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PHENOLIC COMPOUNDS OF CHLORELLA VULGARIS AS ANTIMICROBIAL AGENTS

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Among the primary and secondary metabolites of microalgae, there are compounds that are potential antimicrobial substances: polyphenols, carotenoids, saponins, tannins, etc. It is interesting that these BARs can be both localized in algomass and accumulate in the culture liquid during the cultivation of algae.

In the study, an algologically pure culture of Ch. vulgaris, maintained in the NN collection of the Institute of Biology, Chemistry and Bioresources of the ChN University. Algae were grown on the classic Tamiya medium at a temperature of 24 ± 2 °C for 14 days. Hydroxycinnamic acids, flavonoids and polyphenolic compounds were detected in the culture fluid and alcoholic extract of biomass. The alcoholic extract of Chlorella vulgaris, in contrast to the culture liquid, had a higher amount of polyphenols, hydroxycinnamic acids, azulenes and different classes of flavonoids. Spectral analysis of alcohol extracts from algal biomass and culture liquid confirmed the presence of flavonoids, phenolic acids and coumarins.

The following microorganism test cultures were used: Bacillus subtilis and Sarcina flava (Gr+), Microcystis pul-verea (Gr-), Rhodotorula rubra (eukaryote). One-day test cultures of microorganisms were standardized according to McFarland to 0.5. The study of antimicrobial activity was carried out by the method of diffusion in agar: 100 µl of the test culture of microorganisms was sown with a lawn on a solid medium of MPA, 25 µl of culture liquid Chh was introduced into the wells. vulgaris. On the third day, the result of cultivation was evaluated.

It was noted that the sensitivity of the test cultures to the culture liquid and biomass extract of Ch. vulgaris depends on the structure of the cell wall of microorganisms. Yes, gram-positive microorganisms and yeast are more sensitive. For them, the diameter of growth retardation zones is determined in the range from 20 to 25 mm. The most sensitive were Sarcina flava and Rhodotorula rubra.

At the same time, gram-negative microorganisms were not sensitive to the culture liquid and biomass extract of Ch. vulgaris, the diameter of the zone of growth retardation did not exceed 5 mm. The revealed antimicrobial activity of green algae Ch. vulgaris is associated with the presence of polyphenolic compounds in the biomass. Therefore, culture fluid and biomass of Ch. vulgaris can be recommended for use in feed as a natural antifungal and antibacterial component.

Keywords: antimicrobial activity, test cultures of microorganisms, hydroxycinnamic compounds, flavonoids, polyphenolic compounds, Chlorella vulgaris

Introduction. Algae are a valuable source of biologically active compounds that are synthesized in the process of their vital activity. Most of them can not only improve metabolism, digestion, skin condition, well-being, but also fight bacterial diseases. This new approach to the use of algae is based on ecological observations and mechanisms of environmental control (Andriopoulos et al., 2022). During the study of algae, the following compounds were found that are capable of exhibiting antimicrobial effects, including proteins and peptides, carbohydrates, pigments, phenolic compounds, and triacylglycerols (Bulut et al., 2019; Goiris et al., 2012).

Among the huge number of compounds, phenols are the largest and most valuable group of secondary metabolites found in algae. These compounds are considered good antioxidants that protect the human body from damage by reactive oxygen species that can lead to health disorders (Arguelles et al., 2018; Freili-Pelegrin and Robledo, 2014).

All phenolic compounds contain at least one aromatic phenolic ring with one or more hydroxyl substituents. Today, more than 8,000 different structures of phenols are known (Fernando et al., 2016; Goiris et al., 2014). So, among the simple phenols of algae there are phenolic acids (gallic, hydroxybenzoic, gentisic, ferulic, caffeic, chlorogenic) (Alshuniaber et al., 2020; Besednova et al., 2020), catechol, hydroquinone and flurogluc in. The latter is characteristic exclusively for the biomass of different departments of algae (Cichonski et al., 2022).

