DETERMINATION OF ANTIMICROBIAL ACTIVITY OF EXTRACTS OF ENDOPHYTIC FUNGI ISOLATED FROM THE ROOTS OF *CYNARA SCOLYMUS* L GROWING IN THE UZBEKSITON REGION

Kh.Kh. Dolimov,

Ferghana Medical Institute of Public Health

Abstract

Today, the increasing need for antibiotics is the reason to search for new sources of antibiotics and conduct research on them. We think that endophytic fungi living in symbiosis with plants serve as a natural source of antibiotic substances.

Methods and Results: Wash the healthy roots under tap water to remove adhering soil particles and epiphytes. After that the healthy roots sample is cut into small pieces of approximately 0.5 cm × 0.5 cm. Immerse the roots samples in 0.1% mercuric chloride solution for 10 min. Followed by 2 min in 70% ethanol. Wash the samples with distilled water for several times and keep them in a sterile blotting paper for dryness. The object of our research work is extracts of endophytic fungi isolated from the root of *Cynara scolymus* L, belonging to the *Asteraceae* family. Microorganisms used in research (bacterial and fungal strains) cell culture. Conditionally pathogenic microorganism strains stored in the Laboratory of Molecular Genetics of the Institute of Chemistry of Plant Substances of the Republic of Uzbekistan : *Bacillus subtilis UzMT - 5, Pseudomonas aeruginosa UzMT - 225, Staphylococcus aureus UzMT - 91, Escherichia coli UzMT – 221, Candida albicans UzMT – 247*. To date, three endophytic fungi have been isolated and extracted from the roots of the plant *Cynara scolymus* L belonging to the *Asteraceae* family. The obtained extracts showed significant activity against harmful bacteria and fungal strains.

Conclusion : Endophytic fungi will serve as the main natural source of antibiotics in the future. The obtained research results serve as an important source for future experimental processes.

Keywords*:* *Cynara scolymus L, Asteraceae,* biological properties, secondary metabolites, endophyte, fungus, antibiotic**.**

Introduction

With the increasing demand for antibiotics, it is necessary to search for their new sources. We can point out the following reasons for the increase in demand: the formation of resistance to antibiotics, changes in the strains of microorganisms, negative effects of antibiotics on healthy cells, etc [1]. Endophytes are microorganisms that live inside the plant in a symbiotic relationship with its vegetable host [2]. They provide the host with important benefits, while the plant offers them nutrients and a protected ecological niche [3-5].

The symbiotic relationship between the plant and its microbiome has been well documented in agricultural studies. It includes natural phytopathogen, inhibition, nitrogen fixation, phytohormones production,nmineral acquisition, and production of growth promoter compounds [6-8]. In addition, secondary metabolites produced by fungi have been found to be effective against many harmful microorganisms [9-11]. Although hundreds of antibiotics have been discovered so far, only a few dozen of them are used in medicine and veterinary medicine; this is because most antibiotics are toxic and cannot be used to treat the disease [12].

Active antibiotics are prepared from penicillium and aspergillium groups of mold fungi. Penicillin (crystalline, potassium salt, phenoxymethyllin, bicillin, etc.) is prepared from penicillium mold, aspergillin, fumigacin, and clavacin antibiotics are made from aspergillium mold. Bacitracin, polymyxin, gramicidin and subtilin are among the antibiotics that produce bacteria. The

effect of these antibiotics on microbes is much weaker than that of antibiotics obtained from fungi [13].

Materials and Methods

Healthy fresh roots.

Sterile polythene.

Methanol.

Distilled water.

Potato dextrose agar (PDA) (peeled potato – 200 gm, dextrose –20 gm, agar – 16 gm, distilled water – 1000 ml).

Malt extract agar (MEA) (malt extract – 30 gm, mycological peptone – 5 gm, agar – 16 gm, distilled water – 1000 ml).

Petri plate.

Conical flask.

Spirit lamp.

Hot air oven.

Wash the healthy roots under tap water to remove adhering soil particles and epiphytes. After hat the healthy roots sample is cut into small pieces of approximately 0.5 cm × 0.5 cm. Immerse the roots samples in 0.1% mercuric chloride solution for 10 min. Followed by 2 min in 70% ethanol. Wash the samples with distilled water for several times and keep them in a sterile blotting paper for dryness. Prepare the potato dextrose agar medium or malt extract medium by sterilizing at 121 °C for 15 min in an autoclave. Pour the medium in a sterile Petri plate supplemented with streptomycin sulfate (20 mg/L) to suppress the growth of bacteria. After solidification, place a small piece of roots on the surface of potato dextrose agar or malt extract agar by using sterilized forceps. Isolation of Endophytic Fungi from Roots Incubate the plates at room temperature for at least 10–15 days. After the incubation period, observe the plates for fungal growth, purify them by using the standard method and maintain them as individual colonies in an agar slant.

The object of our research work is extracts of endophytic fungi isolated from the root of Cynara scolymus L, belonging to the Asteraceae family. Microorganisms used in research (bacterial and fungal strains) cell culture. Conditionally pathogenic microorganism strains stored in the Laboratory of Molecular Genetics of the Institute of Chemistry of Plant Substances of the Republic of Uzbekistan : *Bacillus subtilis UzMT - 5, Pseudomonas aeruginosa UzMT - 225, Staphylococcus aureus UzMT - 91, Escherichia coli UzMT – 221, Candida albicans UzMT – 247*. To date, three endophytic fungi have been isolated and extracted from the roots of the plant *Cynara scolymus* L belonging to the *Asteraceae* family. The obtained extracts showed significant activity against harmful bacteria and fungal strains.

