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Comparative study of spirulina extracts from the regions of Algeria and Egypt

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بسم الله الرحمن الرحيم
١٤٢٠

شكر وعرفان

عن أبي هريرة – رضي الله عنه – قال – صلى الله عليه وسلم :

{ مَن لا يشكُر الناس، لا يَشكُر اللّهّ }

وفاء وتقديرا واعترافا منا بالجميل نتقدم بجزيل الشكر لأولئك المخلصين الذين لم يألو جهدا في مساعدتنا في بحثنا هذا ،ونخص بالذكر الأستاذ الدكتور الفاضل : عمار بن ميه على توجيهه ونصحه لنا جزاه الله كل خير .

ولا يخفى علينا أن نشكر جميع أساتذة قسم البيولوجيا بجامعة الشهيد حمه لخضر على ما قدموه لنا طيلة فترة دراستنا و إلى كل تقنيي المخابر بكلية علوم الطبيعة والحياة و إلى كافة عمال وعاملات جامعة الشهيد حمه لخضر .

إهداء

(وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

" لِـذة الخِتَّام "

الحمد لله الذي بنعمته تتم الصالحات في طياتها الكثير من الصعوبات والمشقة والتعب اليوم نقطف ثمارها بالتخرج.

لم تكن الرحلة قصيرة, ولا ينبغي لها أن تكون ,لم يكن الحلم قريبا , ولا الطريق كان محفوفا بالتسهيلات ,لكني فعلتها .

أهدي تخرجي إلى من كلله الله بالهيبة والوقار إلى من علمني العطاء من دون إنتظار , إلى من أحمل إسمه بكل إفتخار إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم إلى "أبي العزيز".

إلى ملاكي في الحياة قرة عيني وأعز ما أملك ....غاليتي .... إلى بسمة الحياة وسر الوجود التي سهرت وكانت معي في كل حالاتي وضغوطاتي إلى من كان دعائها سر نجاحي "أمي الغالية " .

إلى من رزقت بهم سندا وملاذي الأول والأخير إخوتي
(أحمد ,ونيس ,شعبان ,الهاشمي ,ياسين) حفظهم الله لي ورعاهم .
إلى أختي وحيدتي توتة انتِ يا مصدر الضوء في عمري .
إلى أعز من قلبي صغيرتي و أميرتي أميمة .

إلى رفاق الخطوة الأولى والأخيرة إلى من كانو في سنوات العجاف سحابا ممطرا إلى كل من له الفضل علي وكل من ساهم معي في إنجاز هذا العمل أنا ممتنة جدا .
في الختام أتمنى أن تكون هذه الخطوة إلا بداية لطريق طويل مليئ بالتوفيق والنجاح والسداد .

فالحمد لله على التمام .

سامية

إهداء

الحمد لله وكفى والصلاة والسلام على الحبيب المصطفى وأهله ومن وفى .

الحمد للّه الذي وفقنا لتثمين هذه الخطوة في مسيرتنا الدراسية بمذكرتنا هذه.
أهدي هذا العمل المتواضع إلى الوالدين الكريمين حفظهما الله تعالى و لكل العائلة الكريمة التي ساندتني ولا تزال من إخوة وأخوات وإلى صديقاتي و إلى كل من ساهم وشجعني لإتمام هذه المرحله أهديه لهم سائلة الله أن ينفعنا به .

مسعودة

Abstract

The aim of our work is to scientifically and nutritionally evaluate Algerian and Egyptian spirulina to show their nutritional value.

The spirulina used in this work belongs to Arthrospira, the Spirulina that was brought from Algeria specifically from the Tamanrasset region, and the other from Egypt from the Khatahtba region.

Chemical sorting is done through chemical detection of extracts prepared in two ways: decocted and macerated. In this examination, the results were similar for Spirulina A and Spirulina E (because each of them contains active compounds: flavonoids, tannins, alkaloids, phenols, and others).

We extracted some biologically active compounds by soaking (methanol) and boiling (distilled water). The yield of the extracts showed that the results of the methanolic extract (total phenols) were superior to the rest of the extracts. Among them, the methanolic extract of spirulina E (24.3%) is superior.

By measuring the content of total phenols, flavonoids, and tannins, the methanolic extract exceeded the total phenols of Spirulina E (64.48 micrograms GA/g).Spirulina E recorded a higher percentage of chlorophyll A (19.31 mg/g), chlorophyll B (8.09 mg/g) and carotenoids (3.15 mg/g) than Spirulina A.

We evaluated the antioxidant activity of our extracts using the DPPH test. The DPPH free radical test showed that the results of the methanolic extract (flavonoids) of Spirulina E were superior to the rest of the extracts (IC50=1.65 μg/ml).

We have evaluated the physical and chemical analyzes of spirulina. This evaluation showed that: the content of protein (62.81%), fat (6.39%) and ash (10.87%) in Spirulina E is higher than that of Spirulina A while carbohydrates (22.23%), pH (7.69) and humidity(8.77%) in Spirulina A is higher than Spirulina E.

Keywords: Arthrospira, spirulina, chemical screening, total phenols, flavonoids, tannins, antioxidant, physical and chemical analyses.

الملخص

الهدف من عملنا هو التقييم العلمي و الغذائي للسبيرولينا الجزائرية و المصرية لإظهار قيمتهما الغذائية.

السبيرولينا المستعملة في هذا العمل تنتمي إلى Arthrospira ، Spirulina التي أحضرت من الجزائر تحديدا من منطقة تمنراست و الأخرى من مصر من منطقة الخطاطبة .

يتم الفرز الكيميائي من خلال الكشف الكيميائي للمستخلصات المحضرة بطريقتين :الغلي، النقع ،في هذا الكشف كانت النتائج متقاربه في سبيرولينا A وسبيرولينا E (لإحتواء كل منهما على مركبات نشطة فلافونويدات، عفص،ألكلويدات،فينولات و غيرها).

قمنا بإستخلاص بعض المركبات النشطة بيولوجيا عن طريق النقع (ميثانول) والغلي (ماء مقطر). وضح مردود المستخلصات أن نتائج المستخلص الميثانولي (الفينولات الكلية) تفوقت على باقي المستخلصات. فيما بينها، تفوق المستخلص الميثانولي لسبيرولينا E(24,3%) .

من خلال قياس محتوى الفينولات الكلية و الفلافونويد والعفص، تفوق المستخلص الميثانولي للفينولات الكلية لسبيرولينا E (64,48ميكرو غرام GA /جم).

سجلت سبيرولينا E نسبة من الكلوروفيل أ (19,31ملجم/جم) ،الكلوروفيل ب (8,09ملجم/جم) والكاروتينات (3,15ملجم/جم) أعلى من سبيرولينا A .

قمنا بتقييم النشاط المضاد للأكسدة في مستخلصاتنا عن طريق إختبار ال DPPH .أظهر إختبار الجذور الحرة DPPH أن نتائج المستخلص الميثانولي (الفلافونويدات) لسبيرولينا E تفوق على باقي المستخلصات (IC50=1,65 ميكرو غرام /مل).

تم تقييمنا للتحليل الفيزيائية والكيميائية للسبيرولينا. أظهر هذا التقييم أن : محتوى البروتين (62,81%) والدهون(6,39%) والرماد(10,87%) في سبيرولينا E أعلى من سبيرولينا A بينما الكربوهيدرات (22,23%)ودرجة الحموضة (7,69)والرطوبة (8,77%) في سبيرولينا A أعلى من سبيرولينا E.

الكلمات المفتاحية : Arthrospira ، سبيرولينا ،الفرز الكيميائي ،الفينولات الكلية ، الفلافونويدات ، العفص ، مضاد للأكسدة ، التحاليل الفيزيائية والكيميائية .

ABREVIATIONS LIST

- Spirulina ”A” :Spirulina Algeria .
- Spirulina ”E” :Spirulina Egypt .
- ROS: Reactive oxygen species .
- LPOs : Lipid peroxides.
- MDA:malondialdehyde
- SOD:superoxide dismutase .
- GPx : glutathione peroxidase .
- MGDG:monogalactosyl diacylglycerol.
- DGDG:digalactosyldiacylglycerol .
- SQDG:sulfoquinovosyldiacylglycerol .
- E.coli :Escherichia coli.
- IL-1β:Interleukin-1β .
- IL-6:Interleukin 6 .
- TNFα:Tumor necrosis factor alpha.
- COX-2 : cyclooxygenasee .

- GSH:reduced glutathione .
- HIV-1:human immunodeficiency virus .
- HSV-1 :virus Herpes simplex .
- AGL:gamma-linolenic acid .
- PGE1 : prostaglandin E1.
- SARS-CoV-2 :Severe acute respiratory syndrome coronavirus 2 .
- DPPH: 2,2-diphenyl-1-picrylhydrazil .
- IC50 :50% inhibitory concentration .

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INTRODUCTION

Since the beginning of time, countless people all across the world have used medicinal herbs. Because they contain unique biologically active components (such as alkaloids and terpenoids) that have been employed experimentally in traditional medicine, plants are now frequently exploited to generate a variety of valuable products for human health, including medications. To combat diseases brought on by free radicals linked to oxidative stress, such as heart disease, cancer, and others that affect the central nervous system. Cancer is one of the world's leading causes of death, with an estimated 20 million people having been diagnosed with it and 10 million dying from it. Traditional medicine was created by humans to heal illnesses with medicinal plants and helpful microbes like microalgae. Modern medicine was then developed to replace traditional medicine**(Simpore et al., 2006)**.

One of the nutritional supplements beneficial to the human body, Spirulina platensis is a cyanobacterium that thrives in salt water and offers a balanced diet. It is renowned for its high nutritional value in proteins (60–70% in dry weight), vitamins (B1, B2, B12, E, A, and K), minerals, and essential fatty acids. It also serves as a good source of chlorophyll and carotenoids, particularly phycocyanin, the pigment most often found in plants that gives them their blue color. It is prevalent in spirulina and makes up more than 15% of its weight**(Lafri et al., 2017)**.

It is distinguished by the presence of active and biochemical substances with potential therapeutic advantages and qualities. is abundant in antioxidants, which are crucial for protecting against cancer, boosting the immune system, memory, and kidney, liver, and heart health. It functions as an alternative remedy. gives the body an almost immediate energy boost. Boost endurance and lessen tiredness.**(Uddin et al., 2018)**

Spirulina is grown across the world in three distinct methods (traditional, semi-industrial, and industrial), where it may develop naturally because the right circumstances (pH, salinity, and temperature) are present for its growth. Five thousand tons are produced there annually. Approximately 487,000 EGP (four hundred and eighty thousand Egyptian pounds) would be its economic value based on current market rates. 90% of it is utilized as human food, while 10% is used as animal feed for things like fish and fowl.**(Ahsan et al., 2008)**

In Algeria and Egypt we find farms that produce spirulina, as this microorganism grows in environments that provide moderate temperatures and very high levels of sunlight. It also grows in salt lakes and oceans with a subtropical climate. It is used as a whole food because it contains many nutrients, as well as in animal production. Its production capacity is 30 tons annually.**(Zidane et al., 2022)**

In order to extract secondary metabolites, we focused our work on different methods of extraction, including: extraction by (water, methanol, acetone).

The topic of our study revolves around: Is there a difference between spirulina extracts produced from the regions of Algeria and Egypt?

- Therefore, our work aims to study the chemical examination of spirulina and test its secondary metabolites.
- Extracting some biologically active compounds using different methods and determining the yield and content of total phenols, flavonoids, and tannins.
- Study of the biological activity of spirulina as an antioxidant
- Identify the chemical and physical composition of spirulina produced from the regions of Algeria and Egypt.

Our work will be divided into two parts The theoretical part which contains three chapters

- The first chapter General information on spirulina .
- The second chapter Biochemical composition of spirulina
- chapter three: Biological activities of spirulina
- The practical part, which contains two chapters: The

- first chapter : Materials and Methods The second chapter : Results and Discussion
- And finally, Conclusion

The theoretical part

CHAPTER I

GENERAL INFORMATION ON SPIRULINA

I.1 History

Spirulina is a primitive life form that emerged approximately 3.5 billion years ago **(Olguín et al., 1997)** . It played a significant role in enriching the Earth's oxygen levels and has been consumed by ancient civilizations such as the Aztecs and Mayas **(MEHREZ, 2021)** . While spirulina has been traditionally utilized as a dietary supplement **(Bensehaila, 2016)** , it is important to note that it was one of the earliest organisms to undergo photosynthesis. It was harvested and consumed for centuries by the Kanembous people of Chad in Africa and the ancient Aztecs in the Texcoco Valley of Mexico. The first written account of spirulina appears in the memoirs of Cortès, a conquistador in 1521, describing its consumption in the form of sun-dried pancakes. Rediscovered in Chad in 1930 by a pharmacist from the colonial troops, spirulina attracted the attention of Brandilly, an anthropologist and filmmaker, who published an article with a prophetic title: "Since ages, an African tribe in Chad has been exploiting the food of the year 2000." Despite its early recognition, spirulina only piqued the interest of Western scientists much later.Since the 1980s, numerous scientific studies have been conducted by researchers worldwide, aiming to uncover the various beneficial effects of daily spirulina consumption **(Adiba et al., 2011).**

In the twentieth century, the history of spirulina is closely associated with an intriguing figure, Christopher Hill. Hill, an American, recognized the extraordinary potential of spirulina, a seemingly insignificant algae at the time, to address modern nutritional challenges. Spirulina is remarkably easy to cultivate, thriving in environments with minimal water and sunlight. As a result, it is considered one of the most remarkable food sources on our planet . **(LAFRI, 2018)**

