ISBN: 978-628-95207-8-1. ISSN: 2414-6390. Digital Object Identifier: 10.18687/LACCEI2024.1.1.568

Comparative study between different extracts and brands of spirulina regarding antioxidant activity

Elvis Gilmar Gonzales-Condori, D.Sc.[1], Veronica Remigia Jara-Quille B.Sc.[2], Jonathan Gonzales-Condori, B.Sc.[3], Rosa Alvarez-Gonzales, B.Sc.[2].
[1]Universidad Tecnológica del Perú (UTP), Av. Tacna y Arica 160, Arequipa, Perú, elvgonzalesc@gmail.com
[2] Centro de Investigación Kodama S.A.C., Urb. Leoncio Prado U10, Paucarpata, Arequipa, Perú, veronicaremigiajaraquille@gmail.com, rosalizethag@gmail.com
[3]Universidad Católica de Santa María (UCSM), Urb. San José s/n Umacollo, Arequipa, Perú, jogonzaco@gmail.com

Abstract: Spirulina is a seaweed consumed worldwide and studies related to its biological properties have increased in recent years. However, comparative studies on the antioxidant activity of spirulina are currently scarce. For this reason, this study compared the antioxidant activity of aqueous, hydroalcoholic, and ethanolic extracts of four brands of spirulina (B1, B2, B3, and B4), which are frequently consumed in the city of Arequipa, Peru. For this purpose, fluid extracts of spirulina were prepared using ultrasound. All four brands of spirulina studied were found to exhibit significant antioxidant activity. The aqueous extracts obtained antioxidant activity values of 51.70 ± 0.52, 48.24 ± 0.52, 36.82 ± 0.46, and 13.52 ± 0.61 µmol TE/mL for brands B1, B2, B3 and B4, respectively. In the hydroalcoholic extracts, the antioxidant capacity was 6.66 ± 0.53, 6.48 ± 0.95, 3.25 ± 0.17, and 2.85 ± 0.26 µmol TE/mL for B1, B2, B3, and B4, respectively. The ethanolic extracts showed antioxidant activity of 2.50 ± 0.26, 2.33 ± 0.26, 1.06 ± 0.26, and 1.00 ± 0.17 µmol TE/mL for B1, B2, B3, and B4, respectively. Statistically, the aqueous extracts of the four brands were found to be significantly superior to the hydroalcoholic and ethanolic extracts. Finally, a significant difference was found between the brands of spirulina studied, suggesting that the production processes of spirulina may differ in terms of antioxidant activity. For this reason, further studies could emerge from this study such as the search for the composition-brand relationship of spirulina, as well as, the standardization of the production process for a more uniform production of spirulina.

Keywords: Spirulina, antioxidant activity, brand, ultrasound, extract.

I. Introduction

Most diseases nowadays have a complex origin involving various factors such as genetics, lifestyle, and environmental pollution by different chemical substances such as nitrogen oxides, polycyclic aromatic hydrocarbons, heavy metals, pesticides, plasticizers, polychlorinated biphenyls, dioxins, furans, food additives, hormones and antibiotics [1]. Therefore, environmental pollution is a global problem with diverse and substantial implications for public health [2, 3] producing an increase in morbidity and mortality since pollutants can cause degenerative diseases such as respiratory, cardiovascular, central nervous system, reproductive, cancer[4, 5, 6] and diabetes [7].

An alternative to cope with these degenerative diseases is the consumption of plant species [8, 9] which are rich in antioxidant compounds capable of counteracting free radicals [10, 11] as is the case with macroalgae and microalgae [12], [13]. Several species of microalgae are approved by the FDA (Food and Drug Administration) as edible for human consumption like *Generally Recognized*, *Spirulina platensis*, *Euglena gracilis*, and *Chlorella vulgaris* [14]. Specifically, spirulina has been widely used for its bioactive compounds, essential amino acids, vitamins, and minerals [15] for the food, agricultural, pharmaceutical [16, 17], medical and cosmetic industries [18, 19]. For this reason, investments are now being made in the conservation, micropropagation, and overproduction of these edible algae [20].

Spirulina is a cyanobacterium commonly known as blue-green algae [21] unbranched, helical, and filamentous, and has been found in various aquatic environments [22]. Spirulina grows in fresh and saltwater and is known for its high protein and micronutrient content [21, 23]. Commercially produced in large open-air ponds under controlled conditions [24]. Spirulina products are highly promoted for their high vitamin B12 content [13]. Spirulina contains functional compounds, such as phenolics, phycocyanins, and polysaccharides, with antioxidant, anti-inflammatory, and immunostimulant effects [25]. Among the components of spirulina, c-phycocyanin has demonstrated anticarcinogenic activity [26]. Hypolipidemic, hypoglycemic, and hypotensive effects have also been reported [18]. In addition, spirulina has proven to be well accepted for its organoleptic properties and has shown no acute or chronic toxicities, which makes it safe for consumption [27], thus, its processed products are used in agriculture, food industry, pharmacy, perfumery and medicine [28].

