DETERMINATION OF BIOLOGICAL ACTIVITY OF CYNARA SCOLYMUS L. ROOT EXTRACT AGAINST HARMFUL MICROORGANISMS IN THE TERRITORY OF UZBEKISTAN

[1]Kh.Kh. Dolimov, [2]I.J. Jalolov, [3]M.Y.Imomova, [4]M.R.Mamadaliyev

[1]Lecturer at Department of Botany and Biotechnology, Fergana State University

[2]Doctor of Philosophy on Chemical Sciences (PhD), Associate Professor, Fergana State University

[3]Doctor of Philosophy on Chemical Sciences (PhD), Associate Professor, Department of Botany and Biotechnology, Fergana State University

[4]Student in the Field of Medicine, International Faculty of Fergana Public Health Medical Institute

https://doi.org/10.5281/zenodo.8029301

Abstract. *The biologic activity of extracts obtained from the leaves of Cynara scolymus L., belonging to the Asteraceae family, using ethyl alcohol and low polar solvents, was investigated against Gram-positive bacteria (Staphylococcus aureus ATCC 25923 and Bacillus subtilis RKMUz-5), Gram-negative bacteria (Pseudomonas aeruginosa ATCC 27879 and Escherichia coli RKMUz-221), and yeast (Candida albicans RKMUz-247). As a result of the research, it was found that extracts obtained using ethyl alcohol exhibited high activity against Gram-positive bacteria.*

Keywords: *Cynara scolymus L., Asteraceae, biologic properties, secondary metabolites, Gram-positive, Gram-negative, bacteria, extract.*

I. INTRODUCTION

In recent years, there has been a significant increase in the importance of utilizing approximately 20% of extracts and natural compounds derived from plants for their biological activity [1]. Research in the field of creating new and effective antibacterial drugs is a challenging and crucial task. Antibacterial or antimicrobial resistance leads to the emergence of treatment-resistant mutations, resulting in prolonged treatment duration, increased medical costs, and higher mortality rates [2]. The resistance to antibiotics occurs naturally as a result of identifying random mutations related to antibiotic effects [3]. Literature indicates a sharp shift in the attitude of healthcare professionals towards herbal plants in recent years, emphasizing their importance in maintaining public health [4]. Herbal plants and their derived substances have been shown to possess highly active biological compounds for both higher organisms and humans [5]. According to the data collected by the Pan-American Health Organization's Joint Secretariat for Surveillance and Treatment of Antibiotic Resistance (JSST America), Escherichia coli shows significant resistance to cephalosporins and third-generation fluoroquinolones. Fluoroquinolones are considered one of the most important and widely used types of antibacterial drugs [6]. In many European Union countries, it has been reported that 60% of Staphylococcus aureus infections are methicillin-resistant, indicating unsuccessful battles against this microorganism using standard antibiotics (JSST Information Bulletin, October 2020).

In the Colombian Ucumari region, extracts from Cynara scolymus L., a member of the Asteraceae family, and 30 other extracts from seven additional plants were subjected to testing for antibacterial activity. The antibacterial activity of these extracts was evaluated against two Gram-

positive and two Gram-negative bacteria, as well as three fungi. The results revealed the biological activity of the extracts against Bacillus subtilis and Staphylococcus aureus bacteria, as well as Candida albicans and Fusarium solani fungi. Additionally, the extracts from Asteraceae species exhibited the highest cytotoxic activity. Notably, Gonzalagania rased standl (rubuaceae) was the most significant species in this study, as its methanol and dichloromethane extracts demonstrated potent and moderate biological activity against C. albicans and F. solani, respectively. However, no specific information was provided regarding the biological activity of the other eight plants mentioned [5,6]. Moreover, certain Astraceae species, such as Marigold flowers, have been patented as part of a plant series for the treatment of hepatitis B virus (HBV) [7,8,9]. C. arvensis extract exhibited antibacterial activity against golden staphylococcus at concentrations of 10-25 mg/ml [10]. Sesquiterpene glycosides derived from C. arvensis have the potential to inhibit HBV [11,12]. The ethanolic extract of C. officinalis flowers showed an effect against OIV [13].

