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By

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Entitled

**Characterization of *Opuntia ficus-indica*  
extracts**

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## *DEDICATION*

*I dedicate this work to:*

*My mother, who worked for my success, with her love, her support, all the sacrifices made and her precious advice, for all her assistance and her presence in my life, receives through this work, however modest it maybe, the expression of my feelings and my eternal gratitude.*

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## ملخص

توجهات الفلاحين الجزائريين تعز تنمية المناطق المحاصيل التي تتكيف مع الاحتباس الحراري. يتكيف مع المناخ الجاف ولا تنافس في. تحتوي مضارب التين الشوكي على مكوناته بيولوجيًا ، آثار صحية مفيدة. كان العصير المستخرج من مضارب وزيت بذور التين الشوكي (ابونتيا فيكوس انديكا) موضوع هذا العمل

تم حصاد المضارب في منطقة دائرة عين الهاجل الواقعة غرب المسيلة في الجزائر عام 2022 لتقييم التركيب الفيزيائي والكيميائي وإجمالي محتوى الفينول ومحتوى الفلافونويد ونشاط مضادات الأكسدة والنشاط المضاد للبكتيريا. يبلغ مردود عصير المضارب حوالي 79.02 ٪. القيمة التحليلية الفيزيائية أن عصير مضارب غني بالبوليفين (4.6 جم / 100 جم) ، وفي الفلافون (38.35 TPC والكيميائية و أعطانا عصير مضارب DPPH. مجم / 100 جم) ، وتم إجراء النشاط المضاد للجراثيم باختبار المستخرج من الأنواع الشوكية قيمًا عالية في نشاط مضادات الأكسدة ، وله تأثير على بعض السلالات البكتيرية مثل: المكورات العنقودية الذهبية ، و الإشريكية القولونية ، و السرانيا التيفية ، و السرانيا. نتائج هذه التحليلات أن عصير المضارب غني بالعناصر الغذائية وله نشاط مضاد للأكسدة

في صيف 2021 في المنطقة الغربية (عين الدفلة) ، يجب أن يكون حجم البذور الكبيرة حوالي 30-35 كجم للحصول على لتر واحد فقط. نحن فعلنا النشاط المضاد للبكتيريا. كانت الثلاث عينات ، الزيت المعصور على البارد ، المضغوط المضغوط بزاوية 90 درجة ، والزيت المضغوط بدرجة حرارة 160 درجة ، من الزيوت التي أثبتت فاعليتها ضد الإشريكية القولونية ، والزائفة والسالمونيلا ، والكلبسيلا الرز. قطرها بين 11 و 19 ملم

الكلمات الرئيسية صبار الإجا ص الشائك. عصير مضارب ، زيت بذور ، (ابونتيا فيكوس انديكا) ، التركيب الكيميائي الفيزيائي ، محتوى الفلافونويد ، نشاط مضادات الأكسدة ، النشاط المضاد للبكتيريا ، التركيب ، سوكللي

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## Abstract

The direction of agriculture in Algeria encourages the development of dry areas and the development of crops adapted to global warming. Prickly pear cactus is a dry climate-adapted plant that remains unexplored in Algeria. Prickly pear cladodes and the seeds contain bioactive components that are beneficial to health. The juice obtained from the cladodes and the seed oil of the *Opuntia ficus indica* are the subject of this work. The leaves were harvested in 2021 in the Daira d'Aïn El Hadjel region, west of M'sila, Algeria, to evaluate the physicochemical composition, TPC, flavonoid content, antioxidant activity and antibacterial activity. The yield of cladodes juice was about 79.02%. Physicochemical and TPC analysis showed that cladodes juice was rich in polyphenols (4.6 g/100 g) and flavonoids (38.35 mg/100 g), and the anti-radical activity was determined by DPPH test. Leafy sap from spiny species gave us high marks for antioxidant activity, and has got an effect on some of bacterial strains like: *Staphylococcus aureus*, *E coli*, *Salmonella typhimurum*, *Serratia marcescens*. Results of these analyzes showed that cladode juice is rich in nutrients and has a potential antioxidant activity. The prickly pear seeds are harvested in summer 2021 in the western region (Ain Defla), and the seed oil yield is weak (5%), so a large seed volume of around 30-35 kg is required to have only 1 liter of oil. we did The antibacterial activities and results were as follows: *Escherichia coli*, *Pseudomonas*, *Salmonella*, and *Klebsiella pneumoniae* strains were relatively resistant to Soxhlet oil, and *Escherichia coli* and *S. marcescens* strains were relatively resistant to cold-pressed oil. Resistance is the same. The 3 samples, cold pressed oil, 90° pressed oil and 160° pressed oil, were the most proven oils to be effective against *Escherichia coli*, *Pseudomonas*, *Salmonella* and *Klebsiella pneumoniae*; between 11 and 19 mm inhibition zone diameter.

**Keywords:** prickly pear cactus; cladode juice, seeds oil, *Opuntia ficus-indica*, flavonoid content, antioxidant activity, antibacterial activity.

# GENERAL INTRODUCTION



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## General introduction

Originally, nature, consisting mainly of plants, was the food for the animals and humans that inhabited the earth. But in addition to this nutritional function, man has also discovered many other functions that these plants can offer him, medicinal, ecological, economical and industrial. Future trends typically show reduced precipitation and warmer temperatures across the country. Extreme events such as prolonged droughts and heavy rainfall are also likely to occur in dry regions. These future trends, as well as existing impacts, will lead to the loss of dryland biodiversity and increase its vulnerability to desertification (**Gommes *et al*, 2009**).

Cacti can be assessed in marginal areas as well as in arid and semi-arid areas that have not yet been planted. Adapting to different climates and soils allows plants to respond effectively when used to resist erosion. Cactus is a suitable species for sustainable agriculture in Algeria because of its drought resistance, desertification resistance and use as food for humans and livestock. It is an important cash crop but is rarely used. Can be recycled in food, cosmetics, and pharmaceuticals; these are products with high added value for farmers and local populations. These products will play an important socio-economic role for farmers and rural populations and will contribute to sustainable development in rural areas (**Arba, 2009**).

Agriculture in Algeria has always been a strategic sector for the socio-economic development of the country. Growing consumer demand for local products in domestic and international markets creates important opportunities for their commercialization. Currently, the economic importance of prickly pear cultivation lies in the commercialization of its fruit and its animal feed (**Arba, 2009**). Other uses of cactus (jams, preserves or dried fruit, leaf powder, etc.) are still at the cooperative or small and medium-sized enterprises stage. Prickly pear cladodes are still underexploited in Algeria and therefore need to be evaluated as numerous benefits have been demonstrated in several studies, so it should be very encouraging for Algerian industrialists to study this axis to develop this nascent industry. To highlight the prickly pear, we turned to cladodes branches, still little known to the public. Much research has been done on cladodes, but the sap of the cladodes has not been studied. Thus, research on Algerian cladodes juice and industrial evaluation of its uses was initiated. This will provide a solid scientific foundation for

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industrialists looking to invest in the field and will lead to business and employment opportunities. Aspects of prickly pear mining, namely, the development of a new product "cladode juice".

This present work is composed of four main parts: The first part deals with the physicochemical analysis of the frond sap of a species found in Algeria: *Opuntia ficus indica*. The second part is for phytochemical screening. The third part involves a major upgrade of cladodes juice, which is the determination of flavonoid content, TPC and analysis of antioxidant activity. Part four focuses on biological activity, evaluating the antibacterial properties.

After that we extracted the oil from prickly pear seed flour with two solvents, and then we did the antibacterial activity and the antifungal activity on this oil and that which comes from the extraction by the pressure of the seeds prickly pear.

# CHAPTER I: LITERATURE REVIEW



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## Introduction

The prickly pear is a succulent plant belonging to the cacti family, more specifically the genus *Opuntia*. It grows in arid climates such as the Mediterranean and Central America (**Ginestra et al., 2009**). The semi-arid regions of Mexico have the largest variety of cacti in the world (**Pimienta-Barrios, 1994**). The genus *Opuntia* includes about 300 species, many of which produce very tender and edible stems and fruits (**Hegwood, 1994**). One of these species is *Opuntia ficus indica* (single or thornless), commonly called prickly pear, which is the subject of our study.



**Figure 1-** the species *Opuntia ficus indica*

## I. Generalities on prickly pear

### 1. Cacti

#### 1. Definition

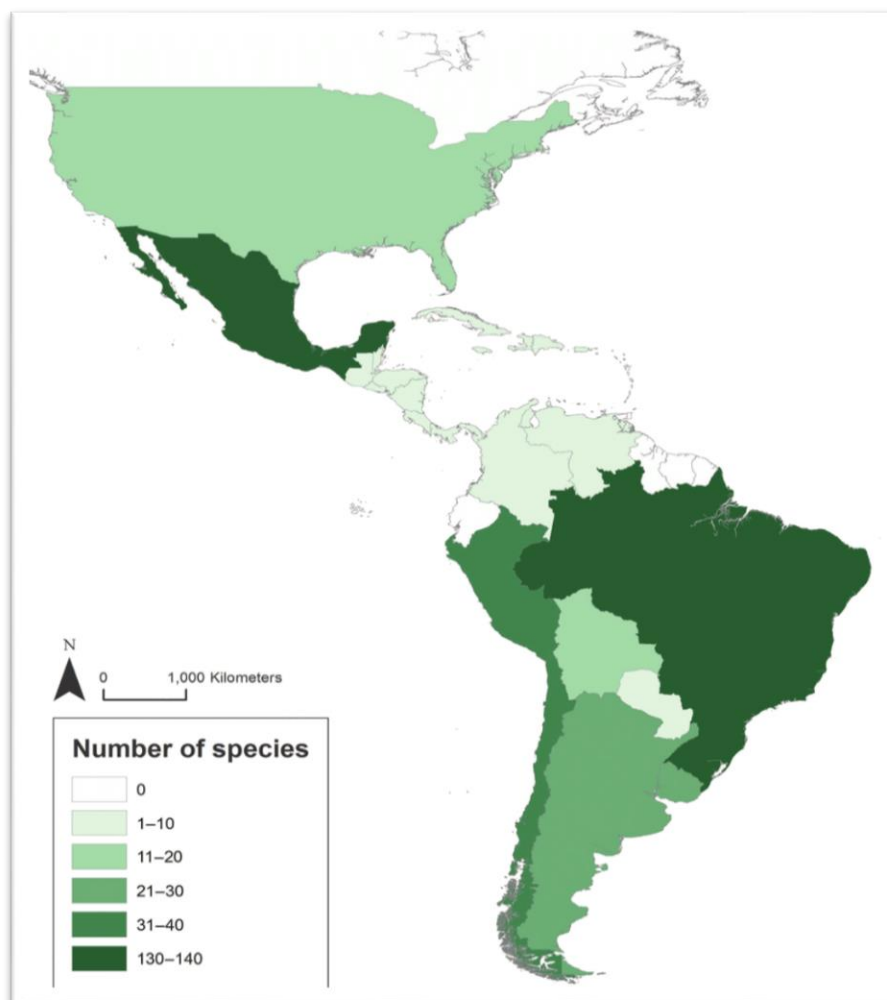
Cacti are succulent plants. Thanks to the water storage system, they can survive on their own reserves during temporary dry periods. Under similar conditions, most other plants wither and die from lack of water (**Mace, 2003**). Their often odd appearance is related to mutations they have evolved to store water in their very succulent stems, leaves or roots. Other, more important developments made it possible to increase water reserves by reducing evaporation. They usually have a fleshy or hairy cuticle, or a waxy coating that reduces evaporation. The dense

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growth of thorns plays a vital role in this and limits the effects of intense solar heat by providing partial shade to the plants. The cells of these plants are able to withstand greater fluctuations in water content than other "normal" plants (Mace, 2003). Il existe de très vieux mythes sur les cactus. Le cactus était un symbole important des Aztèques du Mexique et des Incas du Pérou, et des illustrations de ses propriétés curatives et divinatoires peuvent être trouvées sur de nombreux bâtiments, poteries et autres objets du quotidien (Mace, 2003). There are very old myths about cacti. The cactus was an important symbol of the Aztecs of Mexico and the Incas of Peru, and illustrations of its healing and divination properties can be found on many buildings, pottery, and other everyday objects (Mace, 2003).

## 2. Origin and spread

Almost all cacti are native to the New World, with some species extending as far as Alberta, Canada, and others as far north as Patagonia (Figure 1).



**Figure 2-** map illustrating the distribution area of the cactus at its origin

The only exceptions are some species found in East Africa and Madagascar, which may have been introduced by migratory birds and continued to evolve there (Mace, 2003). Cactus cultivation originated in the highlands of Mexico, where the climate is hot and dry. The species' food use dates back to prehistoric times thanks to the discovery of fossil seeds dating back to the seventh millennium BC. Advertising (Mullas *et al.* 2004).

### 3. Morphology

Cacti come in a variety of shapes, ranging from miniature plants to large trees reaching nearly 25 m in height. However, all other genera are very similar except for *Preskia* and related genera, which have normal leaves, spines, and basic structures of flowers and fruits. These other genera have few leaves and the process of photosynthesis takes place on fleshy green stems (Lamb, 1991). At the areola layer, thorns, new shoots and flowers appear. There are some exceptions, such as *Echinocereus*, whose buds pierce the stem wall close to the *areola*, *Mammillaria* and *Corphanta*, where the buds emerge from the axillary region between the tubers or from the grooved outlet of the tuber areola. Cactus flowers are sessile, with the sole exception of *Pereskias*, which are stalked flowers (figure 2).

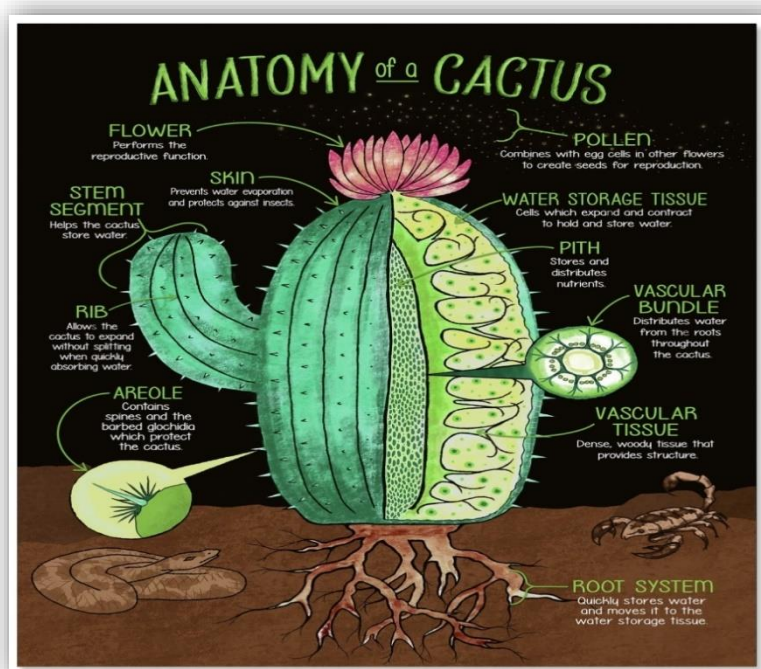


Figure 3- diagram illustrating anatomy of the cactus.

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The fruits are usually succulent berries, but in some genera, they are dry capsules from which the seeds drain through basal holes or irregular fissures (**Lamb, 1991**).

#### **4. Classification**

The Cactaceae family is subdivided into eight groups, each of which includes several genera (**Lamb, 1991**): Pereskia Group, Opuntia Group, Cereus Group, Echinopsis group, Hylocereus group, Neoporteria Group, Melocactus Group, Echinocactus Group.

## **II. Prickly pear**

### **1. Definition**

Native to Mexico, cacti are adapted to arid and semi-arid regions (**Reynolds et al., 2003**) and are important for human nutrition and can also be used as livestock feed. It is an interesting plant due to the environmental conditions in which it grows and its resistance to extreme climatic conditions (**Hernández-Urbiola et al., 2011**). *Ficus indica* belongs to the cacti family, which includes about 1600 species, with the greatest center of diversity in Mexico, where 669 species are native (**Benkaddouri, 2011**).

### **2. Distribution**

The cactus was introduced to North Africa (Algeria, Tunisia, Morocco) around the 16th century, and its cultivation quickly spread throughout the Mediterranean. It has also spread to the southern hemisphere, including South Africa, Madagascar, Reunion, Mauritius, India, Ceylon and Australia. It is also found in Canada, Argentina and Peru at altitudes up to 5100 meters. It is currently introduced and grown in more than 30 countries for fruit production, animal feed and as a worldwide crop. In Algeria, the introduction of cacti is similar to Morocco and Tunisia. Today, over 30,000 hectares of cacti are cultivated, 60% of which are located in the municipality of Sidi-Fredj (45 km north of Souk Ahras) and the rest in Ouled Mimoun, Taoura, Dréa and Ouilène (**Huffposte Algérie, 2015**). In the north, cacti are used as fences around houses and towns. Plant fences are also used to produce fruit for human consumption, sold in local markets, and as a source of forage during the dry season. In the south, cactus foliage is evolving into food for small ruminants and camels (**FAO, 2018**).

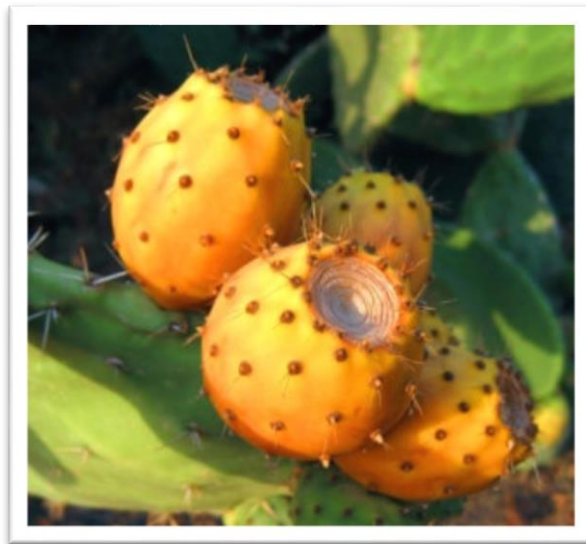
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### 3. Taxonomy

Many authors have developed classifications of the Genus *Opuntia*. The classification considered the most valid to date is undoubtedly that established by

#### **Britton and Rose in 1963:**

- Kingdom: *Plants*.
- Order: *Caryophyllales*.
- Subclass: *Caryophyllidae*.
- Family: *Cactaceae*.
- Group: *Opuntiaeeae*.
- Genus: *Opuntia*.
- Subgenus: *Platyopuntia*.
- Species: *Opuntia ficus-indica*.



**Figure 4-** Prickly pear (*Opuntia ficus indica* L.)

*Opuntia ficus-indica* is among the cacti that have the greatest agronomic importance, both for its edible fruit and for the rackets that could be used as fodder or as vegetables (Mulas *et al.*, 2004).

