ANALYSIS OF THE COMPOUNDS PROVIDING ANTIHELMITIC EFFECTS OF CHICHORIUM INTYBUS THROUGH FRACTIONATION

[1]Shodiyeva Dildora G`iyosovna, [2]Shernazarov Farrukh
[1]SamDTU, Assistant of the Department of Microbiology, Virology and Immunology
[2]Samarkand State Medical University
https://doi.org/10.5281/zenodo.7632365

Abstract. *Increasing resistance to anthelmintic drugs has necessitated the search for alternative treatments to control gastrointestinal nematode parasites. Animals fed Cichorium intybus L, a temperate (pasture) crop, reduce their parasite burden, making C. intybus a potentially useful source for new anthelmintic compounds or a diet-based prophylactic-therapeutic option. Here, we used in vitro bioassays with the parasitic nematode Ascaris suum and molecular network methods with five species of yeast to identify putative active compounds. Network analysis predicted sesquiterpene lactones (SL) as the most likely group of anthelmintic compounds. Subsequent biomass-based fractionation supported these predictions, and isolation of pure compounds showed that SL 8-deoxylactusin (8-DOL) was the compound most strongly associated with antiparasitic activity. In addition, 8-DOL was observed to have an anti-parasitic effect in synergistic combination with other SL. Finally, it was found that extracts obtained from the mushroom showed activity against two ruminant nematodes (Teladorsagia circumcincta and Cooperia oncophora) in in vitro assays. Overall, our results confirm the antiparasitic activity of the mushroom against a range of nematodes and pave the way for targeted isolation of active compounds or selective breeding of specific cultivars to optimize its future use in human and veterinary medicine.*

Keywords: *Teladorsagia circumcincta, Cooperia oncophora, Ascaris suum, sesquiterpene lactones, Cichorium intybus L, Asteraceae.*

Introduction

Parasitic infections have a major detrimental impact on humans worldwide, causing disease, reducing nutrition and quality of life, and reducing the productivity of affected individuals. Globally, an estimated 900 million people are infected with nematode gel mites, mostly in tropical regions. Livestock are also significantly affected by helminth parasites with subclinical effects on welfare and productivity as well as clinical disease. Plants are a rich source of new antiparasitic compounds. This has been particularly well demonstrated in animal husbandry where wild plants or crops can be used as dietary interventions to reduce intestinal helminth infections. One of the most promising plant-based treatments is *Cichorium intybus L*, a perennial plant from the *Asteraceae* family. The leaves are highly nutritious and can be used as fodder for livestock grazing or as a salad for human consumption, while the roots are a rich source of the polysaccharide inulin, which is widely used as a feed additive and prebiotic. Over the past two decades, there has been consistent evidence of anthelmintic effects in cattle fed chicory leaves. Cattle fed a diet rich in fresh or applied chicory by grazing had significantly reduced infestations with the stomach nematode Ostertagia ostertagi when compared to a mixture that preferred ryegrass and others. Similarly, feeding with the spray was found to reduce nematode infection in the stomach of lambs.

Thus, fungi can be a natural source for potential new generation anthelmintic compounds or used as nutritional agents to control infections. Although it has been suggested that the active compound group is SL, there is no clear evidence for this. Here, we used a combination of bioactivity-based molecular networking and bio-guided fractionation to identify potentially active compounds in C.intybus. Using material from several chicory cultivars, we used a robust anti-parasitic assay with A. suum larvae to evaluate the activity of extracts and fractions as well as purified compounds. This approach allowed us to clearly identify SLs as key anti-parasitic compounds and resolved the question of whether this activity resulted from a single individual or the synergistic effect of several individual compounds. In addition, we conducted a bioassay with parasitic nematodes from different hosts to determine the broad-spectrum anti-parasitic activity of the mushroom extract.

Materials and methods

Root and leaf materials from five different *Cichorium intybus* cultivars (cv.) were collected for SL extraction. Industrial Fresh leaves and roots from four cultivars of *Cichorium intybus (Cichorium intybus L.),* cv. "Benulit", cv. "Goldine", cv. "Larigot" and Cv. "Maestoso" was taken and the roots were collected.