Also, according to the authors, all 13 classes of flavonoids, coumarins and isocoumarins can be found in algae biomass (Frazzini et al., 2022; Haoujar et al., 2019). Among the polymeric phenolic compounds of algae, tannins and lignans are distinguished (Bulut et al., 2019; Adhomi et al., 2016). Particular attention is paid to the detection of phlorotannins - these are oligomers of phloroglucin, which is contained exclusively in the biomass of algae (Andrade et al., 2018). What is

characteristic, its content can reach up to 25% of the dry biomass of algae (Zakaria et al., 2017).

Algae are considered a natural and attractive biotechnological source of new drugs. The high antimicrobial activity of their polyphenolic compounds is a promising basis for the development of innovative medicines. They can become both a serious alternative to traditional antimicrobial agents and an effective addition to antibiotic therapy (Pina-Perez et al., 2017; Ibanez and Cifuentes, 2013).

Chlorella vulgaris is a single-celled green alga that has become widespread due to its high adaptive potential, rapid accumulation of biomass, and ability to suppress the growth of other representatives of phytoplankton (Shaima et al., 2022). *Chlorella* biomass synthesizes many biologically active compounds, including phenolic compounds. It is likely that their presence will determine the manifestation of the antimicrobial activity of this algae.

The purpose of this work is to study the antimicrobial potential of phenolic compounds of the green microalga *Chlorella vulgaris*

Materials and methods. In the study, an algologically pure culture of *Ch. vulgaris*, maintained in the NN collection of the Institute of Biology, Chemistry and Bioresources of the ChNU. Algae were grown on the classic Tamiya medium at a temperature of 24 ± 2 °C for 14 days.

Algal biomass was separated from fugat by centrifugation at 3500 rpm for 10 min on a Micromtd CM-3M centrifuge. Determination of the qualitative and quantitative composition of phenolic compounds was carried out in the culture liquid and in biomass *Ch. vulgaris.*

Phenolic compounds were extracted with 40% ethanol. To determine the UV spectra of the extracts, spectroscopy was performed after 5 nm in the range of 180–400 nm on a CaryWin UV 60 spectrophotometer (Agilent, USA).

Determination of the amount of phenolic acids and polyphenolic compounds was carried out through the specific absorption index of chlorogenic acid, the content of flavonoids with $AlCl_3$ through the specific index of rutin.

The following microorganism test cultures were used: *Bacillus subtilis* and *Sarcina flava* (Gr+), *Microcystis pulverea* (Gr-), *Rhodotorula rubra* (eukaryote). One-day test cultures of microorganisms were standardized according to McFarland to 0.5. The study of antimicrobial activity was carried out by the diffusion method in agar: 100 μl of the test culture of microorganisms was sown with a lawn on a solid medium of MPA, and 25 μl of culture liquid or biomass extract *Ch. vulgaris* was introduced into the wells. Tamiya's medium (in the experiment with culture liquid) or 40% ethanol (in the experiment with extracts from biomass) was used as a comparison solution (Balouri et al., 2016). On the third day, the result of cultivation was evaluated.

For static data processing, quantitative indicators were processed by mathematical methods used in biology on a personal computer using the Microsoft Excel data analysis package. The mean (M) and standard error of the mean (m) were estimated. Student's t-test was used for parametric data. The results were considered reliable at $p \leq 0.05$.

Results and discussion. Among the huge number of primary and secondary metabolites of algae, it is phenols that exhibit high antimicrobial activity. Such high acantimicrobial activity of phenolic compounds of algae is probably due to modification of the permeability of cell membranes, changes in various intracellular functions, or modification of the rigid cell wall of prokaryotes with loss of its integrity due to various interactions with the cell membrane (Zakaria et al., 2020).

Depending on taxonomic, ecological and exogenous aspects, there may be differences in the content and composition of phenols in algae. Physiological changes in response to ambient light, temperature, and nutrient conditions directly affect the chemical composition of algae.

UV spectra of the culture liquid and alcohol extracts indicated the presence of a huge number of groups of phenolic compounds (Fig. 1). The alcoholic extract of Chlorella vulgaris, in contrast to the culture liquid, had a greater amount of flavonoids, oxycinnamic acids, and azulenes.

