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MEMORY

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Theme:

Biological activity of polysaccharides from Allium ampeloprasum variety Porrum

Defended on 30/06/2025, before the jury composed of:

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ملخص

الكراث (*Allium ampeloprasum* var. porrum) هو عضو في عائلة Amaryllidaceae ، وهو غني بالمركبات النشطة بيولوجيًا، وخاصة البوليفينولات والفلافونويدات ومركبات الكبريت، وبالتالي فهو ذو قيمة في مجالات الطب التقليدي والتغذية وخاصة البحث الصيدلاني. تكمن أهمية هذه النبتة في خصائصها المضادة للأكسدة والمضادة للبكتيريا وأفعالها المضادة للسرطان والمضادة للسكري.

في هذه المذكرة، يتم استخدام السكريات المستخرجة من الكراث وتقييم النشاط المضاد للأكسدة باستخدام قوة الاختزال الحديدية (FRP) وكمية السكريات. تم استخراج نوعين من المستخلصات عن طريق التسخين، تلا ذلك ترسيب بالإيثانول والتجفيف.

أسفر هذا العمل عن عائد بنسبة 0.87 % لأفضل عينة ، مع محتوى إجمالي من السكر يبلغ 381 ± 36.6 ملغ/غ. أظهرت النتائج أن المستخلصات كانت لها قدرة ارجاع كبيرة للحديد (Fe^{3+}) إلى(Fe^{2+} ، مع أقصى قدرة تقليل تبلغ 0.49 ملغ مكافئ حمض الأسكوربيك/غ من المستخلص في عينة.P2V60

تشير هذه النتائج إلى أن سكريات الكراث تساهم بشكل فعال في مقاومة الجذور الحرة، مما يدعم استخدام كفئة واعدة ناشئة من مضادات الأكسدة المستمدة من الطبيعة والتي يمكن تطويرها لاحقًا، سواء لصناعات معالجة الأغذية المتقدمة أو للمكملات الصحية.

الكلمات المفتاحية : *Allium ampeloprasum* var. *porrum*، السكريات ، النشاط المضاد للأكسدة، FRP

Abstract

Leek (*Allium ampeloprasum* var. *porrum*), is a member of the Amaryllidaceae family, and is rich in bioactive compounds, specifically polyphenols, flavonoids and sulfur compounds, and is therefore of value in the areas of traditional medicine, nutrition and especially pharmaceutical research. The significance of this plant lies in its antioxidant, antibacterial properties and anticancer and antidiabetic actions.

In this memory, the use of polysaccharides extracted from leeks and the evaluation of the antioxidant activity of the polysaccharides from leeks using the FRP (Ferric Reducing Power) and the sugars quantification were studied. Polysaccharides were extracted by heating followed by precipitation with ethanol and drying.

The yield is of 0.87% for the best sample of polysaccharides, with a total sugar content of 381 ± 36.6 mg/g. The results showed the extracts had a significant reducing capacity for iron (Fe^{3+} to Fe^{2+}), with the maximum reducing capacity of 0.49 mg ascorbic acid equivalent/g extract in the P2V60 sample.

These results indicate that leek polysaccharides could contribute to the resistance to free radicals, supporting the use of leek polysaccharides as a emerging promising class of antioxidant derived from nature which could then be further developed, either for higher food processing industries or for health supplements.

Keywords: *Allium ampeloprasum* var. *porrum*, polysaccharides, antioxidant activity, FRP

Résumé

Le poireau (*Allium ampeloprasum* var. *porrum*) est un membre de la famille des Amaryllidacée, il est riche en composés bioactifs, notamment en polyphénols, flavonoïdes et composés soufrés, et est donc précieux dans les domaines de la médecine traditionnelle, de la nutrition et surtout de la recherche pharmaceutique. L'importance de cette plante réside dans ses propriétés antioxydantes, antibactériennes et ses actions anticancéreuses et antidiabétiques.

Dans ce mémoire, l'utilisation des polysaccharides extraits des poireaux et l'évaluation de l'activité antioxydante en utilisant le FRP (Ferric Reducing Power) et la quantification des sucres ont été étudiés. Les polysaccharides sont extraits chauffage, suivis d'une précipitation avec de l'éthanol et d'un séchage.

Ce travail a donné un rendement de 0,87 % pour le meilleur échantillon de polysaccharides, avec une teneur totale en sucres de 381 ± 36,6 mg/g. Les résultats ont montré que les extraits avaient une capacité réductrice significative pour le fer (Fe^{3+} à Fe^{2+}), avec une capacité réductrice maximale de 0,49 mg équivalent acide ascorbique/g extrait dans l'échantillon P2V60.

Ces résultats indiquent que les polysaccharides de poireau contribuent efficacement à la résistance aux radicaux libres, soutenant l'utilisation des polysaccharides de poireau comme une classe émergente et prometteuse d'antioxydants d'origine naturelle qui pourraient ensuite être développés, soit pour les industries de transformation alimentaire, soit pour les compléments alimentaires.

Mots-clés : *Allium ampeloprasum* var. *porrum*, polysaccharides, activité antioxydante, FRP

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Special thanks go to all the professors and academic staff who have accompanied us throughout our university studies. Each of you has played a role in broadening our knowledge, sharpening our critical thinking, and nurturing our passion for scientific discovery. Your dedication and professionalism have been a constant source of inspiration.

DEDICATION

I begin by thanking those who did not believe in me; unknowingly, they gave me the strength and determination to succeed.

To those who doubted me and tried to hold me back — thank you. Your challenge was the spark that fueled my drive to reach this achievement.

I dedicate this work:

To the two people I love most in the world and whose existence never ceases to fill my life with happiness

*To my dear father **DJILALI**, my first mentor since birth, you have always been my support in life and always encouraged me, I wish with all my heart to make you proud, God willing.*

*To my mother **OUASSILA** for her love, unwavering support and countless sacrifices, which allowed me to pursue my dreams.*

*A special dedication to my dear brother **ADEM** and my sisters **ROUWAYDHA** and **MOUNIRA,** and my grandmothers who have encouraged me endlessly and believed in me. Words are not enough to express the attachment, love and affection I have for you.*

To all my uncles and aunts

To all my family, near and far.

*To my partner **KHALED**, with whom I was able to complete this project.*

To my teachers throughout my studies who guided me towards success.

To my friends.

To my colleagues.

To all those who know me.

I dedicate this modest work.