II. METODS

The research focuses on the extract of the plant Cynara scolymus L, which belongs to the Asteraceae (composite) family. The research utilizes microbial cultures of bacteria and yeast strains as the test organisms. The strains of microorganisms used in the research are maintained in the Molecular Genetics Laboratory of the Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan. The extract of Cynara scolymus L. root was obtained using several different solvents, namely ethanol, ethyl acetate, and chloroform. The extraction process involved drying and grinding the root, followed by extraction with the respective solvents in a 1:5 ratio. The resulting extracts were combined, concentrated, and dried using a vacuum rotary evaporator. The initial identification of the obtained extracts was performed using thin-layer chromatography on Silufol plates and comparing them to known standard samples. The extracts were tested for their antimicrobial activity against two Gram- positive bacteria, Bacillus subtilis (O'zMT-5) and Staphylococcus aureus (O'zMT 25923), two Gram-negative bacteria, Pseudomonas aeruginosa (O'zMT 27879) and Escherichia coli (O'zMT 27879), and two pathogenic yeasts, Candida albicans (O'zMT-247) and Pichia anomala (O'zMT).

The microbial strains used in the experiments were obtained from the Microbial Strain Collection of the Institute of Microbiology of the Academy of Sciences of Uzbekistan. The antimicrobial activity of the synthesized compounds was determined using the modified agar disk diffusion method. Sterile solid agar (LB Agar) and Petri dishes were prepared, and bacterial cells were inoculated onto the agar plates. For the yeasts Candida albicans and Pichia anomala, sterile Mueller-Hinton agar was used. Sterile filter paper discs (Whatman No.1, diameter 6 mm) impregnated with 40 µl of the extract solution (equivalent to 0.2 mg/disk) were placed on the agar surface. Positive control antibiotics, ampicillin (for Gram-positive bacteria), ceftriaxone (for Gram-negative bacteria), and fluconazole (for yeasts) were used. The plates were incubated at appropriate temperatures for specific durations, and after incubation, the diameter of the inhibition zones was measured and recorded.

III. RESULTS

The following table presents the extracts obtained from Cynara scolymus L. roots collected within the territory of Uzbekistan for conducting this research (Table 1).

Table 1.

Information about the plant extracts used in the study

№	Plants used in research	Parts of examined plants	Alcoholic extract (ethanol)	Ethyl acetate extract	Chloroform extract
1	*Cynara scolymus* L	Root part	+	+	+

In the study conducted, the activity of the extract obtained from the aerial part of the Cynara scolymus L plant was investigated against various strains of Gram-positive and Gram-negative bacteria, including Staphylococcus aureus ATCC 25923, Bacillus subtilis RKMUz-5, Pseudomonas aeruginosa ATCC 27879, and Escherichia coli RKMUz-221. As a result, the alcoholic extract of the Cynara scolymus L plant exhibited the highest activity against the Staphylococcus aureus ATCC 25923 strain, with a diameter of inhibition zone of 15.08±0.12 mm. During this phase of the research, Ampicillin antibiotic was used as a positive control against Gram-positive bacteria, and it showed inhibition zones of 27.08±0.12 mm and 28.04±0.10 mm against Staphylococcus aureus ATCC 25923 and Bacillus subtilis RKMUz-5 strains, respectively. The obtained plant extracts showed significant activity against Gram-negative bacteria as well. As a positive control, Ceftriaxone antibiotic exhibited inhibition zones of 26.12±0.13 mm and 27.12±0.13 mm against Pseudomonas aeruginosa ATCC 27879 and Escherichia coli RKMUz-221 strains, respectively. The antibacterial activity results against Gram-positive and Gram-negative bacteria are fully presented in the following table (Table 2).

Table 2

In vitro activity of extracts from the root of *Cynara scolymus* L. belonging to the *Asteraceae* family against Gram-positive and Gram-negative bacteria n=3

№	Samples	Inhibition diameter (mm, ± SD, P≤0.05)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus ATCC 25923	***B. subtilis*** RKMUz – 5	***P. aeruginosa*** ATCC 27879	***E. coli*** RKMUz – 221
1.	*Cynara scolymus* L (ethanol)	15.08±0.17	13.01± 0.14	10.08± 0.12	12.04± 0.10
2	*Cynara scolymus* L (ethyl acetate)	10.07±0.10	11.02± 0.12	10.11± 0.15	NA
3	*Cynara scolymus* L (Chloroform)	9.08±0.11	NA	9.01± 0.13	NA
4	**Ampicillin**	27.08± 0.12	28.04± 0.10		
5	**Ceftriaxone**			26.12± 0.13	27.12± 0.13

NA*- not active

Determination of antifungal activity of extracts isolated from the root of *Cynara scolymus* L. belonging to the *Asteraceae* family

Cynara scolymus L., which belongs to the Asteraceae family, was investigated for its activity against Candida albicans RKMUz-247, a fungus. The ethyl alcohol and low polar extracts obtained from the Cynara scolymus L. plant were tested for their activity against Candida albicans. According to the obtained results, only the alcoholic extract derived from the plant's root exhibited an inhibition zone of 10.04±0.10 mm against Candida albicans. The remaining extracts did not show significant activity against Candida albicans. As a positive control, Fluconazole antibiotic demonstrated an inhibition zone of 28.04±0.10 mm, confirming the validity of the conducted research. The antifungal activity of the plant extracts against Candida albicans is fully presented in the following table (Table 3).