However, trace amounts of coumarins were found in the culture fluid. In general, the qualitative composition of phenolic compounds of culture liquid and biomass was similar. The differences related to the intensity of expression of the peaks typical for the specific compound. The conducted qualitative analysis gave grounds to determine the key components of phenolic compounds *Ch. vulgaris* – hydroxycinnamic acids (chlorogenic acid), various classes of flavonoids.

To establish the antioxidant potential of algae biomass, the content of polyphenolic compounds was also determined (Fig. 2).

It was noted that the number of investigated biomass components of Ch. vulgaris is 3-12 times higher than that in the culture liquid. Yes, the amount of hydroxycinnamic acids is the smallest. In biomass, this indicator is 1.2 mg/g, while in the culture liquid it is only 0.5 mg/g.

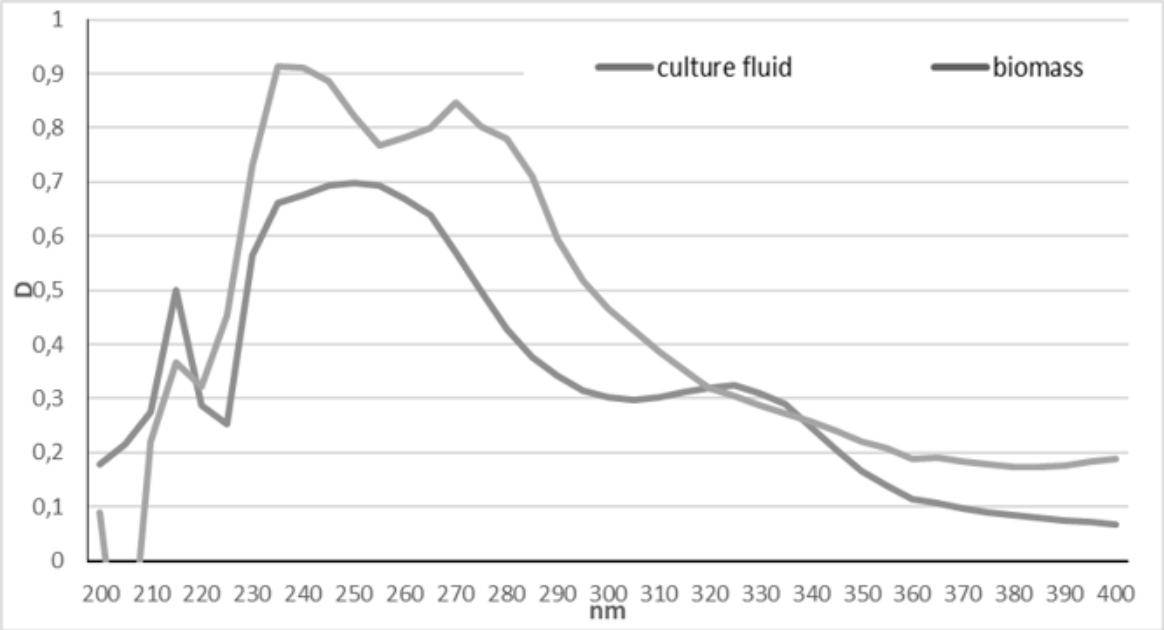


Fig. 1. UV spectra of the culture fluid and biomass of Chlorella vulgaris

The content of flavonoids in the biomass is 10 times higher than that in the culture liquid and is 3.87 mg/g. Flavonoids are polyphenolic compounds containing two aromatic rings connected by a three-carbon bridge (diphenylpropane fragment C6-C3-C6). The classification of these compounds is based on the position of the phenolic ring B and the size of the oxidation cycle. Flavonoids include flavones, flavonols, flavanols, flavanones, isoflavones, proanthocyanidins, anthocyanins and others (). They are well known as antibacterial agents against a wide range of pathogenic microorganisms. With the increasing prevalence of incurable infections caused by antibiotic-resistant bacteria, flavonoids have attracted great interest because they can enhance the effects of traditional antibiotics (Zielinski et al., 2020).