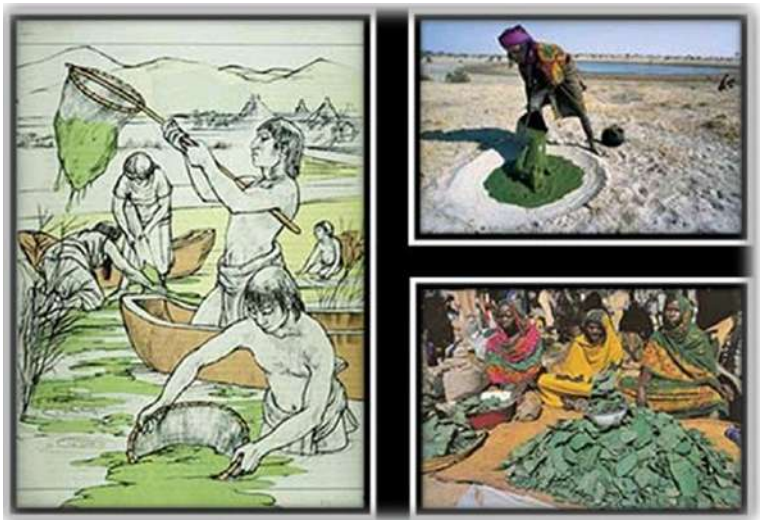

Figure I.1: **Spirulina Harvest (Moutard, 2003)**

I.2 Definition

Spirulina is a species of cyanobacterium that is as ancient as the Earth itself. Its scientific name is *Arthrospira platensis*. Spirulina is characterized by its blue-green color and its unbranched, spiral-shaped pluricellular filament, which is approximately 0.3 mm in length. It is mobile and thrives on photosynthesis, similar to plants. Spirulina is typically found in warm regions of the world, particularly in salt and alkali lakes, where it grows naturally. **(Henrikson, 1989)**

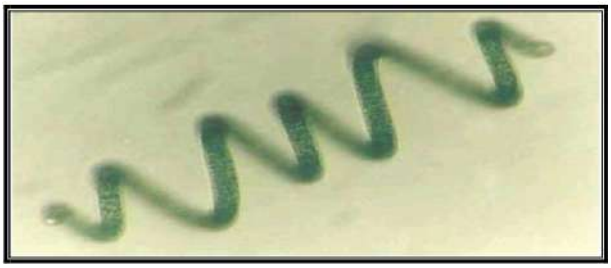

Figure I.2: **Microscopic appearance of *Arthrospira platensis* (Salomez, 2009)**

Spirulina's unique biological characteristics position it at the intersection between the bacterial and plant worlds. As a micro-algae, it obtains energy through photosynthesis. Unlike some other cyanobacteria, however, Spirulina lacks specialized structures called heterocysts, which means it cannot fix nitrogen in the air. This characteristic sets it apart from other cyanobacteria like Anabaena and Nostoc. To grow and develop, Spirulina requires simple mineral elements such as water, mineral salts, carbon dioxide, and oxygen, which it draws directly from its environment. Using sunlight as a source of energy through its pigment system, Spirulina synthe-

sizes biomass through autotrophic photosynthesis.Spirulina is recognized as a highly nutritious food source, thanks to its high protein content (up to 70%) and its superior digestibility, as well as its richness in phycocyanin. Additionally, Spirulina has a chemical composition that is rich in vitamins such as provitamins A and β-carotene, as well as vitamin B1, and unsaturated fatty acids such as linoleic acid and linolenic acid.**(Henrikson, 1989)**

I.3 Structure

Spirulina is a prokaryotic micro-algae that can exist as single or multicellular filaments. Its name is derived from its spiral and helical physical configuration. The filaments take a helical shape only under favorable liquid or culture medium conditions, and gas vacuoles allow them to float and protect themselves from excessive sunlight while also reaching nutrient-rich bottoms. When the temperature is too high, spirulina goes into a resting state and no longer reproduces. The filaments take irregular or linear shapes, and the color changes from blue-green to pearly white. Spirulina is sweet-tasting due to the transformation of proteins into polysaccharide sugars when exposed to heat. It is a microscopic cyanobacteria, with a size ranging from 50 to 500 µm in length and 3 to 4 µm in width. The filaments are composed of vegetative cells regularly rolled up and wrapped in a thin sheath forming constrictions. One teaspoon of spirulina contains millions of spirals. Spirulina moves at a speed of 5 μm/s and can be carried by flamingos in their feathers or beak. **(Ali and Saleh, 2012)**

I.4 classification

According to **(AlFadhly et al., 2022)**, spirulina is classified as:

Domain :Bacteria

Kingdom :Eubacteria

Phylum :Cyanobacteria

Class :Cyanophyceae

Sub-class :Oscillatoriophycideae

Order :Oscillatorlales

Family :Osellatorlaceae

Genus :Arthrospira

Species :*Arthrospira platensis*

I.5 Morphology and Physiology

Spirulina exhibits a wide range of morphological features that can vary greatly depending on the culture conditions and strain type. It is composed of trichomes or filaments consisting of vegetative cells with visible walls that are stacked end to end. The defining morphological characteristic of Spirulina is the arrangement of these trichomes, which are not branched and have a diameter of around 10 to 12 μ m and an average length of 250 μm when they have seven coils. The filaments are enveloped by a very thin sheath and exhibit reductions at the sides of varying degrees. Spirulina displays a broad range of morphologies, including Spirals, which describe strains with filament shapes resembling a spring, Undulations, which refer to strains with filaments in a drawn spiral shape, and Straight, which indicates strains with filaments so stretched that they appear almost straight. Environmental factors, such as temperature, physical conditions, and culture medium, can influence the geometry of the filaments. **(Vonshak, 1997)**

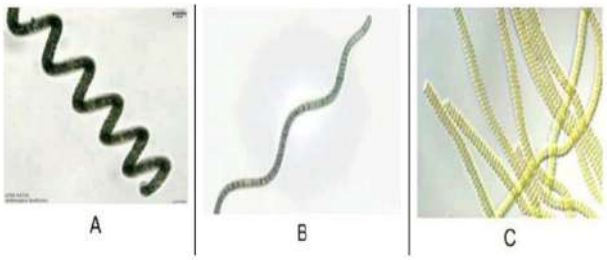

Figure I.3: **the different aspects of spirulina. (A) spiral, (B) wavy, (C) straight.(Goulamabasse, 2018)**

Spirulina's filaments acquire a helical shape only when the environmental conditions are favorable. Some strains that were initially helical can become wavy or straight. Once a strain is converted to a straight form, whether naturally or after physical or chemical treatments such as UV radiation or chemicals, it cannot revert to the helical form. this is due to a mutation affecting certain trichomes during specific growing conditions. Spirulina's cellular structure resembles that of bacteria because it lacks a plant cell wall, and it is characterized by the absence of nuclei and intracellular organelles. Additionally, it shares characteristics with the animal kingdom because it contains complex sugars similar to glycogen on its cell membrane.**(Vonshak, 1997)**

Spirulina exhibits two types of movements: motility and buoyancy. Its mode of

motility involves screwing through the water at a speed of 5 µm per second. In addition, it can produce gas vesicles that are approximately 70 nm long and 10 nm in diameter. These vesicles fill with gas when sunlight is present.The United Nations (UN) declared spirulina the best food source of the future at the 1974 World Food Conference . **(Doumenge et al., 1993)**

I.6 Habitat and Geographic

Cyanobacteria, including spirulina, are widely distributed and have a high growth rate in water, utilizing solar energy, carbon dioxide, and minerals. Spirulina naturally thrives in alkaline waters of lakes, as well as in sea and freshwater regions of Asia, Africa, Southern Europe, and North America. It can also be found in arid deserts, particularly in the salt and alkaline lakes of warm regions around the world. Today, spirulina is cultivated in various countries including the USA, India, China, Thailand, the Dominican Republic, Honduras, France, Algeria, Tunisia, Ethiopia, Peru, and Mexico, as indicated in Figure 6. **(Fox, 1999)**

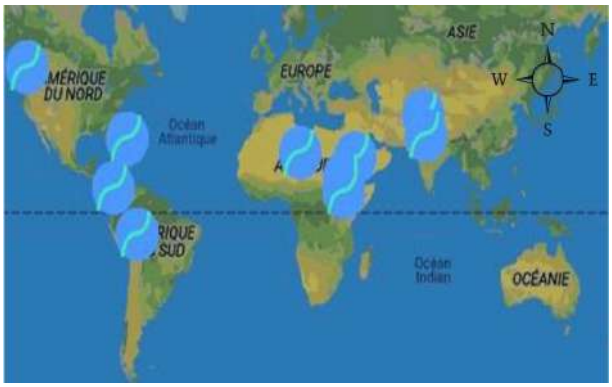

Figure I.4: **Geographic distribution of spirulina (Fox, 1999)**

I.7 Life cycle and Reproduction

There are three basic stages: the fragmentation of trichomes from the necrids which are spirulina filament cells and which differ from other cells by their biconcave appearance and are assimilated to separation disks. The trichome splits to give new filaments of 2 to 4 cells called hormogonies. These cells will grow in length by binary division (each cell will give two cells by scissiparity) and take the typical helical shape . Under experimental conditions, the maximum generation time (from one generation to another) of spirulina is around 7 hours.**(Ali and Saleh, 2012)**

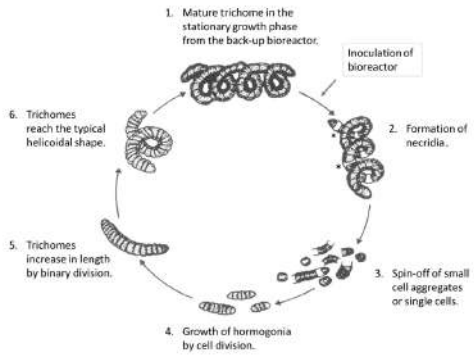

Figure I.5: **Life cycle of *Arthrospira platensis*. The asterisk (*) indicates necridia (Ciferri, 1983)**

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Spirulina grows by 25% every day, doubling in 4 days. Its reproduction is vegetative (asexual) and is carried out by simple split, binary or multiple fission, by budding or fragmentation at random. The 3 fundamental stages of its life cycle are: the fragmentation of trichomes, then the cells expand, the mature trichome, and divide into filaments by binary fission, these filaments take a helical shape.**(Manet, 2016)**

I.8 Cultivation and production of spirulina

I.8.1 Cultivation of spirulina

Spirulina platensis is a widely cultivated cyanobacterium in the world. The world production of this first can exceed 3000 tons of biomass per year **(BENAHMED, 2012)**.

I.8.1.1 Culture Parameters

One of the greatest advantages of cultivating spirulina compared to other types of cultures is its growth under extreme conditions (salinity and alkalinity), thus making it possible to exclude the proliferation of most other microorganisms **(BENAHMED, 2012)**.

Temperature Spirulina is a mesophilic cyanobacterium that grows at temperatures varying between 25 and 40°C **(Fox, 1999)**, with an optimum of 30°C **(Ogbonda et al., 2007)**. At temperatures below 20°C, the growth rate is slowed down or practically stopped ((**Jourdan, 2006**);(**Ogbonda et al., 2007**)). Beyond 40°C, the culture will wither away from excess heat and the spirulina will eventually die **(BENAHMED, 2012)**.

Hydrogen potential (pH) Spirulina proliferates in alkaline waters whose pH varies between 8.5 and 11 with an optimum of 9.5 ((**Ciferri, 1983**).; **(Fox, 1999)**). Alkalinity is usually provided by sodium bicarbonate (NaHCO3), but the latter can be replaced, in part; by caustic soda or sodium carbonate (Na2CO3) to raise the initial pH of the culture medium**(Fox, 1999)**.

Salinity The growth of spirulina Seems to be directly linked to the concentration of salts in the medium. It grows in saline waters at concentrations varying between 20 and 70 g/l**(Ciferri, 1983**).

Light Spirulina Is a photosynthetic organism, to grow it needs light with an intensity between 30 and 40 klux. However, at high light intensities, photolysis may occur **(BENAHMED, 2012)**.

Oxygen and agitation Like any other aerobic microorganism, spirulina needs oxygen (O2) to breathe. However, this O2 can be toxic when it is oversaturated during active photosynthesis **(Jourdan, 2006)**. During the day, agitation is necessary to homogenize, promote the elimination of oxygen and ensure a good distribution of light **(Jourdan, 2006)**.

I.8.2 Production of spirulina

The production of spirulina is done on several scales: artisanal, semi-industrial and industrial.

I.8.2.1 Artisanal production

Historically, this mode of cultivation was initiated by Ripley FOX to fight against malnutrition in developing countries. In recent years, this mode of production has continued to grow, supported by many NGOs. These are systems requiring low energy inputs. The means implemented can be rustic, appealing to good engineering sense. Nevertheless, some artisanal farms may present semi-industrial farm characteristics. Production quality is controlled throughout production. They are intended for humanitarian aid or partly for marketing**(Charpy et al., 2008**).

I.8.2.2 Semi-industrial production

In developing countries, semi-industrial farms use the same technologies as artisanal farms. They are intended for humanitarian and commercial purposes. Their goal is to be sustainable and autonomous through the sale of their product **(Charpy et al., 2008)**.

I.8.2.3 Industrial production

Represented for more than twenty years by large companies such as Earthrise or Cyanotech, they are distinguished from the previous ones by the importance of the means implemented, their production capacity and their clearly commercial objective. Quality is controlled automatically by computerized systems **(Charpy et al., 2008)**.

