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CHARACTERIZATION OF OIL AND CAKE FROM *Spirogyra porticallis*

D. D. Gaiya [1, 3]
L. L. Ashafa [4]
M. E. Entonu [1, 2, 3]
S. K. Udu [1, 2, 3]

[1]Department of Biology, Air Force Institute of Technology Kaduna Nigeria
[2]African Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria
[4]Department of Biochemistry, Kaduna State University, Nigeria

E-mail: gaiyadaniel@gmail.com

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Aim. Search of healthy and edible alternative oils from algae. Such oil provides many health benefits mainly because of docosahexaenoic acid (DHA) form of omega-3 fattyacids and some other micro nutrients in smaller amounts.

Methods. Soxhlet extraction method was used to extract the oil with n-hexane as the solvent. The proximate composition was determined by AOAC methods, while the mineral contents were determined by AAS. FTIR and UV-Visible spectra of the oil were run using Agilent-FTIR Spectrometer and UV-Visible Spectrophotometer respectively.

Results. The oil yield was very low (1.05%). The proximate composition reveals carbohydrate as the major nutrient in the residue (79.18%), others include lipid (8.03%), crude protein (5.00%), moisture (2.78%), crude fibre (3.01%) and ash (2.00%). The mineral composition reveals high amount of potassium (1602.5 mg/100 g) and calcium (632.5 mg/100 g) with low levels of phosphorous (14.9 mg/100 g) and sodium (12.8 mg/100 g). The FTIR spectrum of algae oil is similar to the normal vegetable oil. Stretching vibrations at 2922.2 cm^{-1} and 2855 cm^{-1} are attributed to methylene ($-CH_2-$) and methyl ($-CH_3$) groups while absorption bands at 1710 cm^{-1} and 1744 cm^{-1} showed carboxylic groups for algae oil and vegetable oil (control) which was attributed to C=O stretching vibrations (esters). The UV-Visible spectrum of algae oil showed two peaks at 408 nm and 660 nm for carotenoids and chlorophyll A respectively, which corroborate with previous studies.

Conclusions. We conclude that the oil and cake characterized from *Spirogyra porticallis* has great potential for medicinal and nutritional usage.

Key words: *Spirogyra porticallis*; Algae, Docosahexaenoic acid; Carotenoid; Chlorophyll; Soxhlet extraction; Agilent- FTIR Spectrometer.

Algae have received a lot of attention as new biomass source for the production of renewable energy due to their photosynthetic nature, fast growth rate, biomass and lipid production efficiency [1]. They can be produced through autotrophic or heterotrophic cultivation under photo heterotrophic or chemo heterotrophic conditions by using solar energy or artificial light source [2]. Most of their essential nutrients can be supplied by waste water and CO_2 from the atmosphere; leading to high productivity and an associated high lipid content making them a very attractive option [3, 4].

Algae yield per unit area does not require agricultural lands, promoting their photosynthetic nature, utilizing atmospheric CO_2, have the ability to adapt to any hostile condition and maintain productivity [5]. Under favorable conditions, the growth rate is very high and they are harvested daily or every few minutes due to the fact that most of them divide every 1–2 days or once every 3–4 hours which serves as a basis for their potential biomass producers [6, 7].

The use of edible oil to produce biodiesel in the developing countries is not feasible in view of a big gap in demand and supply of such oils in developing countries [8]. Algae contain lipid and fatty acids as membrane component, storage products, metabolites and sources of energy. The lipid and fatty acids contents of algae vary with culture conditions. Algae stimulated under environmental stress [9]. Algae are rich in high value compounds and specially lipids, including; astaxanthin, neurotoxins, ω-3 long chain polyunsaturated fatty acids (PUFAs) and beta carotene [10].

Algae can be channeled into more useful forms such as feeds for animals. It is also interesting to know that algae comprises of lipids which can be used in the production of biofuels, and edible oil which this work is targeting [8]. The aim of this research is to extract oil from algae (*Spirogyra porticallis*), characterize the oil, and determine the proximate and mineral composition of the algae cake.