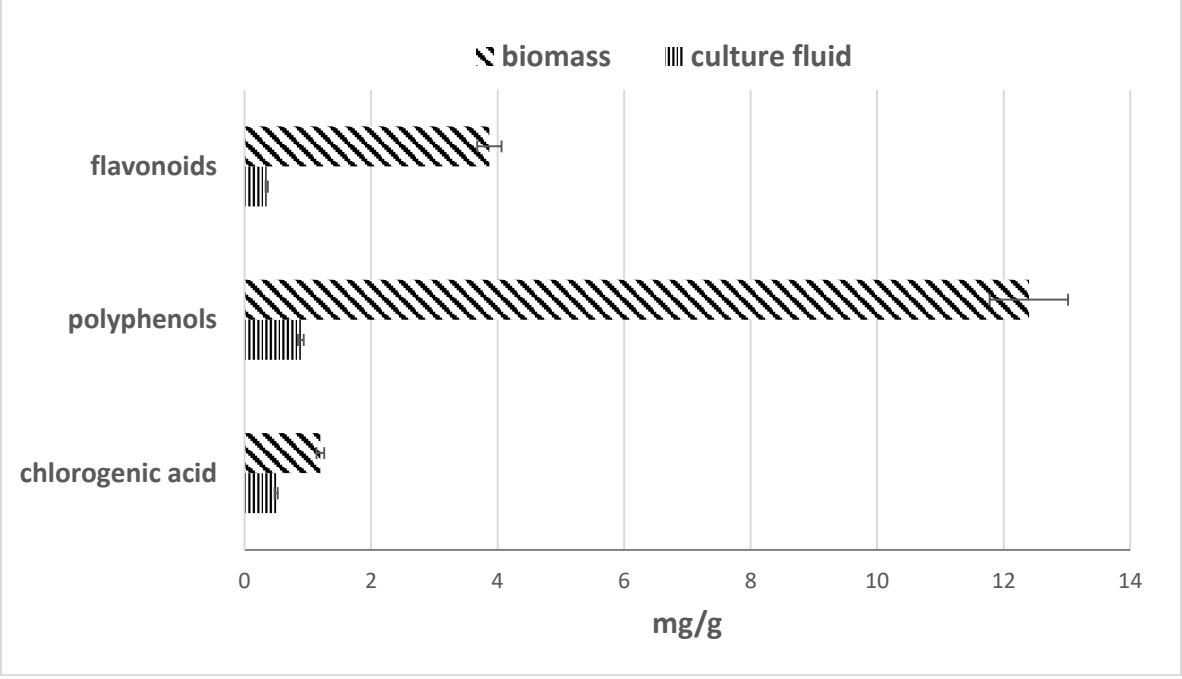


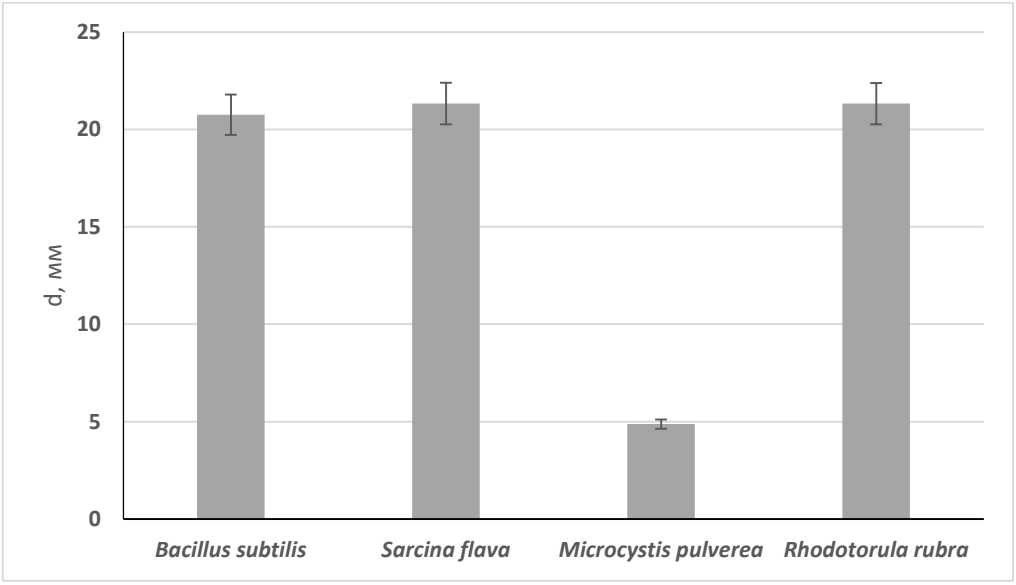
Fig. 2. Phenolic compounds of culture fluid and biomass of Chlorella vulgaris