### 4. Cactus botany (morphological description):

According to the botanical classification, the cactus family or cacti are dominant plants belonging to the angiosperms, dicots, petals. They are perennial shrubs and trees with succulent stems, flowers, and conspicuously leafless. Its flowers are large, red, and hermaphroditic. They are part of the chance order

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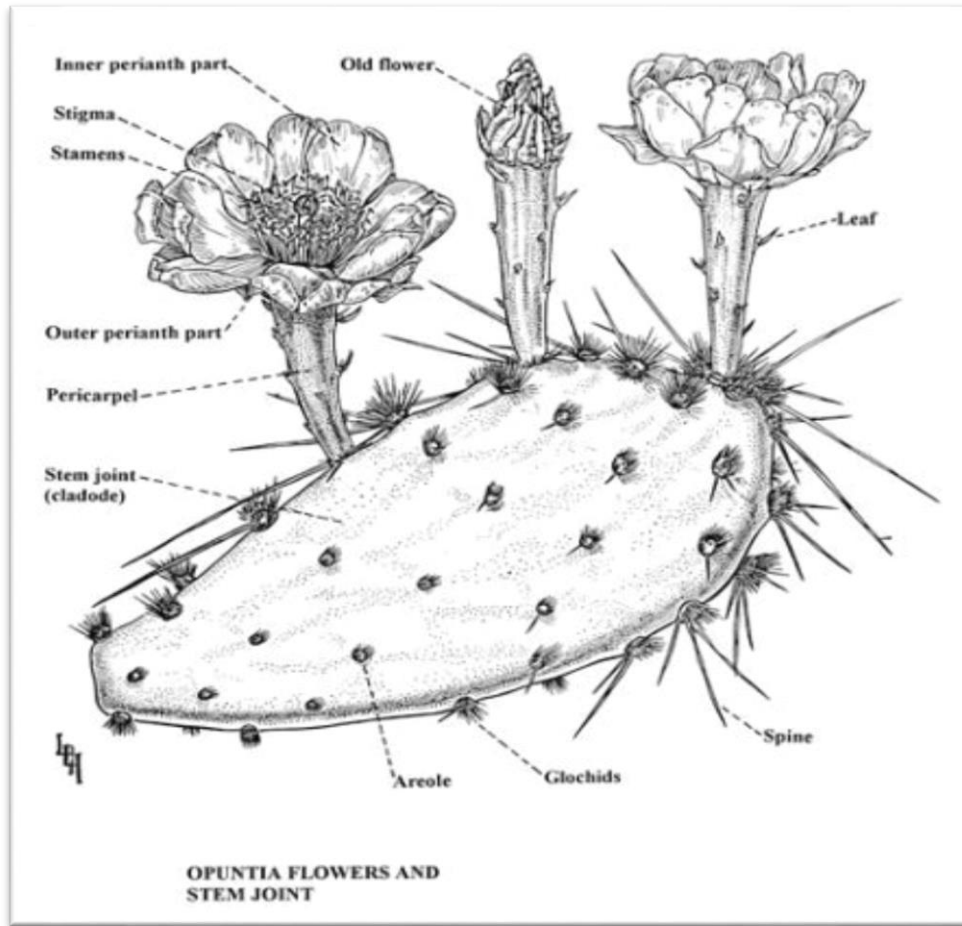
(Schweizer, 1997). *Opuntia ficus-indica* is a robust tree-like plant (3 to 5 m) tall with a stout woody trunk and flattened tissue, oval or ovate, dark green, and (30 to 50) long cm), width (15 to 30 cm) and thickness (1.5 to 3 cm) are called shoots or bats. Branches and leaves provide chlorophyll function and are covered with a waxy cuticle (cuticle) that limits transpiration and protects them from predators (Wallace and Gileson, 2002). On the fronds there are so-called areoles. These areolae are oval in shape and are located 2 mm below the surface of the skin. Under suitable environmental conditions, new thallus, flowers or roots develop from the meristem of the areola. In *O. ficus-indica*, the areola is spirally distributed and grows spines (rather than leaves like most plants) (Powell and weedin, 2004). The presence of spines is a distinguishing feature of the areola, and their morphology is of potential taxonomic importance. Two types can be distinguished: spines and glochids.

Glochids and Spines are best thought of as the morphological equivalents of leaves, and the differences between the two are quantitative; both species are derived from capsules and bodies, as are leaf primordia (FAO, 2018). Emerging from a receptacle with green sepals, the stemmed flowers have many thick petals covered with light fuzz. They are androgynous. Their short stamens are usually dominated by stigmas (Schweizer, 1997).

Cacti bloom and bear fruit. Appearing on the top of bats, the flower is 4 to 10 cm in diameter and is yellow, orange or red in color and is a large oval and fleshy berry whose yellow-green skin is also dotted with small spines (Boutakiout, 2017).

Root organs are superficial and concentrated in the first 30 cm of the soil (Mulas and Mulas, 2004), but roots differ from those of other plants because they have heteromorphic properties that allow plants to survive prolonged droughts. Roots can contribute to improving drought tolerance in a number of ways, by:

- Restricts the root surface and reduces its water permeability
- Rapid uptake of small amounts of water in a light rain - "rain roots" - form within hours of a heavy downpour and disappear as the soil dries out or the surface area of the roots that seep water decreases.
- The decrease in twig transpiration due to the very negative root potential.



**Figure 5-** diagram illustrating the different parts of the prickly pear

## 5. Habitat

Cacti have evolved and adapted to all types of biomes. The generally accepted definition is that a desert is a place where the average annual rainfall does not exceed 250 millimeters. By this definition, only a very small number of cacti grow in true deserts. The vast majority of species live in biomes that receive at least twice as much water each year. Some cactus species even prefer wetter habitats, especially many epiphyte species of tropical evergreen forests where humidity levels are consistently high. Cacti colonize biomes from coast to inland, from sea level to altitudes of nearly 4000 m. Some cacti have adapted to severe cold and snow, as well as cacti that grow in sand a few meters above sea level (**Lamb, 1991**). From an ecological point of view, cacti, even found in temperate climates, are typical species of arid and desert regions and thus exhibit a range of morphological and physiological adaptations to their original features environment (**Mulas et al., 2004**).

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Cacti have the special property of storing large amounts of water in their succulent organs. This plant can efficiently take advantage of short rainfall events of only a few millimeters of rain, as its superficial but very extensive roots can absorb water from the soil at low humidity, rendering most species grown nearby unviable (Mulas *et al.*, 2004).

In the absence of permanent leaves, the photosynthesis process occurs in green parts. These also contain aqueous parenchyma that can effectively store and preserve water. Due to the presence of a thick epidermal and intraepidermal wax coating, the efficiency of limiting water loss through perspiration through the skin can be improved. The morphology of the stomata, as well as the overall photosynthesis process, helps limit transpiration losses. In fact, this may occur after the CAM (sedum acid metabolism) cycle, which is known to allow stomata to open at night for gas exchange, thereby reducing sweat loss (Mulas *et al.*, 2004).

## **6. Interests and use of prickly pear**

The prickly pear is named in some countries fruit of the devil. Others, who do not attach importance to its thorns, call it the fruit of paradise as it has miraculous benefits on human and animal health and the environment (Agroligne, 2016)

- **Human food**

### **Fruit:**

The prickly pear performs an essential function inside the eating regimen of ruminants in arid and semi-arid regions (De Waal *et al.*, 2015), the principle use being the consumption in the fresh fruit of the end result which can be sold in different places of the world (Mulas and Mulas, 2004). For the underprivileged populations of Latin America, Africa, all the poor and wilderness areas of the sunny international in which it has become acclimatized, the prickly pear is a providential plant. By itself represents a complete survival meal (Schweizer, 1997).

Its sweet and juicy fruit is wealthy in nutrition C and their nutritional content is much like that of most fruits including oranges, apples, pears, apricots, cherries, and so on. (Arba, 2009).

**Table 1--** composition of the prickly pear *opuntia ficus-indica* (anonymous, 1993)

Constituents	Fruit (%)	Pulp and seed (%)	Seedless pulp (%)
Water	80.0	84.5	83.6
Protein	1.0	1.3	0.8
Total lipids	0.7	1.3	0.3
Carbohydrates available	14.8	8.0	10.8
Crude fibers	2.3	4.4	3.6

➤ **Juice:** Numerous studies were achieved on prickly pear juice and feature proven that this product has a nice taste and perfume. Another possibility is the manufacturing of juice concentrates that show appropriate microbiological stability (Bhira, 2012). In line with the FAO, several tries have been made to produce a quality and sable juice.

➤ **Jam:** In step with the national middle for Textual and Lexical assets (CNRTL), jam is a preparation which include fruit, or more rarely other veggies, left entire or having undergone treatment, and cooked with sugar to preserve them (CNRTL, 2012). The preparation of jams combines warmth treatments with a lower in water (and occasionally additionally in pH to permit less extreme warmth remedy) (FAO, 2018).

➤ **Vinegar:** Another product with interesting potential is vinegar and some manufacturing experiences are worthy of note (FAO, 2018). This gourmet vinegar with its rich, tasty and fruity aroma, its slight acidity and its very refined taste, has several nutritional and cosmetic virtues. It stimulates the intestinal flora, improves digestion and strengthens natural defenses and is extracted from fig pulp (Agroligne, 2016). Now in Algeria, the NOPALTEC prickly pear processing plant in the municipality of Sidi Fredj, in the wilaya of Souk Ahras, produces orange vinegar from prickly pears.

➤ **Food coloring:** In the agro-food industry, beetroot is the only source of betalaines exploited to obtain natural food colors, generating several shades of red-violet. However, preparations of beetroot are obtained from the roots. Therefore, unfavorable taste characteristics could also have an effect on their marketing. The usage of prickly pear as a source of betalains is very interesting; these are strongly fragrant and display higher nutritional properties than beetroot roots (Bhira, 2012).

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**Seeds:** The seeds represent about 15% of the edible part of the fruits and they have a variable content of oil (on average, 9.8 g 100 g<sup>-1</sup> of seeds) (**Ramadan and Morsel, 2003**). The seeds of the fruit give edible oil which has a high degree of unsaturation, with a high content of linoleic acid and a low content of linolenic acid. Thanks to these and other chemical and physical characteristics, the oil is in the same category as soybean, corn and sunflower seed oils (**Mulas and Mulas, 2004**).

**Seed oil:** Seed oil is rich in unsaturated fatty acids (**Sepúlveda and Sáenz, 1988; Ennouri and al., 2005; Ghazi et al., 2013**); therefore, it is interesting for the pharmaceutical and cosmetic industries, for example, in Morocco and Tunisia. Given the low oil yield from the seeds, it is neither economical nor attractive as consumer oil. The presence of tocopherol, recognized as an antioxidant natural, varies from 3.9 to 50%. **Matthäus and Özcan (2011) and Özcan et al Juhaimi (2011)** report that fibre and minerals are also important components seeds, with 12.5% crude fibre and high amounts of calcium, potassium and phosphorus, among other minerals. The relatively high protein content (approximately 6%) means that the seeds prickly pear are a source of protein for human consumption (**Tlili et al., 2011**)



**Figure 6-** prickly pear seeds (web site: kajuard)

**Seed flour:** Those are actually the seeds or pips of the fig, the residue of which, once the oil has been extracted, is overwhelmed and then dried to make completely best flour. This whitish powder, wealthy in protein, could be used in the preparation of pastries, improve soups and used in the area of cosmetics (**Agroligne, 2016**).

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**Cladodes:****➤ Use in complementation and properties:**

The mucilages which constitute a dietary fiber intake are also of interest for the food industry thanks to their gelling and thickening properties (**Trachtenberg and Mayer, 1981; Sepúlveda et al., 2007**). Cladodes in powder form are even offered as an additive in dairy products (gelled desserts), at a rate of 10%. This share is even raised up to 20% in soups to increase thickening. They are also offered as a raw material for cereal breakfasts and as a substitute for wheat flour (**Saenz-H et al., Saenz C., (2002)** cited by **Stintzing and Carle, (2005)**).

The work of **Ayadi et al., (2009)** on smooth and spiny cladode powders (2 to 3 years old) from Tunisia, with a view to improving the food value (fibre enrichment) of flours from cereals, have shown in parallel that the addition of 5% of powder does not affect the properties technological and organoleptic aspects of pasta and cakes (cake type). In addition, mucilages can constitute an edible coating film of appreciable quality, as shown by **Del-Valle et al., (2005)**, after strawberry preservation trials. Other studies that have addressed the effect of mucilages on emulsions (**Rwashda cited by Garti, 1999**) have shown that they stabilize oil/water emulsions. Adsorbed at the water-oil interface, they do not significantly modify the viscosity of the system, and do not cause flocculation **Garti and Leser (2001)** quoted by the same author, later specified that the mucilage of *Opuntia ficus indica* has a good emulsifying and stabilizing capacity, if it is used in water/oil emulsions. While the work of **Espinosa (2002)** on foaming properties showed that the addition of 0.5 to 0.8% of mucilage has a stabilizing effect on egg foam and reduces syneresis to a minimum. Its addition also limits crystallization and can form gels (**Anon, 1991 cited by Saénz C. et al., 2004**).

Supplementing this work, **Medina-Torres and al., (2000)** adds that these polysaccharides (or mucilage) in solution in water at 10% and 2% xanthan have comparable rheological properties. At a concentration of 5% and at a temperature of 35°C the solution tends strongly towards the formation of a gel as is the case for i- and j-carrageenan (**Medina-Torres and al., 2003**).

Recent work by **Ayadi, et al., in 2009**, confirm that cladodes powder has an interesting technological potential thanks to its water retention capacity (**WBC**) with

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3.15g per gram of dry matter and its lipid adsorption capacity (**FCC**). These results remain comparable to those of other plant sources such as carrots, apples, wheat (**Thebaudin et al., 1997 cited by Ayadi**). Thus, wide possibilities of use of *Opuntia ficus indica* are opened thanks to the cladodes by the processes of extraction of the mucilage. Their use has diversified thanks to their thickening properties.

➤ **Medicinal properties of cladodes:**

The juice from "nopalitos" (cladodes) is also the subject of growing interest in the southern United States (Texas) and Mexico for the preparation of beverages. **Rodriguez (1999)** developed several formulations including that consisting of the addition of citric acid and aspartame as a sweetener. At pH 3.5 and pasteurized at 76°C, the drink showed that the nutrients or heat-labile constituents were little affected.

Young shoots are eaten as a vegetable because they are soft and fibrous. Their nutritional value is similar to many vegetables and leaves. They are rich in water, carbohydrates, protein, vitamin C, and beta-carotene, a precursor to vitamin A. These young leaves are called "nopolitos" in Mexico, and they have been a traditional vegetable for centuries. Century, they can be eaten fresh or cooked as green vegetables. They are recommended for diabetic patients with non-insulin-dependent diabetes mellitus, as their consumption improves glycemic control and reduces blood cholesterol levels in these patients (**Arba, 2009**). There are other products derived from fronds: jams, pickles, and candied fronds. Although traditionally used as a meat substitute during Lent, today they are eaten with a meal similar to green beans (**Stintzing and Carle, 2005**).

Infusions of fruit, cladodes and flowers are traditionally used to treat and soothe ulcers, allergies (**Lee. and al., 2000; Lee et al., 2002 cited by Feugang, 2006**), and as anti-uric acid and diuretic by its effective effect on hypertension (**Galati, et al., 2002**). The juice extracted by pressing from the cladodes is recommended for the treatment of liver, rheumatism, scurvy and kidney disease (**Saéñz-Hernandez, 1995; Galati et al., 2001**). In the form of powder in capsules, cladodes are currently used to stabilize weight (**Stintzing and Carle, 2005**).

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## Flowers:

The prickly pear flower is very rich in vitamin C, calcium, iron, magnesium and zinc. Dried, they are prepared in herbal tea for their diuretic, vermifuge and astringent properties. They are also a natural treatment for benign prostatic hypertrophy and are used as an anti-inflammatory and antihemorrhoid (Agroligne, 2016).

Cactus is a prolific flowering plant with a flowering cycle that could last from 3 to 6 months, depending on the region and variety. Its flowers attract swarms of bees with their large yellow blooms, rich pollen and nectar. It ensures the activity of bees over a period of time, while other species of bees ensure their activity during other seasons (Arba, 2009).

- **Fodder production for livestock**

The cactus is considered a standing fodder reserve; it can constitute a food supplement for the transition periods in summer and autumn and during years of drought (Shoop *et al.*, 1977). Fodder is the second major use of the cactus, indeed many countries such as Mexico, Brazil, the United States, Peru, South Africa, and Tunisia already produce significant quantities of livestock feed from opuntia (Araba *et al.*, 2000).

**Table 2-** comparison of the composition of cladodes with other foods (poupon, 1975).

Type of fodder	Dry matter (%)	Nitrogen matter %	Carbohydrate %	Fat %
Lucerne hay	91.4	10.6	39.0	0.9
Triplex	23.3	2.8	5.9	0.1
corn silage	26.3	1.1	5.0	0.7
Sugar beet pulp	9.4	0.2	6.4	0.1
Cladodes of <i>Opuntia</i>	10.4	0.6	5.8	0.1

- **Medicinal use**

The fruits sought and appreciated for their taste are the subject of great interest by scientists, have justified the orientation of numerous research works on their nutritional and medicinal quality and in particular their conservation and transformation.

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**Opuntia ficus-indica** is one of the most used plants in traditional medicine (Welegerima *et al.*, 2018). Its use applies to the different parts of the plant. The fruits are known everywhere in Algeria for the fact that they stop colic and diarrhea. In Australia and North Africa, the hypoglycemic effect of Napolitos is used for the treatment of non-insulin-dependent diabetes. In Sicily, tea prepared with flowers is used as a treatment for kidney ailments. Mucilage isolated from snowshoes helps reduce total cholesterol in the blood. The cladodes are used to treat inflammations, and even flower preparations are used as an antidiuretic substance (PNTTA, 2000).

In addition, the vegetable proteins with which Nopal is abundantly provided help the body eliminate excess water from certain cellular tissues, thus reducing water retention, of which cellulite represents one of the most unfortunate consequences (Schweizer, 1997).

- **Industrial use**

The cactus plant is currently used in the United States and Mexico for industrial purposes in the form of sticky and anti-rust material in oil wells. It is also used as a coating to remove salt from offshore oil installations (salt facilitates the formation of rust). Research has shown that the juice of the cactus plant is a factor that prevents iron from wearing down, oxidizing, and rusting.

In Chile, the cactus rackets, after their natural fermentation, are used as raw material and an important source in the production of a vital gas (biogas). They are productive, their yield is high, and they are a source of vital energy. Their uses are many. The gelatinous material they isolate is one of the components that produce chewing gum and wax. In addition, they are used as a reinforcing element in the weaving of garments made of cotton. (Stintzing, 2001).

- **Impact of the cactus on the evolution of soils:**

The cactus considerably improves the organic fertility of the soil by relatively quickly producing a humus horizon which creates an organo-mineral complex thick enough to give rise to a dynamic soil.

This dynamism results in an intense microbial activity, a rapid melanization of the organic matter released especially by the tracing roots which bring into play the action of humic and fulvic acids which are sufficiently aggressive concerning the

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bedrock. They accelerate chemical weathering and thereby deepen the soil (15 to 30 cm). Consequently, the cactus can be considered a pioneer in the production of soils, particularly in arid zones. **(Reguieg Yssaad A.)**

➤ **Impact of cactus on water treatment**

The use of cactus mucilage as a natural flocculant for water treatment allows the elimination of bacteria (*Bacillus cereus*). The results show good efficacy of this mucilage during the microbiological analysis of water contaminated with *Bacillus cereus*. The effectiveness of this mucilage, comparable to that of an industrial flocculant, offers an economical alternative in terms of wastewater treatment and is less toxic **(Reguieg Yssaad A.)**

• **Other uses**

➤ **Protection:**

This plant constitutes living hedges that allow the fixing of the grounds ravined by the rains and stabilize the sandy grounds and are impassable to the wild animals. They require little maintenance while offering the richness of their fruits and rackets. Nevertheless, the invasive nature of this plant must be taken into account **(Revue nature et santé, 2011).**

➤ **Fuel:**

It is a plant that provides excellent firewood and an illuminating flame **(Revue nature et santé, 2011).**

## **IV. Prickly pear cladodes**

### **1. Morphology:**

The term prickly pear leaves is frequently used in the literature to refer to the flattened stem segments of the plant that replace the leaves in their functions. These cactus stems, cactus raquettes or cladodes are the correct terms, synonymous with 'nopales'**(Anderson, 2001)**. The prickly pear cladodes are mainly composed of epidermal cells, parenchyma and chlorinated wall tissue. These different tissues have been individualised by alkaline extraction and treatment with sodium chlorite **(Malainine et al., 2001)**.

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The latter is covered with multicellular spines (modified leaves) and hairs or trichomes, which form the areole and is a characteristic of members of the Cactaceae family (**Anderson, 2001**). Glochids are composed of 100% crystalline cellulose (**Waldron *et al.*, 1996**). The cellulose microfibrils are 0.4 mm in length and 6 to 10 µm in diameter, and are embedded in an arabinose matrix. The arabinose matrix is present as a solid gel, woven tightly with the cellulose. The spines consist of 96% polysaccharides, which in turn are divided into 49.7% cellulose and 50.3% arabinose, the rest being ash, fat, wax and lignin (**Malainine1 *et al.*, 2003**). They are 1 to 3 cm long and make up 8.4% of the total weight of the cladode. Their functions include mechanical protection from herbivores, reflection of light, shade for the stem, and thus reduce water loss as well as condensation of mist (**Anderson, 2001**).



**Figure 7-** prickly pear cladodes (website: plantes& santé).

## **2. The chemical composition:**

The chemical content of a cladode varies depending on the edaphic factors, location, and season. There are also differences in nutrient composition between species and varieties, so it can't be taken with absolute certainty as an average of what is provided by the food (**Stintzing and Carle, 2005**).

Young cladodes are very rich in water, 91 to 93%, and have high mineral contents. Minerals represent more than 13% of the dry matter in young cladodes. The young cladodes are rich in calcium, magnesium, potassium and copper but have low phosphorus content. The content of Ca<sup>++</sup>, Mg<sup>++</sup>, K<sup>+</sup>, Cu<sup>++</sup> is comparable to that of spinach and tomato. Young cladodes can thus make an important contribution to the satisfaction of human nutritional needs. Young cladodes are also rich in fibre

(8 to 11% of dry matter), total sugars (1.66 to 8.79% DM) and vitamin C, the content of which is (9 to 15mg/100g fresh matter). The total polyphenols known for their antioxidant activities are also present at levels ranging from 41.6 to 23.4 mg/100 g of fresh cladodes. Fresh cladodes. Condensed tannins represent 6.45 to 6.93 mg/100 g of fresh material and carotenoids 77 µg to 47 µg per 100 g of fresh material (**Hadj Sadok T., et al., 2008**).