1. Enriched extraction method

2. Quantification and identification of sesquiterpene lactones for a multivariate network

3. Fractionation and purified compounds

4. LC-MS analysis for fractions and pure compounds

The extract and fractions were dissolved in MeOH at a concentration of 1 mg/ml. Pure compounds were prepared in MeOH at a concentration of 0.2 mg/ml. Ultra high performance liquid chromatography–high resolution mass spectrometry (UHPLC–HRMS) was performed on a Dionex Ultimate 3000 RS equipped with a diode array detector and coupled to a Bruker QTOF Maxis with an electrospray ionization source.

Results and discussion

Cichorium intybus is a common plant with many varieties used for different purposes. Chicory used for fodder and salad consumption, grown for the high nutritional value of the leaves. In contrast, cultivars used for inulin production have been bred for high inulin content in the roots, paying less attention to the chemical composition of the leaves. Thus, there is considerable variation in the chemical profile of different varieties. To cover this variability, we obtained extracts from the lettuce cultivar Spadona and four different inulin-producing cultivars. These extracts were then screened for their antiparasitic activity and analyzed by UHPLC for metabolites such as SL.

Analysis of the antiparasitic activity of *Cichorium intybus* cultivars against *A.suum*

Previous studies have suggested that SL may be responsible for the anthelmintic activity. To answer this question, we first used samples from *Cichorium intybus*. Spadona, we have previously shown activity against *A. suum* and confirmed that semi-purified extracts enriched with SL have stronger activity than the crude extract of the same leaf material. We obtained semi-purified extracts (in triplicate) from four inulin cultivars and leaf and root material from *Cichorium intybus.* Spadona resulted in 30 samples, all of which were tested for antiparasitic activity against *A. suum*, a mortality assay that we have previously shown to be a robust and reproducible tool for assessing the antiparasitic activity of plant extracts. The concentration-response curves of each extract showed clear, concentration-dependent lethality.

We tested a selected number of samples against two ruminant helminths to assess whether the effects observed against *A. suum* would also apply to other helminths of socioeconomic importance. Taken together, the data clearly show for the first time that the antiparasitic activity of chicory is derived from SL. These compounds are known for their biological activity and have been proposed as a promising natural resource for the development of new drugs against a number of infectious diseases. Our results provide motivation for further development of *C. intybus* as a prophylactic or therapeutic measure against parasitic infection, either by targeted selection of SL-rich cultivars or by production of pure single compound(s) that can be effective against a wide range of both nematode species. Taken together, the data clearly show for the first time that the antiparasitic activity of fennel is derived from SL. These compounds are known for their biological activity and have been proposed as a promising natural resource for the development of new drugs against a number of infectious diseases. Our results provide motivation for further development of sachrac as a prophylactic or therapeutic measure against parasitic infection, either by targeted selection of SL-rich cultivars or the production of pure single compound(s) that can be effective against a wide range of both nematode species. animals and people. A clear finding of this study is that SL activity, although attributed primarily to 8-DOL, acts synergistically involving at least two different compounds. This suggests that the most appropriate use of the fungus as an anthelmintic therapy may be in the form of whole plant or targeted extracts rather than as a source for purifying SL, as was done for the production of antimalarial artemisinin. Since *C.intybus* is already widely used as pasture for livestock, this preventive method can be easily incorporated into veterinary medicine. The identification of active compounds has now, for the first time, enabled plant scientists to selectively breed cultivars with high levels of active SL, thereby creating cultivars for the treatment of parasites.

Conclusion

In conclusion, based on the molecular network and the biologically controlled fraction, we identified the compounds responsible for the anthelmintic properties of chicory. Our results confirm previous studies suggesting a role for SL, and we have shown 8-DOL as a key anti-parasitic compound. Finally, the observed synergistic and additive effects of LAC and LACP suggest that the anthelmintic effect is not caused by 8-DOL alone, but by a combination of several compounds. Future research should focus on using this knowledge to design a new generation of anthelmintic treatments for human and veterinary use.