The spirulina business has increased in recent years to meet market demand, and the degree of competition is increasing, thus, companies operating in the business may have multiple options to differentiate their production and to improve their margins to achieve differentiation, being the quality of the product what could represent a way to create a competitive advantage [29].

The latter could have repercussions in that the production processes are increasingly variable, causing the properties of the spirulina sold to vary from one brand to another. For this reason, the present study aims to prove that there is a significant difference in the antioxidant capacity of different brands of spirulina. Likewise, a comparative study of different extracts obtained with different solvents (ethanol, 50 % ethanol, and water) is sought.

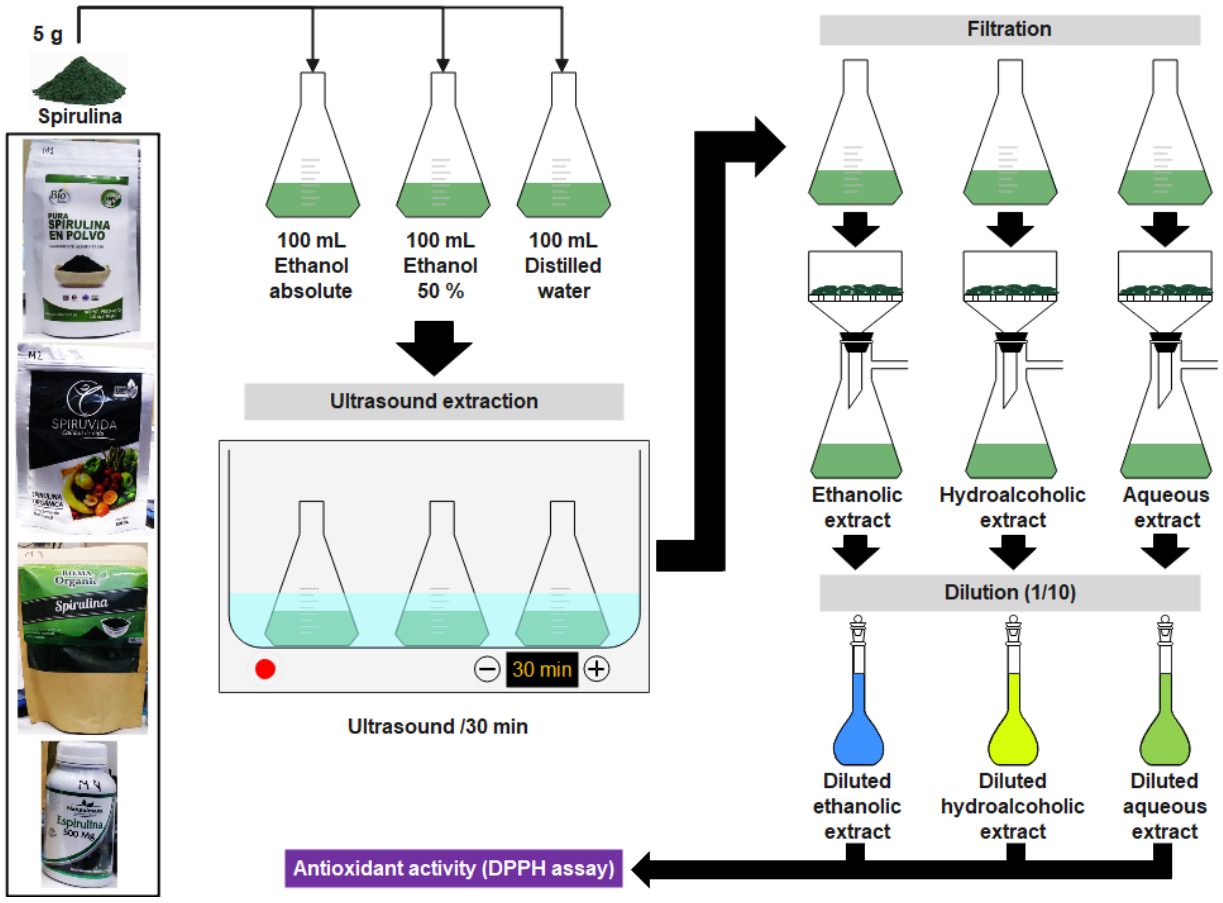

Fig 1. Preparation process of ethanolic, hydroalcoholic, and aqueous extracts by ultrasound.