**Table 3-** Main components of prickly pear cladodes (Stintzing and Carle, 2005).

Component	Rate (g/100g DM)	Rate(g/100g FP)
Water	-	88-95
Carbohydrates	64-71	3-7
Ash	19-23	1-2
Fibres	18	1-2
Proteins	4-10	0.5-1
Fats	1-4	0.2

**DM:** dry matter, **FP:** fresh weight.

## 2.1. Macro composition:

### a) Water and minerals

The water content varies between 80 and 95%, it gives cladodes a food value by being low in calories (27 kcal/100g) (**Murillo-Amador et al., 2002, Bhira, 2012**). In the ricket, minerals are able to reach concentrations at or above 23% DM. Calcium and potassium are prevalent in these levels. There are amounts up to 1.79 and 5.52 g/100g DM respectively (**Feugang and al., 2006; Ayadi et al., 2009**).

Zinc and magnesium exist at levels of  $15.2 \pm 1.2$  and  $78.7 \pm 0.8$  mg/100g DM (**Bakari et al., 2017**). With regard to anions, sulphate, chloride, nitrate and oxalate are the most represented (**Missaoui et al, 2020**).

The oscillation of mineral content in cladodes can be related to environmental conditions, maturity for the stage of clodes, and the presence of spines (**Ayadi et al., 2009; Contreras-Padilla et al., 2012**).

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### **b) Carbohydrates:**

Carbohydrates revolve around 36% to 37%. This is between 64 and 71 grams of carbohydrates per 100 grams of dry weight. There are variations in carbohydrate content due to agronomic and environmental factors, as well as age. Young cladodes have a higher carbohydrate content than older ones (**Ginestra *et al.*, 2009**).

### **c) Protein:**

The total protein content of cladodes varies from 4 to 10% depending on several determinants such as the growth phase (**Astello-García *et al.*, 2015; Belhadj Slimen *et al.*, 2016**). Prickly pear cladodes contain 18 amino acids- 6 of which, glutamine, leucine, lysine, valine, arginine and phenylalanine are well represented (**Brückner *et al.*, 2003; El-Mostafa *et al.*, 2014**).

### **d) Fats:**

From the cactus cladodes, 13.87% of fatty acids are in the form of palmitic acid, 11.16% are in the form of oleic acid, 34.87% are in the form of linoleic acid and 32.83% are in the form of linolenic acid. These four fatty acids represent more than 90% of the total fatty acids found on cactus clades, with 65% being polyunsaturated fatty acids, a percentage that is exceeded only by linoleic and linolenic acids (**Abidi *et al.*, 2009**).

### **e) Organic acids**

Cladodes have high amounts of malic and citric acid, which also changes during the day. This is a result of CAM metabolism (**Stintzing and Carle, 2005**), Fresh leaves of the bitter melon contain malic acid and citric acid, which are at 0.36 and 1.78 mg/100g fresh weight respectively. However, after long periods of storage, the leaves will no longer contain malonic acid. Tartaric and succinic acids only made up a small percentage of their dry weight.

The types of acid found in plants have changed as they have aged. The levels of piscidic and phorbic acids were discussed as looked at how they are amassed over time in plants. Piscidic acid is seen more in younger plants, while phorbic acid is less prominent (CAM) (**Teles *et al.*, 1984**).

Oxalic acid plays an important role in water retention and in the metabolism leading to pectin formation (**Trachtenberg, Mayer, 1982, Stintzing, *et al.*, 2001**).

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## 2.2. Micro composition:

### a) Vitamins:

*Opuntia* has a high content of vitamin C (ascorbic and dehydroascorbic acid) in young cladodes (7 to 22 mg/100g) while vitamin A is less represented with 30µg/100g dry matter. The contents of vitamins thiamine, riboflavin and niacin are relatively low with respectively: 0.14 mg, 0.6 mg and 0.46 mg per 100g of fresh cladodes (**Rodríguez-Félix and Cantwell, 1988; Pimienta-Barrios, 1993, and Teles, 1994 cited by Stintzing, 2005**).

### b) Carotenoids and chlorophylls:

Carotenoids are made up of carotenes and xanthophylls. Among the carotenes, β-carotene and lycopene are the most widespread in fruit and vegetables. β-carotene is a precursor to vitamin A, carotenes represent 11 mg-53.5µg per 100g of fresh cladodes yet the total chlorophyll content is higher at 12.5mg/100g with chlorophyll a dominant at 9.5mg per 100g fresh cladodes (**Guevara *et al.*, 2001 cited by Stintzing *et al.*, 2005**).

### c) Phenolic:

The content of phenolic compounds, known for their antioxidant role, varies from 41.6 at stage 1 to 23.4 mg/100 g of fresh cladodes at stage 5. Stage 1 to 23.4 mg /100 g of fresh cladodes at stage 5. Downward trend during growth (**Hadj Sadok T.,and *al.*, 2008**). **Guevara-Figueroa *and al.*, (2010)** found the following phenolic and flavonoid acids in avocado extracts: ferulic acid, p-Coumaric acid, 4-Hydroxybenzoic acid, caffeic acid, salicylic acid, gallic acid. The flavonoids detected are: rutin, iso-quercitrin and nicotiflorin. Narcissin was not detected.

### d) Alkaloids:

Biologically-based compounds, which are made of carbon atoms and nitrogen, are grouped together into organocycles. Alkaloids fit this category as they belong to the same plant family and have a similar synthesizing pathway. (**Yubin *et al.*, 2014**). Despite the high toxicity of many of these compounds, they have significant pharmacological activity, which explains their wide use in herbal medicine or as pharmaceuticals (**Petruczynik, 2012**). **Buckingham *et al.* (2010)** reports that, in some cases, Mescaline, Opuntine B and asterredione are present in the *Opuntia* cactus plant.

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### e) **Betalains:**

Betalains are natural water-soluble pigments found only in a limited number of plant families. They are found in the order Caryophyllales and exist in the genus *Opuntia* (**Gandía-Herrero and García-Carmona, 2013; Betancourt *et al.*, 2017**). betalamic acid as the basic structural unit, which forms the betaxanthin class by condensation with different amino acids or free amino groups, while the class of red-purple betacyanins after conjugation with indoline derivatives (**Gandía-Herrero and García Carmona, 2013**). In addition to the usual anthocyanins, there are also a wide range of betalains found in different parts of an *Opuntia*. These include indicaxanthin, neobetamine, betanin, isobetamine, and Gomphrenin I and Vulgaxanthin I and II (**Belhadj Slimen *et al.*, 2016**).

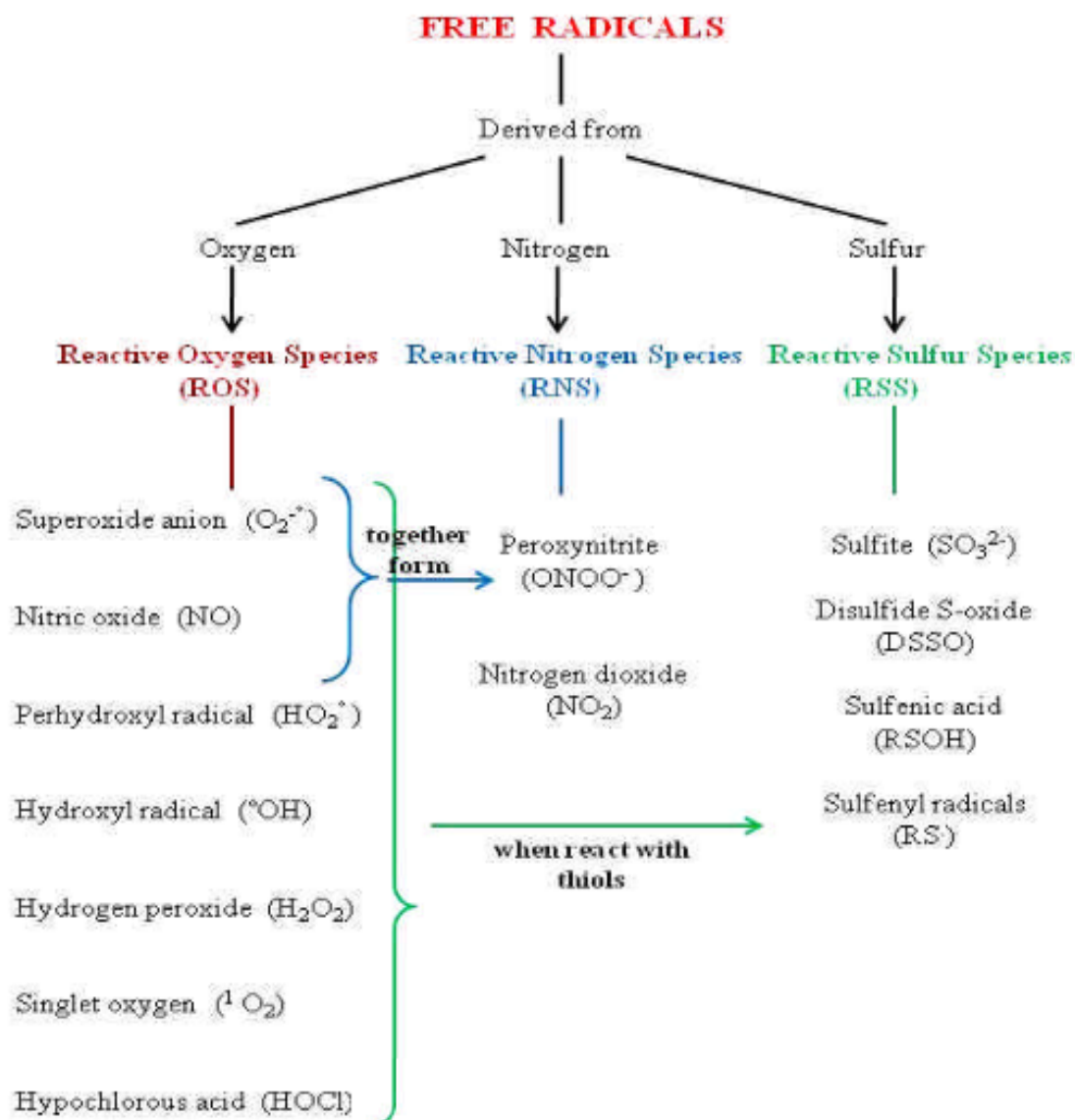
## **V. Antioxidant properties**

### **1. Free radicals and oxidative stress:**

When our body is under oxidative stress, it starts to accumulate free radicals. These are chemically highly oxidized substances that are made when you take in oxygen-which is normal for any organism that consumes oxygen. However, when the cells can't fight against these elements anymore and can't use their natural defenses- this creates great harm instead of benefits (**Gardès-Albert *et al.*, 2003**).

#### **1.1. Free radicals:**

Free radicals and reactive oxygen species (ROS) have been associated with the etiology and/or progression of a number of diseases and in aging. Many of the proteins oxidatively modified by free radicals contain side-chain carbonyl derivatives, which can be used as markers for protein oxidation. The protein carbonyl content has been quantitated as a function of age for human cultured dermal fibroblasts, lens, and brain tissue. These data were analyzed using a simple auto-catalytic model with the assumption that free radicals randomly oxidize proteins or peptides to form carbonyl derivatives and lead to their inactivation. The carbonylated proteins and peptides are highly susceptible to proteolytic degradation. Implication of free radicals in aging and in age-dependent susceptibility to neurodegenerative diseases will be discussed in light of this simplified kinetic model (**Jackob *et al.*, 2002**).



**Figure 8-** Types of free radicals (Blessy B. M. *et al.*, 2011).

## 1.2. Oxidative stress:

At high concentrations, ROS can cause structural damage to cells, proteins, nucleic acid, membranes and lipids, which exhibits a displacement towards the pro-oxidants due to an increment in oxidative metabolism. This results in a stressed condition at the cellular level, which occur due to many factors such as toxins, infections, illness, radiations, medication, chronic inflammation, arduous physical activity, exposure to alcohol and insecticides, smoking and poor diet. The cells produce oxidants in various ways, such as by - xenobiotic metabolism in which toxic substances are detoxified, normal aerobic metabolism in which approximately 10% of oxygen remains unutilized by the cell and by oxidative burst from phagocytes in

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which foreign components like proteins are denatured and destroyed (**Percival M. 1998**).

### **1.3. Defenses against free radicals:**

Antioxidant defense against free radicals. Antioxidants can be endogenous or exogenous. The antioxidants that break the chain are strong electron donors and react with free radicals before the major molecules are damaged. The first line of defense against free radicals includes the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx). Exogenous antioxidants are compounds provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals and polyphenols. These compounds represent the second line of defense against free radicals and consequent pathological disorders and diseases, acting with a scavenging effect, a stimulatory effect on endogenous antioxidant enzymes or both (**Giovanni et al., 2022**).

## **2. Antioxidants**

Any molecule which has the ability to deactivate or immobilize the free radicals by stabilizing them, before they approach the cells for damage are known as antioxidants. (**Mates JM et al., 1999**) But when the antioxidants are limited in the body, then the damage caused becomes massive, but when present in the right amount, they decrease the abnormal cell division (**Percival M. 1998**). To counteract the oxidative damage, the cells have an antioxidant defense system, both enzymic and non-enzymic, consisting of endogenous and exogenous components which work together to protect the cells by neutralizing the harmful radicals (**Mates JM et al., 1999; Percival M. 1998**).

Some of the antioxidants are present on both the sides of the mitochondrial membrane which work together to minimize the stress induced by ROS (**Harman D. 1956**). An ideal antioxidant is the one which can work in membrane as well as aqueous domains constituting the redox metals, can suppress the free radicals readily at physiologically relevant levels and can be absorbed quickly affecting the gene expression. These antioxidants can be endogenous being readily available or can be obtained exogenously for the normal cellular functioning under stressed conditions, which could also be done through diet (**Mates JM et al., 1999**).

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### 2.1. Enzymatic antioxidants:

Such as catalase, superoxide dismutase and glutathione peroxidase require cofactors such as zinc, iron, copper etc. for the metabolization of oxidative intermediates that are toxic. They catalyze quenching reactions of free radicals, (Percival M. 1998). Reduce hydrogen peroxide to water and alcohol (Agarwal *et al.*, 2005).

### 2.2. Non-enzymatic antioxidants:

Non-enzymatic antioxidants or nutrient-derived antioxidants or synthetic antioxidants consists of carotenoids which protect lipid rich tissues, lipoic acid which has the ability to chelate with pro-oxidant metals and quenches free radicals in aqueous and lipid domains, (Packer L, Witt E.H. 1995) Vitamin C which stops the propagation of the per oxidative process, (Agarwal *et al.*, 2005) and neutralizes ROS before its activity initiation, natural flavonoids which offer anti-aging and anti-inflammatory activity, melatonin etc... (Mates JM *et al.*, 1999; Percival M. 1998). Vitamin E which protects membrane fatty acids from lipid peroxidation.

### 2.3. Endogenous antioxidants:

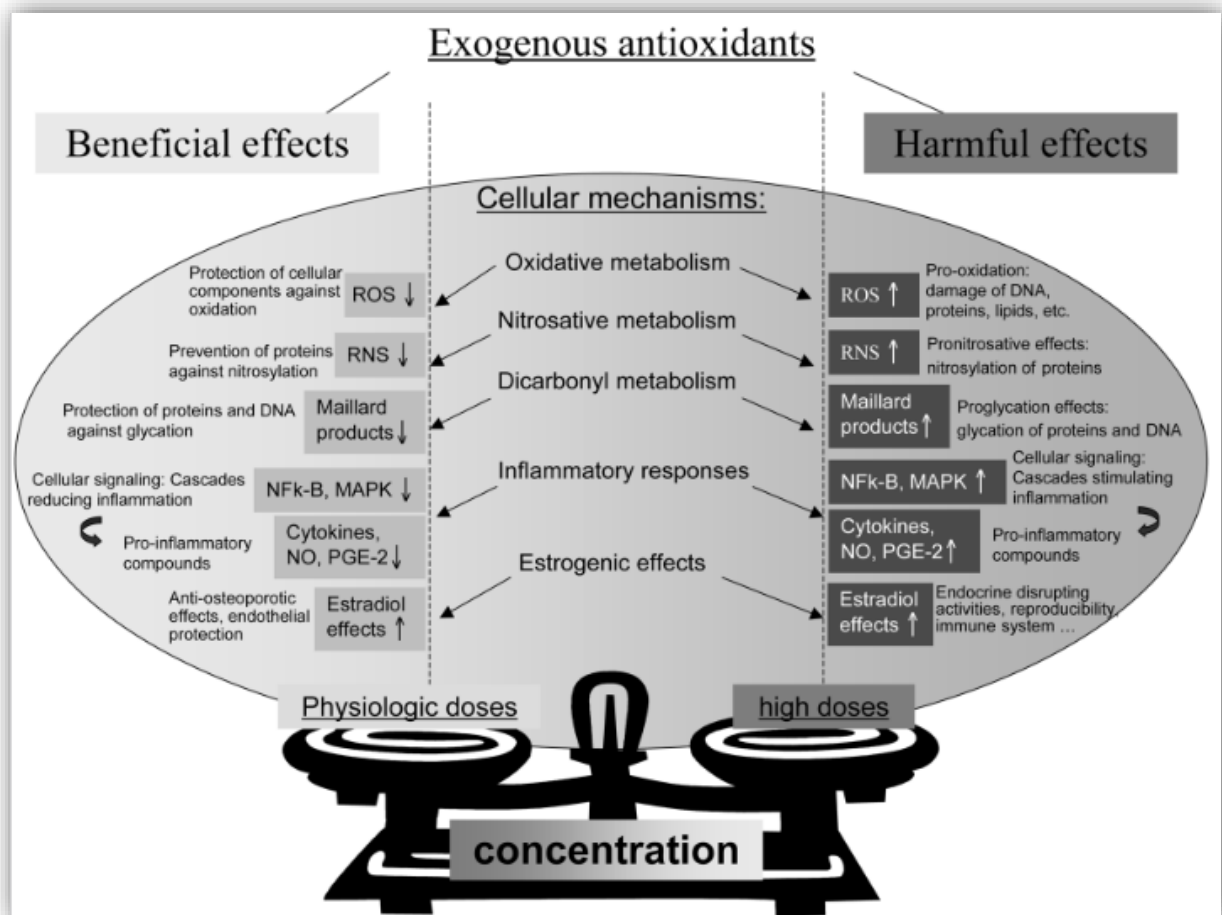
Endogenous non-enzymatic antioxidants include many thiols:

- **Glutathione:** majority thiol, largely present in reduced form, which is capable of reacting, in vivo, with  $\text{OH}^\bullet$ ,  $\text{RO}_2^\bullet$ ,  $\text{RO}^\bullet$ ,  $^1\text{O}_2$ , ONOO- radicals, radicals centered on carbon, but also acid hypochlorous HOCl (Delattre *et al.*, 2005).
- **Uric acid:** present in the form of urate at physiological pH, it has antioxidant properties in vitro against  $\text{OH}^\bullet$  and  $\text{RO}_2^\bullet$ , just like bilirubin, melanin and melatonin (Delattre *et al.*, 2005).
- **Lipoic acid:** compound belonging to thiols, its two oxidized and reduced forms have antioxidant properties in vitro by trapping  $\text{OH}^\bullet$ ,  $\text{RO}_2^\bullet$ , HOCl and  $^1\text{O}_2$ . By binding to metals such as iron and copper, it allows them to be deactivated from a catalytic point of view, and has the ability to regenerate certain endogenous and exogenous antioxidants (Packer *et al.*, 2001).

### 2.4. Exogenous antioxidants:

According to Jaouad and Torsten (2010) the principal dietary antioxidants from fruits, vegetables and grains:

- **Vitamins:** vitamin C, vitamin E
- **Trace elements:** zinc, selenium
- **Carotenoids:**  $\beta$ -carotene, lycopene, lutein, zeaxanthin
- **Phenolic acids:** chlorogenic acids, gallic acid, caffeic acid, etc.,
- **Flavonols:** quercetin', kaempferol', myricetin'
- **Flavanols:** proanthocyanidins and catechins
- **Anthocyanidins:** cyanidin' and pelargonidin'
- **Isoflavones:** genistein', daidzein' and glycitein'
- **Flavanones:** naringenin', eriodictyol' and hesperetin'
- **Flavones:** luteolin' and apigenin.



**Figure 9-** Double-edged effects of exogenous antioxidants on cellular responses including oxidative, nitrosative and dicarbonyl metabolisms and other pathways such as inflammatory processes depending

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potentially on their concentrations: physiologic doses leading to beneficial effects whereas high doses may result in harmful effects (Jaouad and Torsten, 2010).

### 3. Hydrogen proton transfer reactions:

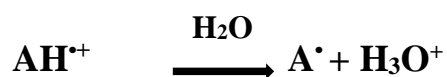
They measure the ability of an antioxidant to neutralize free radicals by transferring a hydrogen proton ( $AH = H^+ + A$ . donor) (Prior *et al.*, 2005).



