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# Polyphenolic Compounds And Antioxidant Activity Of Algerian Spirulina

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***Abstract***
*This study investigated Spirulina collected in Oran Algeria. Spirulina, a blue-green microalga and one of the oldest life forms on the planet, serves as a food source for fish, marine mammals, and humans. It grows naturally in alkaline, mineral-rich lakes in hot, sunny, tropical areas.Phenolic compounds were extracted from the Spirulina using three different organic solvents through a 48-hour cold extraction. Samples of each extract were then prepared alongside laboratory standard solutions for analysis via High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). This analysis identified eight phenolic compounds.The antioxidant activity of the extract was then assessed using a DPPH assay, and its inhibition rate was compared to standard antioxidants. Results showed that Spirulina has strong activity against free radicals, as evidenced by its lower $IC_{50}$ values relative to the standard antioxidants.*
***Keywords:*** *Spirulina.; medicinal plants; polyphenolic compounds; antioxidant activity.*

## INTRODUCTION

*Spirulina* is a filamentous, multicellular microalga with a prokaryotic structure and a multi-layered membrane(Fig1). The filaments, which measure 5-10 µm in width and 50-500 µm in length, are observed as blue-green coils under a light microscope [1,2]. These filaments are composed of regularly coiled vegetative cells encased in a thin sheath, which gives the filaments their characteristic spiral shape.

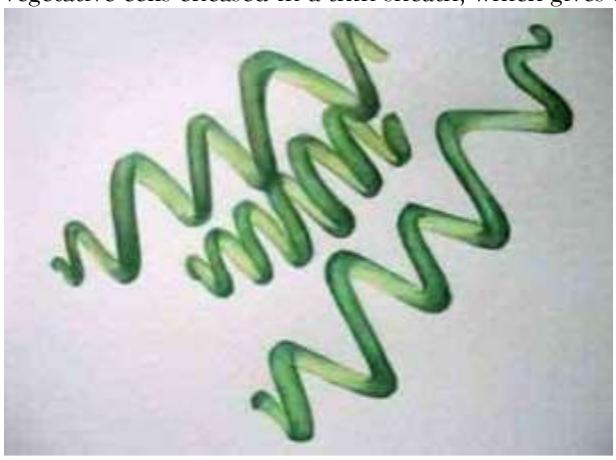


**Figure1** : *Spirulina*

*Spirulina* is considered one of the most ancient forms of life on Earth, with its origins tracing back to the Precambrian Era. Approximately 3.6 billion years ago, the first forms of life, including cyanobacteria like *Spirulina*, appeared [3-6] . These microorganisms were crucial in transforming the planet's atmosphere. They performed photosynthesis, converting carbon dioxide ($CO_2$) into oxygen ($O_2$), which made the atmosphere breathable for future aerobic life. . This process also led to the formation of the ozone layer ($O_3$), which shielded the Earth from harmful ionizing radiation, enabling the evolution of more complex

organisms. Evidence of ancient cyanobacteria has been found in stromatolites, limestone rock formations dating back more than one billion years, discovered in South Africa and Australia [7].

**Historical Use by Humans**

Historically, *Spirulina* has been a food source for humans. A notable example comes from 15th-century Mexico, where the emperor Montezuma enjoyed fresh fish despite his palace being over 300 kilometers from the Gulf of Mexico and 2,000 meters above sea level. To solve this logistical challenge, a relay system of "fish runners" was established. These runners, who carried the fish from the sea to the palace kitchens in record time, relied on *Spirulina* as a significant part of their diet to sustain their endurance[4,8].

The rediscovery of *spirulina* is due to Turbin who isolated it in 1227 from a sample of fresh water, then in 1211 researchers Wittrock and Nordstedt reported blue-green microalgae near Montevideo.

In the 20th century, During World War II, several countries including Japan and Germany attempted to "cultivate" algae to feed soldiers in the face of a blockade. For Japan, it was chlorella, another cyanobacteria that was used and incorporated into many foods[9].

Then in 1911, a French physiologist, Pierre Dangerd, took an interest in the consumption of dehi, a Chadian bread made with spirulina, by people living in Lake Chad and the Rift Valley lakes whose children were not malnourished[10].