II. Materials and Methods

A. *Reagents and equipment*

All reagents used in the present study were obtained from Merck. Spectrophotometric analyses were performed on the Thermo Scientific™ GENESYS™ 150 UV-Vis spectrophotometer.

B. *Obtaining spirulina*

Four frequently consumed brands of spirulina were purchased in the city of Arequipa, Peru (-16.4040524, -71.5390115). Brand 1 (B1) corresponds to Bionature, brand 2 (B2) corresponds to SPIRUVIDA, brand 3 (B3) corresponds to RO.MA. Organic and mark 4 (B4) corresponds to Naturalmaxx.

C. *Extract preparation*

Ethanolic, hydroalcoholic, and aqueous extracts were prepared according to the methodology proposed by Chu *et al.* and Wu *et al.* [30, 31] with some modifications. The preparation of the extracts is shown in Fig. 1. The procedure consisted of weighing 5 g of spirulina of each brand in 250 mL flasks, where 100 mL of absolute ethanol, 50 % ethanol, and distilled water were immediately added to obtain ethanolic, hydroalcoholic, and aqueous extracts, respectively. Subsequently, each flask was subjected to ultrasound at 40 kHz for 30 minutes in a Branson ultrasonic bath. Then, each fluid extract was filtered. Finally, all the extracts were diluted by measuring 1 mL in 10 mL flasks where they were diluted with their respective solvent. Each extract was prepared in triplicate.

D. *Antioxidant activity determination*

The antioxidant activity of each extract obtained in triplicate was determined by the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay. For this purpose, standard solutions of Trolox 0.1, 1, 1, 2.5, 5, 7.5, and 10 µmol/mL were prepared. From these solutions, 10 µL were measured into test tubes and reacted with 3 mL of DPPH 0.05 mg/mL in the dark. The absorbance at 517 nm was read after 30 minutes. The equation of the line was determined by plotting the concentration of Trolox vs. the absorbance of DPPH at 517 nm after the 30 minutes of reaction. To analyze the extracts, 10 µL of each diluted extract (Figure 1) was measured in test tubes, then 3 mL of DPPH 0.05 mg/mL was immediately added and allowed to react in the dark for 30 min. Subsequently, the resulting absorbances were measured at 517 nm [31]. Antioxidant activity was expressed in Trolox equivalent micromoles per milliliter of extract (µmol TE/mL). This was calculated using the linear equation.

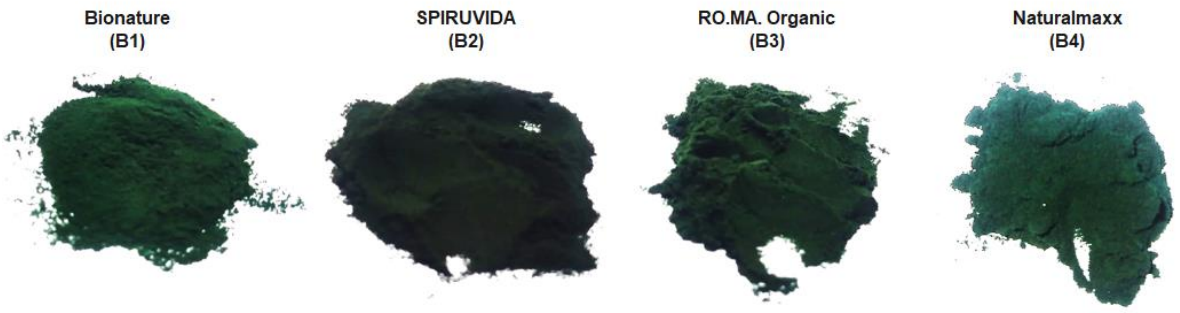

Fig. 2. Spirulina powders of the brands Bionature SPIRUVIDA, RO.MA. Organic and Naturalmaxx.

E. Statistical analysis

Statistical analysis consisted of a Two-way analysis of variance and a Tukey post hoc test. A probability less than 0.05 ($p<0.05$) was considered as a significant difference. The significance level was 95 %.

III. Results and Discussion

A. Spirulina extracts

Fig. 2 shows the spirulina powders corresponding to brands B1, B2, B3, and B4. It can be seen that there is a difference in their coloring, with brands B1 and B4 being lighter in color and B2 and B3 darker.

Fig. 3 shows the diluted extracts of the four brands of spirulina studied. It is observed that the ethanolic extracts (E-Ex) present a blue-green coloration in B1, dark blue in B2, and light blue in B3, and B4. On the other hand, the hydroalcoholic extracts (H-Ex) present a yellow coloration in B1, B3, and B4, however, in B2 the coloration is light brown. The aqueous extracts (A-Ex) are green in all four brands.