Where  $X^\bullet$  is the free radical and  $AH$  the antioxidant. They are independent of pH and solvent and are generally quite fast and last a few seconds to a few minutes. The presence of reducing agents, including metals, complicates such reactions and can lead to a very strong apparent reactivity (Prior *et al.*, 2005).

### 4. Electron transfer reactions:

They measure the potential of an antioxidant to transfer an electron and reduce any compounds, namely metals, carbonyls and radicals (Prior *et al.*, 2005).



Where  $X$  is the free radical,  $AH$  the antioxidant and  $M(III)$  a metal. these reactions are pH dependent and the termination reaction is often very slow. Trace compounds and contaminants, especially metals, interfere with electron transfer reactions and can cause high variability in results (Prior *et al.*, 2005).

There are several chemical methods for the *in vitro* determination of antioxidant activity. The three methods used for our study are: FRAP test (Antioxidant activity) and the two DPPH and ABTS tests (anti-radical activity).

## VI. Industrial production of juices

Low consumption of fruits and vegetables is associated with an increased risk of cancer and other chronic diseases. According to the French study

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**SU.VI.MAX (2000)**, the average consumption of fruit and vegetables is 330 g per day for men and 300 g per day for women, which corresponds to 60% of the population in below nutritional recommendations.

To compensate for this under-consumption of fruits and vegetables, due among other things to a short shelf life, short consumption seasons, high prices, fruit and vegetable juice appears to be a good alternative. Moreover, global nutritional recommendations clearly include fruit and vegetable juice as a portion of daily fruit and vegetable consumption.

## **1. Definition**

### **1.1. Fruit Juice**

According to the Codex General Standard for Fruit Juices and Nectars (**codex, 2005**). Fruit juice is the unfermented but fermentable liquid obtained from the edible part of sound, sufficiently ripe and fresh fruit or fruit maintained in good condition by appropriate means, including post-harvest surface treatments applied in accordance with the provisions of the Codex Alimentarius Commission. Some juices may be processed with pips, seeds and rinds, which are usually not incorporated into the juice, but certain parts or components of pips, seeds and rinds, which cannot be removed by Good Manufacturing best practices (GMPs) will be acceptable.

The juice is prepared by appropriate processes, which maintain the essential physical, chemical, organoleptic and nutritional characteristics of the juices of the fruit from which it is made. The juice may be cloudy or clear and may have released aromatic substances and volatile flavor components, all of which must be obtained by appropriate physical means, and all of which must be recovered from the same type of fruit. Pulp and cells obtained by suitable physical means from the same type of fruit may be added. Only one juice is obtained from one type of fruit. A mixed juice is obtained by mixing two or more juices or juices and purees, from different kinds of fruit. The fruit juice is obtained as follows:

1.1.1 Fruit juice directly expressed by mechanical extraction processes.

1.1.2 Fruit juice from concentrate by reconstituting concentrated fruit juice with potable water

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## **1.2. Fruit juice concentrate**

A fruit juice concentrate is produced after physical removal of water in sufficient quantity to carry. The Brix value at a level at least 50% higher than the Brix value established for the reconstituted juice of the same fruit. For the production of the juice intended to be concentrated, suitable processes are used and can be associated with the concomitant diffusion of cells or fruit pulp in water, provided that the soluble solids of the fruit from which the water has been extracted are added to the original juice before concentration. Fruit juice concentrates may contain flavoring substances and returned volatile flavoring compounds, all of which must be obtained by suitable physical means and must come from the same type of fruit. Pulp and cells obtained by suitable physical means from the same type of fruit may be added.

## **1.3. Water extracted fruit juice**

Water Extracted Fruit Juice is the product obtained by diffusion with water of:

- Pulpy whole fruit whose juice cannot be extracted by any physical means, or
- Dehydrated whole fruit.

Such products may be concentrated and reconstituted.

## **1.4. Fruit puree intended for the production of fruit juices and nectars**

This is the non-fermented but fermentable product obtained by appropriate processes, for example by passing through a sieve or by grinding the edible part of the whole or peeled fruit without extract the juice. The fruit must be healthy, reached a degree of ripeness suitable and fresh or otherwise preserved by physical means or by one or more of the treatments applied in accordance with the relevant provisions of the Codex Alimentarius Commission. Fruit puree may contain aromatic substances and volatile flavoring compounds provided that they have been obtained by suitable physical means and from the same type of fruit. Pulp and cells obtained by suitable physical means from the same type of fruit may be added.

### 1.5. Concentrated fruit Purée for use in the manufacture of Fruit Juices and nectars

The concentrated puree used for the production of juice and nectar is physically extracted. Remove a sufficient amount of water from the puree to raise the Brix to at least one value Brix value higher than 50%, i.e. reconstituted juice made from the same fruit as reported in the report appendix. Puree concentrate may have recovered Flavourings and Flavour Volatiles, All Close must be obtained by appropriate physical methods and must all be recovered from the same species from fruit.

### 1.6. Fruit nectar

Fruit nectar is a non-fermented product obtained from water with or without added sugar and/or food additive sweeteners according to the General Standard for Food Additives (GSFA), General Standard for Flavorings, Volatile Matters, Flavor Components, Pulp and Cells But fermentable products. Must be obtained from the same type of fruit, and ingredients obtained by appropriate physical methods may be added. Mixed fruit nectar is obtained from two or more different types of fruit.

## 2. Production of juice maintaining

The organoleptic and nutritional properties of fruit juices during development and commercialization require continuous adjustment of production tools. Obtaining ready-to-eat juice requires optimization of many operations to ensure adequate production without compromising quality or safety. Fruit juice production implemented on an industrial scale can be modelled in major continuous production stages (Table 4).

**Table 4-** The main stages of fruit juice production

Fruits		
Prepared fruit		
(pulp)		
Residue	Brut juice	
(pectines)	Clarified juice	Turbid juice
Concentrate		

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On an industrial scale, most of these steps are automated. The extracted juice is then processed to ensure its microbial and colloidal stability before or after packaging. Juices are expensive to transport and store, and it is often more economical for manufacturers to concentrate the juice and dilute it in the sales area.

### **2.1. Fruit preparation: selection, washing, grading**

Upon arrival at the plant, the fruit picked when ripe is usually stored for several days under conditions that limit its spoilage. The storage time depends on the fruit: apples can be stacked on a concrete surface of no more than 1 meter for a week, citrus fruits for 5-6 days. The small red fruits are very fragile, so they are processed upon receipt to avoid fermentation, or they are frozen to delay processing.

Pick fruit at the press line entry and discard damaged and/or non-standard fruit. For example, flotation tests make it possible to sort healthy apples in hydraulic chutes. The first selective sorting is carried out by this principle. The fruit is then automatically washed and sorted according to the size of the pressing system. Pretreatment of fruit varies by variety: cut apples and pears into strips with a grater. Only citrus is rated. After an initial heat treatment with steam, the stone fruit is destoned by means of a rotary destoner or pulper. These process steps are well defined. One of the extraction processes in plum pits (**Will and Dietrich, 2006**) is to heat the plums to 90 °C in heat exchanger tubes and then leave them in the tank for 20 minutes. The preparation for pressing the fruit then ends with pitting in the pulper.

### **2.2. Extraction**

Juice is obtained in several methods:

#### ➤ **Squeeze: includes squeezing juice**

The juice is contained in the vacuole of each parenchymal cell of the edible fruit tissue. Each cell is surrounded by a main wall composed of a rigid gel of pectin, cellulose and hemicellulose. Therefore, in order to extract the sap, it is necessary to disrupt the tissue to obtain a pulp composed of individual cells, fragments of these cell walls bathed in the fluid produced by the vacuoles. After extraction, juice is separated from solid particles (**Drilleau, 1988**). Numerous parameters must be taken into account when pressing fruits and vegetables: pulp thickness, porosity, mechanical strength, viscosity, density of juice and applied pressure. The formation

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of a layer of sawdust after pressing will restrict drainage. In order to limit the thickness of the Rapure layer, it is necessary to limit the pressure too fast (**Singh and Kulshreshtha, 1996**). There are currently two main types of presses used in the industrial processing of fruit and vegetable pulp: batch or basket presses and continuous belt presses.

➤ **Batch presses**

Packaging presses are still used on an artisanal scale, such as for apple juice (Figure 10). However, due to their low productivity, they have disappeared from factories in Western countries. They allow juicing of previously chopped or whole fruit. Under the action of the hydraulic piston, a compressive force is generated, so that the fruits and vegetables are squeezed between the different packages. This type of pressing is preferably used for small amounts of fruit or vegetables.



Figure 10- Package press.

Basket presses can handle almost any type of plant. Their programmable control allows the pressure to be adjusted according to the physical properties of the raw material for optimum performance. The press body consists of a basket with a capacity of up to 6 cubic meters. At the level of the pressing mechanism, the pulp fed into the basket is acted upon by the piston and pressed against the skirt (step 1). The juice flows through the drain element (step 2). During the Rebêchage, the rotation of the press body kneads the pulp, favouring its contact with the drain (step 3). When these are passed through a sieve, the juice obtained contains a small amount of sludge. At the end of the pressing process, the piston and basket retract

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and the residue falls into a hopper equipped with an Archimedes screw (step 4) (Figure 11). The system, completely closed from the introduction of the fruit or vegetable until the outlet of the juice, ensures good hygienic quality of the product.

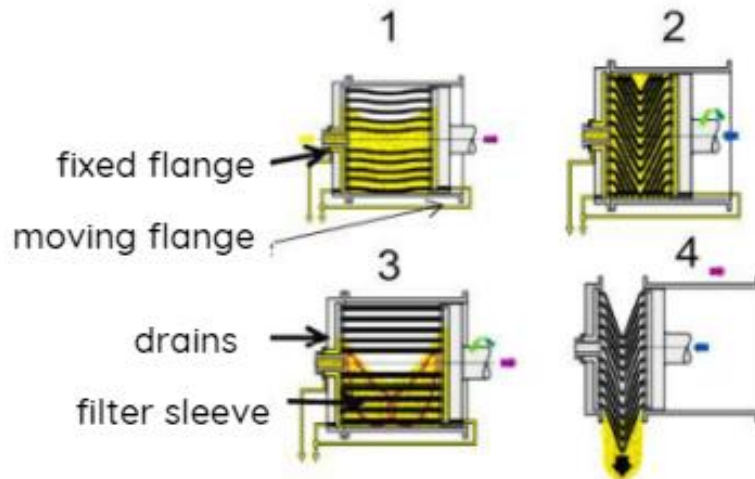


Figure 11- Operating principle of a basket press.

### ➤ Continuous press

The band presses derive from the techniques of the bundle presses. The pulp bed (stage 1) is driven between two endless webs by a series of rolls and counter rolls (stages 2 and 3), which apply progressively increasing pressure to the pulp by reducing its gap. The juice flows through the subband into the drip tray (step 4). At the end of the device, the paper moves aside, allowing the residue to be discharged into the discharge hopper. The agitator and nozzle ensure the cleaning of the fabric (figure 8).

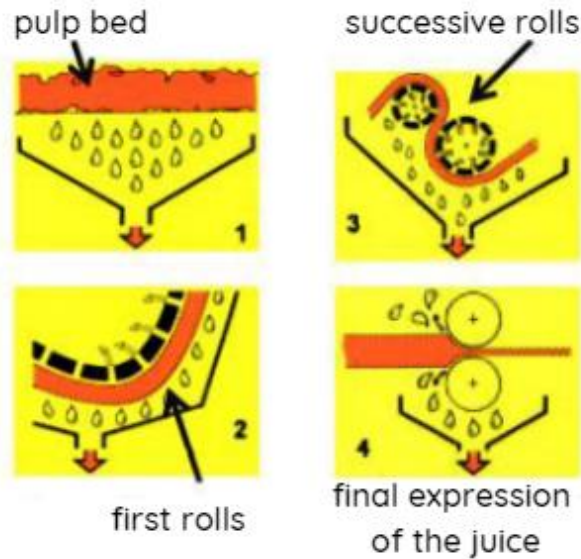


Figure 12- Explanatory diagram of the operation of a band press.

In citrus fruits, the presence of essential oils in the epidermis prevents the whole fruit from being pressed. There are quality restrictions in the production of citrus juice. The standard states that the essential oil content is less than 0.02% of the final juice volume.

### 2.2.1. News Pressing Technology

Juice technology began to develop in the mid-1930s, and with it came difficulties in clarification and yield. Pressing alone does not maximize juice extraction, which is an economic loss for the producer. In addition, some fruits such as mangoes and plums are not suitable for pressing (**Chang, Siddiq *et al.*, 1995; Chauhan, Tyagi *et al.*, 2001; Will and Dietrich, 2006**). Developments in research made possible the first application of pectinases in apple juice clarification technology in 1930 (**Mehlitz, 1930**).

For juice extraction, it is necessary to perform physical and/or enzymatic manipulations prior to pressing in order to increase yield by increasing fruit deconstruction to facilitate extraction, and/or to eliminate turbidity and clarification downstream to obtain juice.

#### a) Mechanical and physical action

Evaporating water, especially rich in aromatic compounds, can be reintroduced into the products after instant relaxation. Mango purees are more

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consistent and stickier, with a higher color intensity, allowing for a higher degree of dilution to prepare nectars. After dilution, more stable nectars are formed, characterized by slower phase separation. The bleaching stage ensures almost complete inhibition of endogenous enzymatic activity (polyphenol oxidase, PME), while the higher viscosity of the product reduces the phenomena of turbidity in the finished product (**Brat, 2001**).

Flash detente <sup>TM</sup> is a technique. Its principle is to ensure continuous, in a minimum amount of time, thermal processing of the fruit and its rapid cooling with high vacuum. This technique allows a better extraction of pellicle compounds (anthocyanins and tannins) by more efficiently destroying the peel of the fruit. Consequently, this technique was also developed for the extraction of pulp and essential oils from citrus fruits by the Center for International Cooperation in Agricultural Research (CIRAD) in Montpellier. This technique consists in introducing the plant material under a vacuum (approx. 30 mBar) after the blanching step. The temperature of water evaporation in these vacuum conditions is about 30 ° C, which causes the immediate evaporation of part of the water in the system. This loss of water causes a fine grinding as a result of the formation of intercellular microchannels.

#### **b) Combination of processes, support for pressing**

In addition to the use of enzymes to help increase juice extraction yields, processes such as pulsed electric fields (PEC) or microwaves can be applied during maceration.

#### **c) Pressing aid enzymes**

The main treatment supporting fruit juicing is the use of enzymes that degrade the walls. Due to the different intensity and specificity of the action, the following are distinguished: compression-supporting enzymes, clarifying enzymes, maceration-supporting enzymes enabling obtaining essentially nectar, and liquefying enzymes. The purpose of maceration and liquefaction of the pulp is to break up and tear the fruit, and to improve the juice flow. Each fruit has a certain amount of pectin, hemicellulose, and cellulose. The ratio of these polysaccharides will be important in selecting the concentration and incubation time of the exogenous enzyme used (**Grassin and Fauquembergue, 1996**).

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- **Enzymes used for these treatments**

Pectins are the main source of fluid retention in fruits. Without the use of enzymes, insoluble pectins show gelling properties and cause juice retention during pressing. For this reason, commercial extrusion enzymes are essentially pectolytics. The correlation between pectin degradation and juice yield was established by Shalom, **Tyagi *et al.* (1999)**. The use of pectinases addresses two main problems: dissolution of protopectin and degradation of soluble pectin. There are different types of enzyme action depending on the desired juice: extraction, liquefaction and maceration enzymes. The enzymes most commonly used to produce fruit juices are pectinases from the microorganism *Aspergillus niger*. Indeed, *Aspergillus niger* can synthesize both pectin esterases (PE), also called pectin methyl esterase (PME), polygalacturonase (PG) and pectin lyases (PL) (**Pilnik and Voragen, 1993**).

- **Effect of enzymatic treatment on the pH of the juice**

The pH of the juice decreased with increasing enzyme concentration (**Joshi *et al.*, 2011**). **Yusof and Ibrahim (1994)** found that for each enzyme level used, the decrease in pH was not significant during the first hour of incubation. With the increase of the incubation time (2-3 h), the drop in the pH value of the fruit juice differed significantly from the initial value. Nevertheless, the values for 2 and 3 hours of incubation are almost the same. According to **Woodroof and Phillips (1981)**, a drop in pH from 4.5 to 3.0 can extend the shelf life of a juice up to about 3 times.

- **Effect of enzymatic treatment on the content of ascorbic acid in juice**

The total ascorbic acid content was found to be reduced by approximately 21% after the enzymatic treatment. The reduction of ascorbic acid by 16.9-20.7 % occurs during enzymatic clarification of various juices (**Singh *et al.*, 1993**).

- **Effect of enzymatic treatment on turbidity of juice**

The turbidity in the juices may be due to pectin and other plant cell wall substances released during the enzymatic prepress maceration. It seems logical that elevated turbidities may transiently result during enzyme catalyzed cell wall degradation, which can partly explain the positive effect coefficient of the enzyme dosage on the turbidity. Turbidity in fruit juices can be a positive or a negative attribute depending on the expectation of the consumers (**Hutchings, 1999**).

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However, this cloud is desirable and acceptable by the consumers. Turbidity of juice at optimized condition for enzymatic treatment of various fruits and vegetable. Increase in enzyme concentration and incubation time might decrease turbidity. Pectin was the main cause of turbidity (**Grassin and Fauquembergue, 1996**). As the clarification process took place, the amount of pectin in the juices decreased, therefore reducing the turbidity of the juices (**Alvarez et al., 1998**).

### **3. Heat treatment**

#### **3.1. General**

Thermal treatment of the product involves heating and cooling to obtain a product with a long shelf life that is free of pathogenic organisms and does not cause food spoilage. The heating and cooling states are called a process (**Simpson, 2009**). A process designed to inactivate microorganisms is often referred to as sterilization, although it is not the same as a medical process that requires the complete removal of microbial species. Often, we don't have to eliminate thermophilic organisms that are not important to our health, which is why we talk about commercial sterilization. The only downside is storing the product above 32°C as microorganisms can germinate and destroy the product. If the ambient storage temperature exceeds this temperature, such as in countries with hot climates, the product must undergo a more stringent process (**Simpson, 2009**).

The most important factor in determining the procedure to follow is the pH of the product, which can vary from neutral at pH 7 to acidic at pH 2.8 - sporulation stops at pH 4.5 (or slightly above  $\leq 4.7$ ) and is inhibited. Therefore, this number is usually considered as mild processes (such as pasteurization) with temperatures below 100°C and more severe processes (often called botulinum processes) (temperatures between 118°C and 125°C (**Simpson, 2009**)). Foods are grouped into four categories based on their pH:

- **G1:** Low acid products (pH  $\geq 5$ ) - meat products, seafood, milk, soups and most vegetables.
- **G2:** Moderately acidic products (pH 4.5-5) - meat, vegetables, pasta, soups, cactus pears and pears.
- **G3:** Acidic products (pH 4.5-3.7) - tomatoes, pears, pineapples and other fruits.

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- **G4:** Products with high acid content ( $\text{pH} \leq 3.7$ ) - grapefruit, citrus fruits and juices.

Sterilization is required for most foods, such as meat, fish and vegetables. On the other hand, other products on the dividing line, such as tomatoes and pears, depend on variety and maturity. Products in this pH range require extensive testing to ensure that they are negative for food poisoning microorganisms. For formulated products, the inhibitory effect of the ingredients also needs to be studied. Another factor to consider is the initial microbial load of the product. It can be controlled by paying attention to handling procedures, preparation and hygiene. In addition to heat treatment, it is important to consider nutrient destruction, vitamin loss and overall quality deterioration. There is a need to determine the optimal process that will provide the necessary sterilization conditions and minimize the degradation of nutritional quality (**Simpson, 2009**).

### **3.2. Pasteurization**

Spallanzani performed the first work on pasteurization in 1765. He uses heat treatment to delay spoilage and preserve the meat. From 1862 to 1864 Pasteur showed that short-term heating at temperatures between 50°C and 60°C prevents microorganisms from destroying wine (**Simpson, 2009**). The term "pasteurization" comes from the work of French researcher Louis Pasteur and refers to a mild treatment method (50°C - 90°C) designed to destroy the vegetative form of pathogenic microorganisms. Or change (**Lund, 1975**). Pasteurization was recently redefined by the U.S. Department of Agriculture in 2006 as "any process, treatment, or combination thereof applied to food to reduce the most resistant microorganisms to those unlikely to pose a risk to public health under normal distribution. Level" and storage conditions." Therefore, the definition includes non-thermal pasteurization processes such as high pressure (HP).