The rediscovery of *Spirulina* is attributed to Maurice Turbin, who isolated it in 1960 from a sample of fresh water. Later, in 1962, researchers Jean Léonard and C.A. Wittrock reported the presence of these blue-green microalgae near Lake Chad.

During World War II, several countries, including Japan and Germany, attempted to cultivate algae to feed their soldiers in the face of blockades. While Japan focused on Chlorella, another type of microalgae, incorporating it into various foods, other researchers were taking an interest in *Spirulina's* nutritional potential[11-14].

In 1965, a French physiologist, Pierre Dangeard, became interested in the "dihe", a bread made from Spirulina and consumed by the people living near Lake Chad and the Rift Valley. He noted that the children in these communities showed no signs of malnutrition, highlighting the high nutritional value of *Spirulina*. This observation was key to its modern rediscovery and subsequent commercial development.

The modern rediscovery of *Spirulina* began in the mid-1960s. During a Belgian expedition to Africa, botanist Jean Léonard observed the sale of "dihe" cakes made from *Spirulina* in Chadian markets. His curiosity led to the identification and analysis of this microalga, revealing its exceptional nutritional profile, rich in proteins, vitamins, minerals, enzymes, and pigments[15].

At the same time, the Sosa Texcoco company in Mexico, which was exploiting the alkaline waters of Lake Texcoco for sodium carbonate extraction, found their equipment being clogged by a type of microalgae. After identifying the organism as *Spirulina*, they recognized its immense nutritional value. This led to Sosa

Texcoco becoming one of the first commercial Spirulina producers, a venture they undertook with the American company Riplex Fox, aiming to provide an exceptional nutritional supplement to help combat global hunger.

In 1967, the International Association of Applied Microbiology named *Spirulina* the "superfood of the future." Its potential was further recognized in 1974, when the United Nations World Food Conference declared it the "best food for the future."

The U.S. Food and Drug Administration (FDA) recognized Spirulina's benefits in 1979, allowing it to be legally marketed as a food or dietary supplement, provided it is properly labeled and free of contaminants. This approval allowed it to be incorporated into various food products, such as pasta, cereal bars, and as a food coloring[16-19].

By 1979, *Spirulina* first appeared on store shelves in the United States. That same year, the Earthrise *Spirulina* Company was established in California, which remains a world leader in the market today. This marked the beginning of a global trend, with the establishment of large industrial farms and smaller artisanal farms, particularly in developing countries, to combat malnutrition[20].

The Case of France

In France, it took a while for Spirulina to gain official recognition. The High Council of Public Hygiene gave a favorable opinion on the consumption of algae in 1982. In 2011, the Federation of *Spirulina* Makers in France was established. Its members are united by a commitment to production quality, environmental respect, consumer protection, and mutual support among producers. The number of producers in France has grown from 11 to 115 between 2009 and 2011, with a total production of 10 to 50 tons per year, primarily for the French market. However, between 70% and 80% of the *Spirulina* consumed in France is imported. As of 2012, there were 110 *Spirulina* farms in France, with a large concentration in the south where the cultivation is significantly easier due to the climate[19].

Unlike many other algae, *Spirulina* naturally thrives in alkaline lakes rich in mineral salts, located in hot, sunny, and tropical regions, generally between 15°N and 15°S latitudes.

The regions where it is found naturally include:

- **Africa:** Chad, Kenya, Tanzania (e.g., Lake Natron), Djibouti, Ethiopia, Congo, Zambia, Algeria, Sudan, and Tunisia.
- **Europe:** France (especially in the southwest) and Spain, where it is primarily cultivated rather than native.
- **Asia:** India, Thailand, Myanmar, Sri Lanka, Pakistan, and China.
- **America:** Peru, Mexico, Uruguay, Ecuador, California, Haiti, and the Dominican Republic.

***Spirulina* Reproduction**

*Spirulina*exhibits a high growth rate, with itsquantity doubling approximately every 24 hours. Its reproduction is asexual (vegetative) and occurs through fragmentation or fission.