Both hydroxycinnamic acids and flavonoids contribute to the total amount of polyphenolic compounds. They are determined at the level of 12.4 mg/g in biomass, while only 0.89 mg/g in the culture liquid. The high content of polyphenols in the studied material may indicate the high antioxidant activity of extracts from this material.

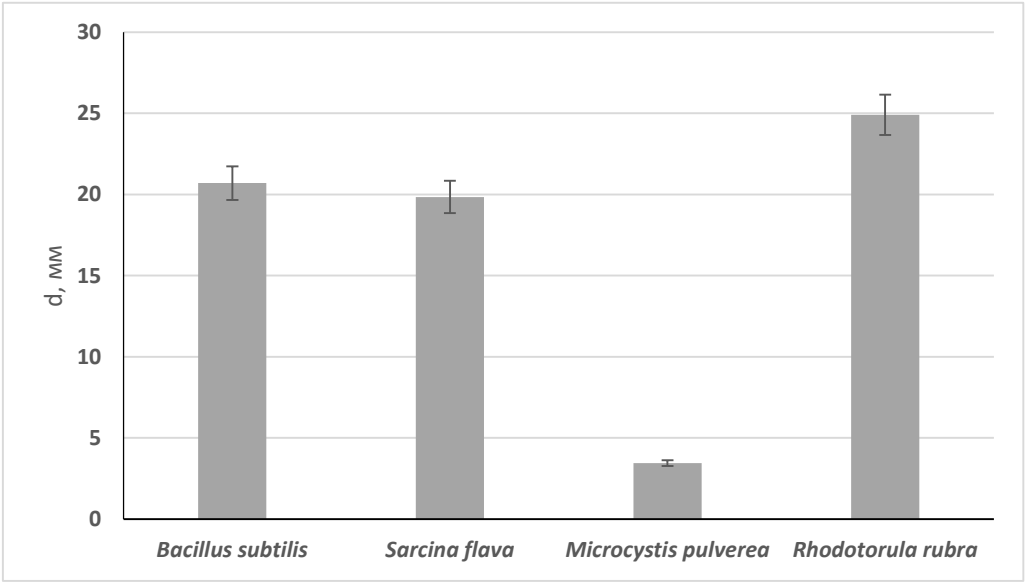
Potentially biomass and culture liquid Ch. Vulgaris should show pronounced antimicrobial activity against a number of microorganisms. That was verified by us in the experiments on determining the diameter of the growth retardation zones of test cultures of microorganisms (Fig. 3).

When conducting an analysis of antimicrobial activity by diffusion method in agar culture fluid and biomass Ch. vulgaris, it was noted that the sensitivity of test cultures of microorganisms depends on the structure of their cell wall. Fig. 3 shows that the culture liquid Ch. has the greatest antimicrobial activity. vulgaris showed to the yeast Rhodotorula rubra, the diameter of the zone of growth retardation was 24.9 mm ± 5%. Whereas for biomass extract Ch. vulgaris, the largest zones of growth retardation were noted for Sarcina flava and Rhodotorula rubra – 21.33 mm ± 5% and 21.32 mm ± 5%, respectively. At the same time, gram-negative microorganisms were not sensitive to the action of this algae, the diameter of the growth retardation zone did not exceed 5 mm.