I.9 Main applications of spirulina

I.9.1 In human food

Spirulina is primarily known for its use as human food and is highly regarded for its exceptional nutritional profile, offering several benefits. Humanitarian workers and doctors use it in powder form to combat severe malnutrition in children, and it has been found to be more effective than drugs in treating deficiencies and diseases such as starvation, kwashiorkor, protein-energy malnutrition, iron deficiency anemia, and hypovitaminosis. For athletes, spirulina facilitates effort and aids in recovery due to its high content of vitamins B9 and B12 as well as iron. Pregnant women can also benefit from spirulina consumption, as it contains phycocyanin, which increases muscle oxygenation and limits uterine cramps, allowing for better preparation for childbirth and post-breastfeeding recovery. Spirulina is highly suitable for children and adolescents, as well as for babies of age to consume protein, due to its high assimilability and essential quality elements. It also adds to the quality of the skin. In the field of dietetics, spirulina is used as a protein supplement beneficial for health, acting as an appetite suppressant, reducing appetite, and optimizing energy intake. Just three to five grams per day of spirulina can help avoid deficiencies and eliminate toxins related to fast food consumption by teens **.(TOUDERT and BOUZIDI, 2020)**

I.9.2 In cosmetics

Spirulina, due to its high concentration of natural active ingredients such as amino acids, trace elements, antioxidants, minerals, vitamins, nucleic acids, proteins, and essential fatty acids, has been introduced by some cosmetic care laboratories into their marketed creams, shampoos, and serums . Its antioxidant properties make it beneficial in improving skin flexibility and elasticity, delaying aging, and providing shine and resistance to nails and hair. It is considered an exceptional "beauty" food and used in various anti-aging treatments with a marine connotation, spa and thalassotherapy products such as face masks, body wraps, poultice, marine wrapping, body conditioner, and face mask for its restorative and fortifying effects on hair and nails.**(BANKS, 2007)**

I.9.3 In medicine

The unique composition of spirulina has led to numerous therapeutic applications, including strengthening the immune system to fight opportunistic diseases and treating certain skin conditions. It has also been shown to be effective in relieving rheumatic pain, osteoarthritis, osteoporosis, excess cholesterol, hypertension, and allergies, while also protecting the heart and promoting the regeneration of brain cells. Spirulina is now available as a dietary supplement for these therapeutic purposes.**(BOUDAOUD, 2016)**

In addition, spirulina is used in food processing as a natural coloring agent due to its phycocyanin content, which is one of the few natural blue pigments. It is used in chewing gum, sorbets, sweets, dairy products, and non-alcoholic beverages. Spirulina is also mixed with salt and tagliatelle in a range of algal products. In Switzerland and Japan, spirulina bread has been popular for some time.**(BOUDAOUD, 2016)**

I.9.4 In animal feed

Spirulina is not only beneficial for humans but also for animals. It enhances their natural defenses and helps to maintain their immune system, which aids in fighting certain diseases and combating aging and fatigue. Dogs, cats, fish, and horses are some of the common animals for which spirulina is used. In horses, it is frequently consumed during growth, competition, or convalescence phases. Poultry farmers often add spirulina to chicken feed to enhance egg laying quality, a practice that is well-known among experienced farmers .**(Casal, 2019)**

CHAPTER II

BIOCHEMICAL COMPOSITION OF SPIRULINA

II.1 Composition of spirulina platensis

Spirulina is a biomass extracted from cyanobacteria that both humans and animals can consume. Its use does not stop at the nutritional supplement, but it can be a complete food. It is effectively used as a food supplement in aquaculture, fish ponds and poultry. It contains a high percentage of proteins 60-70%, so it is used to treat malnutrition. and lipids 6.5%, carbohydrates 15% and minerals 9%.**(Robin, 2017)**

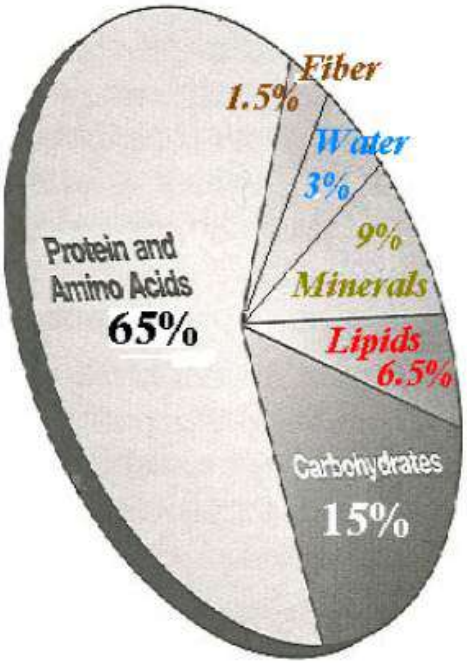


Figure II.1: **Chemical composition of spirulina (Robin, 2017)**

The proteins found in spirulina are considered its main asset because no other food concentrates as much protein.However, this composition may vary according to the culture conditions, the period of It should be noted that the composition of spirulina may vary depending on several factors, such as the culture conditions, harvesting period, geographical origin, and other parameters, as pointed out.**(Niangoran, 2017)**

II.1.1 Protein

Spirulina is particularly rich in proteins since they represent 50 to 70% of its dry weight. These values are quite exceptional. On the other hand, by comparison with other vegetable protein sources which are all less rich, spirulina is consumable in its entirety **(Falquet and Hurni, 2006)**. From a qualitative point of view, the proteins of this micro-algae have a value very high

biological because they contain all the essential amino acids for adults (II.1); these represent 47% of the total protein weight(**Falquet and Hurni, 2006**). TableII.1 shows, by way of example, the amino acid content in 1 kg of " Flamant Vert" brand spirulina (according to the instructions).

Table II.1: **Quantity of Spirulina proteins and other foods.(Ali and Saleh, 2012)**

Amino acids	Content in g/kg of spirulina (dry weight)
Alanine	47
Arginine	43
Aspartic acid	61
Cystine	06
Glutamic acid	91
Glycine	32
Histidine	10
Isoleucine	35
Leucine	54
Lysine	29
Methionine	14
Phenylalanine	28
Proline	27

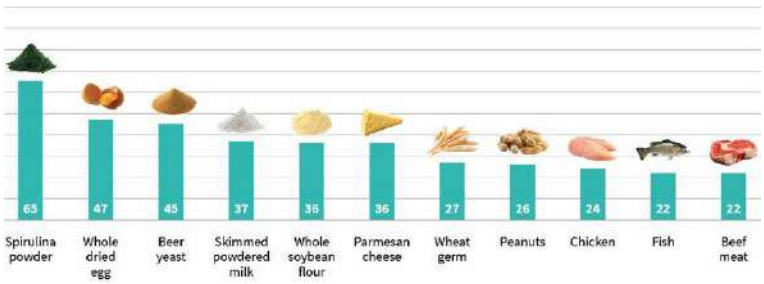


Figure II.2: **Positioning of Spirulina in relation to other foods in terms of protein levels (%)(Vrenna et al., 2021).**

II.1.2 Lipids

II.1.2.1 Total lipids

In total they represent less than 10% of the dry weight **(Bujard et al., 1970)**. These total lipids can be separated into a saponifiable fraction (83%) and an unsaponifiable fraction (17%) containing essentially paraffins, pigments, terpene alcohols and sterols ((**Clément, 1975)** ; **(Santillan, 1974)**).

II.1.2.2 Fatty acids

The -linolenic acid represents in Arthrospira platensis 40%, or about 4% of the dry weight of spirulina; therefore, Arthrospira can be considered one of the best known sources of -acid.linolenic, after human milk and some uncommon vegetable oils **(Ciferri, 1983)** Other essential fatty acids are also present, such as linoleic acid, 18:2 (12%). Note also a high proportion of palmitic acid, 16:0, about 25%. As for sulfolipids such as sulfoquinovosyldiglycerides (5% of the saponifiable fraction), they are currently giving rise to new research since a protective activity against the infection of cells by HIV was attributed to them**(Gustafson et al., 1989)**. Note also the absence of fatty acids with an odd carbon number**(Clément, 1975)** and a very low content of branched chain fatty acids **(Bujard et al., 1970)**, two types of lipids not metabolizable by higher animals.

II.1.3 Carbohydrates

Carbohydrates represent between 15 and 25% of the dry matter. Simple carbohydrates are in very low quantity (glucose, fructose and sucrose), most of the assimilable carbohydrates are made up of polymers such as that :

- amino glucosan (1.9% dry weight).
- amino rhamnosans (9.7%).
- glycogen (0.5%) .

From a nutritional point of view, the only interesting carbohydrate substance by its quantity in Arthrospira is meso-inositol phosphate which is an excellent source of organic phosphorus as well as inositol(350-850 mg/kg dry matter) . Polysaccharides have multiple therapeutic interests, particularly in the stimulation of DNA repair mechanisms **(Pang et al., 1988)**, in its radioprotective effect and in the neutralization of free radicals **(Pang et al., 1989)**.

II.1.4 vitamins

II.1.4.1 Beta-carotene (provitamin A)

ß-carotene represents 80% of the carotenoids present in Arthrospira, the rest being composed mainly physoxanthin and cryptoxanthin **(Palla and Busson, 1969)**. There are between 700 and 1700 mg of ß-carotene and about 100 mg of cryptoxanthin per kilo of dry spirulina.

II.1.4.2 Tocopherols (vitamin E)

From 50 to 190 mg per kilo, **(Clément, 1975)**, content comparable to that of wheat germs. Daily vitamin E requirements would be 15 U.I. or 12 mg of free tocopherols.

II.1.4.3 Group B vitamins

Although less rich than yeast in group B vitamins (except B12), Arthrospira constitutes yet a good source of these cofactors (II.2).

Table II.2: **vitamin content (Falquet and Hurni, 1986)**

Vitamin	Content (mg/kg)	Need/day (adult)
Thiamine (B1)	34-50	1.5mg
Riboflavin (B2)	30-4	6 1.8mg
Pyridoxine (B6)	5-8	2.0mg
Cyanocobalamin (B12)	1.5 - 2.0	0.003 mg
Niacin	130	20mg
Folate	0.5	0.4mg
Panthotenate	4.6 - 25	6 - 10 mg
Biotin	0.05	0.1 - 0.3mg
Ascorbic acid (C)	traces	5 - 30 mg

II.1.4.4 Vitamin B12

We must emphasize the exceptional content of vitamin B12 (cobalamin), which is by far the most difficult to obtain in a meatless diet because no common vegetable contains it. Arthrospira is four times richer than raw liver, long given as the best source. It should be noted, however, that there is a controversy about the real bioavailability of the B12 complex of Arthrospira ((**Hayashi et al., 1996); (Rule et al., 1994**)).

II.1.5 Minerals

he minerals of particular interest in spirulina are magnesium, calcium, phosphorus, potassium, iron and zinc. The first three are present in the spirulina at levels comparable to those found in milk **(Falquet and Hurni, 2006)**. During cultivation, spirulina absorbs several minerals from the medium. Thus, its content in minerals varies depending on the growth medium and the minerals present in the water **(Henrikson, 1997)**.

Table II.3: **mineral content (Falquet and Hurni, 2006)**

mineral	content(mg/kg)
calcium	1300 - 1400
iron	580 - 1800
Phosphorus	6700 - 9000
zinc	21 - 40
magnesium	2000 - 2900
copper	8 - 10
chromium	2.8
sodium	4500
potassium	6400 -15400
manganese	25 - 37

II.1.6 Nucleic acids

Nucleic acids represent 4 to 6% of the dry matter. The proportion of DNA would be a quarter to one-third compared to RNA. The nucleic acid content of Arthrospira is much lower than that of unicellular generality **(Ciferri, 1983)**.

II.1.7 Pigments

Spirulina's color is attributed to its three primary pigments: chlorophyll, phycocyanin, and β-carotene.

Table II.4: Pigments in Spirulina powder(Ali and Saleh, 2012)

Pigments	mg 100g-1
Carotenoids	370
Chlorophyll α	1000
Phycocyanin	14000

II.1.7.1 chlorophyll

Spirulina contains approximately 1% of chlorophyll, which is one of the highest concentrations found in nature. Its structure is closely related to mammalian hemoglobin, which has earned it the nickname of "green blood". Despite not being the most abundant pigment in spirulina, chlorophyll is responsible for its green color due to its strong coloring power. Chlorophyll

has been the subject of numerous studies and has been shown to have several beneficial qualities, including restoring acid-base balance, improving cardiac function, regulating intestinal transit, increasing red blood cell count, and promoting internal and external healing . **(Casal, 2019)**.

II.1.7.2 Phycocyanin

Phycocyanin, which gives spirulina its characteristic bluish color, is considered the most remarkable pigment present in spirulina . **(Sguera, 2008)** It is known to be the most potent antioxidant and anti-radical substance available, providing a boost to the body's natural defenses. Phycocyanin has been found to stimulate the production of red blood cells, promote muscle activity, inhibit the growth of cancer cells, and eliminate harmful chemicals from the body .**(Gabr et al., 2020)**

II.1.7.3 Beta-carotene

Beta-carotene is an orange pigment and a precursor of vitamin A that is abundant in spirulina. It plays a vital role in cell renewal and supports immune defenses, as stated by **(Charpy et al., 2008)**. This nutrient has numerous antioxidant properties that combat cell aging, reduce the risk of cancer, promote wound healing, and protect the skin from external aggressors, according to **(Asghari et al., 2016)**.

CHAPTER III

BIOLOGICAL ACTIVITIES OF SPIRULINA

III.1 Antioxidant activity

III.1.1 Definition of antioxidant

The term ”antioxidant” refers to substances that counteract the process of oxidation. An antioxidant is a compound present at lower concentrations compared to the oxidizable substrate, which effectively delays or prevents the oxidation of that substrate. Antioxidants play a crucial role in preserving food quality and maintaining human health.**(Sehwag and Das, 2013)**

III.1.2 Need of antioxidants

Oxidation reactions have specific consequences depending on their occurrence site. In food systems, oxidation leads to food deterioration, while in biological cell systems, it can cause cell damage or death. The oxidative deterioration of fats and oils in food contributes to rancid odor, flavor deterioration, and a decrease in nutritional quality, sensory appeal, and safety. This deterioration is attributed to the formation of primary hydroperoxides and secondary potentially toxic compounds through the auto-oxidation of unsaturated fatty acids. Overcoming the spin barrier of oxygen requires initiators or catalysts, such as light, metals, singlet oxygen, and sensitizers. Even seemingly spontaneous or uncatalyzed reactions often involve undetected or unconsidered catalysts or contaminants. Therefore, the addition of antioxidants is necessary to control oxidative deterioration.**(Sehwag and Das, 2013)**

III.1.3 Classification of antioxidant

Antioxidants can be broadly classified into five major types:

- Primary antioxidants or chain-breaking antioxidants: These compounds, mainly phenolic substances, terminate free radical chains in lipid oxidation by acting as hydrogen and electron donors. They can also chelate transition metals that act as catalysts in lipid oxidation.
- Oxygen scavengers: Substances that react with oxygen and can remove it from closed systems, such as ascorbic acid (vitamin C).
- Secondary antioxidants: Compounds that decompose lipid hydroperoxides into stable end products.