The reproductive process involves the following stages:

- Fragmentation: A filament (trichome) breaks into smaller pieces, each capable of growing into a new filament.
- Cell Division: The cells within these fragments grow and lengthen.
- Binary Fission: The cells divide into two equal daughter cells, which then continue to grow and form new filaments with the characteristic spiral shape[20].

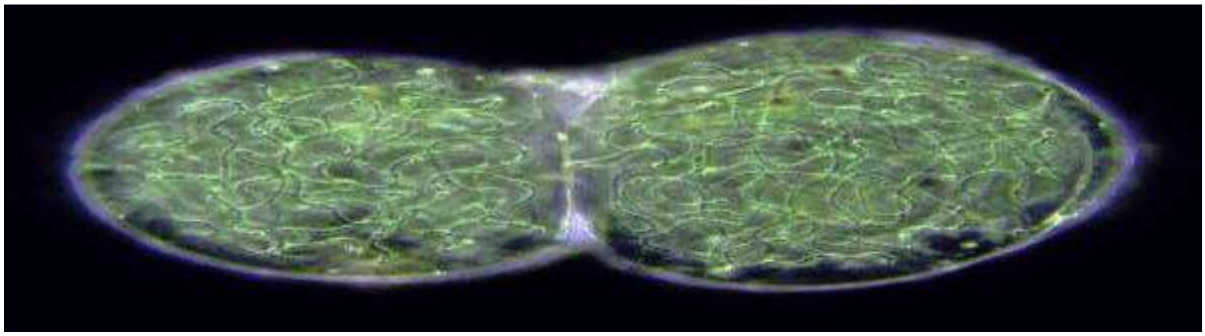
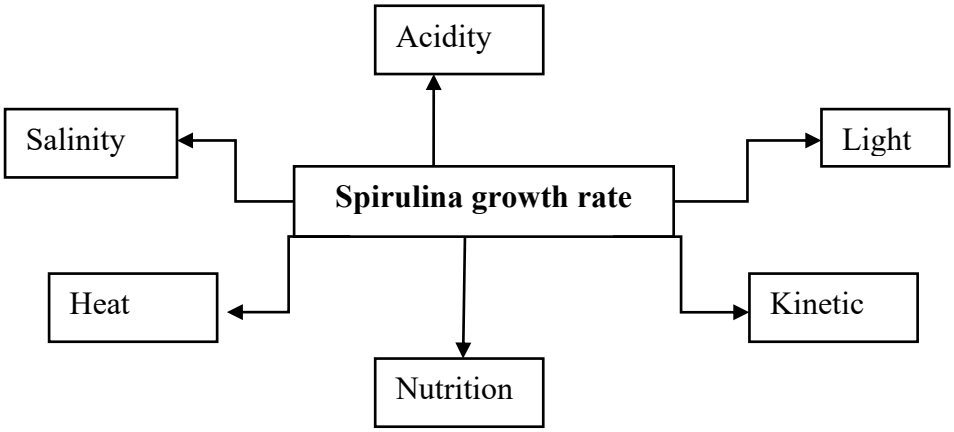


**Figure 3**: Propagation of *Spirulina*

**Factors affecting *spirulina* production:**




**1- Qualitative and quantitative nutritional analysis**

The nutritional composition of *Spirulina* can vary significantly depending on several factors, including:

- Cultivation conditions: This includes the composition of the culture medium.Some producers enrich the media to yield *Spirulina* that is particularly high in specific nutrients like iron, zinc, or fatty acids.
- Harvesting and processing methods: The techniques used for harvesting, drying, crushing, and packaging can all influence the final product's nutrient content.
- Geographical origin and sunlight exposure.

On average, the composition of *Spirulina* is as follows:

- 60-70% Protein: *Spirulina* is an excellent source of protein, containing all eight essential amino acids (leucine, isoleucine, valine, phenylalanine, methionine, tryptophan, threonine, and lysine), as well as many non-essential amino acids (cysteine, glycine, alanine, aspartic acid, glutamic acid, arginine, histidine, proline, and serine).