Materials and Methods

Chemicals and reagents

The following chemicals and reagents which are of analytical grade were used; n-Hexane (Sigma-Aldrich, USA), Hydrogen Tetraoxosulphate (iv) Acid (Sigma-Aldrich, USA), Sodium Hydroxide (Thermo Fisher Scientific Inc, USA), boric indicator, Hydrochloric Acid (Sigma-Aldrich, USA), Ammonium Sulphate (Thermo Fisher Scientific Inc, USA), Distilled water.

Equipment and apparatus

Fourier Transformed Infrared spectroscopy (FT-IR) (PerkinElmer L160000A, USA), Soxhlet extractor (DWK Life Sciences 2400540, USA), Oven (Thermo Scientific ™ 510288113, USA), Muffle furnace (Thermo Scientific ™ FD1530M, USA), Ultraviolet-Visible (UV-Visible) Spectroscopy (LAMBDA 1050, USA), Kjeldahl apparatus (PYREX™ 3340500, USA), Fume hood (Thermo Scientific ™ 1323, Series Class II), Titration apparatus (PYREX™), distillation apparatus (PYREX™ C362220), desiccator (PYREX™).

Sample collection

The algae sample was collected from an open pond located at Narayi, Kaduna state during the raining season in the month of June.

Identification

The algae sample was identified in the Department of Biological Sciences, Kaduna State University with a voucher number 311.

Sample preparation

The algae were dried at a room temperature for two weeks. The algae were grinded into fine homogenous particle.

Extraction of algae oil

510 g of the sample was weighed, wrapped with a filter, and then fixed into the thimble. The boiling flask was then filled with 2 litres of n-hexane, the soxhlet apparatus was assembled and set at a temperature of 40 °C and allows to reflux for 7 hours. The cake was allowed to dry in an open air, while the extract was fixed in a water bath to evaporate the n-hexane present in the extract. After extraction, the algae oil was weighed to determine the oil yield in grams and in percentage.

$$\%\ \text{yield of oil} = \frac{\text{Weight of oil}}{\text{Weight of sample used (510 g)}} \times 100.$$

Determination of moisture content (AOAC 1994).

Aluminium or plastic dishes were washed and dried to a constant weight in an oven at 100 °C. They were later removed and cooled in a desiccator and weighed (W_1). 2 grams of the grinded (powdered) sample was placed in the weighed moisture dish (W_2). The dish containing the sample was kept in an oven for about 3 hours, the sample were removed and cooled in the desiccator and weighed W_3.

$$\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100.$$

Determination of Ash content

Crucibles were cleansed and dried in the oven, after drying; they were cooled in the desiccator and weighed (W_1). 2 g of the powdered sample was placed in the crucibles and weighed (W_2). They were transferred into the muffle furnace for about 550 °C, then removed and cooled in the desiccator and weighed (W_3).

$$\text{Percentage of ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100.$$

Determination of fibre

2 g of the sample was placed in a beaker containing 1.2 ml of H_2SO_4 per 100 ml of solution and boiled for about 30 min, the residue was filtered and transferred to a beaker containing 1.2 g of NaOH per 100 ml of solution and boiled for about 30 min, the residue was washed with hot water and dried in an oven and weighed (C_2), the weighed sample was incinerated in a furnace for at 550 °C, removed and allowed to cool, and weighed again (C_3).

$$\text{Percentage of fibre} = \frac{C_2 - C_3}{W} \times 100.$$

Determination of lipids (fat)

250 ml boiling flask was cleaned and dried in an oven, transferred into desiccator and allow to cool. An empty filter paper was weighed (W_1). 2 g of sample was weighed into labeled thimbles (filter paper) W_2. The boiling flask was filled with petroleum spirit or n-hexane. The soxhlet apparatus was assembled and allowed to reflux for 8 hours. It was then removed and transfer to an oven to dry, from the oven it was transferred into a desiccator and allowed to cool and was then weighed W_3.

$$\text{Percentage of fat} = \frac{W_2 - W_3}{W_2 - W_1} \times 100.$$

Digestion

2 g of sample was weighed into a kjeldahl flask, a catalyst was added (copper) and 15 ml concentrated sulfuric acid (H_2SO_4), was kept in a fume cupboard, and was heated till solution assumed green colour. It was cooled and black particles showing at the mouth and neck of the flask was washed down with distilled water. After cooling, the digested sample was transferred with several washings into 100ml with distilled water.