REFERENCES

1. Annayeva, D. (2022). CICHORIUM INTYBUS LISOLATION OF ENDOPHYTIC MICROORGANISMS FROM PLANTS AND IDENTIFICATION OF BIOTECHNOLOGICAL POTENTIAL. Eurasian Journal of Medical and Natural Sciences, 2(6), 54–61. извлечено от https://www.in-academy.uz/index.php/EJMNS/article/view/1755
2. Annayeva, D. G. Y., Azzamov, U. B., & Annayev, M. (2022). ODDIY SACHRATQI (CICHORIUM INTYBUS L) O‘SIMLIGIDAN ENDOFIT MIKROORGANIZMLAR AJRATIB OLISH. Oriental renaissance: Innovative, educational, natural and social sciences, 2(5-2), 963-972. https://cyberleninka.ru/journal/n/oriental-renaissance-innovative-educational-natural-and-social-sciences
3. Azimovich, A. U. B., G‘iyosovna, S. D., & Zokirovna, M. M. (2022). XLAMIDIYANING INSON SALOMATLIGIGA TA’SIRINI MIKROBIOLOGIK TAHLILLI VA

DIOGNOSTIKASI. Talqin va tadqiqotlar ilmiy-uslubiy jurnali, 1(11), 153-161. https://doi.org/10.5281/zenodo.7305057

4. Giyosovna, S. D. (2023). ODDIY SACHRATQI (CICHORIUM INTYBUS L) O'SIMLIK QISMLARIDAN ENDOFIT BAKTERIYALARNING SOF KULTURALARINI AJRATISH USULLARI. Новости образования: исследование в XXI веке, 1(6), 387-393. http://nauchniyimpuls.ru/index.php/noiv/article/view/3573
5. Shodiyeva, D. (2023). BIO-MORPHOLOGICAL CHARACTERISTICS, GEOGRAPHICAL DISTRIBUTION AND USE IN TRADITIONAL MEDICINE OF CICHORIUM INTYBUS. *GOLDEN BRAIN*, *1*(2), 252-256. https://researchedu.org/index.php/goldenbrain/article/view/1337
6. Shodiyeva, D. (2023). SANOAT MIKROBIOLOGIYASINING BIOTEXNOLOGIYADAGI AHAMIYATI. *GOLDEN BRAIN*, *1*(2), 116-120. https://researchedu.org/index.php/goldenbrain/article/view/1310
7. Shodiyeva, D. (2023). INDOLIL SIRKA KISLOTA MIQDORINI ANIQLASH. *GOLDEN BRAIN*, *1*(2), 321-324. https://researchedu.org/index.php/goldenbrain/article/view/1361
8. Dildora, S. (2023). CICHORIUM INTYBUSDAN OLINGAN BACILLUS AVLODIGA MANSUB BAKTERIYALARINING BIOTEXNOLOGIK POTENSIALI VA MIKROBIOLOGIYADAGI ISTIQBOLLARI. *O'ZBEKISTONDA FANLARARO INNOVATSIYALAR VA ILMIY TADQIQOTLAR JURNALI*, *2*(15), 726-732. https://bestpublication.org/index.php/ozf/article/view/3359
9. Annayeva, D. G. Y., Azzamov, U. B., & Annayev, M. O. S. (2022). O'SIMLIGIDAN ENDOFIT MIKROORGANIZMLAR AJRATIB OLISH.
10. G'iyosovna, S. D., & Muxriddin G'iyos o'g, A. (2023). DOMINANT MICROORGANISMS IN CICHORIUM INTYBUS. https://researchedu.org/index.php/goldenbrain/article/view/1492
11. Boltayev, K. S., & Jamalova, F. A. (2023). MIKOZLARGA MIKROBIOLOGIK MIKROSKOPIK TASHXIS QO 'YISHNING O 'ZIGA XOS XUSUSIYATLARI. *GOLDEN BRAIN*, *1*(3), 35-40. https://researchedu.org/index.php/goldenbrain/article/view/1466
12. Жамалова, Ф. А., Болтаев, К. С., & Шодиева, Д. Г. (2023). ВОЗБУДИТЕЛИ МИКОЗОВ СЛЕПНЕЙ НА ТЕРРИТОРИИ РАЗЛИЧНЫХ РЕГИОНОВ УЗБЕКИСТАНА. *GOLDEN BRAIN*, *1*(3), 28-34. https://researchedu.org/index.php/goldenbrain/article/view/1465
13. Jamalova, F. A., & Boltayev, K. S. (2023). BACILLUS THURINGIENSIS BAKTERIYALAR ASOSIDA YARATILGAN BIOPREPARATLAR. *GOLDEN BRAIN*, *1*(3), 23-27. https://researchedu.org/index.php/goldenbrain/article/view/1464
14. Hamza, S., Muzaffar, A., Dildora, S., & Ulugbek, A. (2023). BACILLUS THURINGINGIENSIS BAKTERIYA SHTAMMLARINING PHASEOLUS VULGARIS OSIMLIGI BIOMETRIK KORSATKICHLARIGA VA RIVOJLANISHIGA TASIRI. *Scientific Impulse*, *1*(6), 327-332. http://nauchniyimpuls.ru/index.php/ni/article/view/4355
15. Dildora, S., & Mekhriniso, B. (2023, January). APPLICATION AREAS OF BIOLOGICALLY ACTIVE METABOLITES PRODUCED BY ENDOPHITE BACTERIA. In *E Conference Zone* (pp. 92-95). http://www.econferencezone.org/index.php/ecz/article/view/1941