**13- Therapeutic activities of *spirulina***

Numerous studies, including both preclinical and clinical trials, are currently investigating the potential therapeutic effects of *Spirulina*. This microorganism has demonstrated several beneficial properties, including:

- Antioxidant Activity: It helps combat oxidative stress by neutralizing free radicals.
- Immune System Modulation: *Spirulina* can improve immune function.
- Anticancer and Antitumor Effects: Studies have indicated its potential to inhibit tumor growth.
- Antihypercholesterolemic Activity: It has shown the ability to lower cholesterol levels.
- Antiviral and Antibacterial Activity: Spirulina possesses properties that fight against various viruses and bacteria.
- Nephroprotective Effects: It can reduce kidney damage caused by heavy metals and certain medications.

- Radioprotective Effects: It offers protection against radiation.

Additionally, *Spirulina* has been shown to increase the population of beneficial intestinal lactobacilli.
The water-soluble components of Spirulina have been found to lower blood glucose levels. Furthermore, some studies have indicated that supplementation with 2.2 grams of *Spirulina* taken twice daily for four weeks can lead to a reduction in body weight in obese individuals.

**The role and utility of phenolic compound**

**II 1-6- In plants**

Phenolic compound play a crucial role in various aspects of plant physiology and ecology. They are involved in growth regulation and in molecular interactions with symbiotic and parasitic microorganisms, such as in lignification processes. Furthermore, these compound help plants interact with their biological and physical environment by providing resistance to bacteria, fungi, insects, and ultraviolet radiation.
In addition to their natural functions, phenolic compounds influence the quality standards of harvested plants and derived products, such as fruits, vegetables, and tubers. They affect qualities like color, acidity, and bitterness, which guide human consumption choices. These compounds can undergo transformations during technological treatments like the preparation of fruit juices or fermented drinks, often due to enzymatic reactions that modify the final product's quality[21].

**In Humans**

The role of phenolic compounds in human health is well-documented, primarily due to their ability to interact with various enzymes and their potent antioxidant properties.
Specifically, flavonoids, a major class of phenolic compounds, are attributed with numerous health benefits:

- Antioxidant and Anti-inflammatory Effects: They act as anti-free radical agents and possess significant anti-inflammatory properties.
- Cardiovascular Health: Flavonoids promote the relaxation of blood vessels and prevent platelet aggregation, thereby reducing blood clotting and lowering the risk of atherosclerosis and blood clots. They also limit the oxidation of fats in the blood, which helps protect arterial walls.
- Other Therapeutic Benefits: They have been shown to have antitumor, antiallergic, antibacterial, analgesic, antispasmodic, and hepatoprotective effects. Additionally, they are known to modulate the activity of many enzymes and cellular receptors and may possess an anxiolytic effect [7, 16].

## MATERIAL AND METHODS

**Plante material and extraction of phenolic compound**

*Spirulina* were collected in Oran western Algeria. 2 grams of dried *Spirulina* powder were measured using a sensitive scale. The weighed sample was placed in a flask, and 100 ml of the selected organic solvent (chloroform, methanol, or ethyl acetate) was added.The flask was then stirred for 12 hours at a low temperature to facilitate the extraction of compounds from the *Spirulina*. The mixture was filtered to separate the liquid extract from the solid *Spirulina* residue. The solvents were carefully evaporated from the extracts at a controlled temperature of 40°C.Finally, methanol was added to the concentrated extracts to prepare them for further analysis . The extracts were stored at a temperature of **4°C** to preserve their integrity and prevent the degradation of the phenolic compound.


**Figure :** Algerian *Spirulina*

**Figure**: **Samples** studied

**2- Antioxidant activity**

The assessment of antioxidant capacity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is performed as follows:

1. Preparation of DPPH Solution: 1 mg of DPPH powder is accurately weighed and dissolved in 100 ml of methanol to create a working solution.
2. Sample Preparation: Different concentrations of each *Spirulina* extract are prepared by diluting them in methanol.
3. Reaction Incubation: 1 ml of the DPPH solution is added to each concentration of the extract. The mixture is then incubated for 30 minutes in the dark at room temperature to allow the reaction to stabilize.
4. Absorbance Measurement: A spectrophotometer is used to measure the absorbance of each sample at a wavelength of 517 nm. Before measurement, the device is "zeroed" or "blanked" using a cuvette containing only methanol. The absorbance of each sample is then measured against this blank.
5. Calculation of Inhibition: The decrease in absorbance, which is directly proportional to the antioxidant activity, is measured. The antioxidant capacity is then calculated as the percentage of inhibition using the following formula:

$I\% = ((A_0 - A) / A_0) \times 100$

$A_0$: absorbence of the solvent "without the presence of any antioxidant"

A: absorbance of the sample measured after 11 minute in the dark

I%: DPPH free radical inhibition rate.