- Enzymatic antioxidants: Enzymes that remove dissolved or headspace oxygen, such as glucose oxidase, or eliminate highly oxidative species, such as superoxide dismutase.
- Chelating agents: Synergistic substances that enhance the action of phenolic antioxidants. These synergists often have little or no antioxidant activity themselves, such as citric acid, amino acids, and phospholipids like cephalin. **(Sehwag and Das, 2013)**

III.1.4 Antioxidant potential of *S. platensis*

Antioxidants are molecules capable of neutralizing rampaging free radicals by donating electrons, thereby reducing free radical damage. Spirulina's antioxidant potential has received considerable attention, with studies indicating its significant reduction of oxidative stress. The antioxidant protective effects are attributed to phycocyanin,β-carotene, and other vitamins and minerals present in spirulina. Reactive oxygen species (ROS) attack and damage DNA, RNA, proteins, and lipids, leading to metabolic disorders, tissue injury, and cell death. Oxidative stress and ROS play critical roles in various diseases, including hypertension, diabetes mellitus, atherosclerosis, ischemic disease, and malignancy. Lipid peroxides (LPOs) and malondialdehyde (MDA) serve as essential markers of oxidative stress. Spirulina's enzymatic and non-enzymatic antioxidant potentials were assessed through aqueous and ethanolic extracts. Key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamin C, and vitamin E, were evaluated. SOD, CAT, and GPx play vital roles in defending against peroxidation activity and maintaining the cell's redox state. Aqueous extract demonstrated higher CAT activity, while both extracts showed significant SOD activity.(**Kumar et al., 2022**)

III.2 Other activities

III.2.1 Antibacterial activity

Preliminary in vitro studies have shown that spirulina extracts exhibit effective antimicrobial potential against some pathogenic bacteria such as E. coli and S.

This suggests that cyanobacteria possess a defense mechanism to fight against pathogenic bacteria, which could potentially lead to the development of plant-based antibiotics with fewer side effects than synthetic drugs. Although the results of various spirulina extracts on different bacte-

ria have not yet identified a specific anti-bacterial substance, they have demonstrated a spectrum of action that supports the potential of spirulina's antibacterial activity against some pathogenic germs .**(Kaushik and Chauhan, 2008)**

III.2.2 Anti-inflammatory activity

The immune system is the primary defense mechanism against physical infections, and cytokines play a central role in initiating inflammation. Pro-inflammatory cytokines, such as TNF α, IL-1β, and IL-6, are produced when the immune system is activated. The benefits of spirulina in enhancing immunity and improving resistance to inflammatory responses have been extensively documented. **(Charpy et al., 2008)**

COX-2 is the main form of cyclooxygenase involved in inflammation, responsible for producing prostaglandins at the site of inflammation. In a full human blood test, Vikocyanin, found in spirulina, was found to significantly inhibit COX-2 with an IC50 value of 80 nm. Phycocyanin's ability to counteract inflammation is partly due to its selective inhibitory effect on COX-2, in addition to its ability to effectively eliminate free radicals and inhibit lipid peroxidation. **(Charpy et al., 2008)**

Furthermore, spirulina is rich in proteins and fatty acids, particularly omega-3 and 6, which the body cannot produce on its own. This makes it biologically significant since these fatty acids serve as precursors to prostaglandins, molecules with anti-inflammatory and immune activity within the body .**(Charpy et al., 2008)**

III.2.3 Antiviral activity

According to a study by **(Hayashi et al., 1996)**, Ca-SP has been found to inhibit the replication of several coated viruses, such as herpes simplex type 1 virus, human cell amplifying virus, measles and mumps virus, influenza A virus, and HIV-1. The study indicates that Ca-SP is more effective in pre-treatment 3 hours before infection, rather than treatment immediately after infection. This suggests that polysaccharides may work early in the reproduction of the virus, during adsorption or penetration. It has been observed that Ca-SP selectively prevents virus penetration in host cells with a concentration of more than 40 ug/mL, thereby blocking almost complete penetration of the HSV-1 virus. The antiviral effect of Ca-SP is primarily protective and acts on the adsorption and penetration of viruses into the cell membrane. The process of removing heavy metal from calcium ion with sulfur groups is necessary for its antiviral ef-

fect because removal of calcium ion or sulfate from polysaccharide reduces its antiviral activity and increases toxicity. Therefore, taking spirulina, and thus calcium-spirulane, in case of possible viral contamination, may be useful in reducing the risk of infection and preventing disease. **(Hayashi et al., 1996)**

III.2.4 Anti-cancer activity

The development of certain types of cancer is often linked to damage to the DNA of cells, resulting in uncontrolled growth. While the body has enzymatic processes in place to identify and correct this damage, exposure to toxins can impair these processes and lead to the development of cancer. In addition to the development of therapeutic anti-tumor drugs, research into cancer prevention has become a major focus, with a growing interest in chemical prevention using synthetic or natural substances that can inhibit carcinogenicity. One such substance is beta-carotene, an antioxidant found in spirulina, which has been shown in various studies to potentially reverse the cancer process and prevent the spread of cancer cells. In fact, a study conducted on individuals with precancerous oral lesions found that daily supplementation with 1 gram of spirulina for a year resulted in an improvement in their condition and prevented the progression of the disease. Phycocyanin, another component of spirulina, has also been found to contribute to this anti-cancer activity by attacking free radicals that are known to promote cancer growth **(Vidalo, 2015)**

III.2.5 Anti-hypercholesterolemic activity

Excess cholesterol is statistically correlated with cardiovascular diseases such as infarction, thrombosis, and arteritis, which are the primary causes of death in the Western world. Multiple factors such as a diet high in salt and sugar, stress, alcohol consumption, tobacco use, and lack of physical activity contribute to this condition. Atherosclerosis occurs when excess cholesterol in the blood is deposited on the inner walls of arteries, typically around the age of 40. In the second stage, the vessels harden, leading to a decrease in arterial diameter, sometimes resulting in complete blockage. A closed coronary artery leads to an infarct. Spirulina, due to its high level of gamma-linolenic acid (AGL), has the ability to lower blood cholesterol levels. AGL is an essential fatty acid that the body cannot synthesize but requires from food. It aids in the production of a vital substance called prostaglandin E1 (PGE1), which helps prevent heart

attacks and strokes, eliminates excess fluid, improves blood circulation, and slows cholesterol production. **(Tsurutani et al., 2018)**

III.2.6 The therapeutic aspect

It is important to note that while spirulina may have potential health benefits, more research is needed to fully understand its effects on various diseases and conditions. It is also important to consult with a healthcare professional before using spirulina as a treatment or supplement, especially if you have any underlying health conditions or are taking medication. Additionally, the suggested daily dose of 15g of spirulina to prevent SARS-CoV-2 infections should be taken with caution and is not yet supported by strong scientific evidence. It is always best to follow the guidance of healthcare professionals and public health organizations when it comes to preventing and treating illnesses**.(Elaya Perumal and Sundararaj, 2020)**

The practical part

CHAPTER I

MATERIALS AND METHODS

I.1 Materials and Methods

I.1.1 Material

I.1.1.1 Biological materials

The biological material used in this work is two samples of dry biomass of the *Arthrospira platensis* strain grown in different regions of Algeria (Tamanrasset)and Egypt (Khatahtba) . The choice of this biological material is mainly due to the richness of this type of bioactive compounds. We grind two samples of spirulina with an electric mill for spirulina powders. They were stored in sealed glass bottles for analysis in the dark.

Figure I.1: **Spirulina powder grown in the "Algeria "region.**

Figure I.2: **Spirulina powder grown in the " Egypt" region.**

Figure I.3: **Tamanrasset location on the map of Algeria.(werbeantrieb, 2020)**

Figure I.4: **Khatahtba location on the map of Egypt (Aburish, 2004)**

I.1.1.2 Non-biological material and equipment

This refers to any material, other than biological, that includes glassware, apparatus and chemical and organic reagents used in the experimental study.

I.1.1.3 Phytochemical screening

During Phytochemical screening we used the tools, apparatus, solutions and reagents listed in table below :

Table I.1: **tools, apparatus, solutions and reagents used in phytochemical tests**

Apparatus	Solutions	tools
Electric crusher	Distilled water(H2O)	Biological material
Electronic scale	Fehling reagent	Beakers
Hot plate	Iron chloride	Funnel
Ph meter	anhydrous(FeCl3)	Paper filter
	Iso amyl alcohol (C5H11OH)	Test tubes
	Anhydrous acetic acid (CH3COOH)	Test tube holder
	Iodine (I2)	Pipette
	Metallic magnesium(Mg)	Spatula
	Sodium hydroxide	Graduated specimens
	1N hydrochloric acid(HCL)	
	Sodium sulphate	
	anhydrous	
	Methanol(CH3OH)	
	Sulphuric acid(S2HO4)	
	Chloroform(CHCL3)	

I.1.1.4 Extraction

When extracting the contained extracts in Table below, we used the tools, Apparatus and solutions included in it.

Table I.2: **tools, apparatus and solutions used to extract extracts.**

Apparatus	Solutions	tools
the glass-works	distilled water	biological material
steamroom	methanol	beakers-funnel
precision	acetone	paper filter
balance		spatula
blades		balloon
grinder		separating funnel
		aluminum foil
		erlenmeyer flask
		Test tubes

I.1.2 Quantitative dosage of phenolic compounds

In quantitative dosage of phenolic compounds we used the tools, apparatus and solutions listed in table below :

Table I.3: **tools, apparatus and solutions used in quantitative determination of phenolic compounds**

Apparatus	Tools	Solustions	
Beakers Electronic scale jenway UV-visible spectrophotometer agitator fridge centrifuge precision balance	Extracts Beakers test tubes Tube holder essay The tanks aluminum foil thermometer	Folin reagent-ciocalteu (10%) Sodium carbonate (Na2 Co3) (7.5%) Gallic acid Methanol	Dosage of total polyphenols (PPT)
		AIC13(2%) Quercitin acid Methanol	Dosage of flavonoid (FVT)
		Solution of vanillin (4% in methanol) acid hydrochloric concentrate (HCL) catechin Methanol	Dosage of condensed tannins (TC)
		Acetone	Chlorophyll a, b and Carotenoids

I.1.3 Evaluation of antioxidant activity

When evaluating antioxidant activity, we used the tools, Apparatus and solutions listed in Table below :

Table I.4: **Tools, apparatus and solutions used in the evaluation of antioxidant activity**

Apparatus	Tools	Solustions	Antioxidant Activity
Beakers Electronic scale jenway UV-visible spectrophotometer agitator fridge centrifuge vortex Analytical balance blades	Extracts Beakers test tubes Tube holder essay The tanks aluminum foil thermometer	Solutions DPPH methanolic (4 mg/100mg MeOH) Ascorbic acid Methanol	Free radical scavenging DPPH (2,2-diphenyl-1-picrylhydrazil)

I.1.4 physico-chemical analysis

To study physical and chemical analyses, we used the tools, devices, and solutions mentioned in the table below.

Table I.5: **Tools, apparatus and solutions used in analyses.**

Apparatus	Solutions	Tools	Analysis
ph meter oven precision balance desiccator rotary evaborator soxhlet	distilled water	crucible beakers aluminum foil spatula iherometre	ph humidity ash protein lipid carbohydrate

I.2 Methods

I.2.1 Phytochemical screening

Phytochemical screening is a set of qualitative techniques that can determine the various chemical groups present in a plant organ through physicochemical reactions. These groups include total polyphenols (such as flavonoids and tannins), coumarins, alkaloids, saponins, sterols, terpenes, and others. To conduct Phytochemical screening , conventional characterization reagents were used, and tests were carried out on prepared plant extracts in both aqueous (decoction) and organic (maceration in methanol) media. The results were interpreted as either positive (+) or negative (-). **(Lendvai et al., 2003)**,

I.2.1.1 Preparation of solutions for analysis

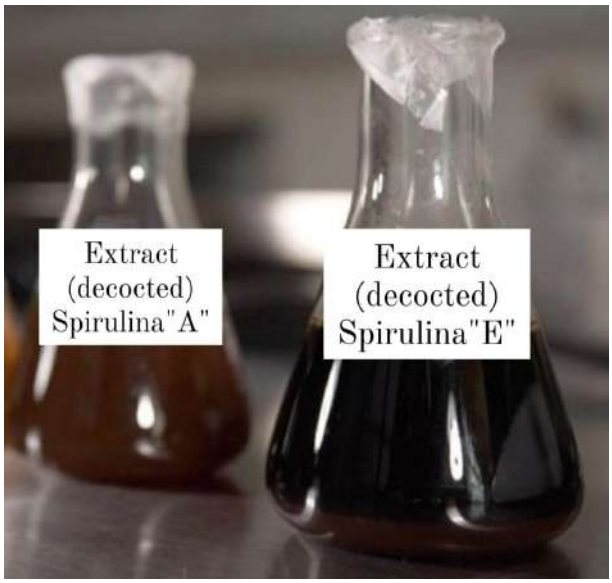
Preparation of the decocted

The decocted of the plant is prepared by adding 10g of spirulina powder to 100ml of distilled water. The whole was boiled for 15 minutes, the mixture is filtered and the filtrate obtained is adjusted to 100 ml with distilled water after cooling.**(Emmanuel, 2012)** The filtrates obtained from this process are depicted in Figures below :

preparation of macerated

The macerated is prepared by adding 10g of spirulina powder to 100ml of methanol. The mixture is macerated for 24 hours, then filtered**(Azzi, 2013)**. The resulting filtrate is shown in Figure below :

Figure I.5: **Decocted at 10% of the two samples of spirulina of two regions "A" and "E".(personal photos)**



I.2.1.2 Test of flavonoids

(A) **Operating mode**

A combination of a little Mg^{2+} and concentrated HCl droplets, placed in a tube, are added to 1 ml soaking.**(Vijayakumari et al., 2013)**.

(B) **Result expression**

The appearance of pink, orange or red refers to the presence of flavonoids **(Vijayakumari et al., 2013)**.

I.2.1.3 Test of tannins

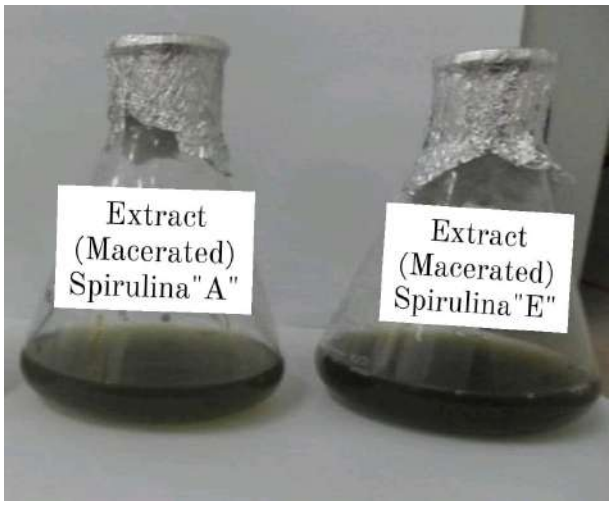
(A) **Operating mode**

1 mL of ferric chloride (5% $FeCl_3$) was added to 1 mL of the Spirulina platensis extract.**(Vijayakumari et al., 2013)**.