BILLAL

DEDICATION

*From the bottom of my heart, I dedicate this final project to my dear parents, who have always encouraged and motivated me in my studies and life in general. Without them, I certainly would not have pursued long studies. This work therefore represents the culmination of the support and encouragement they have given me throughout my education. May they be thanked with this too modest dedication. It is a pleasure to dedicate this work to my sisters as a sign of love, recognition, and gratitude for the dedication and sacrifices you have always shown towards me. And finally, to my friends and my partner "***BILLAL***" who have never stopped supporting me. Not forgetting all the teachers, whether from primary, middle, secondary, or higher education.*

KHALED

List of abbreviations

- **SOD:** superoxide dismutase
- **GPx:** glutathione peroxidase
- **GRx:** glutathione reductase
- **CAT:** catalase
- **AMD:** Age-related macular degeneration
- **ROS:** Reactive oxygen species
- **RL:** Free radicals
- **DNA:** Deoxyribonucleic Acid
- **FRP:** Ferric Reducing Power
- **Fe3+:** Ferric ion.
- **FeCl3:** Ferric chloride
- **Fe2+:** Ferrous iron
- **NaH_2PO_4:** Sodium dihydrogen phosphate
- **PH:** potential of hydrogen
- **G:** gram
- **K3Fe(CN)6:** Ferric potassium cyanide
- **SAB:** serum albumin bovine
- **Mg:** milligram
- **ml:** milliliter
- **TCA:** Trichloroacetic Acid
- **RPM:** revolutions per minute
- **DW:** Distilled Water
- **%:** percentage
- **NMR:** Nuclear Magnetic Resonance
- **GC-MS:** Gas Chromatography–Mass Spectrometry
- **ANOVA:** Analysis of Variance

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General Introduction

General Introduction

Since ancient times, mankind has depended on plant products from its surrounding environment to remedy or cure ailments. Plants are a rich source of diverse secondary metabolites that can be converted to drugs, agrochemicals, flavors, fragrances, dyes, biopesticides, food additives, and more. The harnessing of these properties is still an active and exciting field of research (Kendji et al., 2021).

Algeria has a rich and diverse Flora. Included among the medicinal plants that make up this natural inheritance are the plants of the *genus Allium*. In modern times plants of the *Allium genus* are valued for their unique flavors, subtle aroma, and bold flavor profile. Plants of the *Allium genus* form an important raw material in the food processing sector (Kendji et al., 2021).

Allium ampeloprasum L. var. *porrum* or leek, has been cultivated and consumed for millennia (Bernaert, 2013). Leek is used in many Mediterranean countries not only as food, but also as a condiment, medicinal herb, and a flavoring agent (Strati et al., 2018). The chemical composition of leek contains a variety of bioactive compounds including organosulfur compounds, phenolics, saponins, polysaccharides, flavonoids, minerals, proteins, and vitamins (Camila et al., 2011).

Research has identified the beneficial properties of leek, most notably its antioxidant and antimicrobial properties, which will be explored in detail in the following chapters

This study is organized in the following manner:

Chapter 1: Botanical characteristics of *Allium ampeloprasum* var. *porrum*, and general considerations on plant polysaccharides.

Chapter 2: Polysaccharides: Definitions, structures, classifications, and natural sources. And biological activities: Antioxidant, antimicrobial, and immunomodulatory activities.

Chapter 3: Oxidative stress, antioxidant defenses, and the action of natural compounds against oxidative damage.

Chapter 4: Materials and Methods used for extraction, purification and evaluation of biological activity.

Chapter 5: Results and discussion of experimental findings.

Final section: General conclusion and perspectives for future research.

First part: Literature review

Chapter 1: General Information on the Plant

1. Introduction*:*

Allium is a vegetable plant that can be cultivated in ornamental gardens and mixed herb gardens. Particularly appreciated by peasants in the Middle Ages, it was also widely sought after as a wild species before being cultivated. Its profile, particularly for its strong presence along all paths, was built in the aftermath and during the winter, but it is within reach of its many uses in culinary art, as well as in the renewable energy sector or health. In the realm of biodiversity, it is characterized as well "consumables" the competitor of cultivated garlic (*Allium sativum L*.), wild garlic, and the so-called varieties of garlic cultivated far away, in soil known for having the powerful taste of these herbs grown outdoors or inside gardens. A growth that sneaks into gardens, as needed by infants.

2.Definition*:*

Allium ampeloprasum var. porrum (Figure 1 and 2), commonly known as leek, is a biennial plant from the Amaryllidaceae family with a cylindrical false stem formed by the overlapping of sheath leaves, and a floral stem that only develops from the second year. Unlike the onion, it does not form an intermediate bulb, but for storage reasons, its functionality is assigned to its leaf sheaths. It is commonly confused with elephant garlic (*Allium ampeloprasum var. ampeloprasum*), although their culinary uses are distinct and their morphology differs, and its sweet and slightly sweet flavor makes it a vegetable widely used in culinary preparations (Lidia et al., 2016).

Figure 1*:* Leek plant

Figure 2*:* Leek plant with flower

3. Species classification*:*

The leek is classified as follows:

- **Kingdom: Plantae**
- **Division: Angiosperms**
- **Class: Monocotyledons**
- **Order: Asparagales**
- **Family: Amaryllidaceae**
- **Subfamily: Allioideae**
- **Genus: *Allium***
- **Species: *Allium ampeloprasum***
- **Variety: *porrum***

It belongs to the group of monocotyledonous plants with terminal flowering. This classification highlights its phylogenetic proximity to other species of the *Allium* genus, such as garlic (*Allium sativum*) and onion (*Allium cepa*), which share similar biochemical and medicinal properties (Hanelt et al., 2001).

4. Origin of *Allium ampeloprasum:*

The origins of the leek thus date back many centuries. It is presented as the product originating from the Mediterranean basin where its cultivation first spread to the coastal regions of North Africa on one hand, and on the other hand from the eastern limits of the Mediterranean world to the south of Europe. Its cultivation is attested in ancient Egyptian writings and in Greco-Roman sources that recount its consumption. It is probable that *Allium ampeloprasum* is the wild common ancestor of several cultivated varieties, including the leek, and the domestication of the plant occurred slowly with the appearance of plants with broader leaves, a taste allowing for better adaptation to consumption while ensuring cultivated plants have better climatic resistance (Zohary et al., 2000).

5. Cultivation and Harvesting*:*

The cultivation of leeks requires deep soil, rich in organic matter, well-drained, light, and neutral. It is carried out either in spring or at the end of summer depending on the varieties (summer leeks or winter leeks). The optimal growth conditions are found under moderate temperatures, although they must be able to withstand frost. Blanching techniques (earthing up) are often applied to lengthen the white shaft of the leek. The harvest can take place from

July to February, depending on the sowing period and the variety used. The average yield ranges between 20 and 40 tons per hectare depending on the cultivation conditions (Lespinasse et al., 1997).

6. The chemical components

Leek is an excellent source of multiple phytochemical compounds, including biologically active polysaccharides that have demonstrated antioxidant, immunomodulatory, and antitumor properties (Zhao et al., 2021; Li et al., 2020). It also contains sulfur compounds (thiosulfinate, S-allyl cysteine) that contribute to its distinctive aroma as well as its antimicrobial activity. It is rich in flavonoids, including kaempferol, whose antioxidant properties are well established. Moreover, leek provides various vitamins (A, C, K, B6, folates) and minerals (potassium, magnesium, calcium), as well as soluble and insoluble dietary fibers that contribute to its protective effects against oxidative stress, infections, hypercholesterolemia, and certain forms of cancer (Lanzotti et al., 2006).