The control is the DPPH solution without any extract. The $IC_{50}$ value, which represents the concentration of the extract needed to inhibit 50% of the DPPH radicals, is then determined from the dose-response curve. A lower $IC_{50}$ value indicates a higher antioxidant capacity.

**HPLC /DAD Analysis**

The characterization of phenolic compounds in the different extracts wasperformed using High-Performance Liquid Chromatography with a UV/Diode-Array Detector (HPLC-UV/DAD). The analysis was conducted on an Agilent 1100 apparatus equipped with a Diode Array Detector (DAD). The analysis was carried out in reverse phase with column C18 (250 × 4.5 mm, 5 µm). The flow rate was 0.8 mL/min, and

the temperature was set to 30 °C, and the injection volume selected was 20 µL. The flow rate was fixed at 0.8 mL/min.

The chromatographic conditions consist of solvent A: acetic acid 0.2% and solvent B: methanol (HPLC grade), with the following gradient: 0 min: 95% A + 5% B; 40 min: 30%

A + 70% B at the end 60 min 95% A + 5% B. The DAD detector was used to monitor the separation at multiple wavelengths: 200 nm, 230-260 nm, 320 nm, and 365-380 nm. The phenolic acids and flavonoids present in the extracts were identified by comparing their retention times and UV spectra with those of

pure, commercially available standards. Standards of free aglycones, phenolic acids, and heterosides were injected under the exact same chromatographic conditions as the samples to ensure accurate identification.

## RESULTS AND DISCUSSION

### Antioxidant Activity of *Spirulina* Extracts and Standard Antioxidants

The $IC_{50}$ values represent the concentration of a substance required to scavenge 50% of the free radicals. A lower $IC_{50}$ value indicates a higher antioxidant capacity.

The following table presents the $IC_{50}$ values for the different *Spirulina* extracts and the standard antioxidants used for comparison.

**Table 1:** $Ic_{50}$ values for *spirulina* extracts

| **$Ic_{50}$** | ***Spirulina* samples by solvent** |
|---|---|
| 0.24752±0.04 | Chloroforme |
| 0.43478±0.05 | Ethyl acetate |
| 0.12437±0.08 | Methanol |
| 9,56±0,07 | Vitamin E |
| 7,25±0,03 | Vitamin C |
| 20,11±0,01 | BHT |
| 8,35±0,05 | BHA |