Distillation

The sample was steamed through the Markham distillation apparatus for about 15 min, under the condenser was placed a 100 ml conical flask containing 10 ml of boric indicator. 10 ml of the digest was pipetted into the body of the apparatus via the small funnel aperture; was washed down with distilled water followed by 10 ml of 40% NaOH solution. The digest was steamed through for about 5–7 min to collect ammonium sulphate (about 40 ml), the receiving flask was then removed and the tip of the condenser was washed down into the flask.

Titration

The solution was titrated in the receiving flask using N/100 (0.01 N) hydrochloric acid and the nitrogen content was calculated and hence the protein content of the sample.

A blank was always run through along with the sample.

$$\%\text{ of protein} = \frac{\text{Final reading} - \text{Initial reading} - \text{Blank } (0.2) \times \text{Standart reading}}{\text{Initial weight } (0.5) \times \text{Standart number of protein}} \times 100.$$

Determination of carbohydrate (CHO) (AOAC 1994)

By difference, in this method carbohydrate content was obtained by calculations having estimated all other fractions by proximate analysis.

$$\%\text{ of Carbohydrate} = 100 - (\%\text{ of moisture} + \%\text{ Ash} + \%\text{ Protein} + \%\text{ Fat}).$$

Identification using infrared spectroscopy (FT-IR)

Infrared spectroscopy is a technique used to identify various functional groups in Unknown substances through the identification of different covalent bonds that are present in the compound. By identifying the different covalent bonds that are present in a compound, one can establish the types of functional groups present. By comparing the absorption seen in an experimental spectrum to the literature absorptions in various functional groups, one can determine a list of possible identities for the bond present as previously described [4].

Determination by Ultraviolet-Visible (UV-Visible) Spectroscopy

The ultraviolet-visible spectroscopy utilizes light to determine the abundance or transmission of a chemical species in either solid or aqueous state.

Results and Discussion

The algae oil from *Spirogyra porticallis* was extracted using soxhlet apparatus with n-hexane and the oil yield was 1.05% (Fig. 1). The absorption peaks were found at specific bands characteristic of triglycerides. Band at 3011.7 cm^{-1} was attributed to the stretching vibration of =C-H. Strong band absorption was observed in the region of 3000 to 2800 cm^{-1} due to C-H stretching vibrations. The spectra of algae oil are similar to that of vegetable oil. Bands at 2922.2 cm^{-1} and 2855 cm^{-1} attributed to methylene ($-CH_{2-}$) and methyl ($-CH_3$) groups due to stretching vibrations while absorption bands at 1710 cm^{-1} and 1744 cm^{-1} showed carboxylic groups for algae oil and vegetable oil (control) which could be attributed to C=O stretching vibrations (esters)as seen below (Fig. 2).

The absorption spectra for chlorophyll A and carotenoids were found to be present at 408 nm and 660 nm respectively as shown in Fig. 3. The mineral content of the

Fig. 1. **Oil extract with n-hexane from *Spirogyra porticallis***

algae cake was analyzed and found to be rich in the following minerals: potassium 1602.5 mg/100g, calcium 632.5 mg/100 g, phosphorus 14.9 mg/100 g and sodium 12.7 mg/100 g respectively (Table).

The nutritional composition of the residue (cake) was analyzed and these include the following: ash with 2.18%, moisture with 3.78%, lipid 8.83%, crude protein 6.02%, crude fiber 3.22% and carbohydrate 79.18% respectively (Fig. 4).