(B) **Result expression**

Formation of dark blue or greenish black color indicates the presence of tannins.**(Vijayakumari et al., 2013)**.

Figure I.6: **Macerated of the two samples of spirulina from two regions ”A”and ”E”. (personal photos)**



I.2.1.4 Test of alkaloids

(A) **Operating mode**

2ml of concentrated hydrochloric acid (HCl) was added to 2 ml of extract. A few drops of Myer detector were added. **(Mane and Chakraborty, 2018)**

(B) **Result expression**

The presence of green or white deposits refers to the presence of alkaloids **(Mane and Chakraborty, 2018)**.

I.2.1.5 Test of phenols

(A) **Operating mode**

To 1ml of the extract, 2ml of distilled water were added, followed by a few drops of 10% ferric chloride solution. **(Mane and Chakraborty, 2018)**

(B) **Result expression**

Formation of blue or green color indicates the presence of phenols**(Mane and Chakraborty, 2018)**.

I.2.1.6 Test of quinone

(A) **Operating mode**

1 mL of concentrated sulphuric acid (H_2SO_4) was added to 1 mL Spirulina platensis extract. **(Mane and Chakraborty, 2018)**

(B) **Result expression**

the formation of a red color indicates the presence of quinones. **(Mane and Chakraborty, 2018)**.

I.2.1.7 Test of coumarins

(A) **Operating mode**

10% NaOH was added to 1 mL of Spirulina platensis extract. **(Mane and Chakraborty, 2018)**

(B) **Result expression**

Formation of yellow color indicates the presence of coumarins. **(Mane and Chakraborty, 2018)**.

I.2.1.8 Test of sterols and triterpenes

(A) **Operating mode**

To obtain the extract for testing, 1g of the powder was mixed with 20ml of ether and left for 24 hours through a process called maceration. The mixture was then filtered, and the volume was made up to 20ml with ether. Next, 10ml of the extract was evaporated to dryness, and the residue was dissolved in 1ml of acetic anhydride followed by 1ml of chloroform. The resulting solution was divided into two test tubes, with one being used as a control. In the second test tube, 1 to 2ml of concentratedH_2SO_4 was pipetted into the bottom. **(Boutlelis, 2014)**

(B) **Result expression**

If a brownish red or purple ring is formed at the contact zone of the two liquids, and the supernatant layer turns green or purple, it indicates the presence of sterols and triterpenes. **(Boutlelis, 2014)**.

I.2.1.9 Test of reducing compounds

(A) **Operating mode**

To perform the test, take 5ml of the decoction and evaporate it to dryness in a water bath. Then, add 1ml of Fehling's reagent to the residue.**(Evans, 2009)**.

(B) **Result expression**

The formation of a brick-red precipitate indicates the presence of reducing compounds. **(Evans, 2009)**.

I.2.1.10 Test of saponosides

(A) **Operating mode**

prepare a series of 10 test tubes each with a volume of 16mm and numbered from 1 to 10. In each tube, add 1ml, 2ml, ..., 10ml of the decoction, and then adjust the volume to 10ml with distilled water. Next, shake each tube lengthwise for 15 seconds, with two agitations per second. Allow the tubes to stand for 15 minutes and then measure the height of the foam in each tube. **(N'Guessan et al., 2009)**.

(B) **Result expression**

The tube in which the foam height is 1 cm indicates the value of the foam index. Foam Index =1000 /tube , or foam height is 1 cm .**(N'Guessan et al., 2009)**

I.3 Extraction of phenolic compounds

I.3.1 Preparation of total phenolic extracts

In this study, the maceration method was employed, which is widely recognized and utilized by phytochemists. Maceration is a solid-liquid extraction technique that involves immersing a biological sample in a solvent, either at cold or hot temperatures, to extract the solid or liquid components present in the natural material. Through dissolution in the solvent at room temperature, the desired species or molecules are extracted from the biological matter. **(Bellebcir, 2008)**.

Placed 5g of biological material (Arthrospira platensis) in an Erlenmeyer with 75 ml methanol, for 24 h, after filtration, the methanol solutions are evaporated dry under reduced pressure in a 50°C rotating evaporator .**(Matkowski and Piotrowska, 2006)**(Modified)

I.3.2 Extraction of flavonoids

In the extraction process of *Arthrospira platensis*, 5g of the biological material was placed in an Erlenmeyer flask along with 75 ml of methanol for 24 hours. The resulting mixture

was then filtered, and the methanol solution was evaporated under reduced pressure in a rotating evaporator at 60°C to obtain dry residues.(**Bekkara et al., 1998**)(Modified)

I.3.3 Extraction of tannins

Tannin the extraction process, 15g of organic powder from Arthrospira platensis was mixed with 73ml of a mixture containing acetone and distilled water in a ratio of 51:22 (V/V). The mixture was allowed to stand at room temperature for three days. After the extraction period, the solution was filtered to remove any solid particles. The resulting solution was then subjected to evaporation at 40°C using a rotary evaporator to remove the acetone solvent , resulting in the desired product in a dry form.**(Zhang et al., 2008)**(Modified).

I.4 Determination of extraction yield

The extraction yield is defined as the ratio between the weight of the extract obtained after extraction and the weight of the powder used.**(Wizi et al., 2022)** It is calculated by the following formula:

Yield(%)=Mass of extract / Mass of samble used . 100

I.5 Dosage of phenolic compounds

I.5.1 Dosage of total phenolic

(A) **Principle**

The total polyphenol content in the different extracts is assessed using the Folin-Ciocalteu method, as outlined in the methodology described by **(Yap et al., 2009)**

The Folin-Ciocalteu method is based on the principle of utilizing a reagent known as Folin-Ciocalteu reagent, which is composed of a mixture of phosphotungstic acid ($H_3PW_12O_40$) and phosphomolybdic acid ($H_3PMo_12O_40$). This method relies on the oxidation of phenolic compounds present in the sample, which leads to the concurrent reduction of phosphomolybdic acid

The Folin-Ciocalteu reagent exhibits a change in its colorimetric properties when it forms complexes with specific molecules. This reagent reacts with the hydroxyl (OH) functional groups present in phenolic compounds, leading to the formation of a dark blue coloration. To quantify the concentration of polyphenols, a calibration curve is constructed using known concentrations of polyphenols, as described by **(Khatabi et al., 2016)**.

In the procedure described by **(Nabti and Belhattab, 2016)**, the concentration of total polyphenols in methanol extracts is determined using the Folin-Ciocalteu method.

(B) **operating mode**

The steps involved in the procedure are as follows: Initially, 0.2 ml of the raw extract solution is combined with 1 ml of the Folin-Ciocalteu reagent, which has been diluted 10 times. After allowing a 5-minute incubation period, 0.8 ml of a 7.5% sodium carbonate (Na_2CO_3) solution is added to the mixture. The resulting solution is then incubated for 30 minutes, shielded from light to prevent any potential light-induced reactions.

(C) **Results expression**

- To quantify the concentration of total polyphenols, the absorbance of the solution is measured at a wavelength of 765 nm. Concurrently, a series of standard gallic acid solutions with concentrations ranging from 0.01 mg/ml to 0.12 mg/ml is prepared. These standard solutions serve as a reference for estimating the total phenolic compounds in the methanol extracts.
- Using the calibration curve generated from the absorbance values of the gallic acid standards, the concentration of total polyphenols in the extract is determined. The concentration is expressed in milligrams of gallic acid equivalent per gram of the extract, providing a standardized measure for the polyphenol content **(Khosravi et al., 2013)**.

I.6 Dosage of flavonoids

The total flavonoid content of our extract was estimated by a colorimetric assay based on the method of **(Bouba A et al., 2010)** with some modifications.

(A) **Principle**

Flavonoids possess a free hydroxyl (OH) group in position 5, which has the potential to form a colored complex with aluminum chloride $(AlCl_3)$ through interaction with the carbonyl (CO) group. This complexation between flavonoids and aluminum chloride results in the formation of yellowish-colored complexes due to the phenomenon of metal

chelation. By exploiting this property, a colorimetric assay can be employed to detect and quantify the total flavonoid content in a sample.**(Chang et al., 2002)**

Aluminum chloride ($AlCl_3$) has the ability to form highly stable complexes with the hydroxyl (OH) groups of phenols. This reaction leads to the formation of a yellow-colored complex that exhibits absorption of visible light at a specific wavelength of 430 nm. **(Ribereau-Gayon, 1968)**

(B) **Operating mode**

To establish a reference for flavonoid quantification, a set of standard solutions In the procedure for estimating the flavonoid concentration in the methanol extract, a volume of 0.5 ml of the extract solution is combined with 0.5 ml of a 2% aluminum chloride ($AlCl_3$) solution. The resulting mixture is then incubated for 1 hour, shielded from light to prevent any light-induced reactions. **(Mbaebie et al., 2012)**

(C) **Results expression**

After the incubation period, the absorbance of the solution is measured at a wavelength of 430 nm using a spectrophotometer . containing quercitine acid at concentrations ranging from 0.01 mg/ml to 0.12 mg/ml is prepared. These standard solutions serve as a calibration curve against which the absorbance of the sample can be compared. The concentration of flavonoids in the extract is expressed as milligrams of quercitine acid equivalent per gram of the extract, allowing for standardized measurement and comparison of flavonoid content. **(Mbaebie et al., 2012)**

I.7 Dosage of condensed tannins

(A) **Principle**

The method for determining condensed tannins in Salvia chudaei extracts follows the procedure described by Schofield et al. in 2001. This method relies on the reaction between the vanillin aldehydic group and the carbon 6 of the catechin cycle A, resulting in the formation of a red chromophore complex. This complex exhibits absorption of light at a wavelength of 500 nm. By measuring the absorbance at this specific wavelength, the concentration of condensed tannins in the Salvia chudaei extracts can be determined.**(Schofield et al., 2001)**

(B) **Operating mode**

In the Dosage of condensed tannins, 400 μl of each sample or standard is combined with 3 ml of a vanillin solution (4% in methanol) and 1.5 ml of concentrated hydrochloric acid. The resulting mixture is incubated for 15 minutes, allowing for the reaction to occur.**(Schofield et al., 2001)**

(C) **Results expression**

After the incubation period, the absorbance of the solution is measured at a wavelength of 500 nm using a spectrophotometer. This measurement provides information on the concentration of condensed tannins in the samples or standards.**(Schofield et al., 2001)** The concentrations of condensed tannins are determined using a calibration curve generated with catechin standards ranging from 0 to 0.5 mg/ml. These concentrations are then expressed as milligrams of catechin equivalent per gram of extract (Mg Ec/g E), providing a standardized measure for comparing the condensed tannin content across different samples.**(Schofield et al., 2001)**

I.8 Pigments Chlorophyll a, b and Carotenoids

(A) **Principle**

The isolation of new chlorophyll degradation products remains the key step in understanding the mechanisms of the chlorophyll degradation phenomenon. This is why it will be interesting to try to detect, isolate and characterize one (or more) catabolize(s) present in the spirulina samples.

(B) **Operating mode**

Chlorophyll a, b and carotenoids were determined according to the modified method described by **(El-Sheekh et al., 2009)** The procedure involved the following steps:

- In this process, one gram sample of spirulina is suspended in 50 ml of 90 acetone.
- Stirred vigorously with a magnetic stirrer (Stuart stir SB161, UK).
- The solutions were then placed in the dark at 4°C and centrifuged at 3900(x g) for 15 minutes.

(C) Results expression

The supernatants obtained were used to determine the concentration of chlorophyll a (chla), chlorophyll b (chlb) and total carotenoids (Car) by measuring the absorbance at wavelengths 663.2,646.8 and 470 nm. The optical density is read with a UV/Visible spectrophotometer (Jenway Genova plus, Staffordshire, United Kingdom). The content (mg/g) of each pigment was quantity using equations (01-03).**(Soni et al., 2018)**:

Equation 01

$$\mathbf{Chla=12.25.A663.2-2.79.A646.8}$$

. Equation 02

$$\mathbf{Chlb=21.5.A646.8-5.1.A663.2}$$

. Equation 03

$$\mathbf{Car = (1000\ .\ A470 - 1.82Chla-85.02Chlb)/198}$$

.

WHERE: A663.2, A646.8 and A470 are the respective absorbances of the sample at wavelength 663.2,646.8 and 470 nm. **(Soni et al., 2018)**

I.9 Study of biological activity

I.9.1 Antioxidant activity

To estimate the antioxidant activity of the test extracts, Measurement of the sample's ability to move radicals using the DPPH° (2.2-diphenyl-1picrylhydrazyl) root .

I.9.2 DPPH free radical inhibition test

1. **DPPH root working principle**

The DPPH radical, which stands for 2,2-diphenyl-1-picrylhydrazyl, is a solid substance with a black-violet color. However, when it is stabilized, it exhibits a yellowish-orange color. The stability of the DPPH radical is attributed to the presence of aromatic rings in its structure, which allow for multiple resonant forms. This resonance delocalizes the electrons, preventing them from being confined to a single location. As a result, the

DPPH radical remains stable for several days, exhibiting its characteristic color.**(Mbaebie et al., 2012)**

2. **DPPH free radical inhibition test**

The DPPH assay relies on the ability of extracts to donate hydrogen atoms to the DPPH free radical, resulting in its reduction. The reduction of the DPPH radical can be monitored using a spectrophotometer by measuring the decrease in absorption at a specific wavelength. This decrease in absorption indicates the ability of the extracts to inhibit or scavenge the DPPH free radical. By quantifying the reduction in absorption, we can assess the antioxidant capacity of the extracts and determine their effectiveness in inhibiting the DPPH free radical. **(Ardestani and Yazdanparast, 2007)**

3. **Preparation of DPPH solution:**

Prepare the concentrated DPPH solution (0,4Mol/ml) by dissolving 4mg of DPPH in 100ml of methanol .(Belguidoum et al., 2015)

4. **Preparation of concentrations:**

To prepare the original extract, 0.05 g of the thoughtful extract was taken and dissolved in 10 ml of a mixture of water and ethanol solvent to obtain a 5 mg/ml mother solution. From this mother solution, further diluted concentrations were prepared by adding additional ethanol to the ethanol extract and adding water to the water extract, following the specified ratios.**(Belguidoum et al., 2015)**

C1V1=C2V2

5. **Working Method:**

The antioxidant activity of the water and ethanol extracts is determined by assessing their ability to scavenge the DPPH free radicals. To measure this activity, different concentrations of the ethanol and water extracts are prepared. The procedure involves mixing 1 ml of each extract at concentrations ranging from 0.001 to 1 mg/ml with 2 ml of a DPPH solution with a concentration of 0.177 M.