Antimicrobial activity, which was revealed during the study, is associated with the presence of a huge number of biologically active compounds, in particular, phenolic compounds, which are able to inhibit the growth of microorganisms to one degree or another, occupy an important place.



A



B

Fig. 3. Antimicrobial activity of the culture fluid (A) and biomass (B) of Chlorella vulgaris

Therefore, when screening the antimicrobial activity of green microalgae Ch. vulgaris, a positive result was noted for gram-positive bacteria and yeast. For them, the diameter of growth retardation zones is determined in the range from 15 to 25 mm. At the same time, gram-negative microorganisms were not sensitive to either the culture liquid or biomass extract of Ch. vulgaris. Phenolic compounds of green algae Ch. vulgaris can become a new generation of drugs with antimicrobial action. The use of phenolic compounds of this algae as part of complex preparations with pronounced antioxidant activity is also promising. That is, the biologically active substances of green algae can become a useful additive to the fodder and food ration, which will significantly improve the well-being and endurance of the end user of these compounds.

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ФЕНОЛЬНІ СПОЛУКИ CHLORELLA VULGARIS ЯК АНТИМІКРОБНІ АГЕНТИ

Л.М. Чебан, А.-М.Р. Козак

Серед первинних та вторинних метаболітів мікроводоростей виділяються сполуки, що є потенційними антимікробними речовинами: поліфеноли, каротиноїди, сапоніни, дубильні речовини, тощо. Цікавим є те, що ці БАР можуть бути як локалізовані у альгомасі, так і накопичуватися у культуральній рідині при вирощуванні водоростей.

У дослідженні використовували альгологічно чисту культуру Ch. vulgaris, що підтримується в колекції НН інституту біології, хімії та біоресурсів ЧНУ. Водорість вирощували на класичному середовищі Тамія, при температурі 24±2 °С протягом 14 діб. У культуральній рідині та спиртовому екстракті біомаси виявляли гідроксикоричні кислоти, флавоноїди та поліфенольні сполуки. Спиртовий екстракт Chlorella vulgaris на відміну від культуральної рідини мав більшу кількість поліфенолів, гідроксикоричних кислот, азуленів та різних класів флавоноїдів. Проведений спектральний аналіз спиртових екстрактів із біомаси водорості та культуральної рідини підтвердив присутність флавоноїдів, фенолкарбонових кислот та кумаринів.

Як тест-культури мікроорганізмів використовували: Bacillus subtilis та Sarcina flava (Гр+), Microcystis pulverea (Гр-), Rhodotorula rubra (еукаріот). Однодобові тест-культури мікроорганізмів стандартизували за McFarland до 0,5. Дослідження антимікробної активності проводили методом дифузії в агар: 100 мкл тест-культури мікроорганізмів висівали газоном на тверде середовище МПА, у лунки вносили по 25 мкл культуральної рідини Ch. vulgaris. На третю добу оцінювали результат вирощування.

Відмічено, що чутливість тест-культур до культуральної рідини та екстракту біомаси Ch. vulgaris залежить від будови клітинної стінки мікроорганізмів. Так, більш чутливими є грампозитивні мікроорганізми та дріжджі. Для них діаметр зон затримки росту визначений у межах від 20 до 25 мм. Найчутливішими виявилися Sarcina flava та Rhodotorula rubra.

В той же час грамнегативні мікроорганізми виявилися не чутливими до культуральної рідини та екстракту біомаси Ch. vulgaris, діаметр зони затримки росту не перевищував 5 мм. Виявлену антимікробну активність зелених водоростей Ch. vulgaris пов'язують із наявністю у складі біомаси поліфенольних сполук. Отже, культуральну рідину та біомасу Ch. vulgaris можна рекомендувати для використання в кормах як природного протигрибкового та антибактеріального компонента.

Ключові слова: антимікробна активність, тест-культури мікроорганізмів, гідроксикоричні сполуки, флавоноїди, поліфенольні сполуки, Chlorella vulgaris

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