The characteristics of infrared spectra for algae oil is shown in Fig. 2. The spectra look very similar and showed a typical characteristics of absorption peaks for common tryglycerides; Band at 3011.7 cm^{-1} is attributed to the stretching vibration of =C-H [10, 11]. Strong band absorption was observed in the region of 3000 to 2800 cm^{-1} due to C-H stretching vibrations [11]. The stretching vibrations of methylene ($-CH_{2--}$) and methyl (-CH3) groups can be seen at frequencies of 2922.2 and 2855 cm^{-1}, respectively [11, 12]. Methylene and methyl groups are also observed at 1461 cm^{-1} and 1379 cm^{-1} due to their bending vibrations. The band at 1606 cm^{-1} is attributed to the stretching vibrations of =C-C. The peak around 1710 cm^{-1} is due to C=O double bond stretching vibration [18]. Deformation and bending of C-H and stretching vibration of C-O result in peaks in the 1500–650 cm^{-1} region [18]. The spectra of algae oil is similar to that of vegetable oil as seen in Fig. 2, the differences between the spectra of algae oil and that of vegetable oil was found at peak intensity of 1710 cm^{-1}, due to the C=O stretching vibrations (carboxylic group) for algae oil and 1744 cm^{-1} for vegetable oil attributing to the C=O stretching vibrations (esters) [10, 4]. The UV-Vis spectrum of algae oil showed two peaks at 408nm and 660nm. These peaks are likely to be carotenoids and chlorophyll A respectively which corroborate with previous studies [8]. The oil was extracted using the solvent n-hexane, the percentage of oil gotten from the soxhlet extraction was 1.05% shown in Fig. 1.

Mineral composition of Algae residue of *Spirogyra porticallis* after oil extraction

Mineral	Concentration mg/100 g
Ca	632.5
K	1602.5
Na	12.7
P	14.9

The proximate composition result of the algae residue of *Spirogyra porticallis* after oil extraction is shown in Fig. 3 revealed average moisture of 2.78% [13].The low moisture content of the algae residue showed that the residue is less prone to deterioration since food with high moisture contents are prone to perishability [14]. The percentage ash content was 2.00% which gives an indication of the mineral elements present. Dietary ash has proved helpful in establishing and maintaining acid-alkaline balance of the blood system [15] as well as in controlling hyperglycemia condition [13]. The percentage lipid was 8.03%. Dietary lipids are important not only because of their high energy value but the fat soluble vitamins and essential fatty acids contained in the fats of natural foods [15–17]. Lipids help to regulate blood pressure and play useful roles in the synthesis and repair of vital parts [16, 17].

The percentage protein was 5.00%. Proteins are important in the body for the production of hormones, enzymes and blood plasma. They are immune boosters and can help in cell division as well as growth [18]. The average percentage fibre was 3.01%. Fibres are parts of plants and vegetables which can neither be digested nor absorbed by the human system [19].

Generally, dietary fibre function in the body to slow down the rate of glucose absorption into the blood stream thereby reduces the risk of hyperglycemia [13, 14]. They also reduce the levels of plasma cholesterol and prevent colon cancer and cardiovascular diseases [19]. The percentage carbohydrate was 79.18% and is the major nutrients in the algae residue. They are consumed by man and animals as the major source of energy. Carbohydrates are hydrolyzed in the body to yield glucose