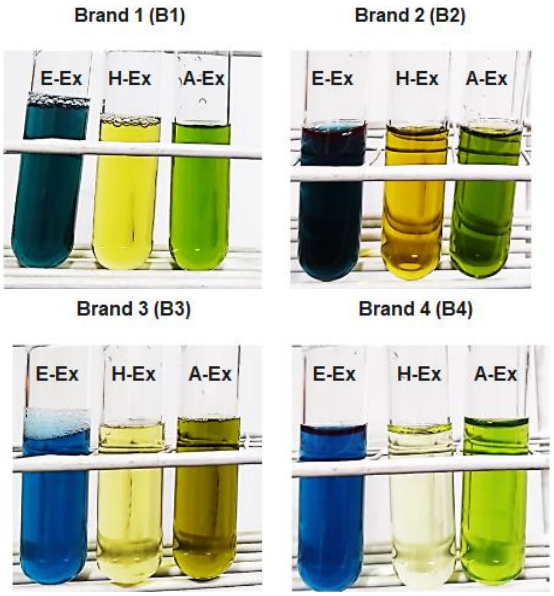

Fig. 3. Ethanolic (E-Ex), hydroalcoholic (H-Ex), and aqueous (A-Ex) extracts of spirulina brands B1, B2, B3, and B4.

The blue coloration of the ethanolic extracts would correspond to a hydrophilic biliprotein known as C-phycocyanin that is formed by a protein and the phycocyanobilin, which is present in blue-green algae such as spirulina [32].

On the other hand, other pigments would also be present in these algae as is the case of chlorophyll a which is dominant in spirulina powders giving it a greenish coloration and the presence of carotenoids would make them take yellow to reddish colors, also, it has been reported that these pigments may vary according to the preparation and storage of spirulina [32]. This would explain the different color intensities of the spirulina powders studied in both powder and extracts.

B. Antioxidant activity of spirulina extracts

Fig. 4 shows the calibration graph for the quantification of antioxidant capacity by the DPPH assay using Trolox as a reference standard at concentrations of 0.1-10 µmol/mL. It is observed that the coefficient of determination (R^2=0.9998) is greater than 0.995 which indicates that the method is linear [33, 34, 35] at the concentrations of Trólox used.

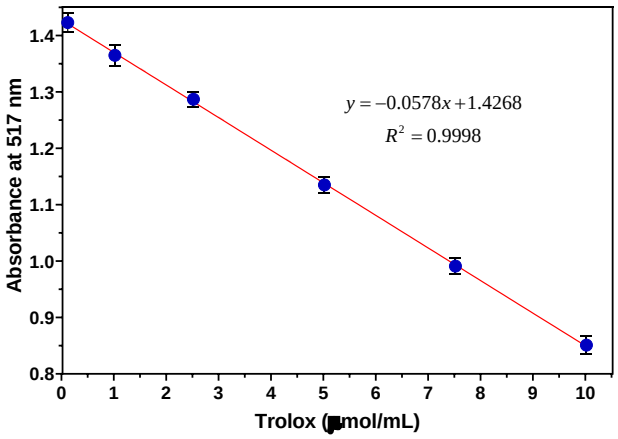

Fig. 4. Calibration graph for the determination of antioxidant capacity by the DPPH method using Trolox as a standard at concentrations of 0.1-10 µmol/mL.

The linear equation is presented in Equation 1.

$$y = -0.0578x + 1.4268 \quad (1)$$

Taking into account that "x" is the concentration of Trolox and in turn represents the antioxidant capacity equivalent to Trolox per milliliter of fluid extract (µmol TE/mL) and "y" is the absorbance of DPPH read in the spectrophotometer at 517 nm, equation 2 is obtained:

$$Antioxidant\ activity(\mu molTE/mL) = \frac{\text{Absorbance at } 517\,nm - 1.4268}{-0.0578} \quad (2)$$

Equation 2 was multiplied by the correction factor corresponding to the 1 in 10 dilutions of each fluid extract, leaving the formula for the calculation of the antioxidant capacity as follows (equation 3):

$$Antioxidant\ activity(\mu molTE/mL) = \frac{\text{Absorbance at } 517\,nm - 1.4268}{-0.0578} \times \frac{10}{1} \quad (3)$$

The absorbances at 517 nm of the 0.05 mg/mL DPPH solutions that reacted with the extracts shown in Figure 3 are presented in Table I.

TABLE I
ABSORBANCES AT 517 NM FROM THE ANALYSIS OF THE ANTIOXIDANT ACTIVITY OF SPIRULINA EXTRACTS BY THE DPPH ASSAY.