Extracts against two gram-positive bacteria, *Bacillus subtilis* (UzMT – 5) and *Staphylococcus aureus* (UzMT 25923), two gram-negative bacteria, *Pseudomonas aeruginosa* (UzMT 27879), *Escherichia coli* (UzMT 27879) tested for antibacterial activity, and tests were also conducted for one pathogenic fungi *Candida albicans* (UzMT - 247) and one strain of yeast *Pichia anomala* (UzMT). UzMT microorganism cultures were obtained from the collection of microorganism strains of the Institute of Microbiology of the Academy of Sciences of Uzbekistan. The antimicrobial activity of the synthesized compounds is determined using a modified agar-disc diffusion method. Bacterial cells are propagated in sterile nutrient agar (LB Agar, Invitrogen, USA, 25 g agar/l distilled water) and solid medium poured into Petri dishes (2 ml of 0.9% NaCl suspension and 200 µl of bacterial cells in 20 ml of medium). *Candida albicans* and *Pichia anomala* (1x106 colony-forming units) were cultured on CLSI-sterile Mueller-Hinton agar for agar disk diffusion assay.

Forty microliters of test material (equivalent to 0.2 mg/disc for individual compound dissolved in methanol) is applied to sterile paper

discs (Whatman No.1, 6 mm diameter). Ampicillin (for gram-positive bacteria), ceftriaxone (for gram-negative bacteria) and fluconazole (for fungi) (Himedia Laboratories Pvt. Limited) were used as positive controls. A laminar was used to create conditions for the evaporation of solvents in the air stream. The Petri dishes were kept in the refrigerator for 3 hours to allow diffusion of the substances into the agar. Petri dishes were incubated at the respective growth temperatures for 24 h at 37 C for bacterial strains and 48 h at 29 C for fungal and yeast strains.

Evaluation of antimicrobial activity is based on the measurement of inhibited zones (including disc diameter) on the agar surface around the disc. After the incubation time, the inhibited level was measured and recorded.

Results and Discussion

In order to carry out this research, a total of 3 endophytic fungi were isolated from the roots of Cynara scolymus L, collected from the territory of Uzbekistan. These isolated fungi were conditionally designated as F1, F2, and F3. In our research, the activity of ethyl acetate extracts obtained from these fungi against Gram-positive and Gram-negative bacteria Staphylococcus aureus ATCC 25923, Bacillus subtilis RKMUz-5, Pseudomonas aeruginosa ATCC 27879 and Escherichia coli RKMUz-221 was studied. As a result, the studied F2 strain extract showed the highest activity of 19.06±0.11 mm against Bacillus subtilis RKMUz-5 strain. At this stage of the study, the antibiotic Ampicillin was used as a positive control against Gram-positive bacteria, and it showed the appropriate activity against the strains of Staphylococcus aureus ATCC 25923 and Bacillus subtilis RKMUz-5 of 26.08± 0.12 mm and 27.04± 0.10 mm. Extracts of all obtained strains showed significant activity against Gram-negative bacteria. Antibiotic Ceftraksion taken as a control showed activity of 25.12 ± 0.13 mm and 26.11 ± 0.10 mm

against Pseudomonas aeruginosa ATCC 27879 and Escherichia coli RKMUz - 221 strains. The results of antibacterial activity against Gram-positive and Gram-negative bacteria are fully presented in the table below (Table 1).

Table 1

In vitro activity of extracts of F1, F2, F3 fungal strains isolated from the roots 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*** RKMU z – 221
.	*F1*	15.08±0.17	13.01±0.14	10.08±0.12	NA
	F2	11.07±0.10	19.06±0.11	13.11±0.15	10.08±0.12
	F3	11.08±0.11	NA	10.01±0.13	NA
	Ampicillin	26.08±0.12	27.04±0.10		
	Ceftriaxone			25.12±0.13	26.11±0.10

NA*- not active

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

The activities of extracts of fungal strains obtained from the root of Cynara scolymus L belonging to the Asteraceae family against Candida albicans RKMUz - 247 yeast were studied. According to the obtained results, only F2 strain extract showed 17.04 ± 0.11 mm activity against Candida albicans yeast. The remaining extracts were found to be inactive against Candida albicans. Antibiotic Fluconazole taken as a control showed activity of 28.04 ± 0.10 mm, which means that the study was conducted correctly. The activity of plant extracts against Candida albicans is fully presented in the table below (Table 2).

Table 2

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

		Inhibition diameter (mm, ± SD, P≤0.05)
		Candida albicans
	F1	NA
	F2	17.04 ± 0.11
	F3	NA
Fluconazole		28.04± 0.10

NA*- not active.

Today, experimental processes aimed at the pure separation of secondary metabolites from the extracts that have shown effective results and the identification of the type of fungal strains are being continued. The results obtained in the course of our research work will be important in the future in obtaining antibiotic substances from endophytic fungi.

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. 3. Yakovlev, S.A. Infectious diseases as a global problem of our time / S.A. Yakovlev // Territory of Science. - 2017. S. 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. 5. Maslennikov P.V., Chupakhina G.N., Skrypnik L.N. The content of phenolic compounds in medicinal plants of the botanical garden // News of the Russian Academy of Sciences. Biological series. 2013. S. 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.