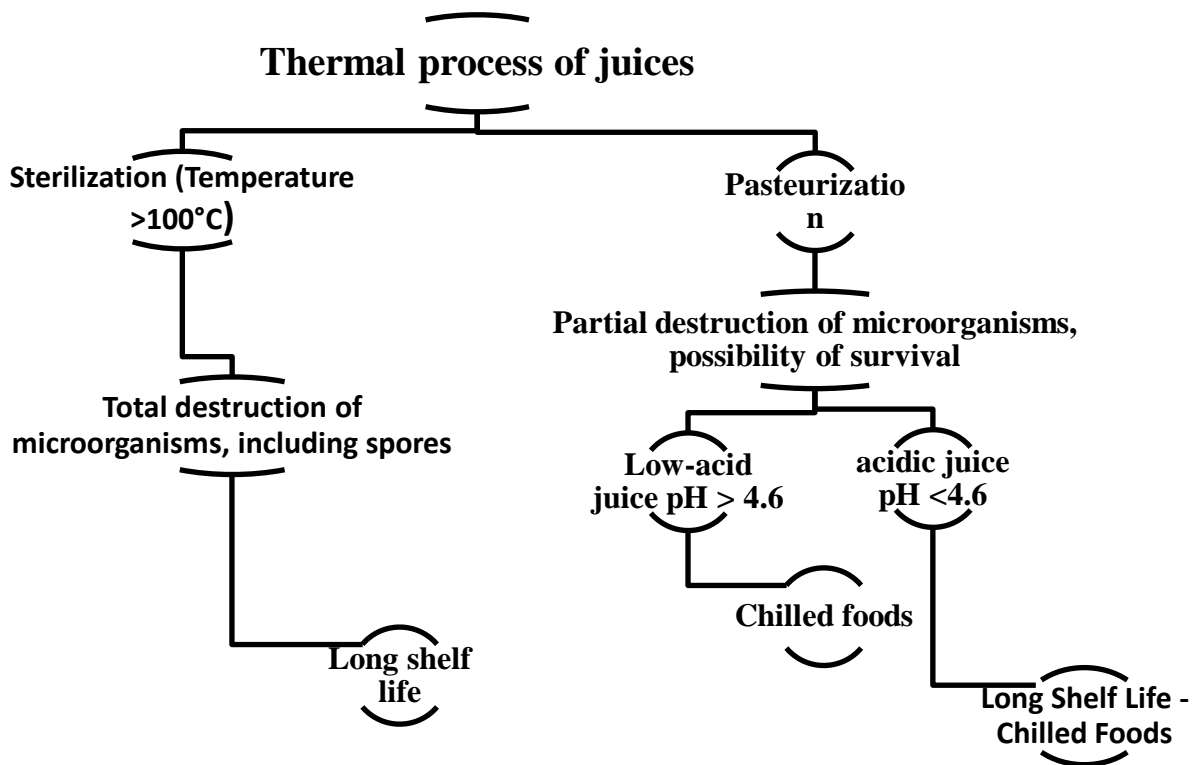


Figure 13- Thermal process of juices (Simpson, 2009).

Heat treatment of heat to food is the oldest method of pasteurization. More recently, the effects of non-thermal pasteurization (eg, high-intensity, high-voltage pulsed electric fields) on microorganisms and food have been studied (Simpson, 2009). Today, pasteurization itself remains a goal (partial destruction of microorganisms), and techniques are varied:

➤ **Heat treatment**

The use of heat treatment is one of the primary means of food decontamination today, with improvements aimed at minimizing changes in product organoleptic properties. Heat transfer occurs by radiation (infrared, microwaves), pipes with direct current channels and by ohmic heating.

➤ **Non-thermal treatment**

There are several techniques for the cells themselves and their destruction, namely: pulsed electric fields, pulsed magnetic fields, high hydrostatic pressure, pulsed light, ultrasound, and cold plasma. Certain processed foods contain

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ingredients or ingredients that have antibacterial properties and inhibit the growth of microorganisms under certain conditions with exceptions: Fermented foods containing alcohol or acid (wine, beer, and pickles), carbonated beverages (soda), very sweet foods < 0.65 or soluble solids > 70° Brix (honey, jams, jellies, dried fruit, fruit concentrate) or savory foods (cured fish or cured meat). Other exceptions apply to highly acidic foods (pH < 4.6) that are stable under ambient conditions after the pasteurization process, as the acidic environment of these foods can adversely affect the growth of harmful microorganisms and microbial spores. In pasteurized foods. For this type of food (pH < 4.6), the pasteurization process allows a long shelf life (months) at room temperature (**Ramaswamy and Abbatemarco, 1996**), on the other hand, if refrigerated storage, medium Pasteurization and product quality will improve and then increase. For low acid foods (pH > 4.6), the shelf life after pasteurization is short (a few days), but refrigeration is necessary to keep the product safe during storage and limit the growth of surviving food pathogens (**Adams and Moss, 1995**).

#### **4. Packaging**

Pasteurized juices are packed under nitrogen (finished products) or in bulk in individual packages: drums (aseptic or frozen), containers (1000 liters aseptic), tanks, cisterns... After the juice is re-pasteurized, the bulk juice is shipped to product developers who remove the design from the final packaging (**Baron, 2002**).

#### **Conclusion**

Prickly pears are native to the highlands of Mexico, where the climate is hot and dry. It belongs to the cacti family, more specifically the genus Cactus. Cactus leaves are rich in minerals, carbohydrates, ascorbic acid, polyphenols and fiber. They contain bioactive ingredients that have a positive effect on our health. They fight several common diseases such as hyperglycemia and lower cholesterol levels. Natural antioxidants play an important role in regulating oxidative stress and are involved in scavenging free radicals that cause many diseases. Therefore, the analysis of the sap obtained from the branches and leaves will provide a new assessment of this plant that has been used for centuries. It is necessary to highlight it by determining its composition, antioxidant activity and stability, i.e. pasteurization and storage time.

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## VII. Oil extraction methods

### 1. Extraction of oil from prickly pear

There are many techniques for extracting high value-added products present in plants. These technologies can be classified as traditional (used for a long time) and new (recently developed). **LUQUE DE CASTRO, M. and GARCIA-AYUSO, L. (1998)**

Prickly pear oil is a very precious and very rare oil, its cost comes from the very low yield because there is only 6% of the oil in the young seeds of *Opuntia ficus-indica* obtained by pressure at cold, so about 800 kg of figs have to be processed for one liter of oil. **BENATTIA, F.K. (2017)**

### 2. Extraction processes

Oils are obtained from natural substances by different extraction techniques, which consist in removing one or more chemical species from a solid or liquid medium. The extraction process is based on the differences in solubility of the compounds of a mixture in a solvent, there are several extraction techniques, among these we can mention the following methods:

#### a) Soxhlet extraction:

Soxhlet extraction is a classic method for solid-liquid extraction, simple and convenient allowing infinite repetition of the extraction cycle with a fresh solvent until complete depletion of the solute in the raw material (**Wang and Waller, 2006**)

The advantage of this technique is that the sample quickly comes into contact with a fresh portion of solvent, which helps it shift the transfer equilibrium towards the solvent. This method does not require filtration after extraction.

The Soxhlet apparatus is composed of a glass column (figure 14), in which is placed a cartridge of thick filter paper or cellulose, a penetrable material for the solvent, a siphon tube and another of distillation. In the assembly, the extractor is placed on a flask containing the extraction solvent surmounted by a condenser. The flask is heated, causing the evaporation of the solvent which passes through the adductor tube, then condenses thanks to the refrigerant, and falls back into the body of the extractor.

The condensed solvent accumulates in the extractor until it reaches the top of the siphon tube and returns to the flask, together with the extracted substances. Thus, the solvent contained in the balloon is gradually enriched with soluble compounds, mainly oil (Benattia, 2017)

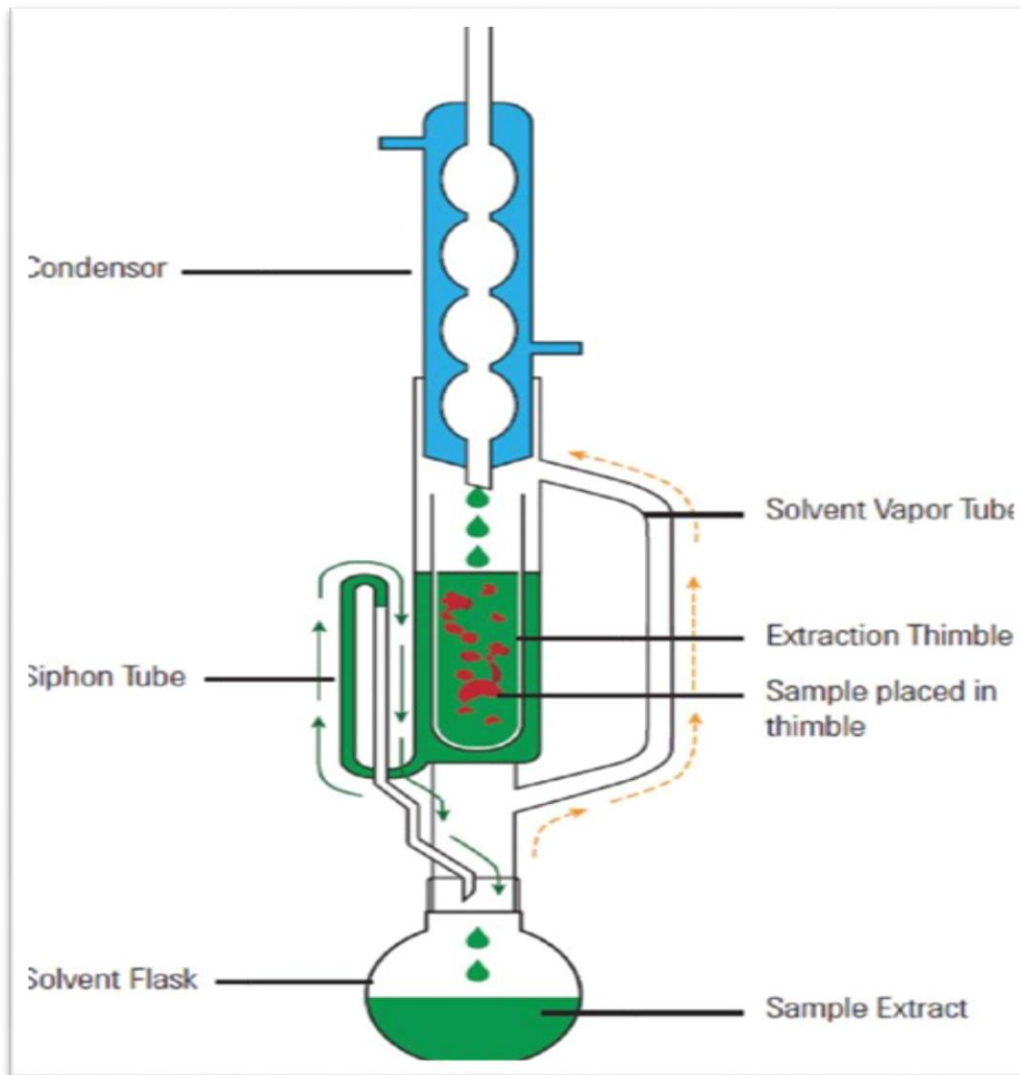


Figure 14- soxhlet extraction

#### b) Extraction by cold pressing

Extraction by cold pressing is used to pass the seeds through a screw oil press which causes an increasing pressure at around 60°C; the recovered oil was decanted, weighed then stored at - 20°C.

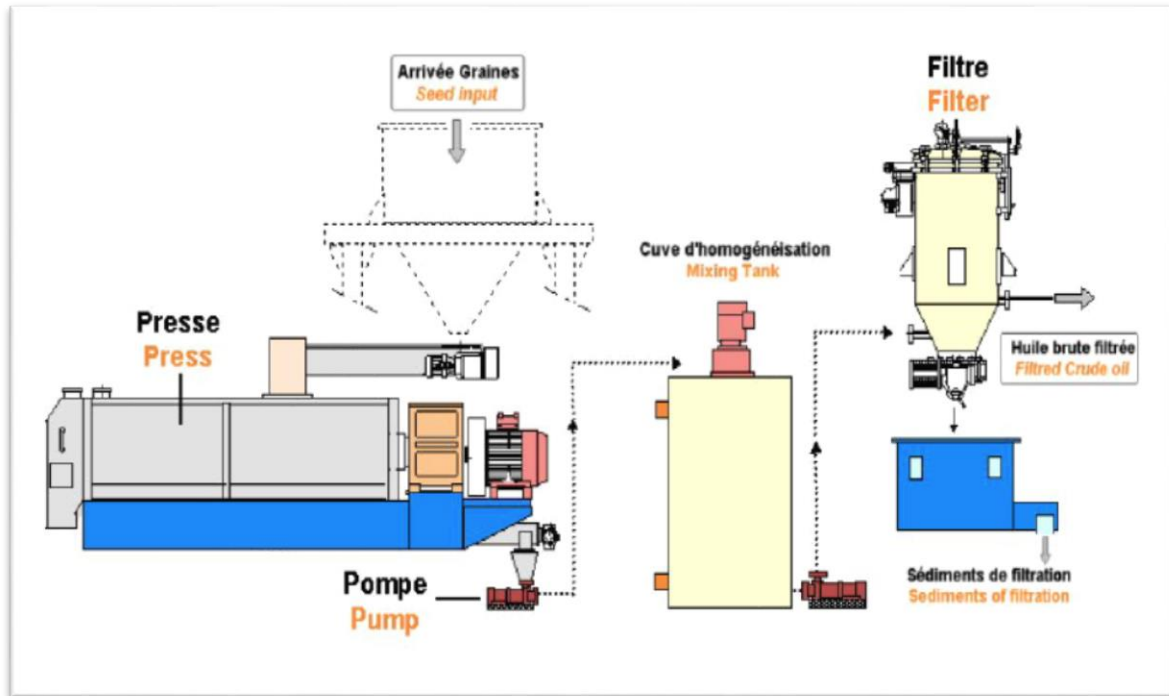


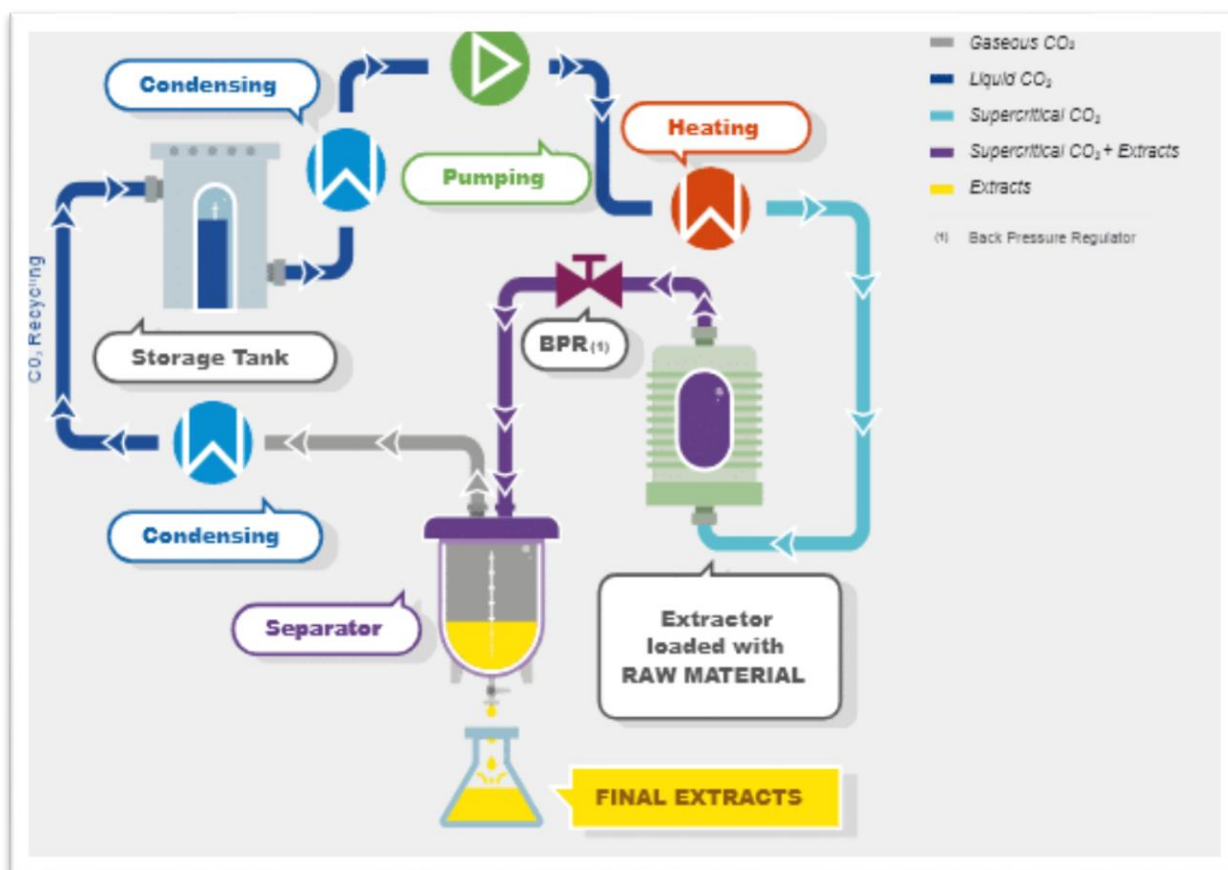
Figure 15- diagram of a cold pressing system

This technique allows the preservation of the content of essential fatty acids and natural anti-oxidants, and consequently avoids an alteration of the properties of the oil. The process of crushing oilseeds to extract the oil they contain is divided into two types of mechanical pressing (continuous and discontinuous). **BENATTIA, F.K. (2017)**

### c) Supercritical Fluid Extraction

Supercritical fluid extraction and more particularly supercritical CO<sub>2</sub> extraction has been introduced as an alternative to these solvent extraction processes. **DANIELSKI, L. et al., (2006).**

It is a technique without residue, which uses CO<sub>2</sub>, presenting the most satisfactory properties it is inexpensive and available at a high purity, its pressure (74 bar) and its temperature (31°C) critical are relatively easy to reach, it is inert, non-flammable and non-toxic, and outside of radioactive applications it is chemically stable. **BOCEVSKA, M. and SOVOVA, H. (2007).**



**Figure 16-** mechanism of supercritical  $\text{CO}_2$  extraction

It also makes it possible to recover the most natural extract possible, without solvent, guaranteeing the preservation of all the active ingredients with a shorter extraction time, high selectivity and the ease of eliminating the solvent after extraction by simple decompression. .

The method is based on the particular properties of fluids under given temperature and pressure conditions, such that they have both gas properties (penetration into the material to be extracted) and liquid properties (dissolution of what is to be extracted, And in particular fat-soluble molecules). Moreover, it makes it possible to avoid any degradation of the molecule sought (the working temperatures are low), the selectivity is better, but the cost is generally very high. **Benattia, F.K. (2017).**

### 3. Factors involving solvent extraction

There are some factors which intervene and act more or less directly on extraction rate, extract concentration and yield.

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## 1. Solvent

A solvent is, by definition, a fluid that has the power to solubilize other substances leading to a homogeneous solution **Gerin (2002)**. The choice of solvent is made according to several criteria: **Aguilera (2003)**

- Solubility of the specific components in the solvent.
- Regeneration of the solvent if it must be reused. It must not form an azeotrope with any of the compounds it solubilizes and its latent heat (boiling) must be low.
- Interfacial tension and viscosity, because the solvent must correctly wet the solid matrix.
- Ideally it should be non-toxic, stable, non-reactive, non-flammable, harmless to the environment and inexpensive. **Poirot, R. (2007)**

The most suitable and widely used solvent in the food industry is water. Lipids are insoluble in water and the oil mill differs from other food industries by using organic solvents. Among these solvents, we have: hexane, the cost price of which is relatively low, but which has the disadvantage of being flammable; trichloroethylene which provides very good extraction yields but which is not very selective and dissolves undesirable compounds (**Mafart and Beliard, 2004**)

## 2. Granulometry

All authors agree on the generally positive effect of grinding on extraction operations. The grinding of the solid makes it possible to intensify the phenomena of transfer of the solvent through the increase in the specific surface (exchange surface between the solvent and the solid), but also by the reduction of the distance of penetration into the material. Indeed, at a given solid content, the contact surface between the solid and the liquid increases when the size of the particle decreases through the increase in the specific surface. **Sovová et al (1994)**.