Extract	Absorbance at 517 nm			
	Brand 1	Brand 2	Brand 3	Brand 4
Ethanolic	1.414	1.415	1.421	1.422
	1.412	1.413	1.419	1.421
	1.411	1.412	1.422	1.420
Hydroalcoholic	1.389	1.395	1.409	1.412
	1.385	1.384	1.408	1.409
	1.391	1.389	1.407	1.410
Aqueous	1.131	1.145	1.216	1.352
	1.128	1.151	1.215	1.349
	1.125	1.148	1.211	1.345

Replacing the absorbances from Table I in Equation 3, the antioxidant activities of the spirulina extracts of the four brands were obtained. The results are presented in Table I.

Fig. 5 shows the bar graph where the aqueous extracts show higher antioxidant activity than the hydroalcoholic and ethanolic extracts. It is also observed that there is a difference in the antioxidant activity of the different brands. Therefore, a two-way analysis of variance was performed, taking into account the results of the antioxidant capacity in Table II.

Table III shows the two-way analysis of variance applied to the data in Table II. It is observed that the brand, the type of extract, and their interaction have a significant effect on the antioxidant activity, achieving significantly different results at 95 % confidence ($p<0.05$).

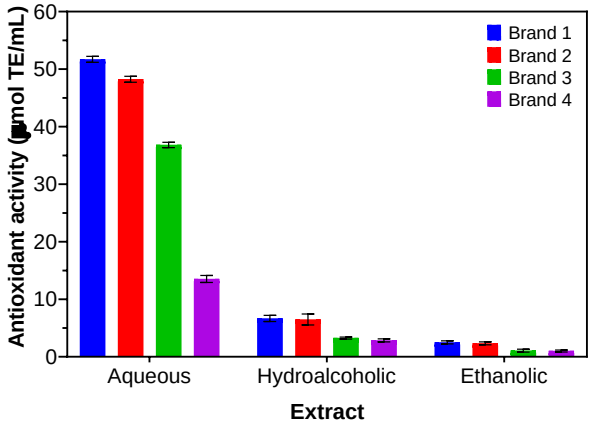

Fig. 5. Antioxidant activity of aqueous, hydroalcoholic, and ethanolic extracts of four brands of spirulina.

TABLE II
ANTIOXIDANT ACTIVITY OF SPIRULINA EXTRACTS DETERMINED BY DPPH ASSAY

Extract	Antioxidant activity (µmol TE/mL)			
	Brand 1	Brand 2	Brand 3	Brand 4
Ethanolic	2.215	2.042	1.003	0.830
	2.561	2.388	1.349	1.003
	2.734	2.561	0.830	1.176
Hydroalcoholic	6.540	5.502	3.080	2.561
	7.232	7.405	3.253	3.080
	6.194	6.540	3.426	2.907
Aqueous	51.176	48.754	36.471	12.941
	51.696	47.716	36.644	13.460
	52.215	48.235	37.336	14.152

To define the equality and difference between brands, a Tukey test was performed in Minitab 19 software. The results are presented in Table IV where equal letters indicate that there is no significant difference and different letters indicate that there is a significant difference. It can be observed that the order from the highest to the lowest antioxidant activity in the different brands is as follows; B1>B2>B3>B4. It can also be extracted that water can extract compounds with higher antioxidant activity than 50% ethanol and absolute ethanol.

The content of carotenoids and chlorophyll present in aqueous and hydroalcoholic extracts could be responsible for their higher antioxidant activity. This could be because the polarity of the solvent used to perform the extractions affects the composition of the extract [36] since the phenolic compounds or secondary metabolites and other compounds present in each extract act synergistically in the antioxidant activity of spirulina extracts [37].

TABLE III
TWO-WAY ANALYSIS OF VARIANCE OF THE RESULTS OF ANTIOXIDANT ACTIVITY IN SPIRULINA EXTRACTS.

Source	Sum of squares	df	Average of squares	F	*p*
Brand	9468.66	2	4734.33	21486.75	*<0.05*
Extract type	1171.61	3	390.54	1772.45	*<0.05*
Interaction	1548.65	6	258.11	1171.43	*<0.05*
Between group	5.29	24	0.22		
Total	12194.21	35			

A similar study was performed using three extracts (water, absolute methanol, and 50% methanol in water) and found that absolute methanol had the highest antioxidant activity against DPPH radicals, followed in descending order by water, and finally aqueous methanol [38]. Similar results were found in this study where water achieved higher antioxidant activity than the hydroalcoholic extract (aqueous ethanol). Considering the above, it could be said that aqueous extracts could have important biological effects, and added to this would be the ease of preparation of an aqueous extract. In addition, the literature shows that aqueous extracts of spirulina have been studied as having antioxidant and antiproliferative effects of activation of hepatic stellate cells (HSC) and human liver cancer cells, HepG2 [31]. Therefore, it would be important to explore the possible application of spirulina incorporation in food and beverage products to improve their antioxidant capacity [30].