7.Usage

Leek is mainly used as a vegetable in the diverse cuisines of various regions around the world. It is eaten raw (young shoots) or cooked (soups, gratins, tarts, etc.). In herbal medicine, it is recognized for its diuretic, purifying, digestive, and expectorant properties. Leek decoctions were used for sore throats, urinary infections, and intestinal disorders. Selected as an ally for the liver and digestion due to its sulfur compounds, leek extracts are now being studied for their potential in therapeutics against metabolic and cardiovascular pathologies (Duke et al., 2002).

8. Role of *Allium ampeloprasum* in some pathologies

8.1. Role of Antimicrobial

In leeks, sulfur compounds are naturally present in the form of Thio sulfinate and cysteine products, which are the subject of ongoing research for their antimicrobial potential, demonstrated for some of them by inhibiting the growth of microorganisms, disrupting the cell membrane or certain enzymatic systems, or even causing a collapse of the cellular content. In vitro studies have shown that these sulfur compounds are active against pathogenic strains such as *Escherichia coli, Staphylococcus aureus, Salmonella spp*., or

*Candida albican*s, with activity similar to that already demonstrated for other members of the *Allium genus* such as garlic (Benkeblia et al., 2005).

8.2. Implication in diabetes and its associated complications*:*

Recent studies have revealed the hypoglycemic effect of aqueous extracts of *Allium ampeloprasum*. Indeed, these extracts are capable of significantly lowering hyperglycemia in diabetic rats induced by alloxan or streptozotocin, probably due to the presence of sulfur compounds and flavonoids that improve insulin sensitivity and are known for their antioxidant capabilities acting against oxidative stress involved in diabetes complications, but some compounds seem to inhibit the digestive enzymes responsible for glucose absorption (Ibrahim et al., 2018).

8.3. The role in digestive problems:

Due to its richness in soluble and insoluble fibers, leeks promote intestinal motility, which helps prevent constipation. Fibers act as prebiotics because they promote the multiplication of certain bacteria in our gut microbiota. Moreover, sulfur compounds provide anti-inflammatory effects and could be useful in alleviating certain symptoms of irritable bowel syndrome (Slavin et al., 2013). Regularly consuming leeks is therefore desirable in a balanced diet to improve digestion and intestinal health

8.4. Anticancer effect*:*

Experimental studies suggest that leek extracts inhibit the proliferation of cancer cells, particularly in the colon, breast, and prostate, and that the active compounds quercetin, kaempferol, and sulfur derivatives can induce apoptosis (programmed cell death) and block tumor angiogenesis while reducing DNA mutation, both in vitro and in vivo, giving this vegetable a broad potential for nutritional cancer prevention (Sengupta et al., 2004).

8.5. Androgenic Effects*:*

Experimental studies have shown in rats that the administration of *Allium ampeloprasum* extracts is accompanied by a significant increase in serum testosterone levels, an improvement in spermatogenesis, and an increase in the weight of reproductive organs. The androgenic effects would be linked to the antioxidant properties of flavonoids and sulfur compounds, which protect Leydig cells from oxidative damage while stimulating their hormonal secretion efficiency, according to Khaki et al., 2012.

8.6. Hypocholesterolemia effect*:*

Leek is often praised for its positive effects on lipid metabolism. It contains sulfur compounds and saponins that help reduce LDL cholesterol levels (also called "bad cholesterol") and slightly increase HDL levels (or "good cholesterol"). Regular consumption of leeks, in reasonable quantities, thus helps to limit the risk of atherosclerosis, high blood pressure, and cardiovascular diseases. In a study conducted on animals, decreases of more than 25% in total cholesterol were observed after oral administration of leek extracts (Hassan et al., 2010).

8.7. The antioxidant capacity of *Allium ampeloprasum:*

The high concentration of polyphenols, flavonoids, and antioxidant vitamins (vitamin C and vitamin E) in leeks contributes to their strong ability to capture free radicals, and thus to reduce oxidative stress, which is one of the triggering factors for chronic diseases such as cancer, diabetes, or neurodegenerative diseases. By trapping reactive oxygen species (ROS), these antioxidants limit cellular damage, thereby helping to reduce cellular aging (Bahmani et al. 2018).

8.9. To conclude*:*

In summary, *Allium ampeloprasum var. Porrum* is a plant with multiple attributes. In addition to its nutritional characteristics, it also has therapeutic properties, particularly in the prevention or treatment of various conditions such as diabetes, infections, cancer, and cardiovascular diseases. Its active constituents – flavonoids, sulfur compounds, vitamins, and minerals – justify its growing interest in the field of biomedical research and functional nutrition. Its regular integration into the human diet is thus a natural strategy, among other things, for public health (Fritsch et al (2002)).

Chapter 2:
Polysaccharides

2.1 Introduction*:*

High molecular weight carbohydrates (Figure 3) called polysaccharides are made up of numerous monosaccharide units connected by glycosidic bonds. One of the four main classes of biological macromolecules, polysaccharides are essential to life and are used in a variety of structural and functional ways by living things. Because of their bioactivities, polysaccharides—which are found in large quantities in bacteria, fungi, plants, and animals—have been extensively studied in pharmaceutical and biomedical research (Zhou et al., 2020).

2.2 Definition and General Structure*:*

A polysaccharide is a polymer that is composed of more than ten monosaccharide units - oftentimes this number ranges from hundreds to thousands. The sugars (monosaccharides) are joined mainly by α- or β-glycosidic bonds. Polysaccharide structure and properties depend on the type of sugar monomer, the type of glycosidic bonds, the degree of branching and the molecular weight (Zhang et al., 2018).

Polysaccharides can be:

- Homopolysaccharides – composed of only one type of monosaccharide (e.g., cellulose, starch).
- Heteropolysaccharides – composed of two or more types of monosaccharides (e.g., pectins, hemicellulose).

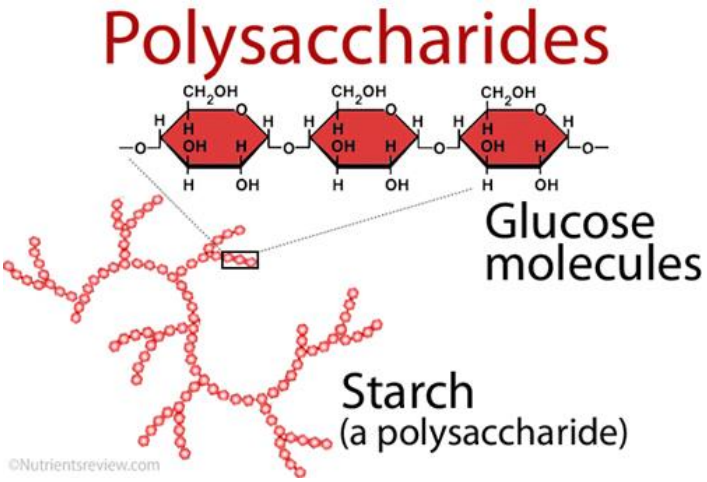


Figure 3*:* polysaccharides structure consisting of glucose unites (by courtesy of nutrientsreview.com)**.**

2.3 Sources of the Polysaccharides*:*

There are several natural sources of polysaccharides, including:

- Polysaccharides from plants: cellulose, starch, pectin, gums.
- Polysaccharides from fungi: β-glucans, chitin.
- Polysaccharides from algae: agar, alginate, carrageenan.
- Polysaccharides from animals: glycogen, chondroitin sulfate.
- Polysaccharides from microorganisms: xanthan gum, dextran.