However, a certain limit should not be exceeded with regard to the fineness of the particles; the presence of fine particles induces an exaggeration in this direction and implies a notable reduction in the permeability of the bed of solids to the solvent, which leads to the establishment of preferential currents thus blocking

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the extraction process in certain places where the solvent does not circulate more  
**Nguyen (2010)**

### **3. Humidity**

As a general rule, plant materials are dried to facilitate their packaging and especially their storage. Excess humidity can therefore deteriorate the substrate. Moreover, when using hydrophobic solvents, the diffusivity is inversely proportional to the water content of the solid (**Leybros and Fremeaux, 1990**)

Therefore, solvent extraction is hampered by humidity, the oil yield is linked to the humidity rate in the material to be treated, but on the other hand, very intense drying produces the contraction of cell membranes and makes difficult the extraction process. The optimum humidity values ensuring maximum extraction yield of soybean and cottonseed flours are 8 to 10% (**Cano Munoz, 1976**)

### **4. Temperature**

The extraction temperature is a factor that influences the extraction efficiency. At high temperature, the solvent sees its diffusion capacity increase, while its tension of surface and its viscosity decrease. **SPARR ESKILSSON, C, BJÖRKLUND, E. (2000)**. It is difficult to identify in a simple way the influence of temperature on extraction. In most cases, high temperature ranges are favourable to extraction efficiency, because heat has four main consequences:

- facilitates extraction by permeabilizing the cell walls by denaturation.
- Increases the solubility of the materials to be extracted, at least in the usual high temperature ranges.
- Increases the diffusion coefficients.
- Decreases the viscosity of the extraction solvents, which facilitates not only the passage of the solvent through the mass of solid substrate, but also the subsequent separation operations.

The upper temperature limit is imposed by the boiling point of the solvent, by the risks of thermal degradation of the solute and extraction of harmful compounds. **BIMBENET, JJ. (2007)**

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## 5. Solvent/solid ratio

The solvent/solid ratio influences in an inverse way compared to the concentration of the miscella. Its increase produces a positive effect on the oil yield in a given extraction time. Nevertheless, there is a value from which the yield is constant. **LEYBROS, J. FREMEAUX, P. (1990)**

## VI. Prickly pear seed oil

### 1. Definition

Prickly pear seed oil is very precious and very rare oil generally obtained by cold pressing. This oil is a true wonder of nature. Its unique composition gives it rapid and deep penetration into the layers of the epidermis. It contains unsaturated fatty acids and sterols, rarely present in vegetable oils, and which are recognized for their performance in revitalizing and protecting against free radicals. Its penetration is fast and total. The skin takes on a radiant and satiny appearance. After a few weeks of application, wrinkles fade and fine lines disappear, the face is luminous and smooth. (Site web: laboratoire emeraude)

Prickly pear seed oil is rich in acids unsaturated fats and has a high content of linoleic acid (57.7-73.4%) and low linolenic acid content.

The oil has a high content of unsaturated fatty acids, as well than other healthy components, such as sterols, tocopherols, vitamin E,  $\beta$ -carotene and vitamin K (**Ramadan and Mörsel, 2003a; Kouba *et al.*, 2015**).

**Table 5- Fatty acid content (%) of prickly pear oil (*Opuntia ficus-indica*) from different countries**

Fatty acid	Country						
	Algeria <sup>f</sup>	Tunisia <sup>a,c,g</sup>	S.Africa <sup>a</sup>	Turkey <sup>b</sup>	German <sup>d</sup>	Chilli <sup>e</sup>	Morocco <sup>a</sup>
<b>Palmitic (C16:0)</b>	13.1	12.2-12.7	13.7	10.6-12.8	23.1	16.2	11.9
<b>Stearic (C18: 0)</b>	3.5	3.2-3.9	3.38	3.3-5.4	2.67	3.3	3.4
<b>Oleic (C18: 1n-9)</b>	16.3	16.4-22.3	15.7	13-23.5	24.1	19.9	21.3
<b>Vaccenic (C18: 1n-7)</b>	5.3	4.8	–	5.1-6.3	–	–	–
<b>Linoleic (C18: 2n-6)</b>	61.8	53.5-60.6	64.38	49.3-62.1	32.3	57.7	60.8

<sup>a</sup> Gharby and *al.* (2015); <sup>b</sup> Mattháus and Özcan (2011); <sup>c</sup> Tlili and *al.* (2011); <sup>d</sup> Ramadan and Mörsel (2003a); <sup>e</sup> Sepúlveda and Sáenz (1988); <sup>f</sup> Chougui and *al.* (2013); <sup>g</sup> Ouerghemmi and *al.*, 2013.

## 2. Chemical composition of seeds

The seeds of the cactus have aroused a lot of interest in recent years and studies have multiplied to characterize their constituents in order to evaluate their nutritional value above all (table 6). However, most attention has focused on the oils contained in these seeds. The seeds that are the object of our work are rich in mineral salts and sulfur amino acids and they are characterized by a high content, protein, crude fibre, ash, carbohydrates and crude oil.

This oil belongs to the category of "polyunsaturated" oils like most vegetable oils. It is composed of 65% of essential fatty acids, mainly linoleic acid and oleic acid (**table 8**). On the other hand, its particularity lies in its richness in unsaponifiable matter (sterols and tocopherols). This characteristic could be a good asset for its exploitation in the field of cosmetology given the beneficial effects of these substances on the elasticity of the skin, the cellular metabolism and the restoration of the cutaneous structure. **BENATTIA, F.K. (2017).**

Table 6-Chemical composition of prickly pear seeds.

Constituent	Percentage (%)
Water	5-6
Oil	7-8.5
Minerals	1.3
Klason Lignin	18
Proteins	11-12
Cellulose	30
Other polysaccharides	27

The cell walls consist mainly of polysaccharides. Acid hydrolysis makes it possible to determine the composition of neutral and acid monosaccharides contained in the pericarp of the seed of the prickly pear; the results are grouped in the following table **BENATTIA, F.K. (2017)**.

Table 7-Composition of neutral and acid monosaccharides of *Opuntia* seed.

Sugar neutrals and acids	The (%) by mass
Uronic acid (UA)	1.1
Rhamnose (Rha)	0.6
Arabinose (Ara)	3.1
Xylose (Xyl)	23.8
Mannose (Man)	1.0
Galactose (Gal)	1.0
Glucose (Glu)	35.6

Table 8-Fatty acid composition of *Opuntia* seed oil.

Fatty acid	Proportion (%)
Lauric acid (C12:0)	0.11
Myristic acid (C14:0)	0.22
Palmitic acid (C16:0)	13.83
Palmitoleic acid (C16:1)	0.91
Heptadecanoic acid (C17:0)	0.03
Heptadecenoic acid (C17:1)	0.04
Stearic acid (C18:0)	3.16
Oleic acid (C18:1)	19.77
Linoleic acid (C18:2)	60.61
Linolenic acid (C18:3)	0.74
Arachilic acid (C20:0)	0.34
Eicosenoic acid (C 20:1)	0.24
Saturated acids	17.69
Unsaturated acids	82.31

### 3. Physico-chemical characteristics

For the physico-chemical characteristics of prickly pear seed oil, according to **ENNOURI, M. and EL MANNOUBI, I.** is given in the following table:

**Table 9-** Physico-chemical characteristics of prickly pear seed oil.

Properties	Values	Authors
Density*	0.903±0.002	Ennouri et al
Viscosity** (Pa s)	0.0531±0.002	Ennouri et al
Refractive index*	1.475±0.002	Ennouri et al
Saponification index	169.0±0.1	Ennouri et al
Peroxide value***	1.46±0.06	El Mannoubi et al
Acid value	1.270±0.005	El Mannoubi et al

\* At 20° C, \*\* at 20° C and  $\tau > 2$  Pa, \*\*\* meq O<sub>2</sub>/kg oil

### 4. Biological role and uses of prickly pear seed oil

The composition of prickly pear seed oil gives it many interesting properties. We distinguish:

First, pure prickly pear seed oil is mainly composed of oleic (16.41%), palmitic (12.76%) and linoleic (60.69%) acid, which gives it its properties very popular in cosmetics. In particular, at the epidermal level, the oleic acid of this oil is known for its great affinity with the intercorneocyte cementum. It facilitates the passage of nutrients or active ingredients through the hydrolipidic film. As such, oleic acid is considered as an activator of cutaneous bioavailability. Also, linoleic acid, a precursor involved in the synthesis of ceramides, intervenes at the level of the cutaneous barrier by its lipid-replenishing action and makes it possible to reduce insensible water loss. It is therefore particularly suitable for the care of dry and / or mature skin and also has soothing properties for sensitive and irritated skin. Secondly, prickly pear seed oil benefits from a record concentration of tocopherols (vitamin E), of which it displays 447.38 mg/kg of oil, and of sterols (16.06 g/kg of oil) **EL MANNOUBI, I. (2009)**. This high dose of vitamin E (powerful antioxidant), sterols and essential fatty acids, therefore constitute the basis of this veritable natural anti-aging cosmetic cocktail. Stigma sterol, contained in prickly

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pear oil, is a very rare active ingredient in the plant world that delays skin aging **Monica, A. (2019)**. The main uses of prickly pear seed oil:

- Anti-aging care for mature skin.
- Anti-aging eye contour serums.
- Preventive care stretch marks.
- Care of scars and cracks.

### **5. Economic study of prickly pear seed oil**

Prickly pear seed oil is very precious and very rare oil, generally obtained by cold pressing. This method makes it possible to avoid the use of any solvent. The amount of this oil in the seeds is only around 5% approximately: obtaining one liter of pure prickly pear oil therefore requires almost 30 kilograms of seeds, or almost a ton of fruit. These low extraction yields therefore explain the high cost, of around €2,000 per liter of oil, in relation to its very high production cost: this vegetable oil can be considered the most expensive in the world **Monica, (2019)**.

# CHAPTER 2: MATERIALS AND METHODS



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## • Juice Part

### I. Plant Material

#### 1. Sampling Area

The sampling was carried out in the Daira of Ain El Hadjel (fig.17) which is located in Wilaya of M'sila, Algeria. It covers an altitude of 544 m and is 67.9 km west of the Wilaya de M'sila, with a population of 39,732 inhabitants (2008).



Figure 17-Localization of the center of Ain El Hadjel on the map of Algeria.

#### 2. Sampling

The species *Opuntia ficus-indica* (prickly fig) is the subject of the study. The species was planted in several years. Rain is the only source of water for these plants and they receive no chemical treatment.



Figure 18- Picture of the species studied and harvested in the Daira d'Aïn El Hadjel located west of M'sila in Algeria, *Opuntia ficus-indica*.

## II. Juice Extraction

The juice of the cladodes was carried out in the lab of Faculty of Science, Mohamed Boudiaf University, M'sila in 2022. The cladodes were thoroughly washed and the thorns were removed by scissors from the thorny cladodes, then cut into 2 cm cubes and ground. The resulting extract mash was centrifuged at 5600 rpm for 20 minutes and the supernatant was recovered. The clarified juice obtained from the species was stored at 4°C before analysis.

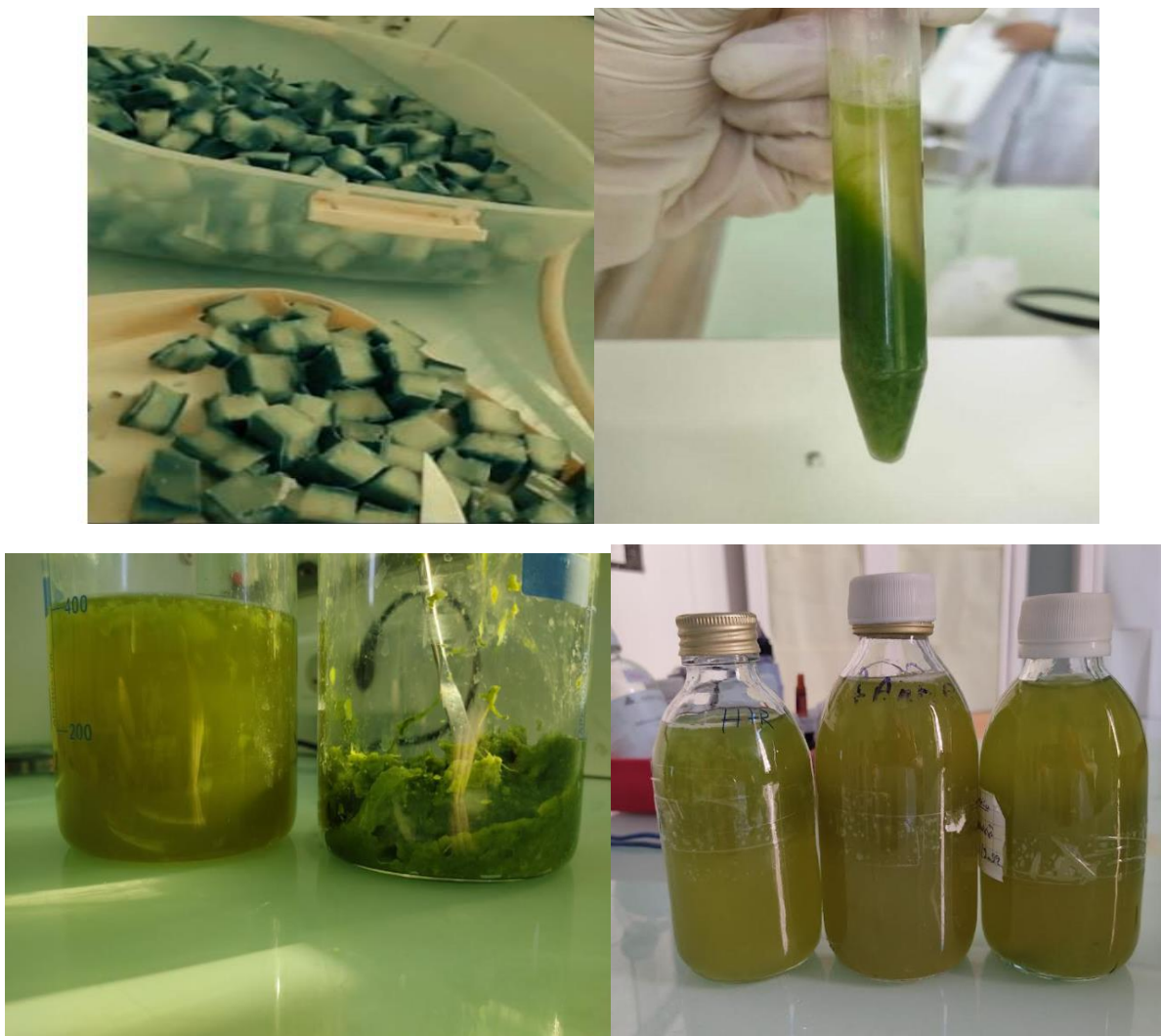


Figure 19- Steps of juice extraction from species cladodes: cutting, centrifugation, separation and storage.

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### III. Physico-chemical and biochemical analyses

#### 1. Basic physico-chemical analyses

The physico-chemical analyses were carried out at the Faculty of Science Laboratory, Mohamed Boudiaf University, M'sila. On the juice extracted from the cladodes after thawing.

##### 1.1. Moisture content

The water content was determined by drying the samples (10g) in an oven ventilated to 105°C for 24 hours.

##### 1.2. Ash Content

The ash content was determined (10g) by oven at 550°C for 3 hours.

##### 1.3. pH

The pH was measured using a pH meter.

##### 1.4. Titratable acidity

Titrate acidity was measured by titration of 25 mL of juice in the presence of a 0.25 N sodium hydroxide solution until a pH of 8.1 was obtained. The result was expressed in g of equivalent citric acid per 1L of juice.

#### 2. Preparation of reactivities for phytochemical screening

##### 1. Detection of alkaloids:

###### • Iodine test:

In a test tube 3 mL of the extract solution (of the plant) is added and a few drops of iodine solution are added. The positive result is the appearance of a blue color, which disappears when boiled and reappears when cooled (**Bhatt and Dhyani, 2012; Basumatary, 2016**).

##### 2. Detection of reducing sugars:

###### • Fehling test:

In a test tube one puts 1mL of filtrate (b) with 1mL of each of the solutions of Fehling A and B, then one boils in a water bath. The positive result is a red precipitate (**Silva and al., 2017; Singh and Kumar, 2017**).

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### 3. Detection of carbohydrates:

#### • Starch test:

Put the aqueous extract with 5 mL of 5% KOH solution. The positive result is the appearance of a cinerary coloration (Audu, 2007).

### 4. Detection of glycosides

#### • Modified Borntrager test:

We put 1ml of plant extract with 1ml of ferric chloride solution, then boil for 5min and cool, then add an equal volume of benzene. Then the benzene layer is separated. Finally, the ammonia solution is added. The positive result is the appearance of a pink to blood-red colored solution (Tiwari *et al.*, 2011; Pandey and Tripathi, 2014).

### 5. Detection of proteins and amino acids:

#### • Biuret test:

In a test tube, put 2ml of filtrate with a drop of copper sulphate balance at 2% and 1ml of 95% ethanol and KOH pellets. The positive result is the appearance of a pink color balance (in the ethanolic layer) (Silva *et al.*, 2017; Raaman, 2006).

### 6. Detection of Flavonoids:

#### • Alkaline reagent test:

Method 2: In a test tube, put 1ml of the plant extract with 10mL of 10% ammonium hydroxide solution. The positive result is the appearance of a yellow fluorescence (Raaman, 2006).

### 7. Detection of phenolic compounds:

#### • Iodine test:

In a test tube, put 1mL of extract with a few drops of diluted iodine Sol. The positive result is the appearance of a transient red color (Singh and Kumar, 2017).

#### • Ferric chloride test:

In a test tube, put the aqueous solution of extract and add a few drops of 5% ferric chloride solution. The positive result is the appearance of a dark green/bluish black color (Silva *et al.*, 2017; Tiwari *et al.*, 2011).

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## **8. Tannin detection:**

### **• 10% NaOH test**

In a test tube, put 0.4mL of plant extract with 4mL of 10% NaOH and shake well. The positive result is the formation of emulsion (presence of hydrolysable tannins) (**Singh and Kumar, 2017**)

## **9. Detection of Anthraquinones:**

### **• Borntrager test:**

In a test tube, put a few mL of the filtrate (g) with 10mL Sol of 10% ammonia then shake vigorously for 30 seconds. The positive result is the appearance of a pink, purple or red colored solution (**Njoku and Obi, 2009; Gul et al., 2017; Uma et al., 2017**).

## **10. Detection of Anthocyanins:**

### **• HCl test:**

In a test tube, put 1mL of the plant extract with 1mL of HCl2N (then add a few mL of ammonia). The positive result is the appearance of a pink-red soil which turns blue-violet after addition of ammonia (**Obouayeba et al., 2015; Savithramma et al., 2011**).

## **11. Carboxylic acid detection:**

### **• Effervescence test:**

In a test tube, put 1mL of the plant extract with 1mL of the sodium bicarbonate solution. The positive result is the appearance of effervescence (**Singh and Kumar, 2017; Kumar et al., 2013**).

## **12. Coumarin detection:**

### **• NaOH test:**

In a test tube, put the plant extract with 10% NaOH and chloroform. The positive result is the appearance of a yellow color (**Kumar et al., 2018**).

## **4. Determination of Total phenolic content (TPC)**

Folin–Ciocalteu’s assay is commonly applied to assess the TPC of natural products (**Singleton et al., 1999**), with some modifications. This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent). After

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oxidation the absorbance of a green blue complex can be measured at 750nm. The total phenolic content was expressed as gallic acid equivalents in mg per g of dry extract (GAE /g DE), obtained from the standard curve based on gallic acid. (See appendix 2 for methods).

## 5. Determination of flavonoid contents

The content of flavonoid was determined according to **Biesaga et al. (2013)** Aluminum chloride chromatography method with slight modifications. The test solution (standard or sample) was mixed with some NaNO<sub>2</sub> (5%, w/v) and after 5 minutes, AlCl<sub>3</sub> (2%, w/v) was added. It was neutralized with 1 M NaOH solution. The mixture was left for 10 minutes at room temperature and then set in the dark, in which the AlCl<sub>3</sub> solution was replaced with water. Quercetin (0-10mg/ L) was the preferred standard for expressing results at 510 nm. (See appendix 2 for methods).

## 6. Antioxidant activity

Free radical scavenging activity (DPPH assay) of extracts. Free radical scavenging activity (DPPH assay) of extracts was assayed using the method of **Srinivasan et al. (2007)** and **Bursal and Gulcin (2011)** with slight modifications. Briefly, put some solution of DPPH in methanol and this solution were added to the equal volume of each of test samples dissolved in methanol at different concentrations. The mixture was shaken vigorously and maintained in dark for 30 min. Then, the absorbance was measured at 517 nm against a blank. Trolox was used as standard references. The scavenging activity was calculated using the formula:

$$\text{Scavenging activity (\%)} = [(A_{517} \text{ of control} - A_{517} \text{ of sample}) / A_{517} \text{ of control}] \times 100.$$

(See appendix 2 for methods).

## 7. Antibacterial activity

### a) Preparation of bacterial strains

To prepare a bacterial suspension according to CLSI Standards, we must:

First, sterilize materials and products completely by autoclaving, and then reactivate the microbial strains; using nutrient broth and nutrient agar, after that keep the strains in an incubator at 37° for 24 hours, finally, they could be used.