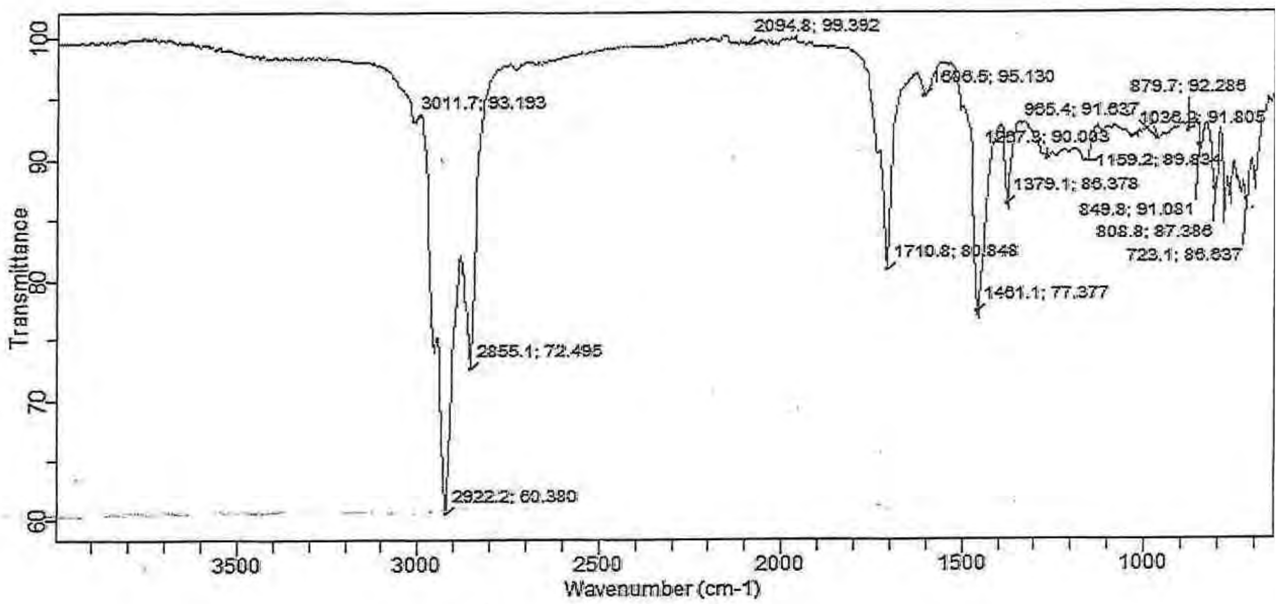

Fig. 2. **FTIR spectrum of oil extracted from *Spirogyra porticallis***

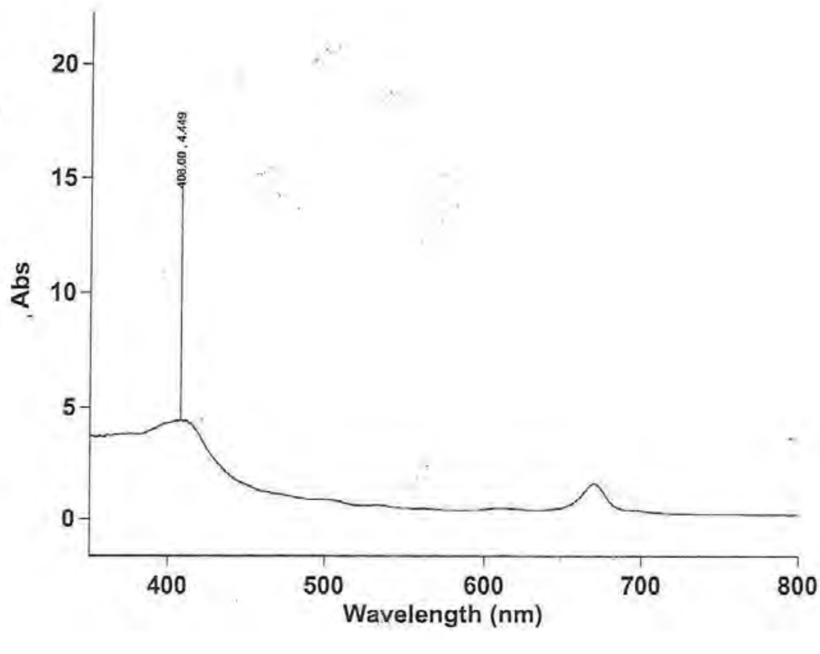

Fig. 3. **UV-Visible absorption spectrum of oil extracted from *Spirogyra porticallis***