In particular, plant polysaccharides have attracted considerable attention from researchers and the research community since polysaccharides from plants are biocompatible, low in toxicity, and have a number of biological functions (Li et al., 2021).

2.4 Biological Roles and Functions*:*

Polysaccharides, in general, have a number of roles:

Structural: cellulose in plant cell walls and chitin in fungal cell walls and insect exoskeletons.

Storage: starch in plants and glycogen in animals

Protective and binding agents: mucilage, gums and pectins in plants. Biological activities: Many polysaccharides have been shown to possess antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and antitumor effects (Wang et al., 2022).

2.5 bioactive polysaccharides*:*

Of interest are bioactive polysaccharides that are isolated from medicinal plants have various biological activities that have been researched, and their activity is correlated to their molecular structure, degree of sulfation, branching & functional groups.

For instance:

• Antioxidant activity: polysaccharides exhibit free radical scavenging, antioxidant enzyme activity;

• Antimicrobial activity: polysaccharides can interfere with bacterial adhesion & biofilm;

• Immunomodulatory: polysaccharides modify the stimulation or suppression of immune responses, interacting with macrophages, dendritic cells or cytokines (Yu et al., 2019).

2.6 Polysaccharides from *Allium* species*:*

Water-soluble polysaccharides, such as fructans and pectic substances, have been elucidated in *Allium* species such as *Allium ampeloprasum* var. *porrum* (leek); their contributions to the antioxidant and antimicrobial actions were identified. This has led researchers to speculate about the possibility of these polysaccharides having a prebiotic effect and also promoting gut health via the promotion of beneficial bacteria (Ramos et al., 2021).

Studies have given some foundation to these speculations as in leek, polysaccharides (inulin-type fructans and galactans) were isolated and shown to modulate immune functions and oxidative stress.

2.7 Conclusion*:*

Polysaccharides are highly promising antimicrobial substances that could be used in addition to or in replacement of antibiotics in light of the current state of multidrug resistance. Those polysaccharides obtained from leeks are well-documented to have many biological effects, efficacy, and low toxicity; thus, they have great value for the pharmaceutical community to continue to explore. Further, there is much research ahead to explore their mechanisms of action, optimize their extraction and purification, and potential efficacy *in vivo*.

Chapter 3: *Oxidative Stress*

3.1 Introduction:

Oxidative stress is considered when there is an imbalance of the formation reactive oxygen species (ROS) and the detoxification of those ROS by the body's antioxidant defenses. It is also implicated in a number of diseases such as cancer, cardiovascular disease, neurodegenerative diseases, and diabetes (Liguori et al., 2018).

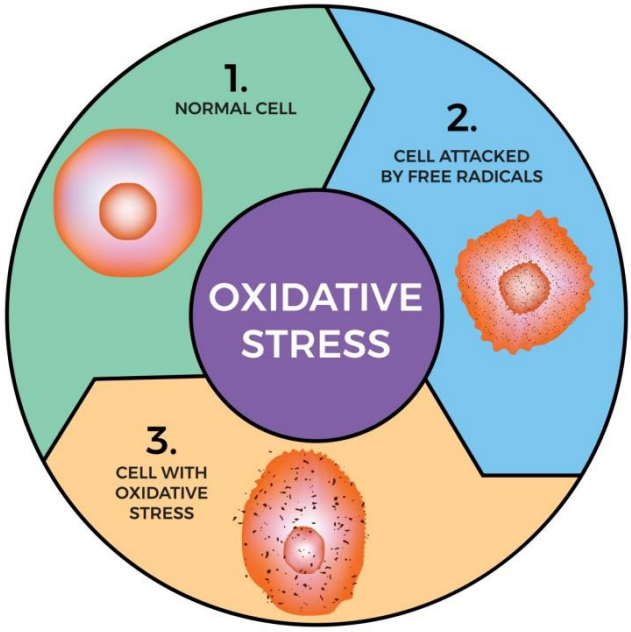


Figure 4

3.2 Sources of Reactive Oxygen Species (ROS):

Reactivity can occur through one or more of the 4 different forms of reactive oxygen species (ROS). ROS are natural by-products of everyday cellular metabolism, particularly in the mitochondria when using aerobic respiration. ROS levels can increase significantly due to factors that generate ROS exogenously (e.g., pollution, smoke, radiation or very high levels of exercise).

Different types of ROS are:

- Mitochondrial superoxide anion, $O_2\bullet^-$
- Hydrogen peroxide, H_2O_2
- Hydroxyl radical, •OH

3.3 Cellular Damage Induced by ROS:

Excessive ROS can damage various cellular components:

- Lipids → lipid peroxidation → membrane damage
- Proteins → oxidation of amino acids → loss of function
- DNA → mutations and strand breaks → carcinogenesis

Such damage is linked to aging and many chronic diseases.

3.4 Antioxidant Defense Systems

To handle oxidative stress, organisms developed two broad groups of antioxidant systems:

a. Enzymatic Antioxidants

• Superoxide dismutase (SOD): turns $O_2\bullet^-$ into H_2O_2

• Catalase (CAT): turns H_2O_2 into H_2O and O_2

• Glutathione peroxidase (GPx): reduces lipid hydroperoxides

b. Non-Enzymatic Antioxidants

• Vitamins: C, E, A; Polyphenols, flavonoids; Glutathione, uric acid

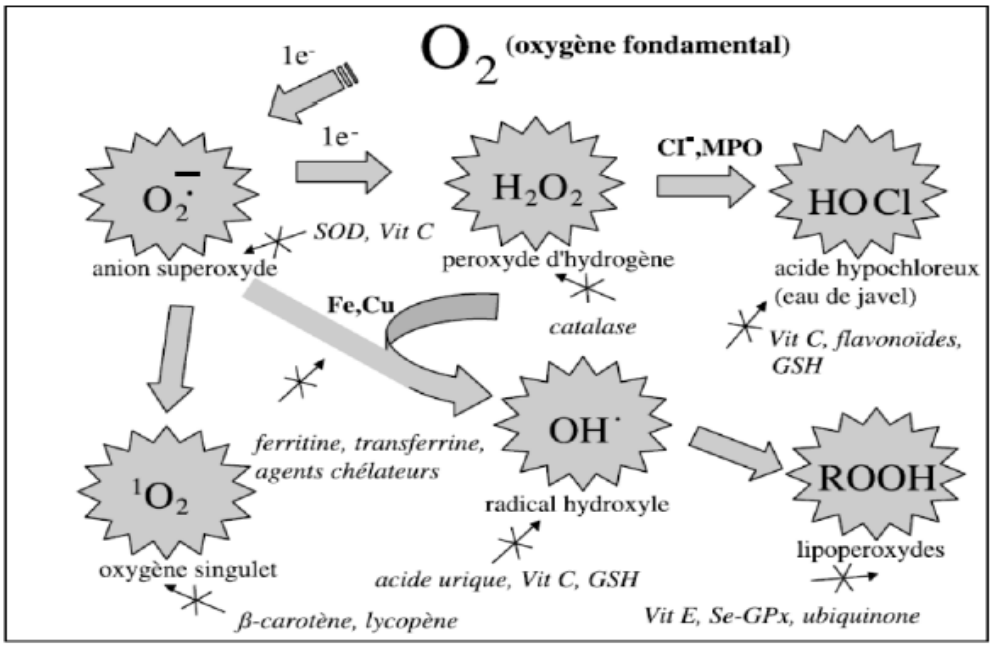


Figure 5*:* Regulation of the production of reactive oxygen species (ROS) by antioxidant defense systems.

3.5 Role of Natural Compounds: Polysaccharides as Antioxidants*:*

Recent studies show that natural polysaccharides from plants, fungi and marine organisms can act as free radical scavengers, stimulate the body to produce endogenous antioxidant enzymes, and protect DNA from oxidative damage (Zhang et al., 2018).

Some polysaccharides from *Allium* species (e.g., leek) have demonstrated desired antioxidant effects through:

- Metal chelation,
- Hydroxyl and DPPH radical scavenging,
- Activation of endogenous superoxide dismutase (SOD) and glutathione peroxidase (GPx) antioxidant enzymes.

3.6 Sources of oxidative stress: endogenous vs. exogenous*:*

Oxidative stress occurs when the amount of reactive oxygen species (ROS) produced exceeds the opportunity by which to detoxify them. Those species can be considered to be of endogenous (internal) or exogenous (external) origin.

Endogenous Sources*:*

Endogenous reactive species are produced naturally within the human body through a variety of physiological processes, primarily aerobic metabolism of glucose, fat and protein oxidation in the mitochondria of cells. During the processes of aerobic cellular respiration, some of the oxygen molecules are incompletely reduced in the electron transport chain producing superoxide radicals ($O_2\bullet^-$) and other sources of ROS. Other cellular processes can also create ROS, including:

- Inflammatory processes (increased ROS from activated neutrophils and macrophages)
- Fatty acid oxidation in the peroxisome
- Enzymatic processes (e.g. xanthine oxidase, NADPH oxidase, cytochrome P450)

For example, mitochondrial respiration in muscle cells during strenuous exercise produces excess ROS.

Exogenous Sources*:*

Exogenous sources of ROS are from outside the body. These may add to the total oxidative load on the body and can become problematic when the antioxidant defenses are overwhelmed. Some major exogenous sources are:

- Pollution (air and water)
- Cigarette smoke
- Irradiation (ultraviolet (UV) light and ionizing radiation)
- Heavy metals (lead, mercury)
- Pesticides and industrial evolution
- Certain medications or xenobiotics

For example, prolonged exposure to ultraviolet (UV) radiation from sunlight can create an enormous oxidation reaction in skin cells resulting in photoaging or damaged skin.

Table 1: Different sources of oxidative stress**.**

Feature	**Endogenous**	**Exogenous**
Origin	Inside the body (metabolism, immune cells)	External environment (pollutants, UV...)
Common Pathways	Mitochondria, peroxisomes, enzymatic reactions	Radiation, toxins, chemicals
ROS Type	Superoxide ($O_2\bullet^-$), H_2O_2, hydroxyl radicals	Same types, often in higher concentrations
Examples	Respiration, inflammation	Smoking, pesticides, UV rays
Impact	Continuous low-level ROS production	Can cause acute or chronic oxidative injury

3.7 Conclusion*:*

Oxidative stress poses a significant danger to cellular integrity and organism health. Antioxidant defenses, both enzymatic and non-enzymatic, work together to neutralize ROS in vivo. In this regard, natural polysaccharides present a novel and safe way to support antioxidant defense mechanisms to mitigate oxidative damage.

Second part: Experimental study

Chapter 4: Materials and Methods

1. Objective*:*

The experimental work we carried out was conducted within the Antifungal Antibiotics: Physio-Chemistry, Synthesis, and Biological Activity laboratory, at the SNV-STU faculty, Abou-Bekr Belkaid University Tlemcen.

The plant material used during the experiment is Allium ampeloprasum which was sourced from the Tlemcen region due to its availability, which we photographed during our experimental practice. The objective of this study is to optimize polysaccharides extracted yield and to test its antioxidant activity. It includes:

Step 1: preparation of extracts and characterization by different conditions.

Step 2: evaluation of the antioxidant power of the extracts obtained by the FRP method (Ferric reducing power)

2. Extraction*:*

Extraction is done using one method, which allows for the obtaining of polysaccharides.

2.1. Polysaccharides Extraction*:*

The extraction is carried out using a method that allows for the obtaining of polysaccharides. This extraction is carried out using the method described by Shan et al., (2008) with modifications, where it is necessary to:

➢ First, cut the plant material into small pieces

➢ Mix 10g of the plant material with 100ml of distilled water in a round-bottom flask, topped with a condenser.

➢ Allow the mixture to boil for 1 hour.

➢ Let the mixture cool for 1 hour.

➢ Filter the mixture through a muslin cloth and collect the filtrate.

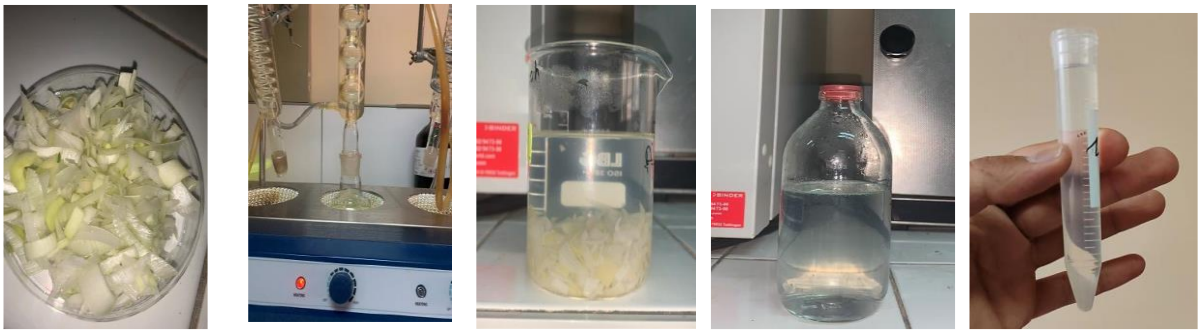
➢ Add the same volume of cold ethanol to 100ml of the filtrate. Ethanol allows the polysaccharides to precipitate. This mixture is placed in the cold (4°C) for 24 hours.