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**b) Disk diffusion methods**

Soak a Whatman sterile 6 mm diameter filter paper disc with juice and distilled water (as a control). Using sterile forceps, place the disc on the surface of the medium inoculated (streaked) with the microbial suspension and incubate the dish at 37°C for 18-24 hours. After incubation, the action of the extract resulted in the appearance of clear circular areas around the discs, corresponding to the lack of growth. The larger the diameter of this region, the more sensitive the bacterial strain.

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## • OILS PART

During this work, we first extracted by two solvents the oil from the flour of prickly pear seeds, and then we did the antibacterial activity and the antifungal activity on this oil and that which comes from extraction by pressure from prickly pear seeds.

### 1. Work equipment

- Material: Glass petri dishes, sterile petri dishes, test tubes, cartridge, platinum handle, beaker, whatman paper, syringes, oven, microbiological hood, autoclave, incubator, precision balance, micro pipette, pipette, sieve, rotavapor.
- Products: Hexane, diethyl ether, distilled water, nutrient broth, sterile physiological water, nutrient agar, sabouraud.

### 2. Plant material

We have worked with two types of oil: virgin oil extracted by cold pressing and oil extracted by solvents. Prickly pear seeds were harvested in summer 2021 in the West region (Ain Defla). Virgin oil extracted by cold pressing comes from a micro-oil mill in the Western region (Mostaganem).

#### 2.1. Pre-treatment of prickly pear seeds

Prior to solvent extraction, the prickly pear seeds underwent the following pre-treatment:

##### a) Washing

It consists of ridding the seeds of the pulp and foreign matter. Washing was done with running water; the seeds are then rinsed with distilled water.

##### b) Drying

The operation consists in reducing the moisture content of the seeds to prevent the development of molds and to facilitate the extraction by the solvent. Drying was carried out in the laboratory at room temperature. The sample was spread on a table; the thickness of the seed layer is approximately 2 cm.

##### c) Grinding

The purpose of grinding is to increase the contact surface between the extraction solvent and the seed powder. It was carried out using a grain mill.

##### d) Sieving

After crushing, we carried out a sieving using a sieve in the opening of the meshes is 2 millimetres, in order to homogenize the sample.

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### 3. Method for extracting oil from prickly pear seeds flour

#### 1. Apparatus

The device with which we performed our extractions is given in figure 21. It made of:

- An extractor fitted with a cartridge (containing the sample) connected:
  - Up to a cooler.
  - Down to a 500ml flat-bottomed flask.
- Heating mantle



**Figure 20-** Apparatus of Soxhlet

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## 2. Solvents used

The solvents we used to extract the oil from prickly pear seed flour are a mixture of hexane and diethyl ether. The characteristics of these two solvents are given in Table 10.

Table 10-Characteristics of Solvents Used

Characteristics/solvents	Hexane	Diethyl ether
Crude formula	C <sub>6</sub> H <sub>14</sub>	C <sub>4</sub> H <sub>10</sub> O
Molecular mass (g/mol)	86.18	74.12
Boiling point (°C)	68.72	34.6
Autoignition point (°C)	225	180
Density (g/ml)	0.6591	0.7134
Polarity	Apolar	Polar
Dielectric constant	1.9	4.3

## 3. Operating mode

The cartridge containing the test portion (about 30 g) is placed in the extractor. We then pour 500 ml of two solvents (mix between the two solvents) into the flask. When the flask is heated, the vapors of the solvent pass through the large tube of the extractor condense at the level of the condenser and are then collected in the extractor. (Fig.22)

The solvent level in the extractor rises as the vapors condense. Once the extractor is filled, a transfer takes place using the siphon. The oil-rich solvent is collected in the flask. The extraction took place during the residence time of the solvent in the extractor. The operation continues until the oil contained in the sample is completely exhausted (approximately 9 hours).

The amount of oil extracted is determined by evaporating the solvent at low pressure using a rotary evaporator. The last traces of solvent and water are eliminated by placing the flask containing the extracted oil in an oven brought to a temperature of  $103 \pm 2^\circ\text{C}$  for 15 min. After cooling, the flask is weighed. The heating and cooling operation continues until a constant weight of the balloon is obtained.

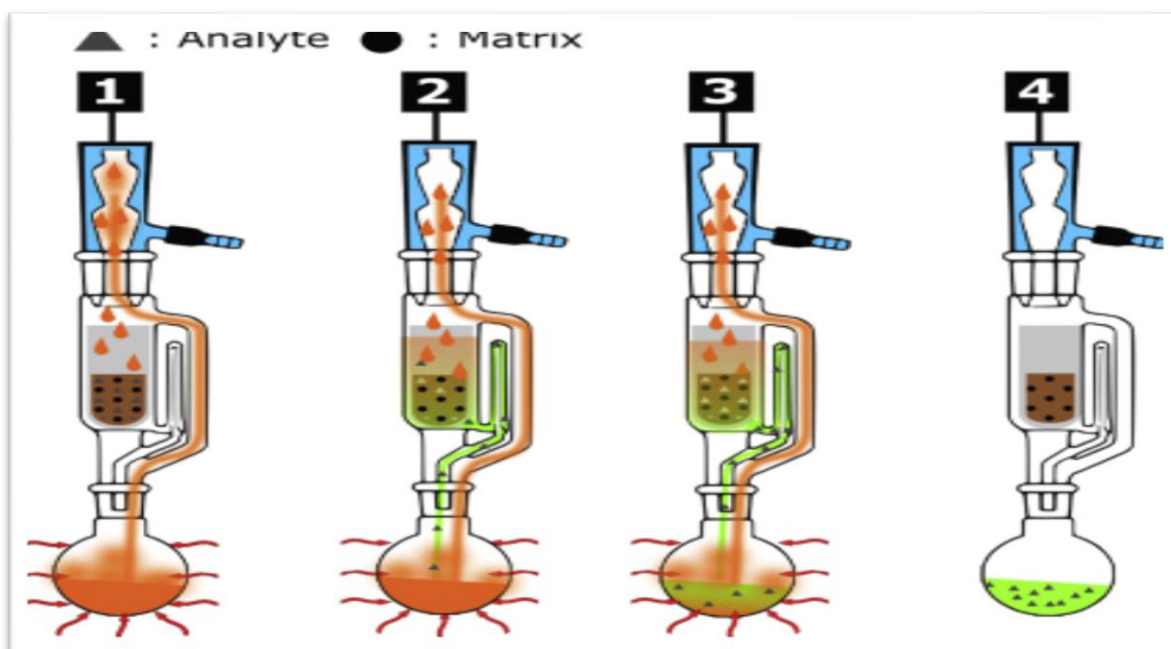


Figure 21- Mechanism of oil extraction by soxhlet

## 8. Antibacterial activity

### a) Preparation of bacterial strains

To prepare a bacterial suspension according to CLSI Standards, we must:

First, sterilize materials and products completely by autoclaving, and then reactivate the microbial strains; using nutrient broth and nutrient agar, after that keep the strains in an incubator at 37° for 24 hours, finally, they could be used.

### b) Disk diffusion method

-Whatman sterile filter paper discs 6 mm in diameter are impregnated with various oil (fig.23) extracts. -Using sterile forceps, the discs are placed on the surface of a medium inoculated (spread) with a microbial suspension, the dishes are incubated for 18 to 24 hours at 37°C.

-After incubation, the effect of the extracts results in the appearance around the disc of a transparent circular zone corresponding to the absence of growth. The larger the diameter of this zone, the more sensitive the strain.



**Figure 22-** A picture showing the method of filling disks with the extracted oil

# CHAPTER 3: RESULTS AND DISCUSSION



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## • JUICE PART

### 1. Yield

The foliage collected in the Aïn El Hadjel area is chosen according to well-defined dimensions: between 15 and 25 cm in length and between 9 and 13 cm in width. According to these measurements, the foliage is less than a year old, and they are called "napolitos" in Mexico and are widely available as vegetables in the country. At this stage of growth, foliage has great nutritional value: mineral balance, good levels of vitamin C, polyphenols and fiber (Hadj Sadok *and al.*, 2008). The juice content of prickly pear cladodes could be expressed,

by the relationship:  $Y (\%) = \frac{m1-m2}{m1} \times 100$

m1: mass of cladodes (g) m2: the mass of the juice (g). **Y % = 79.02 %**

### 2. Basic physico-chemical analyzes

The composition of cladodes varies with soil factors, growing location, season and plant age (Retamal *and al.*, 1987). Therefore, the respective values vary between species and cultivars and should not be considered as absolute values (Rodriguez-Felix, 2002). Basic Physico-chemical analyses on cladode juice: Water content, ash, pH and titratable acidity.

Table 11- Basic physico-chemical analyzes of *Opuntia ficus-indica* species cladodes juice.

Basic Physico-chemical analyses	cladode juice results	% in 100 g
Water content (g/10g of juice)	9.1	91%
Carbon content (g/10g of juice)	0.95	9.5 %
pH	4.8	
Titratable acidity	3.9	

The water content in the cladodes juice is 9.1 g/10g. Generally, the water content of cactus is between 88 and 95% (Murillo-Amador *et al.*, 2002). The ash content in the extract is 0.95 g/10 g. It exceeds the ash content of orange juice, which is  $0.37 \pm 0.01$  g/10 g (Kelebek *et al.*, 2009). Regarding the pH and titratable acidity results, the cladodes juice has a pH of  $4.8 \pm 0.01$ , so the average acidity of the juice exceeds the pH of the other juices, for example, grapefruit juice has a pH of  $3.67 \pm 0.01$  (Cheong *et al.*, 2012) and orange juice has a pH of  $3.52 \pm 0.01$  (Esteve *et al.*, 2005).

The titratable acidity of cladodes juice was  $3.9 \pm 0.01$ , comparable to other juices, but lower than lemon and orange juices:  $0.94 \pm 0.02$  EAC (g/L) for grapefruit juice (**Cheong *et al.*, 2012**) and  $7.8 \pm 0.30$  for orange juice (**Esteve *et al.*, 2005**) and lemon juice  $52.40 \pm 2.50$  EAC (g/L) (**Lorente *et al.*, 2014**) due to high citric acid content.

### 3. Phytochemical screening

Table 12- results of tests of phytochemical screening

Test	Purpose	Resultats	Resultats intrepetation
Iodine test	Alkaloid detection	-	Absence of Alkaloid
Starch test	Carbohydrate detection	+	Presence of Carbohydrate
Fehling test	Detection of reducing sugars	-	Absence of Reducing sugars
Modified Borntrager Test	Glycosides detection	-	Absence of Glycosides
Biuret test	Detection of proteins and amino acids	-	Absence of Proteins and amino acids
Alkaline reagent test	Flavonoid detection	+	Presence of Flavonoid
Iodine test	Detection of phenolic compounds	+	Absence of Phenolic compounds
Ferric chloride test	Detection of phenolic compounds	+	Presence of Phenolic compounds
10% NaOH test	Tannin detection	+	Presence of Tannin
Borntrager Test	Anthraquinone Detection	-	Absence of Anthraquinone
HCl Test	Anthocyanin Detection	-	Absence of Anthocyanin
Effervescence test	carboxylic acid Detection	-	Absence of Carboxylic acid
NaOH Test	Coumarin detection	+	Presence of Coumarin

#### 4. Determination of Total phenolic content (TPC).

The content of TP in our extract was determined using a galic acid calibration curve (see appendix for methods). The results obtained were expressed in mg/g of catechin equivalents in the juice, and the percentage of TPC in the fruit was calculated to be 4.6 g/100 g.

#### 5. Determination of flavonoids contents

The content of flavonoids in our extract was determined using a catechin calibration curve (see appendix 2 for methods). The results obtained were expressed in mg/g of catechin equivalents in the juice, and the percentage of flavonoids in the fruit was calculated to be 38.35 mg/100g. The flavonoid content was higher than that reported by **Haddadi (2005)** for fruit and vegetable flavonoids: orange, grapefruit, apple and strawberry, at 3.22, 7.12, 2.10 and 17.53 mg/100 g, respectively.

#### 6. Antioxidant activity

Prepared five samples at different concentrations from 0.0625 mg/ml to 1 mg/ml of the plant *Opuntia ficus-indica* L. and then prepared pure methanol white 95°. And then prepared the control of the DPPH solution with the methanol solvent. And using the DPPH test from this concentration of the extract obtains the results in the following (**Table 13**). The absorption of DPPH solution with methanol solvent is equal to  $A_{t0} = 0.976$ . In the end get the results in the following table:

Table 13- Presents concentration and absorption and inhibition *Opuntia ficus-indica* L.

Solvent	Extract	Concentration (mg/ml)	Absorption (nm)	Inhibition
Methanol	<i>Opuntia ficus-inca</i>	1- 0.1	0.281	68.0%
		2- 0.05	0.413	54.4%
		3- 0.0125	0.642	30.8%
		4- 0.0062	0.760	18.6%
		5- 0.0015	0.780	16.5%
		Blank	0.976	
		CI50	0.450	

From the results and the comparison of the CI50 of our extract with the blank value, it could be concluded that the the juice of *Opuntia ficus indica* has a high antioxidant activity.

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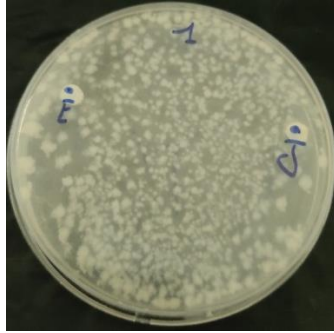
## 7. Antibacterial activity

**Table 14-** The names and the gram of the bacteria and the results of antibacterial activity

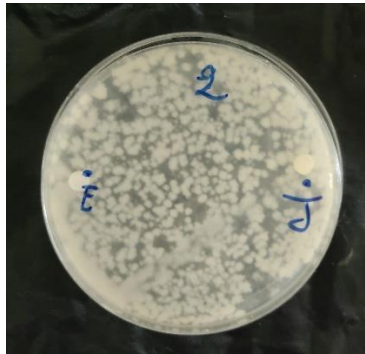
N °	Name	Gram	I zone	Sensibility
1	<i>Staphylococcus aureus</i>	+	11mm	Sensitive
2	<i>E. coli</i>	-	7mm	Sensitive
3	<i>Pseudomonas aeruginosa</i>	-	6mm	Resistant
4	<i>Salmonella typhimurium</i>	-	14mm	Sensitive
7	<i>Serratia marcescens</i>	-	6.5mm	Sensitive
8	<i>Proteus mirabilis</i>	-	6mm	Resistant

The sap extracted from the cladodes of the prickly pear showed antibacterial activity against some of the bacteria tested, such as: *Staphylococcus aureus*, *E. coli*, *Salmonella typhimurium*, *Serratia marcescens*. The reading was made by measuring the diameters of the inhibition halos around the discs using graph paper. The results are expressed by the diameter of the inhibition zone. 6 mm it is disc diameter.

E: Water; J: Juice.



**Figure 23-** *Staphylococcus aureus* antimicrobial activity result



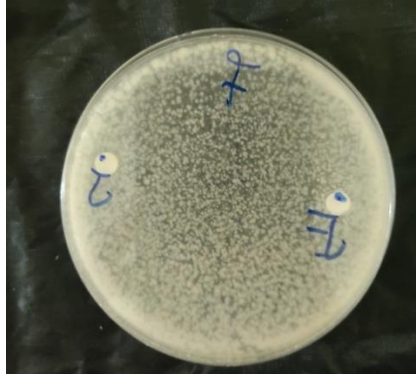
**Figure 24-** *E. coli* antimicrobial activity result



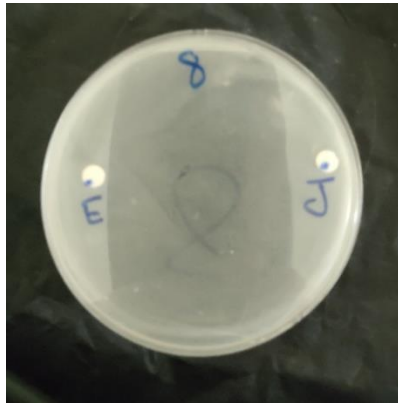
**Figure 25-** *Pseudomonas aeruginosa* antimicrobial activity result



**Figure 26-** *Salmonella typhimurium* antimicrobial activity result



**Figure 27-** *Serratia marcescens* antimicrobial activity result



**Figure 28-** *Proteus mirabilis* antimicrobial activity result

## • OILS PART

### 1. Net weight of oil extracted by soxhlet

- From 30g of prickly pear seeds flour we extracted 1.45g of oil it means 1600µl.

- The oil content of prickly pear seed flour can also be expressed in relation to dry matter, by the relationship:  $\rho_s (\%) = \frac{m1}{m2} \times 100$

m1: mass of oil extracted (g)    m2: the mass of the dry test portion of the prickly pear seeds flour (g).    So:  $\rho_s = 4.83 \approx 5\%$     **Oil yield is 5%**

### 2. Reading samples of antimicrobial activity

The antibiogram consists of looking for the sensitivity of the strains to antibiotics. The table below reports the zones of inhibition by (mm) reached with the various strains studied; 6 mm it is disc diameter.

Table 15-Results reports the zones of inhibition by (mm) reached with the various strains studied.

extracts Bacteria	Gram	Soxhlet oil	cold pressed oil	oil pressed at 90°	oil pressed at 160°
<i>Staphylococcus aureus</i>	+	6	6	6	6
<i>Escherichia coli</i>	-	6	6	13	11
<i>Pseudomonos</i>	-	6	18	19	16
<i>Salmonella</i>	-	6	14	12	6
<i>Sarritia Marcescens</i>	-	16	6	6	6
<i>Proteusmirabilis</i>	-	6	6	6	6
<i>Klebsilla pneumoniae</i>	-	6	11	13	13

The reading was made by measuring the diameters of the inhibition halos around the discs using graph paper. The results are expressed by the diameter of the inhibition zone and could be symbolized by signs of "after the sensitivity of strains to extracts".