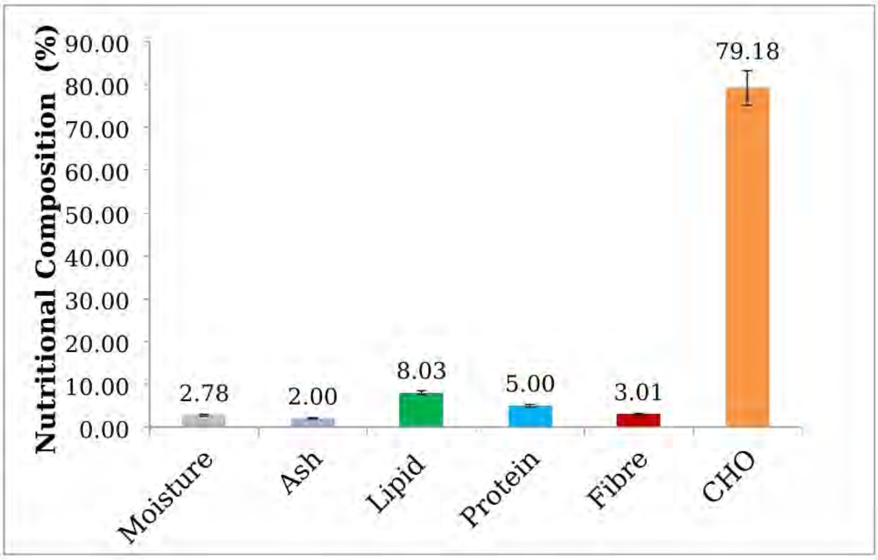

Fig. 4. **Proximate composition of Algae cake**

which can be utilized immediately or stored as glycogen in the muscle and liver for future use [19]. These nutrients in the algae residue make it a good source of energy for animal feeds [20].

The mineral analysis as shown in Table, has potassium (K) serving as the major mineral in the algae residue with a value of 1602.5 mg/100 g. Potassium is a mineral and an essential nutrient needed for a wide range of vital functions. There are incredible amounts of benefits in eating a potassium rich diet [20]. The human body needs 4700 mg everyday because it does so much for the body. Some of the roles played by potassium include brain health support, osmotic balance between cells and the interstitial fluid. Calcium (Ca) is the second major mineral contained in the algae residue with a value of 632.5 mg/100 g. Calcium is essential for bone formation and development [13].

Phosphorous (P) has a value of 14.9 mg/100 g. Phosphorous plays a role in the formation of bones and teeth, it also plays important role in how the body uses carbohydrates and fats. It is also essential to all living things as it forms the sugar-phosphate backbone of DNA and RNA [19]. It is equally important in energy transfer in cells as part of ATP (adenosine triphosphate), and is found in many other biologically important molecules [14]. The mineral with the least value/composition is sodium (Na) with a value of 12.7 mg/100 g. Sodium helps with the body's function of nerves and muscles; it also helps to keep the right balance of fluids in the body.

The results of analysis carried out on algae oil and the residue after extraction has shown that algae have potentials for wider usage. However, it is pertinent to subject the algae oil and residue for further nutritional and toxicity screening to ascertain safety levels. The infrared analysis of the oil studied is in agreement with those of conventional vegetable oil. This research work shows that great potentials exist for the use of algae instead of considering them as waste or pollutants.

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ХАРАКТЕРИСТИКА ОЛІЇ ТА МАКУХИ З *Spirogyra porticallis*

D. D. Gaiya[1, 3], *L. L. Ashafa*[4], *M. E. Entonu*[1, 2, 3], *S. K. Udu*[1, 2, 3]

[1]Департамент біології, Технологічний інститут ВПС Кадуна, Нігерія
[2]Африканський центр вдосконалення тропічних захворювань та судово-біотехнологій, Університет Ахмаду Белло, Зарія, Нігерія
[3]Кафедра біохімії Університету Ахмаду Белло, Зарія, Нігерія
[4]Кафедра біохімії Державного університету Кадуни, Нігерія

E-mail: gaiyadaniel@gmail.com

Мета. Пошук здорової та їстівної альтернативної олії з водоростей. Така олія має багато переваг для здоров'я, головним чином завдяки формі докозагексаєнової кислоти (DHA) омега-3 жирних кислот та деяких інших мікроелементів у менших кількостях.

Методи. Було застосовано метод екстракції Сокслета для вилучення олії з н-гексаном як розчинника. Приблизний склад визначали методами AOAC, а вміст мінералів — AAS. Спектри FTIR та видимі ультрафіолетові проби знімали за допомогою спектрометра Agilent-FTIR та спектрофотометра видимого ультрафіолетового випромінювання. Вихід олії був дуже низьким (1,05%).