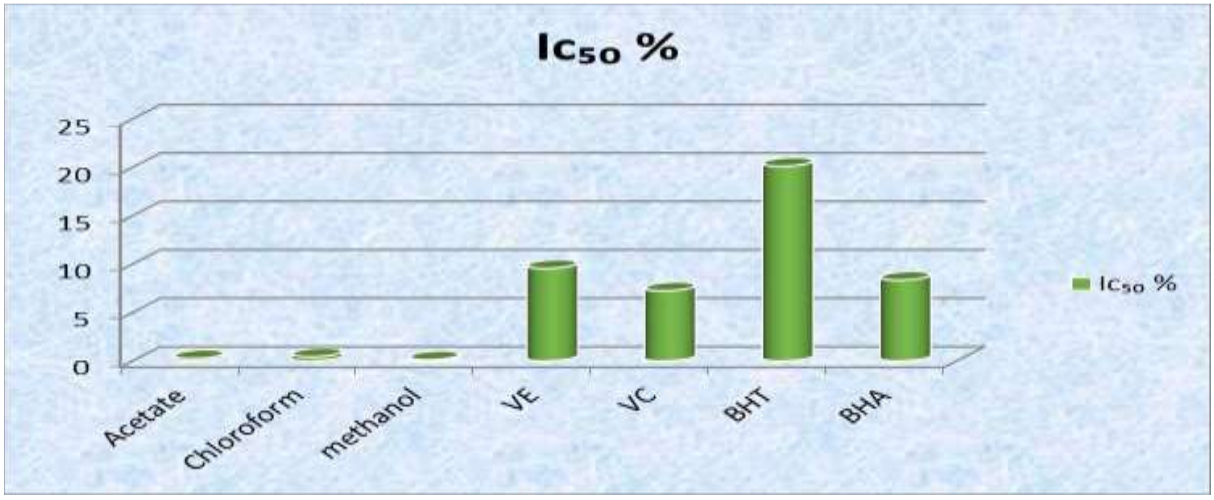



**Figure1 :** $IC_{50}$% for *spirulina* extracts and synthetic antioxidants

The results indicate that the free radical scavenging ability of the phenolic compounds in *Spirulina* is significantly greater than that of the synthetic antioxidants tested(table1, figure1) .

### HPLC/DAD Analysis

### Results of Identified Phenolic Compounds in *Spirulina* Chloroform Extract

The HPLC-DAD analysis of the *Spirulina* chloroform extract identified the following phenolic compounds, with their respective retention times (fig 1, table4):

- Vanillic acid: 16.46 min
- Myricetin: 22.86 min
- Quercetin dihydrate: 25.46 min

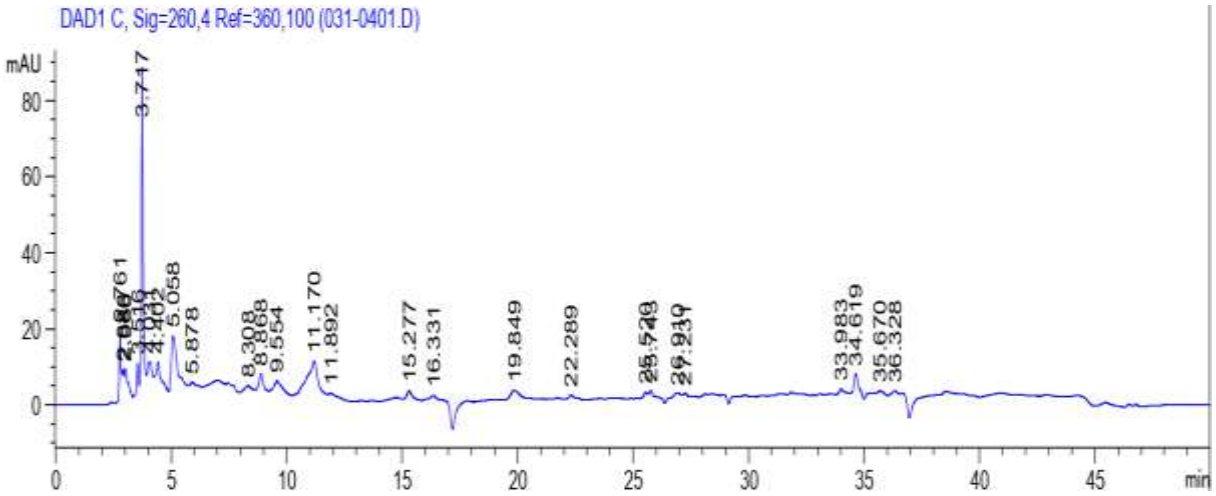



**Figure:** HPLC/DAD chromatogram of the *Spirulina* chloroform extract. The identified peaks are: (1) Vanillic acid, (2) Myricetin, and (3) Quercetin dihydrate.