6. **Calculate I% inhibition ratio for DPPH free root:**

The DPPH free root inhibition ratio for various concentrations of plant extracts and Acide ascorbique is calculated according to the following formula:

$$\mathbf{I\%=(A_0} - A_i)/A_0 \cdot 100$$

(I%): inhibition ratio.

A0: Represents the absorption of the witness.

Ai: Represents sample absorption.

Using Microsoft Excel, we can create a chart depicting the percentage inhibition curve. By analyzing this curve, we can determine the concentration of the extract required to achieve 50% inhibition of the DPPH radicals. This concentration is referred to as the IC50 value, which represents the concentration necessary to inhibit 50% of the free radicals.**(Belguidoum et al., 2015)**

To calculate the IC50 value, we utilize the equation derived from the inhibition ratio change curve (I%) in relation to the concentration of the extracts. The IC50 value is the concentration at which the inhibition ratio is 50%. By identifying the corresponding concentration on the x-axis of the chart where the curve intersects the 50% inhibition mark on the y-axis, we can determine the concentration of the extract required for 50% inhibition of the DPPH radicals.**(Belguidoum et al., 2015)**

I.10 Physico-chemical analysis of spirulina

I.10.1 Determination of pH Principle

The pH gives an indication of the acidity or alkalinity of the medium, it is determined from the quantity of free hydrogen ions (H) contained in the sample. Procedure: Set the temperature of the pH meter to the ambient environment, always rinse the probe using distilled water, then take 01g of sample, mix with distilled water to obtain a solution of 10 % . The pH of the solution obtained was measured at 25°C directly by a pH meter **(AOAC (Association of Official Analytical Chemists), 1998)**.

I.10.2 Determination of humidity level

The determination of humidity was carried out by oven drying **(A.O.A.C. (Association of Official Analytical Chemists), 1990)**. This . The clean crucibles are dried in an oven then cooled in a desiccator. The mass of the empty crucible is then measured. 05g of crushed sample contained in the crucible are then placed in an oven at 110 ± 2 ° C for 03 hours. The crucible assembly plus the dried sample were cooled in a desiccator for 30 min. Then, the mass of the crucible containing the dried sample is determined. vs . Expression of results: The humidity percentage is calculated according to the following formula:

$$(\%)\text{humidity rate } = [(M0 + Pe) - M1]/Pe \cdot 100$$

Of which :

M0: Mass (g) of empty crucible.

Pe: Mass (g) of the sample.

M1: Mass (g) of the whole (crucible + sample) after baking.

I.10.3 Determination of ash rate principle

The ash measurement consisted of the incineration of the spirulina powder using a crucible in a muffle furnace until whitish residues were obtained. The ash content was determined according to the method recommended by $AOAC^2$ **(KAMBOU et al., 2018)** .

$$\text{Ash content } = (P2 - P1)x100/P0$$

P0: mass of the Spirulina platensis sample (in g).

P1: mass of the empty crucible (in g).

P2: mass of empty crucible + ashes (in g).

I.10.4 Determination of protein level

The total protein concentration was determined using the Kjeldahl method (1883) according to the French standard NFV 03 050:1970 **(Anonyme, 1970)**. The principle consists of the transformation of the organic nitrogen contained in the sample in mineral nitrogen as well

as the titration of ammonia by an acid-base dosage. By convention, the protein content of the sample is then obtained by multiplying the total nitrogen content by an empirical conversion factor. This coefficient takes into account the average molar mass of the amino acids making up the proteins to be quantified. It is set at 6.25 in our case. The protein content is expressed as a percentage and is equal to:

$$P(\%) = 6.25 \cdot 14.007 \cdot N(v1_v0)/m \cdot MS\%$$

14.007 g/mol is the molar mass of nitrogen.

N: is the normality of the hydrochloric acid solution (0.1 N) .

V0: is the volume used of the hydrochloric acid solution on a blank sample.

V1: is the volume used of the hydrochloric acid solution (in ml).

M: is the mass of the sample (1 g).

MS: is the dry matter content (%) of the sample.

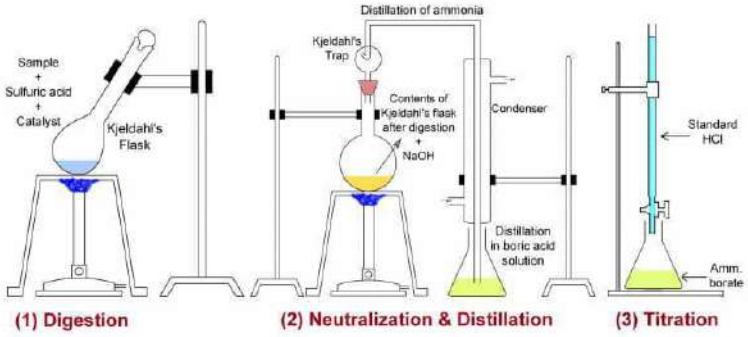


Figure I.7: **Kjeldahl method procedure (Baboo, 2016)**

I.10.5 Lipid content (NF V 03-905)

Fat content is determined in this manipulation according to the SOXHLET extraction method using hexane as solvent. 50 g of sample are placed in the Soxhlet and introduce 500 mL of hexane into the flask, set the temperature to 60°C.

Subsequently, remove most of the solvent using the rotary evaporator to prevent the oil from boiling, which in the long run could modify the acidity indices. The flask containing the lipids is placed in an oven for 30 min at 103° C., then in a desiccator for 30 min. The weight of the lipids is obtained by the difference between the final weight and the initial weight of the

balloon. **(Doumandji et al., 2023)**

The results are given by the following formula:

$$\text{Fat content}(\%DM) = (A - B) \cdot 100/C \cdot DM/100$$

A: weight of the flask + extract in grams .

B: weight of the empty flask in grams .

C: weight of the test sample in grams .

DM: dry matter in percentage.

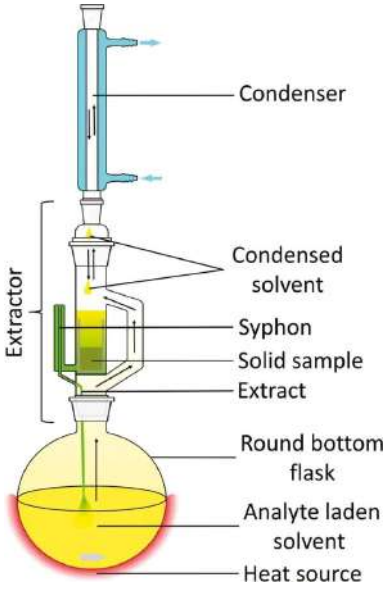


Figure I.8: **Soxhlet extractor (Vrenna et al., 2021)**

I.10.6 The total carbohydrate

The content was calculated following the calculation method recommended by the FAO18, which takes into account moisture, fat, protein and ash contents.**(KAMBOU et al., 2018)**

Total carbohydrates = 100 – (%humidity + %fat + %protein + %ash)

.

CHAPTER II

RESULTS AND DISCUSSION

II.1 Phytochemical screening

A preliminary chemical investigation was performed and various secondary metabolites were identified. Chemical detection tests include the detection of the different active compounds present in spirulina A and E shown in table 1 by qualitative reaction tests, and these reactions are dependent either by the formation of a precipitate or by color change by means of reagents specific to each family of active compounds.

Table II.1: **Results of detection of spirulina active components from two different regions (A and E) for decocted extract.**

Extract	Decocted		macerated	
	A	E	A	E
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Quinones	+	+	+	+
Coumarins	+	+	+	+
Reducing compounds	+	+	+	+
sterols and tri terpene	+	+	+	+
Saponosides	-	+	+	-

II.1.1 Description

Spirulina is a highly nutritious food thanks to its versatility and richness Of its components, this all phytochemicals are biologically important and play a vital role in medicinal applications. The effectiveness of the phytochemical search loom in novel discovery was measured Bioactive compounds from spirulina.

There are two main methods of analyzing the phytochemical assay Such as qualitative and quantitative analysis. Qualitative tests are used to identify components **(Lordan et al., 2011)**. And Quantitative tests are used to measure or determine the amount of active ingredients present **(Mane and Chakraborty, 2018)**.

In this study Spirulina platensis was used for qualitative phytochemical analysis. Phytochemical assay for nine different chemical compounds (alkaloids, sterols and triterpenes, tannins, reducing compounds, Saponins, flavonoids, phenols, coumarins and quinones were

tested). Macerated (methanolic extract) The decocted (aqueous extract) of a sample of spirulina from two different regions A and E.

In the decocted extract (aqueous extract) of a sample of Spirulina A, we find alkaloids, sterols and triterpenes, phenols, tannins, coumarins, quinones, flavonoids, reducing compounds, and the absence of saponins. As for the spirulina E sample, the presence of all the mentioned chemical compounds.

In the Macerated extract (methanolic extract) of a sample of Spirulina E, we find alkaloids, sterols and triterpenes, phenols, tannins, coumarins, quinones, flavonoids, and reducing compounds, and the absence of saponins. As for the spirulina A sample, it contained all of the mentioned chemical compounds

II.1.2 Descussion

All chemical compounds (flavonoids, tannins, reductive compounds, phenols, alkaloids, quinones, coumarins, saponins, sterols, and triterpenes) were positively detected extracts. But each and every Spirulina is varying from each other in the production of these compounds. Somehow these production is depends on environmental conditions such as temperature, pH, nutrients, metal ions and other chemicals **(Lordan et al., 2011)**.

In the decocted extract (aqueous extract) of a sample of spirulina A , we find alkaloids, sterols and tri terpene, phenols, tannins, coumarins, quinones, flavonoids, and Reducing compounds , and this corresponds to the action of both **(Tepal, 2016)** and **(Mane and Chakraborty, 2018)**. And the absence of saponins, and this agrees with the work of **(Tepal, 2016)** and **(Mane and Chakraborty, 2018)**, and opposes the work of **(Ali et al., 2017)**, and **(Boubeker et al., 2018)**. As for the spirulina sample E , the presence of all the mentioned chemical compounds, and this agrees with work **(Tepal, 2016)** and contradicts work **(Mane and Chakraborty, 2018)**.

In the Macerated extract (methanolic extract) of a sample of Spirulina E, we find alkaloids, sterols and triterpenes, phenols, tannins, coumarins, quinones, flavonoids, and reducing compounds, and this corresponds to the action of both **(Tepal, 2016)** and **(Mane and Chakraborty, 2018)**. And the absence of saponins, and this corresponds to the action of **(Tepal, 2016)** and **(Mane and Chakraborty, 2018)**, and opposes action **(Ali et al., 2017)** and**(Boubeker et al., 2018)**. As for the spirulina sample (A), it contains all the mentioned chemical compounds, and this is consistent with Work**(Tepal, 2016)**and contradicts Work **(Mane and Chakraborty,**

2018), **(Ali et al., 2017)**, and **(Boubeker et al., 2018)**.

II.2 Yield of extracts obtained

The values obtained from the yield of the different extracts are represented in the following table .

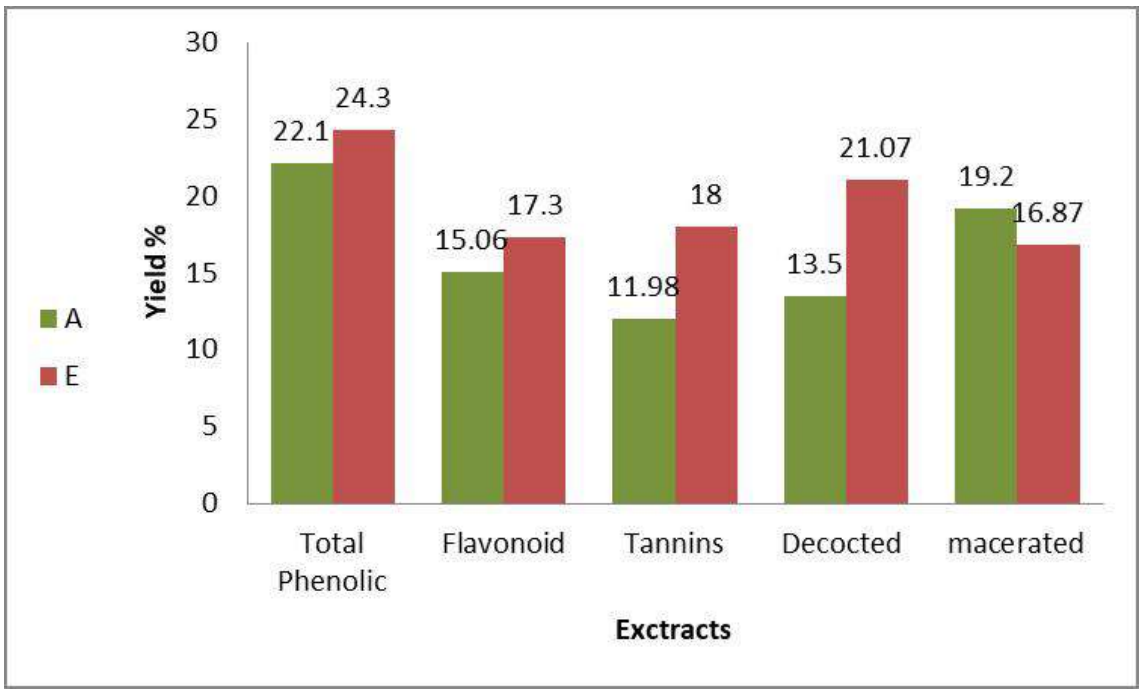


Figure II.1: **Yields of spirulina extracts (A and E).**

II.2.1 Description

After displaying the results of the yield values in Figure II.1, we find that the total phenolic extract gives higher yield values than other extracts, where the Spirulina E sample(24,3 %) contains the highest yield values and the Spirulina A sample (22,1%)has the lowest.

And flavonoid extract, we find the highest yield value in Spirulina E sample (17,3 %)and the lowest in Spirulina A sample(15.06 %).