Table 3

***Cynara scolymus* L. belongs to the *Asteraceae* family In vitro antifungal activity of root extracts n=3**

Samples		**Inhibition diameter (mm, ± SD, P≤0.05)**
		Candida albicans
1	*Cynara scolymus* L (ethanol)	10.04± 0.10
2	*Cynara scolymus* L (ethyl acetate)	NA
3	*Cynara scolymus* L (Chloroform)	NA
Fluconazole		28.04± 0.10

NA*- not active.

For instance, the biological activity of the extracts obtained from the root of Cynara scolymus L., belonging to the Asteraceae family, was investigated against in vitro Gram-positive and Gram-negative bacteria, as well as Candida albicans. As a result, the alcoholic extract derived from the plant's root exhibited a significant positive effect against harmful bacterial strains. Furthermore, only the alcoholic extract obtained from the root showed an inhibition zone of 10.04±0.10 mm against Candida albicans.

IV. CONCLUSION

At present, research work is being conducted on the separation of secondary metabolites from the extracts that have shown promising results and determining the biological activities of the extracts obtained from various parts of the plant against different types of microorganisms.

REFERENCES

1. Antibacterial, antifungal and cytotoxic activites of eight asteraceae and two rubiaceae plants from Columbian biodiversity: Jaim N, Diana M Narvaez, Oscar M.Mosquera. 2007.
2. Paula, J. C. N. V. F., David, J. M., David, J. P. Occurrence, biological activity and 13C NMR data of amides from Piper (Piperaceae). Quim. Nova, 2012, 35, p. 2288-2311

3. Яковлев, С.А. Инфекционные заболевания как глобальная проблема современности/ С.А. Яковлев// Территория науки. - 2017. С. 98-113.
4. Al-Taweel, A.M., Perveen, S., El-Shafae, Fawzy, G.A., Malik, A., Afza., Iqbal, L., Latif, M. Bioactive phenolic amides from Celtis Africana. Molecules 2012, 17, 2675 – 2682.
5. Масленников П.В., Чупахина Г.Н., Скрыпник Л.Н. Содержание фенольных соединений влекарственных растениях ботанического сада // Известия Российской академии наук. Сериябиологическая. 2013. С. 47-62.
6. Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml., Wang, L., Scwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovolovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., Stuppner, H. Discovery and resupply of pharcologically active plant-derived natural products: a review. Biotechnology advances 2015, 1582 – 1614.
7. Ávila, H.P., De Fátima Albino Smânia, E., Monache, F.D, Júnior, A.S. Structure-activity relationship of antibacterial chalcones. Bioorg. Med. Chem. 2008, 16, 9790 – 9794.
8. Balunas, M. J., Kinghorn, A. D., Drug discovery from medicinal plants. Life Sci. 2005, 78, 431 – 441.
9. Brielman, H. L., Setzer, W. N., Kaufman, P. B., Kirakosyan, A., Cseke, L. J. Phytochemicals: The chemical components of plants, in Natural products from plants 2nd.Taylor & Francis Group, Boca Raton. 2006, 22 – 25.
10. Büchi, G., Crombie, L., Godin, P. J., Kaltenbronn, J. S., Siddalingaiah, K. S., Whiting, D. A. The absolute configuration of rotenone. J. Chem. Soc. 1961, 2843 – 2840.
11. Chin, Y. W., Mdee, L. K., Mbwambo, Z. H., Mi, Q., Chai, H. H., Cragg, G. M., Swanson, S.M., Kinghorn, A. D. Prenylated flavonoids from the root bark of Berchemia discolor, a Tanzanian medicinal plant. J. Nat. Prod. 2006, 69, 1649 – 1652.
12. Christenhusz, M. J. M., Byng, J. W. The number of known plants species in the world and its annual increases. Phytotaxa. 2016, 261, 201 – 217.
13. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standart – Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standarts Institute; 2012.