**Table4 :** Phenolic Compounds in *Spirulina* Chloroform Extract

| N°Pics | Retention time of extract | Area% | Retention time of reference | Identified phenolic compounds |
|---|---|---|---|---|
| 16 | 16.331 | 0.5803 | 16.468 | **Vanillic acid** |
| 18 | 22.289 | 0.6731 | 22.863 | **Merictin** |
| 19 | 25.520 | 0.5849 | 25.465 | **Querctin hydrat** |

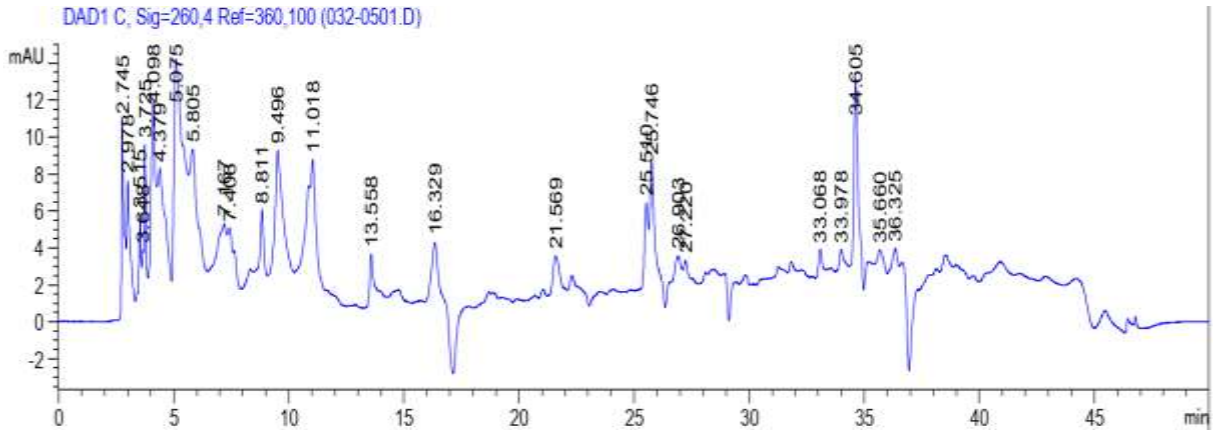



**Figure:** HPLC/DAD chromatogram of the *Spirulina* ethyl acetate extract. The identified peaks are: (1) Boric acid, (2) Catechin hydrate, (3) Vanillic acid, (4) Vitexin, and (5) Quercetin dihydrate. The chromatogram was recorded at a wavelength of 280 nm.

**Results of Identified Phenolic Compounds in *Spirulina* Ethyl Acetate Extract**

The HPLC-DAD analysis of the *Spirulina* ethyl acetate extract identified the following phenolic compounds and their corresponding retention times(fig ; table5) :

- Boric acid: 7.78 min
- Catechin hydrate: 13.93 min
- Vanillic acid: 16.46 min
- Vitexin: 21.67 min
- Quercetin dihydrate: 25.48 min

**Table 5:** Phenolic compounds in HPLC /DAD ***Spirulina*** ethyl acetat extract

| N°Pics | Retention time of extract | Area% | Retention time of reference | Identified phenolic compounds |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 10 | 7.167 | 2.3479 | 7.784 | **Boric acid** |
| 15 | 13.558 | 1.1117 | 13.93 | **Catechin hydrat** |
| 16 | 16.329 | 5.0002 | 16.468 | **Vanillic acid** |
| 17 | 21.569 | 1.4936 | 21.675 | **vitixin** |
| 18 | 25.510 | 1.9674 | 25.485 | **Quercitin hydrat** |

