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Research Article

Extraction of protein and some bioactive compounds from *Westiellopsis prolifica* blue green algae

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ABSTRACT

Isolation of the blue-green alga *Westiellopsis prolifica* from the waters of the Euphrates River (upper Euphrates River in Iraq) in the city of Haditha in October 2019 and was propagated on BG11 culture media. Six types of compounds were identified from it: Acetic acid, anhydride with formic acid, Palmitic acid (Hexadecanoic acid), Tryptophan, Dihydrogeraniol, cisZ-11,12-Epoxytetradecan-1-ol, cisZ-11,12-Epoxytetradecan-1-ol, and Oxirane, (7-octenyl). Using the technique of gas chromatography - mass spectrometry [GC-mass] Gas Chromatography Mass. The compounds were identified and the structural formula of each compound, its molecular weight and concentration were identified. It was found that the compound Dihydrogeraniol, with the structural formula C₁₀H₂₀O, had the largest area of the total area of the compounds detected by this device and was the most concentrated, as its concentration reached 23.27% of the total concentration of the diagnosed compounds, followed by the concentration of the amino acid Tryptophan.

Keywords: Blue-green alga, Euphrates river, Iraq, BG11, oxirane

INTRODUCTION

In addition to the environmental importance of algae, there are new directions to benefit from them, as wide horizons have been opened for their previously unknown uses, including the use of algae in the production of protein and bioactive compounds. Recent studies have tended to exploit the high protein content in the chemical composition of some microalgae to close the so-called protein gap, expected as a result of the needs resulting from increased population growth in addition to the limited resources of agricultural land and fresh water, although the idea of producing protein from algae is still in its early stages. It is accompanied by obstacles, including the unpalatable taste of these proteins and the high cost of their production. However, the very high protein percentages of some algae, which range from 42% to 70% of their dry weight, encouraged these studies. Ismail (2017) confirmed that marine algae contain high levels of proteins, carbohydrates, fatty acids, and vitamins, in addition to biologically active compounds such as phenols, beta-carotene, and antioxidants.

With the world population expected to increase by more than a third (2.3 billion people) by 2050, this requires an estimated increase of about 70% in global food production. This challenge requires several solutions that humanity must follow to avoid famine disasters in the near future (Godfray et al, 2010).). Among the blue-green algae on which some studies have been conducted is *Westiellopsis prolifica*, which has been shown to be one of the algae that has the ability to fix nitrogen in the soil and

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is therefore used in rice fields (Singh & Dhar 2010). As the study of Al-Hussieny, et al, (2019) showed, this species has the ability to produce protein and some important enzymes for living organisms. Therefore, this study aims to reach knowledge of the proteins and biologically active organic compounds that are involved in the composition of the blue-green algae *Westiellopsis prolifica* and the possibility of isolating them from the internal content of the algae and diagnosing them using the GC-MS technology.

MATERIALS AND METHODS

Qualitative samples were collected randomly from the Euphrates River near the city of Haditha, western Iraq, during the month of October 2019, using a conical algae collection net (Phytoplankton Net) with openings the size of 20 microns, by pulling them by boat for 10-15 minutes for the purpose of obtaining the algae *Westiellopsis prolifica*. The samples were brought to the laboratory in plastic bottles for the purpose of diagnosing and isolating the required algae. The samples were examined using an Italian-made Optika optical microscope. Identify the desired moss using a Hemacytometer slide and examine it at 400X using a compound light microscope. Relying on a number of sources in diagnosing algae (Edward, 2010) and (Al-Hussieny, 2018).

Algal isolates were cultured in BG-11 culture medium at 25 ± 2 with 300 micron/m²/s light intensity and 16:8 light:dark ratio in a 1 liter culture. The isolation method was repeated until one moss culture was produced (Stein, 1973). After confirming that it contained just one species of algae, portion of the colony was sterilely transferred to liquid BG-11 media in 250 ml glass flasks. Two weeks in the same circumstances yielded sufficient growth in the flasks. The liquid culture was microscopically checked for additional algae and refreshed every two weeks to guarantee its viability. The Patterson, 1983 approach purified algae from bacteria and fungus, while Sadiq, 2009 extracted amino acids and physiologically active chemical compounds.

The concentration of blue-green algae components was determined using Lowry et al. (1951) and evaluated using a Shimadzu-2010 plus GC-MS equipment.

RESULTS AND DISCUSSION

Protein types differ by digestibility and necessary amino acid concentration. Some animal and vegetable proteins lack histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, Tryptophan, and alanine (Bleakley and Hayes and Bleakley, 2017). Compared to these protein sources, algal protein is superior. Some microalgae generate protein in greater quantities than animal and plant proteins, which is physiologically useful and utilized as an antioxidant. Antihypertensive and antiatherogenic (Fan et al 2014). Microalgae include proteins, carbs, lipids, vitamins, pigments, phycobiliproteins, and more.