For tannins extract, we find the highest yield value in Spirulina E sample(18 %) and the lowest value in Spirulina A sample(11,98 %).

The decocted extract of Spirulina E sample(21,07 %) was lower than that of Spirulina A sample(13,5%) which provided higher yield value.

As for the macerated extract, the Spirulina A sample (19,2%)contains the highest production values, and the Spirulina E sample(16,87%) has the lowest value.

II.2.2 Discussion

Figure II.1, which shows the productive values of the extracts of spirulina samples A and E, the yield of the total phenolic extract reaches the highest value than the other extracts by the methanol extraction method . for the kidneys of two samples of Spirulina A and E. Where we find Spirulina E has the highest yield values in Total Phenolic extracts 24,3% , tannins 18%, flavonoids 17.3%, decocted 21.07% , This is consistent with the work of **(Murugan and Rajesh, 2014)** and contradicts the work of**(Murugan and Rajesh, 2014)**. as well as spirulina A have the highest yield values in extracts of Macerated 19,2% .

This agrees with the work of **(Konkon et al., 2006)** and**(Mahmoudi et al., 2013)** with approximate results . Compared to the results of our a study that we find differs according to various factors such as: the nature of the solvent used in the extraction, pH, temperature, extraction time and chemical nature of the studied sample .**(Ali et al., 2017)**

II.3 Quantitative Analysis of Phenolic Compounds

II.3.1 Determination of total phenolic

The results obtained from the analysis are expressed in micrograms of gallic acid (GA) equivalent per gram of the extract (µg ∈AG/g Ex). This measurement was determined using the linear regression equation derived from the gallic acid calibration curve (as shown in Figure below).

II.3.2 Determination of flavonoids

The determination of flavonoids was carried out using the colorimetric method outlined by **(Bouba A et al., 2010)**. Quercetin was used as the standard, and a calibration curve was generated based on its concentrations. The results are expressed as milligrams of quercetin equivalent (Qu) per gram of extract. The calibration curve, as depicted in Figure below , was utilized to determine the flavonoid content.

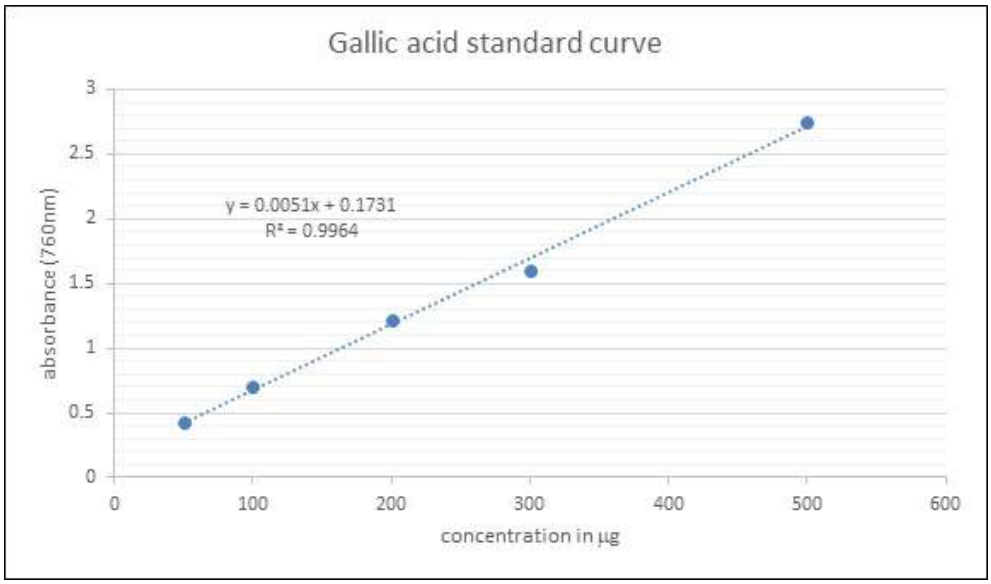


Figure II.2: **Gallic acid calibration curve for the determination of total polyphenols.**

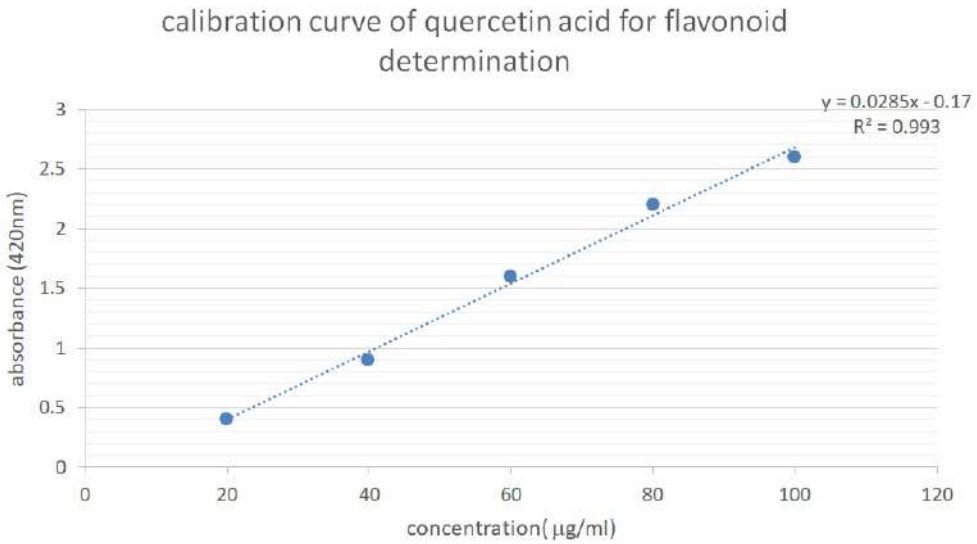


Figure II.3: **Quercetin calibration curve for flavonoid determination.**

II.3.3 Determination of condensed tannins

Tannin quantification was conducted using a method adapted from **(Schofield et al., 2001)**. A calibration curve was generated using catechin as the standard. The results are expressed as equivalent milligrams of catechin per gram of extract (Mg EC/g E). The tannin content of the extract was determined by referencing the calibration curve (as shown in Figure below).

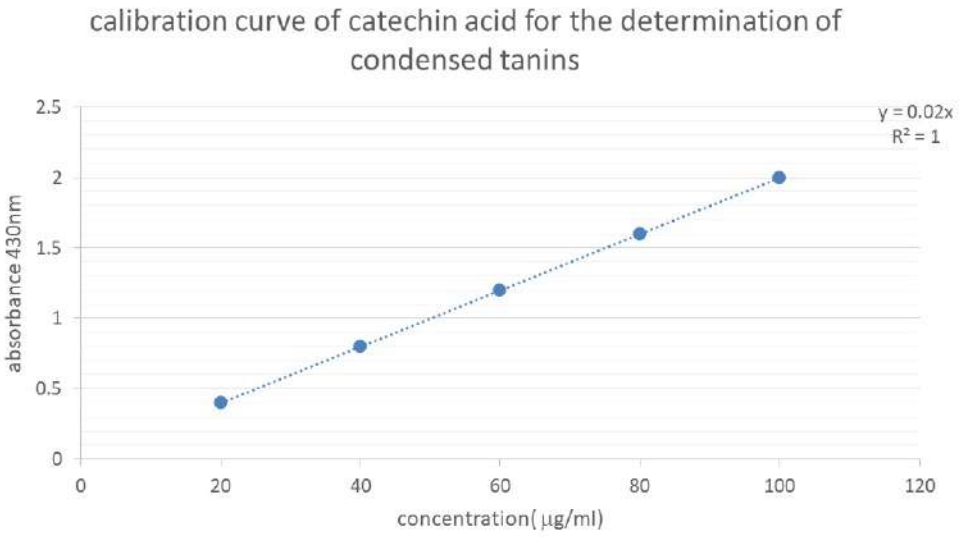


Figure II.4: Calibration curve of catechin acid for the determination of condensed tannins.

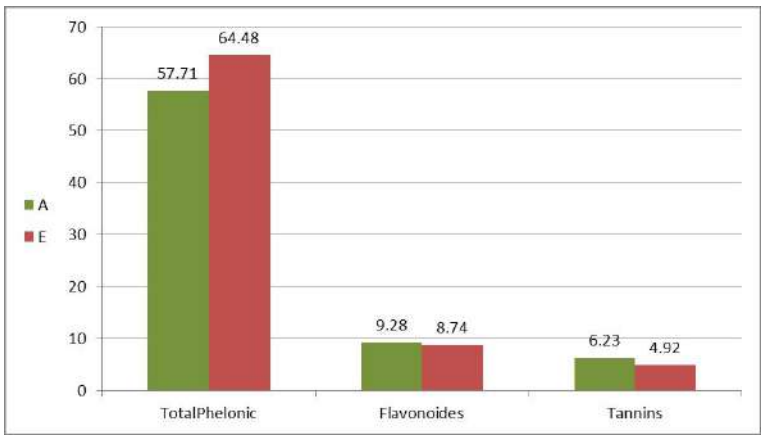


Figure II.5: **Histogram based on the measurements of spirulina extracts A and E.**

II.3.4 Description

Through the results listed in Figure II.5, which represent the quantitative estimation of total phenolics, flavonoids and tannins in mg equivalent of gallic acid, quercetin and catechin, respectively, per gram of extract weight, it was observed that the amount of total phenolics in Spirulina E was variable if its value was estimated by (64.48 μg/g) and spirulina A (57.71 μg/g).

As for the amount of flavonoids and tannins, their value was higher in Spirulina A, their value was estimated at (9.28 μg/g and 6.23 μg/g), respectively. And in Spirulina E, their value was estimated at (8.74 μg/g and 4.92 μg/g) respectively. straight.

II.3.5 Discussion

The results showed that Spirulina E provides a higher total phenolic content (64.48 μg/g) dry weight. This agrees with the work of **(AOUIR, 2017)** and **(Benahmed-Bouhafsoun et al., 2015)** with approximate results.

As for the values of flavonoids in Spirulina A and E, they were close to their values, respectively (9.28 μg/g and 8.74 μg/g). This result is lower than that obtained by **(Dianursanti et al., 2020)** . As for the values of tannins released from Spirulina A and E at levels (6.23 μg/g and 4.92 μg/g), respectively, they were variable. This result is higher than that obtained by **(Hetta et al., 2014)**(2.02 mg EC/g extract).

However, differences in results can be observed and this depends on several factors, for example: Temperature is a non-negligible parameter in biomass production, distribution of secondary metabolites, environmental stress, strong sunlight, etc.

II.4 Content of chlorophyll a, b and carotenoids

The amounts of chlorophylls and e-carotenoids observed in this study are shown in Figure below :

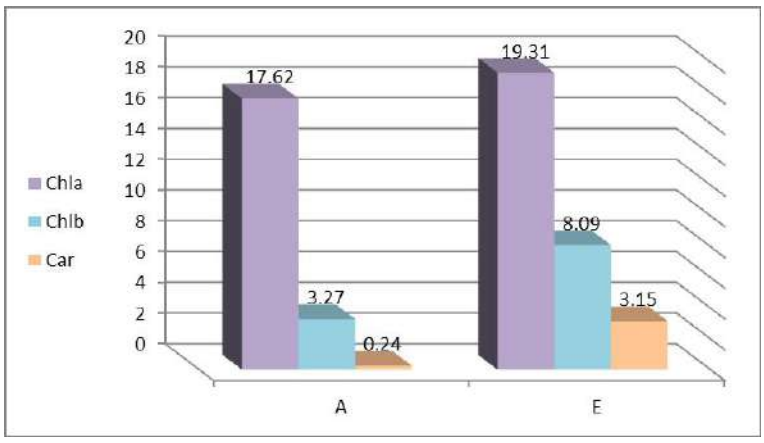


Figure II.6: **content of chlorophyll a, b and carotenoids in (mg/g) in different regioins of spirulina extracts**

II.4.1 Description

Spirulina is known to provide the pure form of chlorophyll at the lowest production costs as most of the chlorophyll (about 90 %) present in spirulina is in the form of chlorophyll

a . In particular , chlorophyll a has recently been in high demand because its biological activity , such as that of an antioxidant , is considered to be higher than that of other chlorophylls , such as chlorophyll b . The chlorophyll content present in the spirulina samples is shown in Figure II.6. The values of chlorophyll and carotenoids were higher in the Spirulina E sample, where chlorophyll a (19.31mg/g), chlorophyll b (8,09mg/g), and carotenoids (3,15mg/g). Were reduced in the spirulina A sample, chlorophyll a (17,62mg/g), chlorophyll b (3,27mg/g), and carotenoids (0,24mg/g)

II.4.2 Discussion

The results presented Chlorophyll A and B and carotenoids present in Spirulina E sample are higher compared to Spirulina A sample, where showed that chlorophyll a was prevalent in the two spirulina samples A and E compared to chlorophyll b. The highest percentage of chlorophyll a was found in spirulina E versus spirulina A to reach their value E = 19,31 mg/g , A=17,62 mg/g and this agrees with the work of**(AOUIR, 2017)** and opposes the work of **(Minchev et al., 2020)** . For chlorophyll b , the highest value appears in Spirulina E, in contrast to Spirulina A, which is low, with two values E=8,09 mg/g, A=3,27mg/g This is consistent with the work of **(Minchev et al., 2020)** . The value of carotenoids in Spirulina E appears in varying proportions compared to Spirulina A, with a value of E = 3,15mg/g A =0,24mg/g , and this agrees with the work **(AOUIR, 2017)** .

II.5 Biological activities

II.5.1 Antioxidant activity

II.5.1.1 DPPH Free Radical Inhibition Test

DPPH is a stable, free radical with an absorbance band at 517 nm used to assess the antioxidant activity of total phenolic, flavonoids and decocted, macerated tannins. In this test, ascorbic acid is used as a standard. The IC50 of each ”A” and ”E” spirulina extract is deduced from the regression equation corresponding to its calibration curve and is expressed in μg/ml. The results are represented graphically on a histogram (FIG II.8).