TABLE IV
TUKEY'S TEST OF ANTIOXIDANT ACTIVITY IN SPIRULINA EXTRACTS OF DIFFERENT BRANDS.

Factor	N	Average Antioxidant Activity (μmol/mL)	Group						
B1-Aqueous extract	3	51.70	A						
B2-Aqueous extract	3	48.24		B					
B3-Aqueous extract	3	36.82			C				
B4-Aqueous extract	3	13.52				D			
B1-Hydroalcoholic extract	3	6.66					E		
B2-Hydroalcoholic extract	3	6.48					E		
B3-Hydroalcoholic extract	3	3.25						F	
B4-Hydroalcoholic extract	3	2.85						F	
B1-Ethanolic extract	3	2.50						F	
B2- Ethanolic extract	3	2.33						F	G
B3- Ethanolic extract	3	1.06							G
B4- Ethanolic extract	3	1.00							G

IV. CONCLUSION

This study compared the antioxidant capacity of extracts of four brands of spirulina sold in the city of Arequipa, Peru. All four brands of spirulina studied showed antioxidant potential. However, a significant difference in antioxidant activity was found in the extracts obtained with water, 50% ethanol, and absolute ethanol. Aqueous extracts showed significantly higher antioxidant activity than hydroalcoholic and ethanolic extracts. It was found that there is a significant difference between brands of spirulina. With the results obtained, the consumption of spirulina for its antioxidant properties could be suggested. On the other hand, further studies could be carried out looking for the antioxidant activity-composition relationship of spirulina in different brands. Likewise, studies could be initiated to standardize the production process of spirulina since, as demonstrated in this research, there is a difference in the antioxidant capacity between brands of spirulina due to different production processes.

ACKNOWLEDGMENT

The authors thank the Universidad Tecnológica del Perú for funding this research under code P-2023-SUR-06.

REFERENCES

[1] E. Konduracka, "A link between environmental pollution and civilization disorders: a mini review," *Rev. Environ. Health*, vol. 34, no. 3, pp. 227–233, Sep. 2019, doi: 10.1515/reveh-2018-0083.

[2] L. Attademo, F. Bernardini, R. Garinella, and M. T. Compton, "Environmental pollution and risk of psychotic disorders: A review of the science to date," *Schizophr. Res.*, vol. 181, pp. 55–59, Mar. 2017, doi: 10.1016/j.schres.2016.10.003.

[3] Y. Jin, S. Wu, Z. Zeng, and Z. Fu, "Effects of environmental pollutants on gut microbiota," *Environ. Pollut. Barking Essex 1987*, vol. 222, pp. 1–9, Mar. 2017, doi: 10.1016/j.envpol.2016.11.045.

[4] I. Manisalidis, E. Stavropoulou, A. Stavropoulos, and E. Bezirtzoglou, "Environmental and Health Impacts of Air Pollution: A Review," *Front. Public Health*, vol. 8, p. 14, 2020, doi: 10.3389/fpubh.2020.00014.

[5] X. Xu, S. Nie, H. Ding, and F. F. Hou, "Environmental pollution and kidney diseases," *Nat. Rev. Nephrol.*, vol. 14, no. 5, pp. 313–324, May 2018, doi: 10.1038/nrneph.2018.11.

[6] G. N. Carpio-Paucar, A. I. Palo-Cardenas, A. N. Rondón-Ortiz, A. Pino-Figueroa, E. G. Gonzales-Condori, and J. A. Villanueva-Salas, "Cytotoxic Activity of Saponins and Sapogenins Isolated from *Chenopodium quinoa* Willd. in Cancer Cell Lines," *Scientifica*, vol. 2023, p. e8846387, Dec. 2023, doi: 10.1155/2023/8846387.

[7] A. Bitam and O. Aissaoui, "Chapter 32 - Spirulina platensis, oxidative stress, and diabetes," in *Diabetes (Second Edition)*, V. R. Preedy, Ed., Academic Press, 2020, pp. 325–331. doi: 10.1016/B978-0-12-815776-3.00033-4.

[8] S.-L. Chen *et al.*, "[Molecular genetics research of medicinal plants]," *Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi China J. Chin. Mater. Medica*, vol. 44, no. 12, pp. 2421–2432, Jun. 2019, doi: 10.19540/j.cnki.cjcmm.20190514.102.