Algae received widespread attention to know its chemical composition and physiological properties, and this coincided with the development of chemical analysis techniques, and one of the most important chemical compounds with nutritional value are proteins (Venkatarman, 1988).

The moss *Westiellopsis prolifica* is considered a blue-green algae, a filamentous, true-branching cyanobacteria. It features two thick main threads and weaker supplementary threads without a sheath. The cells of the alga are barrel-shaped and granular, with a length of 8-12 μ . It contains hormoconia and akinetes. Its presence in the aquatic environment is limited to fresh water. (Abed et al. (2013)) Fig. 1. This type of algae is used to increase the nitrogen content in the soil as it is a nitrogen fixer. It is used in rice growing fields in some countries (Singh & Dhar 2010).

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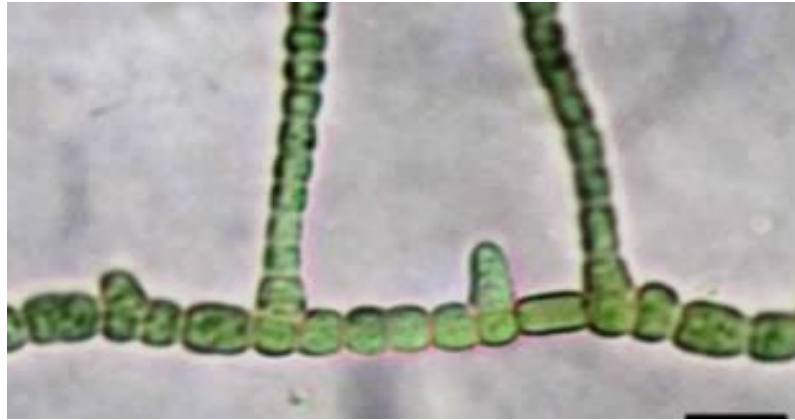


Fig. 1. Westiellopsis prolifica moss colony

Current research found 9.950 mg/L of protein generated by this kind, and this is consistent with what was confirmed by the study of Balasooriya, et al, 2016, that the blue-green alga *Westiellopsis prolifica* reached 40% of its dry weight, followed by carbohydrates at an additional 35% to 15% lipids, 32% ascorbic acid, minerals and vitamins necessary for the vital activities of the body.

The study of Al-Hussieny, et al, (2019) also showed that this type of algae produces protein enzymes that are important for metabolic activities, such as amylase and protease. Its protein content reaches 9.59 mg/l. A study (Santillan 1974) confirmed that some types of algae contain, in addition to their components of essential amino acids, which plants do not produce, various vitamins, including vitamins A, C, and E. They also contain mineral content of iron, calcium, zinc, and selenium. They decompose faster than plants because their cell walls lack cellulose.

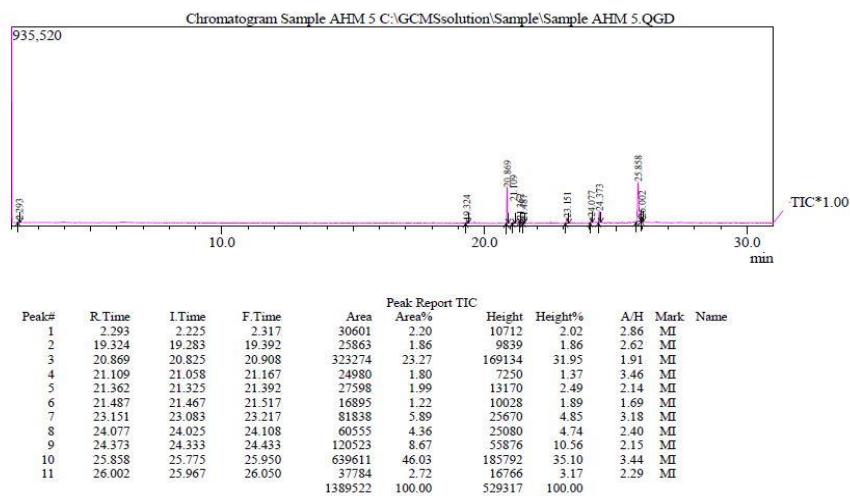


Fig. 2. Examination of Westiellopsis prolifica algae proteins using the GC-MASS device

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Table 1. Protein content of Westiellopsis prolifica moss using the GC-MASS device