➢ Centrifuge the mixture at 3000 rpm for 30 minutes to recover the pellet.

➢ Remove the supernatant

➢ Dry the pellet at 50°C for 168 hours.

➢ The residue obtained is the extract of polysaccharides.



1 2 3 4 5

Figure 6: Protocol for extracting polysaccharides from plant material (taken at laboratory).

Table 2*:* stages of the protocol for extracting polysaccharides from plant materials.

Number	1	2	3	4	5
Practical	**Chopped leek**	**Decoction**	**Cooling**	**Filtrate + ethanol**	**Residue for drying**

3. Procedure of ferric reducing power*:*

0.2 ml of the extract at different concentrations is mixed with 0.5ml of the phosphate buffer solution (0.2M, pH=6.6) and 0.5ml of 1% potassium ferricyanide, then incubated in the oven at 50°C for 20 minutes. After incubation, 0.5 ml of 10% trichloroacetic acid (TCA) is added. A centrifugation at 3000 rpm for 10 minutes may be necessary if turbidity appears.

0.5 ml of the supernatant is mixed with 0.5 ml of distilled water and 0.1 ml of 0.1% FeCl3. The absorbance of the reaction medium is measured at 700 nm using a UV-VIS spectrophotometer with a blank prepared similarly by replacing the extract with distilled water.

Performance

Yield corresponds to the mass of the extract determined after extraction relative to its initial mass, expressed as a percentage. It allows for the evaluation of the efficiency of extraction processes and the comparison of different mixtures or techniques.

The formula used to calculate the yields of polysaccharides from each extract is as follows:

$$R = \frac{M1 - M2}{P} \times 100$$

With:

• R: yield in percentage %.

• M2: the mass of the empty tube in grams.

• M1: the mass of the tube after drying (contains the extract) in grams.

• P: weight of plant material (sample taken) in grams.

3. Characterization Tests*:*

3.1. Phytochemical tests*:*

→ **Tannin test*:***

To characterize the tannins 1ml of each extract was mixed with 0.25 ml of an aqueous solution of $FeCl_3$ (1%). The mixture was incubated at room temperature for 15 minutes. The appearance of a blue-black or greenish color indicates the presence of tannins (Karumi, 2004).

→ **Amino acids*:***

To 1ml of the extract to be tested, add 1ml of the ninhydrin solution prepared in acetone or ethanol with a concentration of 1%; heat in a water bath and observe the color change. The presence of amino acids gives a purple color (Harbone, 1998).

3.2. The pH of polysaccharides*:*

Dissolve an amount of 10 mg of each polysaccharide in 10 ml of distilled water, prepare 3 trials. The pH is measured using a pH meter.

3.2. Determination of total sugars*:*

• Principle

For the analysis of total sugars, we followed the method of Dubois et al., (1956). The principle of this method is that in an acidic and hot environment, the glycosidic bonds of the hydrocarbons are hydrolyzed. The simple sugars thus released undergo intramolecular dehydration to give furfural derivatives. The aldehyde function of furfurals thus condenses in acidic environments with the hydrolysis of a phenolic compound to give reddish-colored acetals or hemi-acetals that absorb in the visible range (450-500 nm).

• Assay

In test tubes, we mix 1 ml of the solution to be dosed, then 1 ml of the phenol solution (5%).

The tubes are carefully shaken. Then, 5 ml of concentrated sulfuric acid are added using a graduated pipette. After a 30-minute stay in the dark, absorbance measurements are taken at 490nm.

Furthermore, the calibration of the spectrophotometer is done with a blank containing: 1ml of distilled water, 1ml of 5% phenol, and 5ml of H_2SO_4.

• Expression of results

The sugar content as a percentage of dry matter is obtained using the following formula:

$$\mathbf{ST = (C_G/C_E) \times 1000}$$

CG: sugar concentration calculated graphically in mg/ml

CE: concentration of the extract solution in mg/ml

ST: total sugars in mg/g of extract

-Glucose is used as a reference molecule to establish the calibration curve in the total sugar assay.

5. Evaluation of antioxidant activity*:*

5.1. Evaluation of antioxidant activity by the FRP method*:*

In our work, we tested the antioxidant activity of different polysaccharide extracts using the FRP (Ferric reducing power) method.

• Principle

It is a quick, simple, and reproducible method. It is a technique that measures the ability of the compounds present in our extracts to reduce ferric iron Fe3+ to ferrous iron Fe2+, which is one of the mechanisms of antioxidant processes (Karagözler et al., 2008).

• Solution to prepare

¬ 0.2 M phosphate buffer solution; pH=6.6 (NaH2PO4, Na2HPO4)

¬ Potassium ferricyanide solution K3Fe(CN)6 at 1%

¬ Solutions of trichloroacetic acid (TCA) at 10%

¬ Aqueous solution of ferric chloride FeCl3 at 0.1%

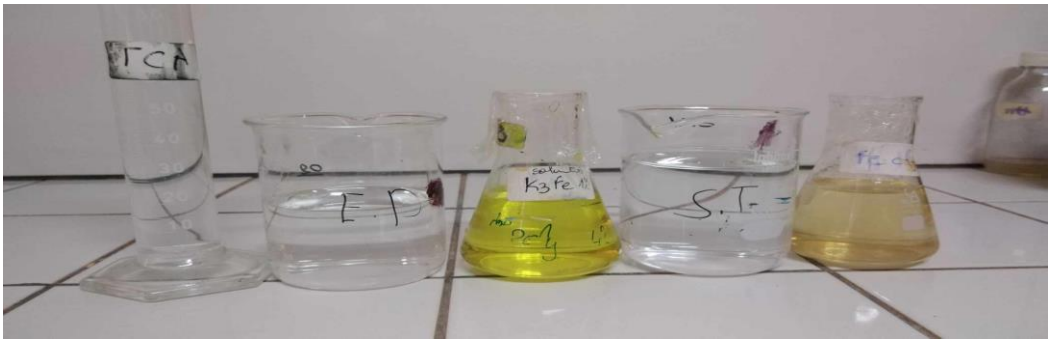


Figure 7*:* the solutions we used for the extract.

• Procedure

A volume of 0.2 ml of the extract at different concentrations is mixed with 0.5 ml of the phosphate buffer solution (0.2M, pH=6.6) and 0.5 ml of 1% potassium ferricyanide, then incubated in an oven at 50 °C for a duration of 20 minutes. Following the incubation, 0.5 ml of 10% trichloroacetic acid (TCA) is added. If there are any disturbances, a centrifugation at 3000 revolutions per minute for 10 minutes is initiated.

In the cuvette, we add 0.5 ml of the supernatant with 0.5 ml of distilled water and 0.1 ml of 0.1% FeCl3. The absorbance of the reaction medium is measured at 700 nm in a UV-VIS spectrophotometer with a blank containing 1ml DW and 0.1ml of 0.1% FeCl3.

