanolic extracts of *Anchusa officinalis* demonstrated higher antimicrobial potential than the other extracts, particularly the petroleum ether and acetone extracts. The ethanolic extract showed the best antimicrobial potential against *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 6057 and *Candida albicans* ATCC 10231 (MIC=3.94 µg/ml). It exhibited slightly weaker antimicrobial potential against *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 13883, *Listeria monocytogenes* ATCC 19112 and *Aspergillus niger* ATCC 1640 (7.875 µg/ml), and the weakest antimicrobial potential against bacteria *Proteus mirabilis* ATCC 35659, *Citrobacter freundii* ATCC 43864, *Pseudomonas aeruginosa* ATCC 27853, *Listeria innocu* ATCC 33090, *Bacillus spieizenii* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus saprophiticus* ATCC 115035 (62.5 µg/ml).

Table 1. Minimum inhibitory concentration (µg/ml) *Anchusa* officinalis L. extracts

Microorganisms/extract	*Ethanol*	*Ethyl acetate*	*Chloroform*	*Petroleum*	*Acetone*	*Amracin*	*Ketoconazol*
MIC µg/ml							
Proteus mirabilis	62.5	62.5	31.25	62.5	125.00	0.49	-
Escherichia coli	7.875	125.00	31.25	125.00	31.25	0.97	-
Klebsiella pneumoniae	7.875	125.00	31.25	125.00	125.00	0.49	-
Citrobacter freundii	62.5	62.5	31.25	62.5	125.00	0.49	-
Pseudomonas aeruginosa	62.5	125.00	31.25	125.00	125.00	0.97	-
Listeria inocuu	62.5	62.5	31.25	62.5	125.00	0.49	-
Enterococcus faecalis	3.94	31.25	7.875	31.25	7.875	0.97	-
Listeria monocytogenes	7.875	125.00	31.25	125.00	31.25	0.97	-
Bacillus spieizenii	62.5	125.00	31.25	125.00	125.00	0.97	-
Enterococcus faecium	3.94	62.5	31.25	125.00	62.5	0.49	-
Staphylococcus aureus	62.5	125.00	31.25	125.00	125.00	0.97	-
Staphylococcus saprophiticus	62.5	125.00	31.25	125.00	125.00	0.97	-
Aspergillus niger	7.875	62.5	31.25	3.94	62.5	-	0.97
Candida albicans	3.94	31.25	7.875	31.25	7.875	-	1.95

The mode of action of antimicrobial agents from plant phenolic compounds depends on the type of microorganism and is mainly influenced by the structure of their cell wall and cytoplasmic membrane, as well as the concentration of the active substance, and so on. A significant mechanism of antibacterial activity of substances is the disruption of the cell's energy status. Ilić (2014) pointed out in his research that the activity of essential oils and their components is directed towards the disruption of the proton gradient and membrane potential of the cytoplasmic membrane. Therefore, the mechanisms of action of active substances (phenols, essential oils, etc.) from plant extracts on different microorganisms are highly complex (Kalemba, Kunicka, 2003; Burt, 2004; Morteza-Semnani et al., 2006).

CONCLUSION

The medicinal properties of plants from the Boraginaceae family have prompted us to investigate the antimicrobial potential of *Anchusa officinalis*. The results of our research have confirmed that the tested extracts of the plant exhibited good antimicrobial activity compared to standard antibiotics - amracin (for bacteria) and ketoconazol (for fungi). In this regard, we believe that further research of plants of the genus *Anchusa* regarding their antimicrobial potential would be of great importance for this scientific field.

REFERENCES

Al Snafi A. E. The pharmacology of Anchusa italica Retz. and Anchusa strigosa Lab. Review, International journal of pharmacy and pharmaceutical sciences, Vol. 6, Issue 4, 2014.

Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine, 2021.

Altundag E., Özer M. Procedia Social and Behavioral Sci., 19, 756, 2011.

Amin GH. Popular medicinal plants of Iran. Tehran University of Medicinal Sciences, Tehran, pp 38–162, 2005.

Bošković I. Antimicrobial and antioxidant activity of plant extracts from family *Boraginaceae*. PhD thesis, University of Montenegro, Podgorica, 2017.

Burt S. Essential oils: their antibacterial properties and potential applications in foods —a review. International Journal of Food Microbiology 94, 2004.

CLSI. Methods for dilution antimicrobial susceptibility tests f or Bacteria that grow aerobically; approved St andard—ninth edition. CLSI document M07-A9, 2012.

Đukić D., Vesković S. Bioprotektori u proizvodnji hrane, HL print d.o.o, Bački Petrovac, 2015.

Fujita T., Tabata M., Yeşilada E., Honda, G., Takeda Y., Tanaka T.,Takaishi Y. Econ. Bot., 49, 406, 1995.

Ilić B. Hemometrijska analiza rezultata hemijskih i bioloških istraživanja farmakološki značajnih biljaka, Doktorska disertacija, Niš, 2014.

Kalemba, D., Kunicka, A. Antibacterial and antifungal properties of essential oils, Current Medicinal Chemistry 10, 2003.

Katz L., Baltz R.H. Natural product discovery: past, present, and future. J. Ind. Microbiol. Biotechnol. 43, 155–176, 2016.

Khare CP. Indian medicinal plants - an illustrated dictionary. Springer Science and Business Media LLC, New York, 2007.

Kliebenstein D.J., Osbourn A. Making new molecules – evolution of pathways for novel metabolites in plants, Current Opinion in Plant Biology, 15, 2012.

Launert, E. Edible and Medicinal Plants, Vol 21. Hamlyn UK, ISBN 0-600-37216-2, 1981.

Morteza-Semnani K., Saeedi M., Akbarzadeh M. Essential oil composition of Teucrium scordium, Acta Pharmaceutica 57, 2007.

Sezik E., Tabata M., Honda G., Takashi Y., Fujita T., Anaka Y. Takeda, T. Economy Botany, 51, 1997.

Su WW., Asali EC., Humphrey AE. Anchusa officinalis: Production of Rosmarinic Acid in Perfusion Cell Cultures. In: Bajaj, Y.P.S. (eds) Medicinal and Aromatic Plants VI. Biotechnology in Agriculture and Forestry, vol 26. 1994.

Yesilada E., Honda G., Sezik E.,Tabata M., Fujita T., Tanaka T., Takeda Y., Takaishi Y. J Ethno-pharmacol., 46**,** 133, 19