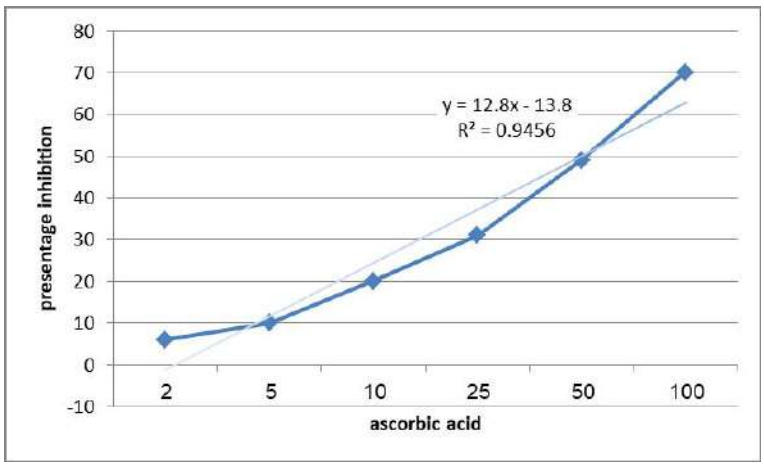


Figure II.7: **Ascorbic acid calibration curve.**

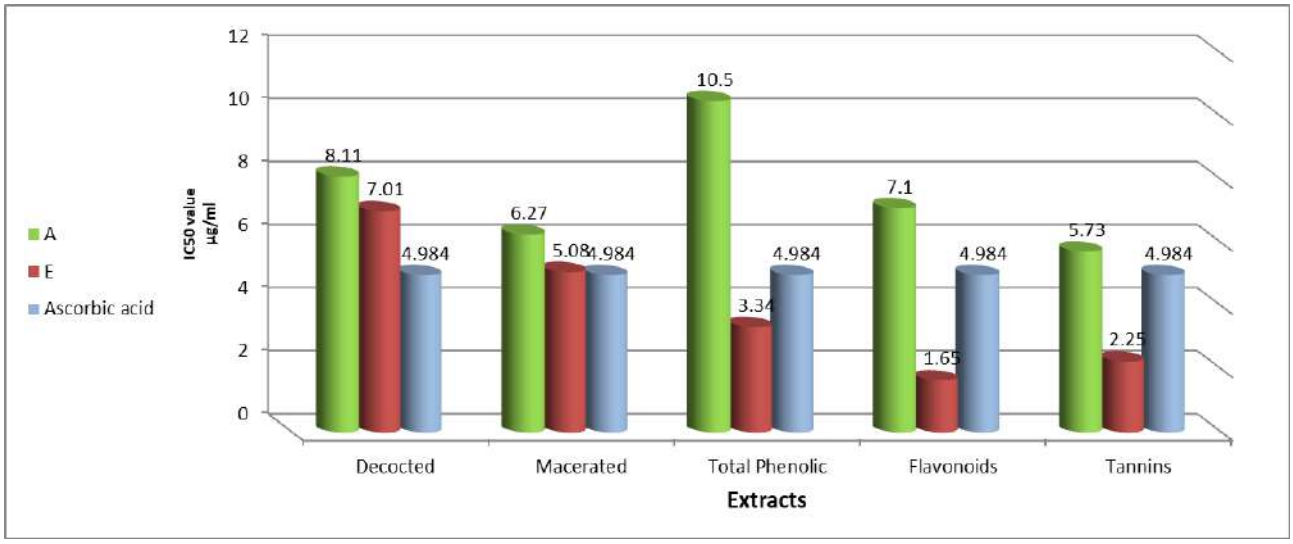


Figure II.8: **the IC50 values of the various extracts and ascorbic acid in (µg/ml).**

II.5.2 Description

According to the results obtained in Figure II.8, which represent the free radical inhibitory IC50 values for the different extracts and the standard (ascorbic acid), we note that the IC50 values for the extracts are in the range of 5.73 micrograms/ml, 6.27 micrograms/ml, and 7.10 micrograms/ 8.11 µg/ml, 10.50 µg/ml, for tannins, marinated, flavonoids, boiled and total phenols for Spirulina "A" respectively, and the IC50 values for Spirulina "E" extracts are about 1.65 µg/ml and 2.15 µg/ml. 3.34 micrograms/ml, 5.08 micrograms/ml, and 7.010 micrograms/ml, for flavonoids, tannins, and total phenols, soaked and boiled, respectively.

We note the anti-free radical power of Spirulina "E" flavonoids compared to other extracts. Regarding our results, the flavonoids, tannins and total phenolics extracts of Spirulina "E" have a very strong anti-free radical potency compared to the valuable ascorbic acid (4,984μg/ml

II.5.3 Discussion

From the results we obtained, it became clear to us that there is an inverse relationship between the percentage of free radical inhibition (IC50) and the antioxidant capacity present in the sample. This is consistent with the work of **(Pokorny et al., 2001)**. It is in line with the work of **(Habibou et al., 2019)** Which means:

The inhibitory concentration (IC50) is inversely proportional to the antioxidant capacity of the compound. It expresses the amount of antioxidant required to reduce the concentration of free radicals by 50%.

The smaller the IC50 value, the greater the antioxidant activity of the compound **(Khoudali, 2014)**.

The stable deep violet DPPH radical is converted to yellow DPPH after reaction with the hydrogen-donating antioxidant: DPPH. + AH $\rightarrow$ DPPH-H + A. (4) Since the 2,2-diphenyl-1-picrylhydrazyl radical takes an electron in the presence of a free radical scavenger, the absorption decreases as a result of the color change which is stoichiometrically proportional to the amount of electrons Acquired**(Zhang et al., 2011)**.

The methanolic extract (tannins and total phenols) of Spirulina "E" converts the stabilized free radical (2,2-diphenyl-1-picrylhydrazyl) into yellow-colored diphenyl-picrylhydrazine with an IC50 of 2.15 µg/ml and 3.34 µg/ml on In a row, which shows a very important antioxidant activity, but it remains low, than the methanolic extract (flavonoids), which brings stability to

DPPH with an IC50 of 1.65 µg/ml. According to these results, the methanolic extract (flavonoids) remains the most effective antioxidant compared to the rest of the extracts. This work is in line with With the work of **(Habibou et al., 2019)**.

II.6 Results of physico-chemical analysis of spirulina samples

The results of the physico-chemical analysis carried out on the spirulina powder of the two samples (A and E) appear in the table below:

Table II.2: **Results of physico-chemical analysis of the two spirulina samples (A and E)**

	Spirulina A	Spirulina E
PH	7.69	6.8
Humidity %	8.77	4.2
Ash%	8.28	10.87
Protein %	54.6	62.81
Lipid %	6.12	6.39
Carbohydrate %	22.23	15.73

II.6.1 Description

Microalgae are considered an alternative source of protein, thanks to their high protein content **(Ahsan et al., 2008)**. The results of this study showed that spirulina is a good food rich in proteins, with a percentage of 62.81% for spirulina E and 54.60% for spirulina A. While it contains 6.39% fat for spirulina E and 6.12% for spirulina A. As for carbohydrates, their value reaches 15.73% for spirulina E and 22.23% for spirulina A. Among its physical factors, we highlight: pH, humidity, and ash, their values were respectively (6.80, 4.20%, and 10.87%) for spirulina E.(7.69, 8.77%, 8.28%) for spirulina A.

II.6.2 Discussion

The results of Table II.2 reveal that there is a difference in the amount of physical and chemical compositions between the two samples (Spirulina A and Spirulina E). Since protein is the main component of spirulina, its percentage was high in both samples, 62.81% in Spirulina E and 54.60% in Spirulina A.

These values represent a high percentage of protein. According to **(Bensehaila et al., 2015)**they measured the protein content at 60.32%, which is higher than the 54.60% and lower than the 62.81% observed in this study. Moreover, **(Sharoba, 2014)**found that spirulina is a rich source of protein, containing 62.84% of its weight in protein, most of which are essential amino acids.

As for the fat content, we find Spirulina E 6.39% higher than Spirulina A 6.12%, and this agrees with the work of **(RaMadaN and Selim aSkeR, 2008)**, who found in his work that the fat content in spirulina powder was 6.38%. The carbohydrate content in Spirulina A is 22.23% higher than that of Spirulina E 15.73%.

Because spirulina is an alkaline food, it combats acidic foods and helps raise the pH level toward the alkaline side of the scale. We find that the pH and humidity of Spirulina A (7.69, 8.77%) are higher than Spirulina E (6.80, 4.20%). This roughly agrees with the work of**(Sharoba, 2014)**, who reached a pH value of (6.84).

As for the ash content, its percentage in Spirulina E was 10.87%, higher than that in Spirulina A, 8.77%. The results were found to be in the range of values reported by **(Liu and Liang, 1999)** who found that green spirulina consists of protein (55-70%), ash (3-11%), moisture (4-9%) and carbohydrates (15-25%).

GENERAL CONCLUSION

Spirulina is a filamentous cyanobacterium. It's part From a particularly interesting bacteria called Spirulina platensis (or Arthrospira platensis) (A. platensis), Known as blue-green algae. Microalgae have been the subject of many studies.

In several countries due to its many benefits Such as its antioxidant properties, strengthening the immune system, and helping with weight .

Its high protein content makes this... Algae is a superfood ,it is rich in nutrients such as: proteins, carbohydrates, fats, vitamins and minerals. In addition, spirulina is rich in phycocyanin, Blue protein stain. It can be used as a dye in food, pharmacy and cosmetics.

In an attempt to identify the presence of these substances and estimate their quantity and effectiveness, we chemically examined samples of Algerian and Egyptian spirulina grown in two different regions (Spirulina A and E), then they were extracted and their antioxidant activity was studied, and their nutritional value and some of their physical indicators were known. Based on the results obtained from chemical examination of extracts prepared in an aqueous medium (decocted) and in an organic medium (maserated), we found that Spirulina A and E contain phenols, flavonoids, alkaloids, tannins, coumarins, reducing sugars, sterols, triterpenes, and saponosides. Entities.

The yield of the extracts was recorded as showing that the results of the methanolic extract (total phenols) were superior to the rest of the extracts. Among them, the methanolic extract of spirulina (E) (24.3%) is superior. By measuring the content of total phenols, flavonoids and tannins, the methanolic extract outperformed the total phenols of Spirulina E (64.48µg GA/g).

Spirulina E recorded high levels of chlorophyll a (19.31 mg/g), chlorophyll B (8.09 mg/g) and carotenoids (3.15 mg/g). We evaluated the antioxidant activity of our extracts using the DPPH test. The DPPP free radical test showed that the results of the methanolic extract (flavonoids) of Spirulina E were superior to the rest of the extracts (IC50=1.65 μ/ml).

We have evaluated the physical and chemical analyzes of spirulina. This evaluation showed that: the content of protein (62.81%), fat (6.39%) and ash (10.87%) in Spirulina E is higher than that of Spirulina A while carbohydrates (22.23%), pH (7.69) and moisture (8.77%) in Spirulina A is higher than Spirulina E.

Finally, it can be said that Spirulina Algeria and Egypt .It can be used as a promising source of nutrition And multi-functional vehicles because Its high content of essential amino acids, Natural colourants, vitamins, proteins, carbohydrates, fats and minerals Play important roles in immune support, And others¨.

In view of the results that have been reached, our work remains preliminary, and it seems that it would be very useful to continue this study while addressing different axes in the future in order to deepen scientific and technological research. From this standpoint, we present some points of view and recommendations:

Raising awareness and encouraging investors to focus on growing spirulina in order to spread it on a large scale and ensure its availability in sufficient quantities to meet the needs of the agri-food industry. It is recommended to consume spirulina against malnutrition, especially in children, and it is also recommended to treat anemia due to iron deficiency. Enriching food products with spirulina to increase their oxidative stability and increase their energy and nutritional value.

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APPENDICES

Appendix 01: Spirulina nutritional sheet

Spirulina

Fiche Nutritionnelle

ceva

Teneurs pour 100 g d'algue déshydratée (produit brut) — *Version du 14/03/2015*

Paramètres	Unité	Teneur Moyenne	Min	Max	Nb données
Energie	kJ	1 553			
Energie	kcal	372			
Eau	g	5,9	1,6	11,0	19
Minéraux	g	7,6	4,4	13,2	12
Protéines (Nx6.25)	g	60,8	43,3	79,3	49
Glucides (par différence)	g	17,5			
Fibres Alimentaires	g	2,2	0,1	7,4	8
Lipides	g	6,0	1,9	9,8	38
AG saturés	g	2,49	2,49	2,49	1
AG monoinsaturés	g	0,64	0,64	0,64	1
AG polyinsaturés	g	1,96	1,96	1,96	1
Phycocyanine	g	10,0	2,2	25,5	21
Sodium	mg	618	28	1 448	10
Magnésium	mg	560	185	1 789	12
Phosphore	mg	1 041	111	3 671	13
Potassium	mg	1 360	932	1 789	9
Calcium	mg	487	61	1 850	14
Manganèse	mg	4,3	1,7	10,8	9
Fer	mg	79,7	26,8	169,4	15
Cuivre	mg	1,3	0,3	2,7	6
Zinc	mg	5,3	1,2	31,1	11
Iode	mg	0,0	0,0	0,0	5
Sélénium	µg	nd			
Chrome	µg	273,0	60,0	580,0	6
Molybdène	µg	nd			
Vitamine A (eq rétinol)	mg	29,4	2,8	79,1	3
Beta-carotène	mg	127,1	21,7	178,9	4
Vitamine D	µg	0,0	0,0	0,0	1
Vitamine E (eq tocophérols)	mg	10	4	18	5
Vitamine K ou phytoménadione	µg	760	24	1 497	2
Vitamine C	mg	11,1	7,5	18,8	4
Vitamine B1 ou Thiamine	mg	3,4	2,2	5,2	6
Vitamine B2 ou Riboflavine	mg	3,6	3,1	4,3	6
Vitamine B3 ou PP ou Niacine	mg	14,4	11,1	22,1	4
Vitamine B5 ou acide panthothénique	mg	0,90	0,43	1,22	3
Vitamine B6 ou Pyridoxine	mg	0,4	0,1	0,8	6
Vitamine B8 ou H ou Biotine	µg	19,0	4,3	37,7	4
Vitamine B9 ou Folates	µg	59,3	37,7	88,5	5
Vitamine B12 ou Cobalamines	µg	236,1	13,7	659,0	10

Elaboré par le CEVA (Centre d'Etude et de Valorisation des Algues), Pleubian, France - www.ceva.fr

Appendix 02: Equipment

Steamroom

Precision balance

rotavapor

Water bath

PH meter

Centrifuge

Stirrer

Vortex

Spectrophotometer

Analytical balance