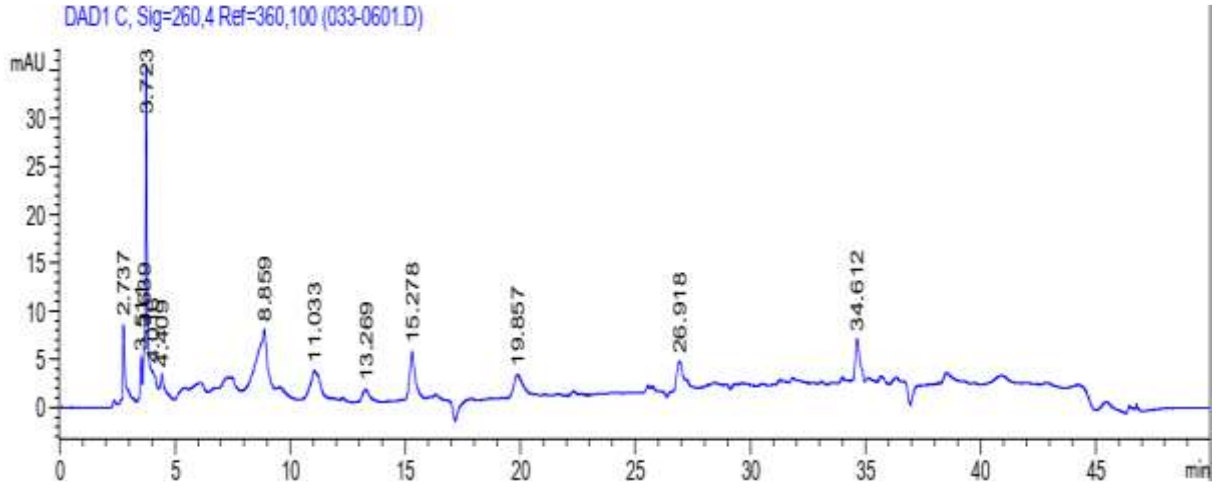



**Figure:** HPLC/DAD chromatogram of the *Spirulina* methanol extract. The identified peaks are: (1) Catechin hydrate, (2) Caffeic acid, and (3) Myricetin. The chromatogram was recorded at a wavelength of 280 nm.

**Results of Identified Phenolic Compounds in *Spirulina* Methanol Extract**

The HPLC-DAD analysis of the *Spirulina* methanol extract identified the following phenolic compounds and their corresponding retention times (fig table6):

- **Catechin hydrate:** 13.93 min
- **Caffeic acid:** 16.83 min
- **Myricetin:** 25.36 min

**Table6 :** Phenolic compounds in HPLC /DAD *Spirulina* methanol extract

| **Pics N°** | **Retention time of extract** | **Area%** | **Retention time of reference** | **Identified phenolic compounds** |
|---|---|---|---|---|
| 9 | 13.269 | 2.6741 | 13.93 | **Catechin hydrat** |
| 10 | 15.278 | 8.4758 | 16.836 | **Cafeic acid** |
| 12 | 26.918 | 26.918 | 25.362 | **Myricetin** |

## CONCLUSION

Due to the growing concerns over the side effects of synthetic drugs, such as toxicity and microbial resistance, there's been a surge in demand for natural, biocompatible products like *Spirulina*. Our work aimed to investigate the chemical properties of *Spirulina* cultivated in Algeria, a resource that could potentially boost the national economy. While numerous studies have explored various aspects of *Spirulina*, there's a notable gap in research concerning its detailed chemical composition. In this study, we analyzed Spirulina using High-Performance Liquid Chromatography (HPLC), and the DPPH assay.

The HPLC-DAD analysis identified several phenolic compounds across different extracts:

- Chloroform Extract: Vanillic acid (tr= 16.46 min), Myricetin (tr= 22.86 min), and Quercetin dihydrate (tr= 25.46 min).Ethyl Acetate Extract: Boric acid (tr= 7.78 min), Catechin hydrate (tr= 13.93 min), Vanillic acid (tR= 16.46 min), Vitexin (tr= 21.67 min), and Quercetin dihydrate (tr= 25.48 min).
- Methanol Extract: Catechin hydrate (tr= 13.93 min), Caffeic acid (tr= 16.83 min), and Myricetin (tr= 25.36 min).

**Antioxidant Activity and Future Perspectives**

Our results from the DPPH assay confirmed that *Spirulina* possesses strong antioxidant activity against free radicals. This finding is highly encouraging and highlights the potential of *Spirulina* in pharmaceutical development.

We recommend several avenues for future research to further explore this "miraculous algae":

- Advanced Analytical Methods: Employing more sophisticated techniques, such as HPLC-MS, to identify a wider range of compounds.
- In Vivo Studies: Assessing *Spirulina's* antioxidant activity in living organisms to confirm its health benefits.
- Antibacterial Activity: Investigating its antibacterial properties, given its classification as a cyanobacterium.
- Anthocyanin Extraction: Developing methods to extract anthocyanins, which *Spirulina* contains in abundance.

These studies could pave the way for a greater understanding and utilization of this valuable natural resource.

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