Результати. Приблизний склад виявив вуглевод як основну поживну речовину у залишку (79,18%), інші включають ліпіди (8,03%), сирий протеїн (5,00%), вологу (2,78%), сиру клітковину (3,01%) та золу (2,00%). Мінеральний склад виявив велику кількість калію (1602,5 мг/100 г) та кальцію (632,5 мг/100 г) з низьким рівнем фосфору (14,9 мг/100 г) та натрію (12,8 мг/100 г). Спектр FTIR олії водоростей подібний до звичайної рослинної олії. Розтягувальні вібрації при 2922,2 $см^{-1}$ та 2855 $см^{-1}$ приписують метиленовій (-CH_{2}--) та метиловій (-CH_3) групам, тоді як смуги поглинання при 1710 $см^{-1}$ та 1744 $см^{-1}$ показали карбонові групи для водоростей та рослинної олії (контроль), що пояснювали розтягувальними коливаннями (ефіри) C = O. УФ-видимий спектр олії водоростей показав два піки при 408 нм та 660 нм для каротиноїдів та хлорофілу А відповідно, що підтверджує попередні дослідження.

Висновки. Ми дійшли висновку, що олія та макуха, що характеризуються Spirogyra porticallis, мають великий потенціал для лікарського та харчового використання.

Ключові слова: *Spirogyra porticallis*; водорості; докозагексаєнова кислота; каротиноїд; хлорофіл; екстракція Сокслета; спектрометр Agilent-FTIR.

ХАРАКТЕРИСТИКА МАСЛА И ЖМЫХА ИЗ *Spirogyra porticallis*

D. D. Gaiya[1, 3], *L. L. Ashafa*[4], *M. E. Entonu*[1, 2, 3], *S. K. Udu*[1, 2, 3]

[1]Департамент биологии Технологического института ВВС Кадуна, Нигерия
[2]Африканский центр передового опыта по забытым тропическим болезням и судебной биотехнологии, Университет Ахмаду Белло, Зария, Нигерия
[3]Кафедра биохимии, Университет Ахмаду Белло, Зария, Нигерия
[4]Кафедра биохимии, Государственный университет Кадуна, Нигерия
E-mail: gaiyadaniel@gmail.com

Цель. Поиск здоровых и пищевых альтернативных масел из водорослей. Такие масла очень полезны для здоровья, главным образом, благодаря форме докозагексаеновой кислоты (DHA), омега-3 жирным кислотам и некоторым другим питательным микроэлементам в меньших количествах.

Методы. Был использован метод экстракции Сокслета для экстракции масла н-гексаном в качестве растворителя. Примерный состав был определен методами AOAC, а содержание минералов — методом AAS. Спектры FTIR и УФ-видимого масла снимали с использованием Agilent-FTIR-спектрометра и УФ-видимого спектрофотометра соответственно.

Результаты. Выход масла был очень низким (1,05%). Примерный состав показывает углеводы как основные питательные вещества в остатке (79,18%), другие включают липиды (8,03%), сырой белок (5,00%), влагу (2,78%), сырую клетчатку (3,01%) и золу (2,00%). Минеральный состав содержит большое количество калия (1602,5 мг/100 г) и кальция (632,5 мг/100 г) с низким уровнем фосфора (14,9 мг/100 г) и натрия (12,8 мг/100 г). Спектр FTIR масла из водорослей аналогичен спектру обычного растительного масла. Колебания растяжения при 2922,2 $см^{-1}$ и 2855 $см^{-1}$ приписываются метиленовым (-CH_{2}-) и метильным (-CH_3) группам, в то время как полосы поглощения при 1710 $см^{-1}$ и 1744 $см^{-1}$ показывают карбоксильные группы для масла водорослей и растительного масла (контроль), которое было приписано валентным колебаниям C = O (сложные эфиры). УФ-видимый спектр масла водорослей показал два пика при 408 нм и 660 нм для каротиноидов и хлорофилла А соответственно, что подтверждается результатами предыдущих исследований.

Выводы. Мы пришли к выводу, что масло и жмых, полученные из Spirogyra porticallis, обладают большим потенциалом для использования в лечебных и пищевых целях.

Ключевые слова: *Spirogyra porticallis*; водоросли; докозагексаеновая кислота; каротиноид; хлорофилл; экстракция Сокслета; спектрометр Agilent-FTIR.