Ascorbic acid is used as a reference molecule.

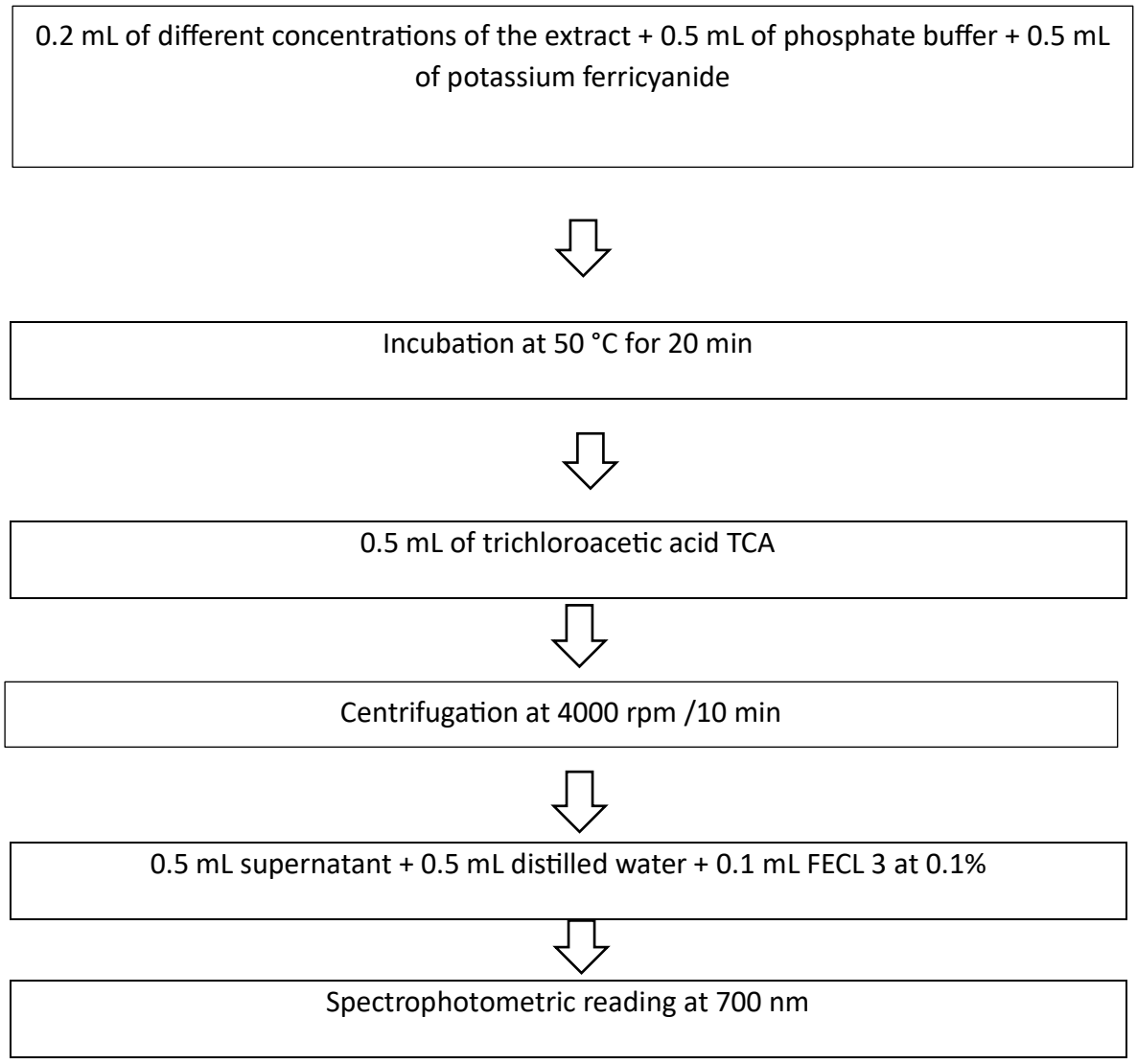


Figure 8*:* Protocol for evaluating the antioxidant power of *Allium ampeloprasum* extracts.

Results and Discussion

1. Extraction of polysaccharides

The addition of ethanol to the filtrate resulted in the precipitation of polysaccharides. After centrifugation and drying, we obtained a solid extract of slightly light brown color. From 10 g of *Allium ampeloprasum L. var. Porrum*. Table shows the yields of the four extracts.

After calculating the yields of each extract, we notice:

- According to the added volume: when the volume of ethanol doubles, the yield increases; in P1V60 and P2V60, there is a yield increase from 0.373% to 0.501%,

- According to the extraction time: increasing the decoction time improves the extraction yield. For the same volume of ethanol, the yield increases from 0.307% (60 min), and for double the volume, an improvement in yield is also observed from 0.874% (60min).

Table 3*:* values of the yields of polysaccharide extracts.

Extracts	**Yield (%) Solubilization**	**solvent**
P1V60	0.307	DW
P2V60	0.8743	DW

When comparing these results with other studies, we find that the best yield is lower than that obtained by Hallis and Hassouni (2022), who worked on the cladodes of *Opuntia ficus indica* and found a mucilage (polysaccharides) yield of 1.34±0.05%.

The yield of the *Ziziphus lotus* extract (6.5%) reported by Bechoua et al. (2020) is also higher than that of our extracts.

We observe that the yield is influenced by the volume of ethanol added.

This result is in agreement with other studies, notably that of Mahamane (2023), which reports a yield of 1.45±1.10% from the fruits of the strawberry tree (*Arbutus unedo*), obtained after a 1-hour decoction followed by the addition of a volume of ethanol equivalent to that of the filtrate.

2. Characterization of polysaccharides*:*

2.1. Quantification of total sugars*:*

Figure 9 represents a linear calibration curve (y=15.097x–0.0314 with R2=0.9938) for glucose. The concentration of glucose is expressed in milligrams per milliliter (mg/mL).

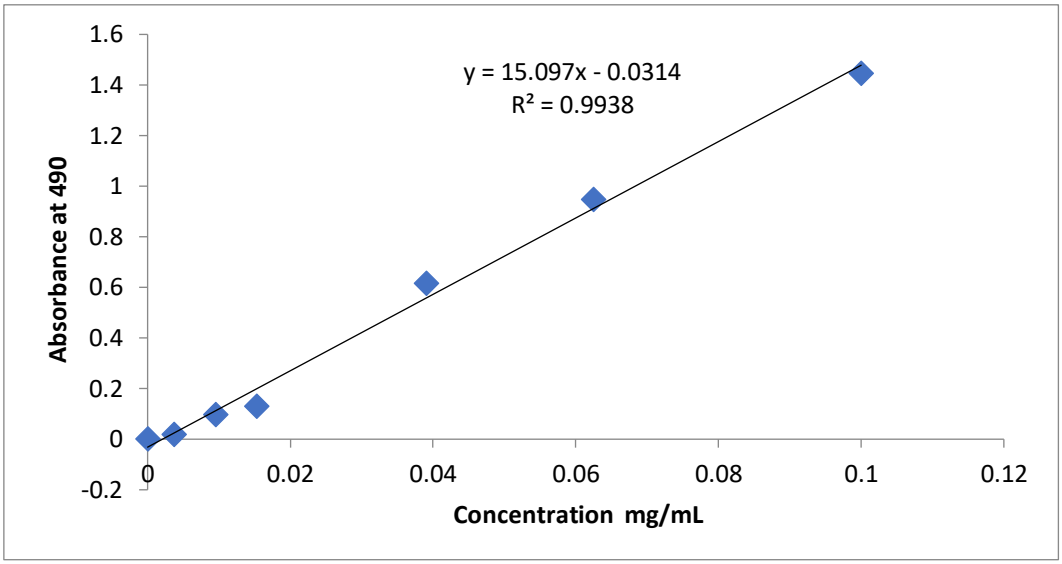


Figure 9*:* glucose calibration curve.