16. Dildora, S., & Mekhriniso, B. (2023). APPLICATION AREAS OF BIOLOGICALLY ACTIVE METABOLITES PRODUCED BY ENDOPHITE BACTERIA. *World Bulletin of Public Health*, *18*, 112-114. https://scholarexpress.net/index.php/wbph/article/view/2073
17. o‘g‘li Shernazarov, F. F., & qizi Tohirova, J. I. (2023). BAKTERIYALARNING IKKILAMCHI BIOLOGIK FAOL METABOLITLAR SINTEZ QILISH XUSUSIYATLARI VA ULARNING FARMASEVTIKADA QO ‘LLANILISHI. *RESEARCH AND EDUCATION*, *2*(1), 269-276. https://researchedu.org/index.php/re/article/view/1455
18. Vahobovna, M. Z., & Dildora, S. (2023). BIOLOGY AND BIOTECHNOLOGY OF ENDOPHITE MICROORGANISMS. *World Bulletin of Public Health*, *18*, 115-117. https://scholarexpress.net/index.php/wbph/article/view/2074
19. Azimovich, A. U. B., & G‘Iyosovna, S. D. (2023). O ‘SIMLIK O ‘SISHI VA RIVOJLANISHIDA FOYDALI MIKROORGANIZMLARNING AHAMIYATI. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(17), 257-260. https://cyberleninka.ru/article/n/o-simlik-o-sishi-va-rivojlanishida-foydali-mikroorganizmlarning-ahamiyati
20. Azimovich, A. U. B., G‘Iyosovna, S. D., & Akmalovich, M. A. (2023). ANTIBIOTIKLAR TA'SIR DOIRASIGA KO'RA KLASSIFIKATSIYASI. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(17), 245-251. https://cyberleninka.ru/article/n/antibiotiklar-tasir-doirasiga-kora-klassifikatsiyasi
21. Shayqulov, H. S., & Mamarasulova, N. I. O ‘TKIR DIAREYALARDA ESHERIXIYALARNING AJRALISHI. *«МИКРОБИОЛОГИЯНИНГ ДОЛЗАРБ МУАММОЛАРИ» МАВЗУСИДАГИ РЕСПУБЛИКА ИЛМИЙ-АМАЛИЙ АНЖУМАНИ*, 103.
22. Shodievich, S. H., & Roziqovna, R. M. (2023). OLIY O ‘QUV YURTLARIDA MASHG ‘ULOTLAR SIFATI VA SAMARADORLIGINI OSHIRISHDA ILMIY MAQOLALARNING O ‘RNI. *PEDAGOGS jurnali*, *25*(1), 52-55.
23. Шодиевич, Ш. Ҳ., & Нарзиев, Д. У. (2023). БОЛАЛАР ИЧАК ЭШЕРИХИОЗИНИ ДАВОЛАШДА АНТИБИОТИКОРЕЗИСТЕНТ ЛАКТОБАКТЕРИЯЛАРДАН ФОЙДАЛАНИШНИНГ САМАРАДОРЛИГИ. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(17), 236-244.
24. Yusupov, M. I., Sh, S. H., & Mamarasulova, N. I. (2022). MASOFADAN TURIB O’QITISHDA MODUL ELEKTRON PLATFORMASINING AHAMIYATI VA AFZALLIKLARI. *PEDAGOGS jurnali*, *9*(2), 47-50.
25. Болтаев, К. С., & Хамза, Ш. Ш. (2022). ЗАМОНАВИЙ УСУЛЛАРДА ЎҚИТИШНИНГ БУГУНГИ КУНДАГИ ЎРНИ. *PEDAGOGS jurnali*, *4*(1), 328-333.
26. Мухамедов, И. М., Юсупов, М. И., & Шайкулов, Х. Ш. (2022). ДИФФЕРЕНЦИАЛЬНЫЙ ДИАГНОЗ ЭНТЕРОКОЛИТОВ У ДЕТЕЙ. *Innova*, (2 (27)), 35-39.
27. Юсупов, М. И., Шайкулов, Х. Ш., Жамалова, Ф. А., & Очилов, У. У. (2021). Иммунный статус детей с коли инфекцией, вызваннойгемолитическими эшерихиями до и послелечения бифидумбактерином и колибактерином. *Биомедицина ва амалиёт. Самрқанд*, *6*, 272-276.
28. Хужакулов, Д. А. (2017). Особенности течения пищевых токсикоинфекций. *Педиатр*, *8*(S).