[9] E. G. Gonzales-Condori, F. Raymi, M. Charca, and A. Paz-Aliaga, "Hepatoprotective effect of *Morus nigra* L. leaves on acetaminophen induced liver damage in rats," *Rev. Cuba. Plantas Med.*, vol. 26, no. 3, Art. no. 3, Apr. 2022, Accessed: Jan. 31, 2024. [Online]. Available: https://revplantasmedicinales.sld.cu/index.php/pla/article/view/1006

[10] J. Chen, J.-W. Wang, and Y.-H. Shu, "[Review on the effects of heavy metal pollution on herbivorous insects]," *Ying Yong Sheng Tai Xue Bao J. Appl. Ecol.*, vol. 31, no. 5, pp. 1773–1782, May 2020, doi: 10.13287/j.1001-9332.202005.035.

[11] H. Alkadi, "A Review on Free Radicals and Antioxidants," *Infect. Disord. Drug Targets*, vol. 20, no. 1, pp. 16–26, 2020, doi: 10.2174/1871526518666180628124323.
[12] M. F. de Jesus Raposo, A. M. B. de Morais, and R. M. S. C. de Morais, "Marine polysaccharides from algae with potential biomedical applications," *Mar. Drugs*, vol. 13, no. 5, pp. 2967–3028, May 2015, doi: 10.3390/md13052967.
[13] S. Grosshagauer, K. Kraemer, and V. Somoza, "The True Value of Spirulina," *J. Agric. Food Chem.*, vol. 68, no. 14, pp. 4109–4115, Apr. 2020, doi: 10.1021/acs.jafc.9b08251.
[14] M. M. Gouda and M. A. Tadda, "Chapter 19 - Microalgae potentials as bioactive phytochemicals for human's health: Novel highlights on their production, applications, and emerging analytical technologies," in *Algal Biotechnology*, A. Ahmad, F. Banat, and H. Taher, Eds., Elsevier, 2022, pp. 377–408. doi: 10.1016/B978-0-323-90476-6.00016-9.
[15] "Spirulina," in *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*, Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, 2012. Accessed: Oct. 04, 2021. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK548312/
[16] M. A. Hernández Lepe, A. Wall-Medrano, M. A. Juárez-Oropeza, A. Ramos-Jiménez, and R. P. Hernández-Torres, "[Spirulina and its hypolipidemic and antioxidant effects in humans: a systematic review]," *Nutr. Hosp.*, vol. 32, no. 2, pp. 494–500, Aug. 2015, doi: 10.3305/nh.2015.32.2.9100.
[17] M.-C. Serban *et al.*, "A systematic review and meta-analysis of the impact of Spirulina supplementation on plasma lipid concentrations," *Clin. Nutr. Edinb. Scotl.*, vol. 35, no. 4, pp. 842–851, Aug. 2016, doi: 10.1016/j.clnu.2015.09.007.
[18] J. Reboleira *et al.*, "Chapter 3.39 - Spirulina," in *Nonvitamin and Nonmineral Nutritional Supplements*, S. M. Nabavi and A. S. Silva, Eds., Academic Press, 2019, pp. 409–413. doi: 10.1016/B978-0-12-812491-8.00055-2.
[19] T. Sen and S. K. Samanta, "Medicinal plants, human health and biodiversity: a broad review," *Adv. Biochem. Eng. Biotechnol.*, vol. 147, pp. 59–110, 2015, doi: 10.1007/10_2014_273.
[20] M. Niazian, "Application of genetics and biotechnology for improving medicinal plants," *Planta*, vol. 249, no. 4, pp. 953–973, Apr. 2019, doi: 10.1007/s00425-019-03099-1.
[21] J. J. DiNicolantonio, A. G. Bhat, and J. OKeefe, "Effects of spirulina on weight loss and blood lipids: a review," *Open Heart*, vol. 7, no. 1, p. e001003, 2020, doi: 10.1136/openhrt-2018-001003.
[22] T.-S. Vo, D.-H. Ngo, and S.-K. Kim, "Chapter 19 - Nutritional and Pharmaceutical Properties of Microalgal Spirulina," in *Handbook of Marine Microalgae*, S.-K. Kim, Ed., Boston: Academic Press, 2015, pp. 299–308. doi: 10.1016/B978-0-12-800776-1.00019-4.
[23] P. Van Hung, "Phenolic Compounds of Cereals and Their Antioxidant Capacity," *Crit. Rev. Food Sci. Nutr.*, vol. 56, no. 1, pp. 25–35, 2016, doi: 10.1080/10408398.2012.708909.