The results obtained, show the extract P2V60 (381±36.6mg/g). The result of this test made it possible to evaluate sugar content compounds of this extract.

When comparing our result with other studies, we cite the results of the total sugar content of garlic (*Allium sativum*) (25.6 g/100g) and onion (*Allium cepa*) (13.16 g/100g) determined by Bellazereg et al. (2015), which remain lower than those obtained in our study. On the other hand, the total sugar content of polysaccharides extracted from *Brasenia schreberi*, reported as 60.86% (or 608.6 mg/g) by Wang et al. (2023), is higher than ours. Regarding the work of Hammadi and Khelkhel (2024) on the fruits of *Arbutus unedo*, they revealed a sugar content of 39.86%. Which remains to be close to the result we obtained.

Therefore, the comparative analysis of the total sugar contents obtained in our study and those reported by other works highlights a significant variability depending on the plant species, extraction methods, and experimental conditions.

2.2. Phytochemical Tests

Table 4*:* Results of the phytochemical tests of the extracts.

The test	P2V60
Tannins	-
Amino acid	+

(+): Average, (-): Absence.

According to the results obtained from the phytochemical tests presented in the previous table, we observed an absence of tannins and we find a presence of the amino acids in our extract. The extracts of polysaccharides from the leek seem to have a less heterogeneous composition.

2.3. pH test

Regarding the pH measurement from our extract show a neutral pH with a 7.226±0.041

Table 5*:* result of the pH test of the extract.

Extract	P2V60
pH	7.226±0.041

3. Evaluation of Antioxidant Activity

3.1. Iron Reduction (FRP)*:*

According to (Figure 10) we observe a linear evolution of the absorbance of ascorbic acid measured as a function of different concentrations. The obtained equation (y=7.5998x + 0.0475, with R^2= 0.9927).

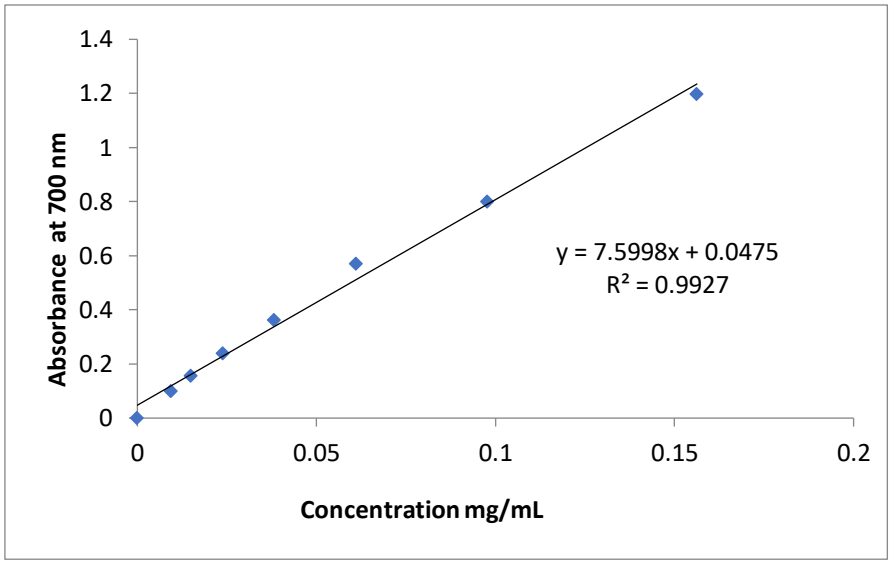


Figure 10: Variation of absorbance of the reducing power of iron of ascorbic acid.

According to the results obtained in Table 6, we observe a higher iron-reducing power in the P2V60 extract (8.06±0.50 mg Eq ascorbic acid/g of extract), whereas the lowest reducing power is marked for the P1V60 extract (1.34±0.06 mg Eq ascorbic acid/g of extract), while the two extracts P1V60 and P2V60 represent intermediate values (0.0983±0.07942

; 0.49±0.09644mg Eq ascorbic acid/g of extract) respectively.

Table 6: Reducing Powers of Iron of Different Extracts.

Extract	P1V60	P2V60
Reducing power of iron (mg Eq ascorbic acid/g of extract)	0.0983±0.07942	0.49±0.09644

These results are confirmed by the ANOVA statistical tests on which we note that;

• P2V60 indicates that (0.49 mg Eq ascorbic acid/g of extract) is significantly higher than the other groups.
• P1V60 means that 0.0983 mg Eq ascorbic acid/g of extract has the lowest reducing power.

General Conclusion and Perspectives

General Conclusion and Perspectives

We examined the biological activity of extracted polysaccharides from the edible plant *Allium ampeloprasum var. porrum*, a member of the *Allium genus* - known for its levels of some phytochemicals and antioxidant effect using the FRP (Ferric Reducing Power). In fact polysaccharides were extracted by heating for an hour followed by precipitation with equal (P1V60) or double volume (P2V60) of ethanol and drying.

The yield is of 0.87% for the best sample of polysaccharides (P2V60), with a total sugar content of 381 ± 36.6 mg/g. The results showed the extracts had a significant reducing capacity for iron (Fe^{3+} to Fe^{2+}), with the maximum reducing capacity of 0.49 mg ascorbic acid equivalent/g extract in the P2V60 sample.

To advance the scientific knowledge-base on leek polysaccharides and to broaden their potential uses, it is a good idea to consider the following potential avenues for future research focusing on:

- Others methods of polysaccharides extraction in order to increase the extracted yield.
- Studies that characterize the structure of leek polysaccharides using modern techniques such as nuclear magnetic resonance spectroscopy (NMR) and gas chromatography mass spectrometry (GC-MS) studies of monosaccharide composition and linkage types.
- *In vivo* studies especially that could assess bioavailability, uptake, metabolism and safety.
- Therapeutic applications of polysaccharides and/or polysaccharides as dietary supplements.
- Studies that investigate polysaccharides in combination with some other natural product or synthetic agent.
- Studies that may evaluate the prebiotic potential of polysaccharides and their potential to affect the gut microbiota.

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