The focus(%)	Molecular weight	Structural formula	Name of the compound	Detention time (min)
2.20	88	C ₃ H ₄ O ₃	Acetic acid, anhydride with formic acid	2.292
1.86	256	C ₁₆ H ₃₂ O ₂	Palmitic acid(Hexadecanoic acid)	19.324
3.50	204	C ₁₁ H ₁₂ N ₂ O ₂	Tryptophan	22.56
23.27	156	C ₁₀ H ₂₀ O	Dihydrogeraniol (Citronellol)	20.869
1.80	228	C ₁₄ H ₂₈ O ₂	cisZ-11,12-Epoxytetradecan-1-ol	21.109
1.99	154	C ₁₀ H ₁₈ O	Oxirane, (7-octenyl)-	21.362

Table 1 shows that the moss *Westiellopsis prolifica* contains six types of protein compounds, and the compound Citronellol (Dihydrogeraniol) with the structural formula C₁₀H₂₀O was the most concentrated, as its concentration reached 23.27% of the total concentration of the identified compounds. It is a compound used in multiple fields. It is used in cosmetics and manufacturing perfumes.

Citronellol has long been used in perfume and cosmetic items to repel mosquitoes. Growing medical research suggests it may be an anti-inflammatory, anti-tumor, and cardiovascular regulator. It has therapeutic and medical benefits, as it is an anti-tumor, anti-inflammatory, and pain reliever

ANTI-INFLAMMATORY

Animal studies have indicated that citronellol may alleviate pain through numerous anti-inflammatory pathways. Citronellol may prevent inflammation in cultured cells by multiple methods, since the body has numerous inflammatory responses and pathways.

ANTI-TUMOR

Since inflammation promotes cancer cell growth, citronellol's anti-inflammatory properties may help it combat tumors. Because citronellol is volatile and may be inhaled, some studies suggest it may treat lung cancer. Citronellol and its components, such as geraniol, may fight malignant tumors. Human studies are needed to confirm this.

BLOOD PRESSURE

Citronellol inhibits smooth muscle cell contraction, which seems to impede the cardiovascular system. Clinical experiments in people are required to use this action to widen blood arteries and reduce blood pressure. Next comes Tryptophan, an important amino acid for protein production that humans cannot produce and must be acquired from diet. It is utilized to make several useful amines.

Tryptophan (TRP) is a precursor to the neurotransmitters serotonin and tryptamine, the antipellagra vitamin nicotinic acid, and the epiphyseal hormone melatonin in addition to being one of the limiting essential amino acids in protein metabolism. Alfieri AB, Cubeddu LX (1994) Effects of serotonin synthesis suppression on healthy persons' 5-hydroxyindoleacetic acid excretion. *J Clin Pharmacol* 34:153–157.

The least concentrated amount was palmitic acid C₁₆H₃₂O₂, a long-chain saturated fatty acid present in milk, its derivatives, and meat and one of the most abundant in animals and plants (Paulpriya, 2014). It is also antibacterial and antioxidant.

As for the compound Acetic acid, anhydride with formic acid, with a structural formula of C₃H₄O₃ and a concentration of 2.20%, it is a formate-forming agent for amines, amino acids and alcohols, in addition to its uses in the food industry. It is also used in the

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manufacture of detergents and other industrial uses, as this biologically active compound was found in some medicinal plants. Such as the plant *f maeruaapetalaroth* (Jacobs) (Capparaceae), which has proven its effectiveness against bacteria (Akilandeswari et al, 2015).

The current study confirmed the importance of this type of blue-green algae in producing amino and fatty acids necessary for feeding living organisms.

According to Roy and Pal (2015), certain microalgae contain more than 50% crude protein and are rich in unsaturated fatty acids, notably the n-3 chain, which is why they were fed to fish and other animals.

CONCLUSION

Westiellopsis prolifica, a blue-green alga from the Euphrates River near Haditha, Iraq, has great nutritional and medicinal promise. Six bioactive substances were found using GC-MS: Dihydrogeraniol, Tryptophan, Palmitic acid, and Acetic acid anhydride with formic acid. Dihydrogeraniol (Citronellol) was the most abundant, at 23.27%, and had many uses, including mosquito repellent, anti-inflammatory, and tumor and cardiovascular disease treatment. This chemical shows algae-derived bioactive compounds' medicinal, cosmetic, and industrial applications.

Tryptophan, an important amino acid, makes *Westiellopsis prolifica* a sustainable supply of high-quality protein. Its role in serotonin and melatonin synthesis emphasizes its biological importance. The algae also included palmitic acid, an antioxidant and antibacterial saturated fatty acid, and acetic acid anhydride, an industrial and antibiotic.

Westiellopsis prolifica's unique biochemical composition—proteins, amino acids, fatty acids, vitamins, and minerals—makes it a valuable resource for human and animal nutrition, according to the study. Its ecological value as a nitrogen fixer and excellent digestion without cellulose make it useful in agriculture and aquaculture. These findings demonstrate blue-green algae's potential as a renewable and sustainable bioresource for industrial and biomedical uses.

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