[24] D. Wan, Q. Wu, and K. Kuča, "Chapter 57 - Spirulina," in *Nutraceuticals (Second Edition)*, R. C. Gupta, R. Lall, and A. Srivastava, Eds., Academic Press, 2021, pp. 959–974. doi: 10.1016/B978-0-12-821038-3.00057-4.
[25] A. Finamore, M. Palmery, S. Bensehaila, and I. Peluso, "Antioxidant, Immunomodulating, and Microbial-Modulating Activities of the Sustainable and Ecofriendly Spirulina," *Oxid. Med. Cell. Longev.*, vol. 2017, p. 3247528, 2017, doi: 10.1155/2017/3247528.
[26] D. Wan, Q. Wu, and K. Kuča, "Chapter 42 - Spirulina," in *Nutraceuticals*, R. C. Gupta, Ed., Boston: Academic Press, 2016, pp. 569–583. doi: 10.1016/B978-0-12-802147-7.00042-5.
[27] G. Gutiérrez-Salmeán, L. Fabila-Castillo, and G. Chamorro-Cevallos, "Nutritional and toxicological aspects of spirulina (Arthrospira)," *Nutr. Hosp.*, vol. 32, no. 1, pp. 34–40, Jul. 2015, doi: 10.3305/nh.2015.32.1.9001.
[28] S. M. Hosseini, K. Khosravi-Darani, and M. R. Mozafari, "Nutritional and medical applications of spirulina microalgae," *Mini Rev. Med. Chem.*, vol. 13, no. 8, pp. 1231–1237, Jun. 2013, doi: 10.2174/1389557511313080009.
[29] C. Santini and A. Cavicchi, "Chapter 9 - Organic and Made in Tuscany Spirulina: the story of Severino Becagli," in *Case Studies on the Business of Nutraceuticals, Functional and Super Foods*, C. Santini, S. Supino, and L. Bailetti, Eds., in Woodhead Publishing Series in Consumer Sci & Strat Market. , Woodhead Publishing, 2023, pp. 161–174. doi: 10.1016/B978-0-12-821408-4.00004-3.
[30] W.-L. Chu, Y.-W. Lim, A. K. Radhakrishnan, and P.-E. Lim, "Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals," *BMC Complement. Altern. Med.*, vol. 10, no. 1, Art. no. 1, Dec. 2010, doi: 10.1186/1472-6882-10-53.
[31] L. Wu, J. A. Ho, M.-C. Shieh, and I.-W. Lu, "Antioxidant and Antiproliferative Activities of Spirulina and Chlorella Water Extracts," *J. Agric. Food Chem.*, vol. 53, no. 10, pp. 4207–4212, May 2005, doi: 10.1021/jf0479517.
[32] A. Barka, A. B. Amira, F. Francis, and C. Blecker, "Physicochemical characterization of colored soluble protein fractions extracted from Spirulina (Spirulina platensis)," *Food Sci. Technol. Int.*, Jul. 2018, doi: 10.1177/1082013218786862.
[33] O. Quattrocchi, S. Abelaira, and R. Felipe Laba, *Introduccion a la HPLC, Aplicacion y Practica*. 1992.
[34] E. G. Gonzales-Condori, R. Alvarez-Gonzales, and S. A. Ramírez-Revilla, "Ultrasound-assisted extraction for the determination of α-linolenic and linoleic acid in vegetable oils by high performance liquid chromatography," *Eclética Quím. J.*, vol. 46, no. 2, Art. no. 2, Apr. 2021, doi: 10.26850/1678-4618eqj.v46.2.2021.p57-66.
[35] J. M. Medina-Pérez *et al.*, "Production of Lactobionic Acid Using Gold Nanoparticles Synthesized with Fruit *Myrciaria dubia* Extract," *J. Nanotechnol.*, vol. 2023, p. e5654802, Jul. 2023, doi: 10.1155/2023/5654802.
[36] M. Herrero, P. J. Martín-Álvarez, F. J. Señoráns, A. Cifuentes, and E. Ibáñez, "Optimization of accelerated solvent extraction of antioxidants from Spirulina platensis microalga," *Food Chem.*, vol. 93, no. 3, pp. 417–423, Dec. 2005, doi: 10.1016/j.foodchem.2004.09.037.
[37] H. E. A. Ali, S. M. M. Shanab, M. A. M. Abo-State, E. A. A. Shalaby, U. M. N. E. Demerdash, and M. A. Abdullah, "Evaluation of Antioxidants, Pigments and Secondary Metabolites Contents in Spirulina platensis," *Appl. Mech. Mater.*, vol. 625, pp. 160–163, 2014, doi: 10.4028/www.scientific.net/AMM.625.160.
[38] E. A. Shalaby and S. M. M. Shanab, "Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis," *IJMS Vol425 Sept. 2013*, Sep. 2013, Accessed: Jan. 31, 2024. [Online]. Available: http://nopr.niscpr.res.in/handle/123456789/24794