29. Boltayev, K. S., & Xo'Jaqulov, D. A. (2021). PIYOZ–ALLIUM CEPA NEMATODALAR FAUNASI. *Academic research in educational sciences*, *2*(11), 1213-1218.
30. Abdikhakimovich, K. D. (2023). ASSESSMENT OF THE QUALITY OF LIFE OF PATIENTS IN MODERN MEDICINE. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(17), 220-224.
31. Isrofilovna, M. N., Qizi, O. B. Q., & Qizi, S. M. R. (2023). ICHAK INFEKTSIYALARINING PATOGENEZI VA DIAGNOSTIKASIDA ALLERGIYANING ROLI. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(17), 252-256.
32. Юсупов, М. И., Курбанова, С. Ю., & Мухамедов, И. М. (2023). ОСБЕННОСТИ СТАФИЛОКОККОВИХ ИНФЕКЦИИ, ВЫДЕЛЕННЫХ ОТ ДЕТЕЙ С ГНОЙНО СЕПТИЧЕСКИМИ ЗАБОЛЕВАНИЯМИ.
33. ЮСУПОВ, М. И., РИЗАЕВ, Ж. А., & ЗИЯДУЛЛАЕВ, Ш. Х. (2022). Esherixioz bilan kasallangan bolalarda sitokinlarning ahamiyati. *Журнал биомедицины и практики*, *7*(4).
34. Хужакулов, Д. А., Юсупов, М. И., Шайкулов, Х. Ш., & Болтаев, К. С. (2019). Состояние внешнего дыхания у больных детей со среднетяжелым течением пищевого ботулизма. *Вопросы науки и образования*, (28 (77)), 79-86.
35. Azimovich, A. U. B., Sultonuvich, B. K., & Zokirovna, M. M. (2022). STREPTOKOKK AVLODIGA MANSUB BAKTERIYALARNING PATOGENLIK XUSUSIYATLARINING TAHLILI. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(13), 95-101.
36. Karabaev, A., & Bobokandova, M. (2022). REACTIVITY OF THE REPRODUCTIVE SYSTEM IN MATURE INTACT RATS IN THE ARID ZONE. *International Bulletin of Medical Sciences and Clinical Research*, *2*(10), 50-55.
37. Mamatkulovna, V. A., Sultonovich, B. K., Abdusalomovna, J. F., Tagirovna, M. Z., & Fazliddinovna, B. M. (2021). Nematodofauna of Retain Plants and Their Seasonal Dynamics. *Annals of the Romanian Society for Cell Biology*, 5455-5462.
38. XUDJAQULOV, D., ORIPOVA, P., & BOBOQANDOVA, M. SURUNKALI BRUSELLOZ KASALLIGIDA FIZIOTERAPIYA MUOLAJALARINI QOLLASH XUSUSIYATLARI. *ЭКОНОМИКА*, 154-160.
39. Худоярова, Г. Н., Муратова, З. Т., Маматова, О. Б., & Вахидова, А. М. (2023). МЕНИНГОЭНЦЕФАЛИТ ОСТРАЯ ВИРУСНАЯ ИНФЕКЦИЯ.
40. Annayeva, D. (2022). CICHORIUM INTYBUS LISOLATION OF ENDOPHYTIC MICROORGANISMS FROM PLANTS AND IDENTIFICATION OF BIOTECHNOLOGICAL POTENTIAL. *Eurasian Journal of Medical and Natural Sciences*, *2*(6), 54-61.
41. Azimovich, A. U. B., G'Iyosovna, S. D., & Zokirovna, M. M. (2022). XLAMIDIYANING INSON SALOMATLIGIGA TA'SIRINI MIKROBIOLOGIK TAHLILLI VA DIOGNOSTIKASI. *Talqin va tadqiqotlar ilmiy-uslubiy jurnali*, *1*(11), 153-161.
42. Farrukh S. TREATMENT OF MYOCARDIAL INFARCTION AND FIRST AID." science and Innovation //International Scientific Journal. ISSN. – 2022. – С. 2181-3337.
43. og'li Shernazarov F. F. CONGENITAL HEART DISEASE-CAUSES, CLASSIFICATION, DIAGNOSIS, TREATMENT, COMPLICATIONS, CONSEQUENCES //Eurasian Journal of Medical and Natural Sciences. – 2022. – Т. 2. – №. 3. – С. 84-89.

44. Shernazarov F. ATHEROSCLEROSIS: CAUSES, SYMPTOMS, DIAGNOSIS, TREATMENT AND PREVENTION //Science and innovation. – 2022. – Т. 1. – №. D5. – С. 7-12.
45. Farrukh S. TREATMENT OF MYOCARDIAL INFARCTION AND FIRST AID //Science and innovation. – 2022. – Т. 1. – №. D3. – С. 317-320.
46. Shernazarov F., TYPES OF HEMORRHAGIC DISEASES, CHANGES IN NEWBOENS, THEIR EARLY DIAGNOSIS. – 2022.
47. Фаррух Ш. и др. ПУТИ УСТРАНЕНИЯ САХАРНОГО ДИАБЕТА //Science and innovation. – 2022. – Т. 1. – №. D3. – С. 313-316.
48. Farrux S. The Structure of the Heart and its Physiology in Regular Athletes //Eurasian Scientific Herald. – 2022. – Т. 8. – С. 102-105.
49. Shernazarov F. ATHEROSCLEROSIS: CAUSES, SYMPTOMS, DIAGNOSIS, TREATMENT AND PREVENTION //Science and innovation. – 2022. – Т. 1. – №. D5. – С. 7-12.
50. ДИАБЕТА П. У. С. То^ ирова Жайрона Иззатилло^ изи //Шерназаров Фаррух «Science and innovation. – 2022. – С. 313-316.
51. Farrukh S. TREATMENT OF MYOCARDIAL INFARCTION AND FIRST AID." science and Innovation" International Scientific Journal. ISSN: 2181-3337, 1 (3), 317-320. – 2022.
52. Farkhod og'li, Shernazarov Farrukh. "CONGENITAL HEART DISEASE-CAUSES, CLASSIFICATION, DIAGNOSIS, TREATMENT, COMPLICATIONS, CONSEQUENCES. EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES, 2 (3), 84-89." (2022).
53. Farrux S. Eurasian Scientific Herald PENACCESS //PEERREVIEWEDJOURNAL https://geniusjournals. org/index. php/esh. – Т. 8.
54. Farhod o'g'li S. F. GASTRIT—SABABLARI, ALOMATLARI, TASHXISLASH, DAVOLASH, DORILAR, ASORATLARI, OLDINI OLISH. The Best Innovator in Science, 1 (1), 103-107. – 2022.
55. Shernazarov F. et al. SYMPTOMS, APPEARANCE, TREATMENT OF VARICOSE VEINS. – 2022.
56. Farrukh S. TREATMENT OF MYOCARDIAL INFARCTION AND FIRST AID." science and Innovation //International Scientific Journal. ISSN. – 2022. – С. 2181-3337.
57. Shernazarov F. F. CONGENITAL HEART DISEASE-CAUSES, CLASSIFICATION, DIAGNOSIS, TREATMENT, COMPLICATIONS, CONSEQUENCES //Eurasian Journal of Medical and Natural Sciences. – 2022. – Т. 2. – №. 3. – С. 84-89.