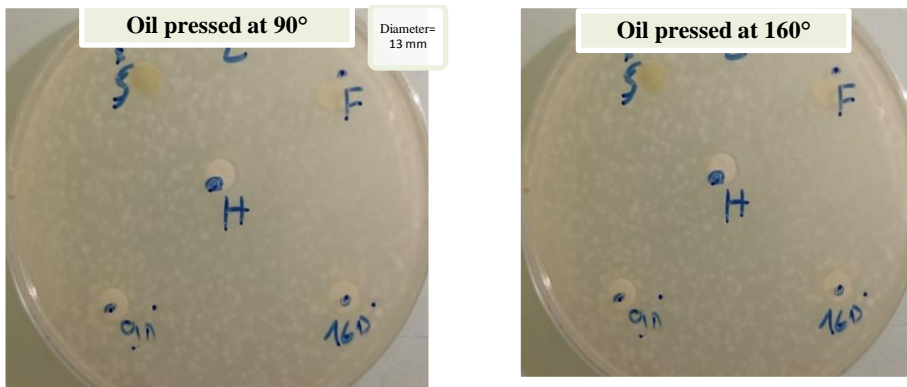


Figure 29- *Escherichia coli* antimicrobial activity result

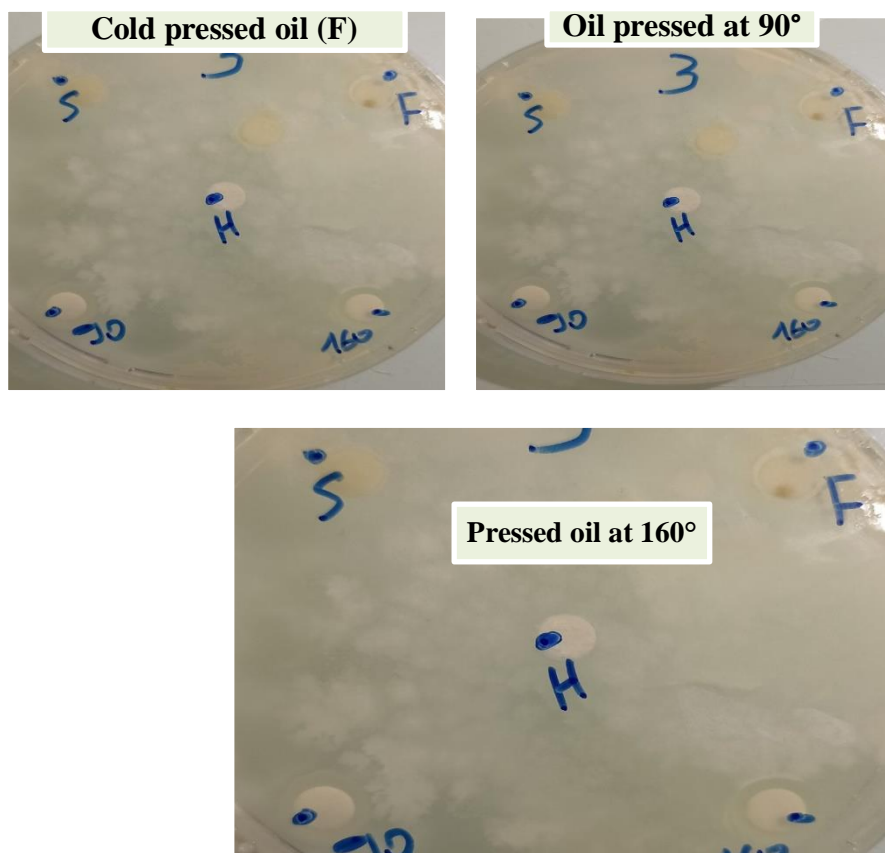


Figure 30- *Pseudomonas* antimicrobial activity result

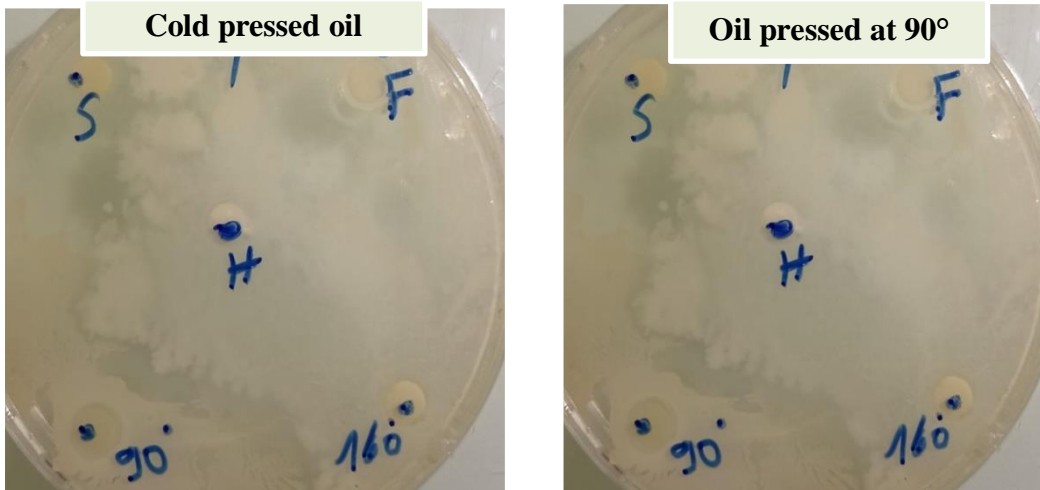


Figure 31- *Salmonella* antimicrobial activity result

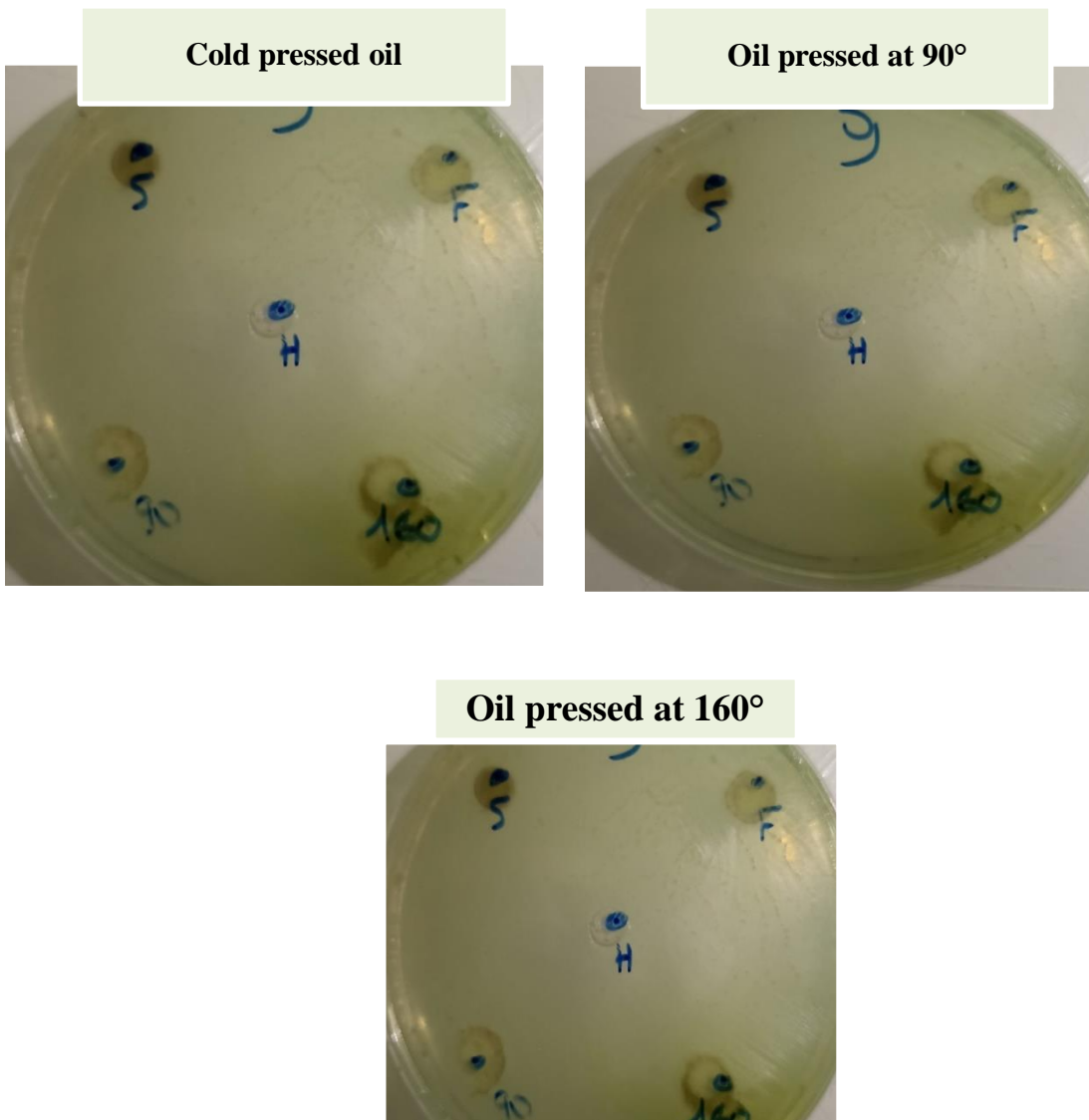


Figure 32- *Klebsilla pneumoniae* antimicrobial activity result

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### Soxllhet oil

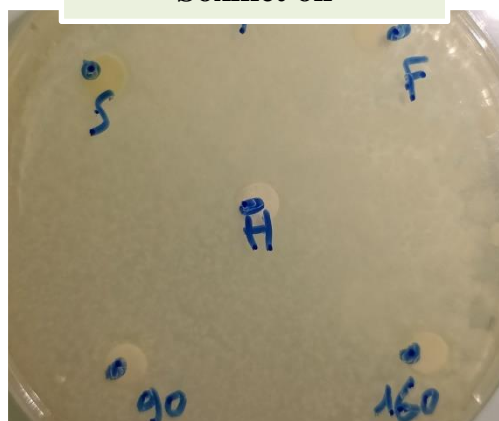


Figure 33-*Serratia Marcescens* antimicrobial activity result

The sensitivity of the bacteria to the oily extracts is determined according to the diameter of the inhibition halo by the agar diffusion method. The results show that all the oily extracts of *Opuntia* seeds proved to be inactive against *Staphylococcus aureus* and *Proteus mirabilis* (6 mm); these bacteria have a very high resistance potential, compared to the results of **Benattia (2017)** her oily extracts of the same species is inactive against more to the two bacteria that we mentioned previously, plus to *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsilla pneumoniae*, and *Micrococcus luteus*. The optimal effectiveness of an extract may not be due to a main active constituent, but to the combined action (synergy) of different compounds at the origin of this extract (**Essawi and Srour, 2000**).

On the other hand, the strain of *Escherichia coli*, *Pseudomonos*, *Salmonella* and *Klebsiella Pneumonia* showed relative resistance to soxllhet oil, the same for the strain *Escherichia coli* and *Serratia Marcescens* against cold press oil. Several studies have highlighted the high sensitivity of Gram (+) bacteria compared to Gram (-) (**Turkmen et al., 2007** and **Falleh et al., 2008**), this can be attributed to the difference in the outer layers of Gram (-) and Gram (+) bacteria. These works are in agreement with our results.

Cold pressed oil, pressed oil at 90° and pressed oil at 160° this 3 samples are the most oils have proven their strength against *Escherichia coli*, *Pseudomonos*, *Salmonella* and *Klebsilla pneumoniae*; diameter between 11 and 19.

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Our results are not in agreement with the results of **(Benattia, 2017)** who worked on prickly pear seeds, his results show that all *Opuntia* seed extracts proved to be inactive against *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. The antimicrobial effectiveness of *Opuntia ficus indica* oil could be explained by its richness in phytosterols and in particular in beta-sitosterol. It has been described that this sterol can inhibit the growth of certain microorganisms, possibly by interfering with the sterols of the cell membrane, thus altering its permeability to nutrients, *which could disrupt vital cellular pathways and thus stimulate pathogenic cellular necrosis (Ogbe et al., 2015)*.

### 3. Reading sample of antifungal activity

The results of the antifungal activity of the oils showed that the extract had no significant effect on the fungus: *Aspergillus Niger*.

**Khemiri et al. (2019)**, who did a study on the antimicrobial and wound healing potential of *Opuntia ficus indica* oil extracted from Tunisia, showed more resistant results.

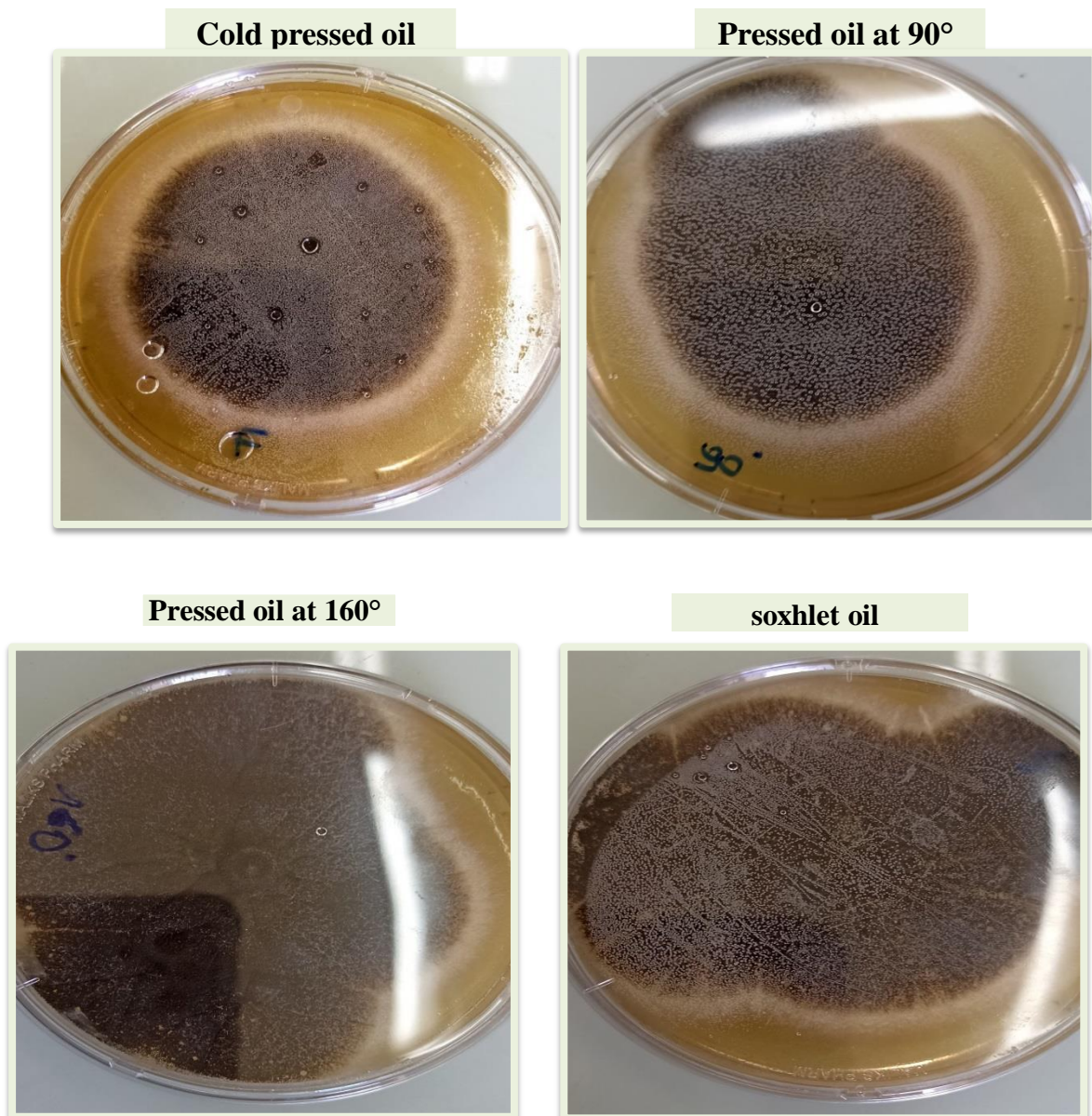


Figure 34- Effects of *Opuntia* seed oils against the fungus *Aspergillus Niger* after 5 days.

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The results presented below support the findings described above regarding antifungal activity; where we see a rapid and terrible spread of *Aspergillus Niger* to the detriment of the oils studied.

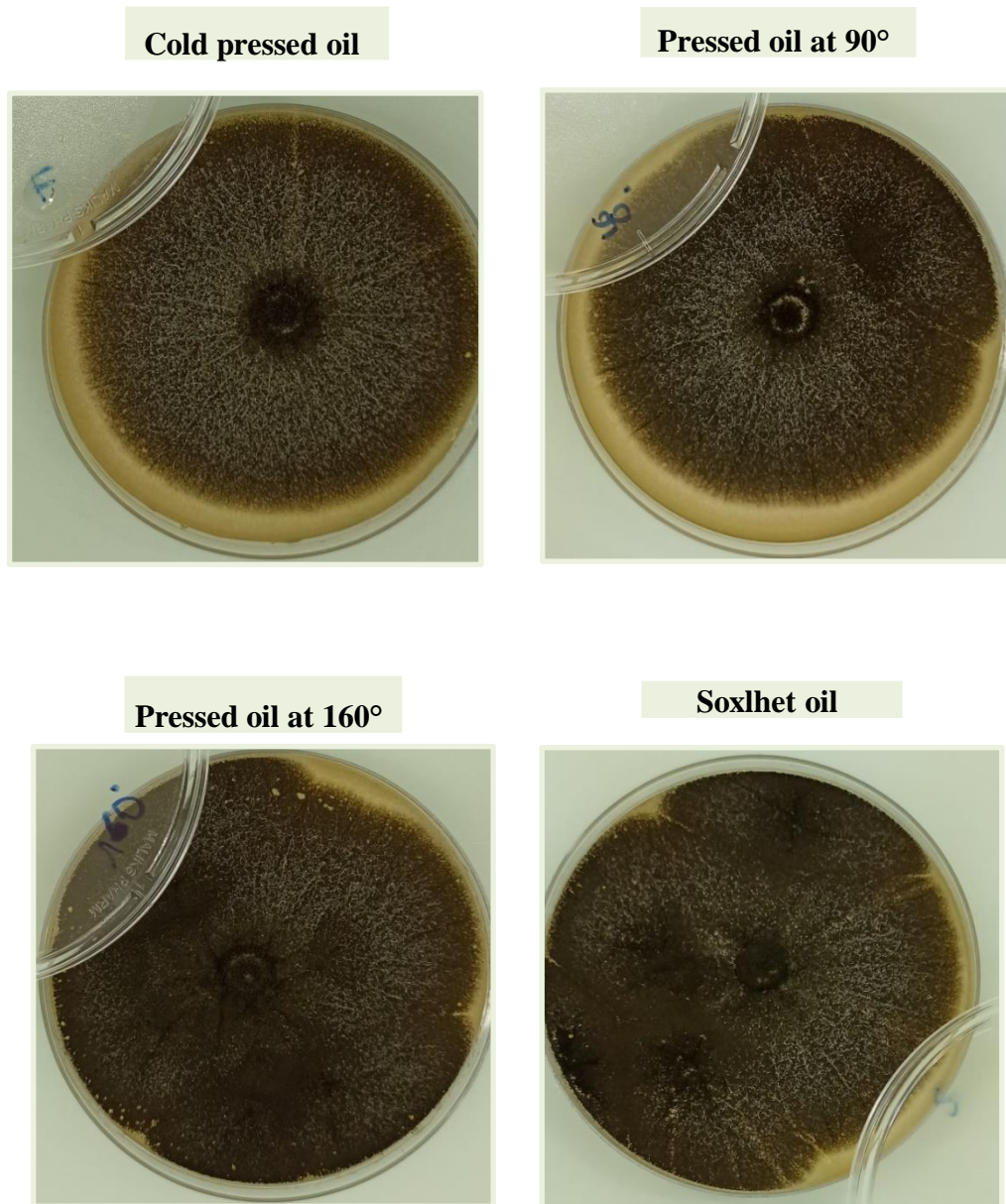


Figure 35- Effects of Opuntia seed oils against the fungus *Aspergillus Niger* after 7 days.

# GENERAL CONCLUSION AND PERSPECTIVES



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## Conclusion

*Opuntia ficus indica* is a type of cactus that can survive with very little water and withstand prolonged droughts. The development of this plant, especially the formulation of the juice extracted from its cladodes, will contribute to the development of drylands by creating businesses and jobs. The cactus *Opuntia ficus-indica* (thornless) is the subject of this work. The yield of cladodes juice is about 79.02%. Rainwater is the only source of water for these plants, thus affecting their water storage capacity. Thanks to its thorns which reduce the surface of exposure to the sun. The Water content in the juice is (9.1g / 10g) and ash content (0.95g / 10g). Its pH varies between  $4.8 \pm 0.01$  therefore the juice has an average acidity. Based on some of the tests we've done with it, phytochemical screening results show that prickly pear juice is rich in carbohydrates, flavonoids, phenols, tannins, and coumarins. To improve fruit juices, analyses have been performed, which are becoming increasingly important in the medical and cosmetic fields, including the determination of TPC, flavonoid content, antioxidant activity and antibacterial activity. The natural antioxidants of fruit juices were tested for their ability to scavenge free radicals using the DPPH method. The results obtained show that the sap from the leafy branches is rich in phenolics and flavonoids, with strong antioxidant and antibacterial activities in some strains, while: the TPC of cladodes juice is 4.6g / 100g and the content of flavonoids is 38.35 mg / 100g, and according to the comparison between IC50 of our extract with the white found that the juice of prickly pear cladodes has great antioxidant activity while our extract showed antibacterial activity on some bacteria. Prickly pear seeds were harvested in summer 2021 in the West region (Ain Defla), the seeds oil yield is too low (5%) so it needs a huge quantity of seeds about 30 kg to have just 1 L of oil. The strain of *Escherichia coli*, *Pseudomonas*, *Salmonella* and *Klebsiella Pneumonia* showed relative resistance to soxhlet oil, the same for the strain *Escherichia coli* and *Sarritia Marcescens* against cold press oil. Cold pressed oil, pressed oil at 90° and pressed oil at 160° these 3 samples are the most oils have proven their strength against *Escherichia coli*, *Pseudomonas*, *Salmonella* and *Klebsilla pneumoniae*; diameter between 11 and 19. This work allowed us to create a new database on Cladodes juice and its uses. It affects three main industries: food, cosmetics and pharmaceuticals. Therefore, cactus cladodes are a versatile raw material.

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# APPENDIX



## APPENDIX 1: Preparation of test solutions

### 1. Iodine Solution

Potassium iodide (KI)	10 g
Iodine	5 g
Distilled water	100 mL

Dissolve KI in about 20-30 ml of distilled water. Add iodine and heat gently with constant mixing until iodine is dissolved. Dilute to 100 ml with distilled water. Store in amber glass-stoppered bottle in the dark. **Bacteriological Analytical Manual, 8th Edition, Revision A, 1998.**

### 2. Fehling's reagent:

copper sulphate	3.47 g
potassium and sodium tartrate	17.3 g
NaOH	5 g
distilled water	20 ml

**Preparation of solution A:** in a beaker, put 3.47 g of copper sulphate and add distilled water to obtain a final volume of 10 ml.

**Preparation of solution B:** in a beaker, put 17.3 g of potassium and sodium tartrate with 5 g of NaOH and add distilled water to obtain 10 mL (**Shaikh and Patil, 2020**).

### 3. 5% KOH solution:

Make sure that KOH you have is in powdered form

KOH	5 g
distilled water	100 ml

- Measure 5 g of KOH using a chemical balance and transfer it to a watch glass.
- Prepare a setup of a volumetric flask (One that's able to measure 100ml) with a funnel.
- Wash the powder into the flask
- Fill the flask with distilled water up to the 100ml mark (The meniscus of the solution should be at 100ml).
- Mix the solution in the flask thoroughly. And then the 5% KOH solution is ready.

#### 4. 10 % NH<sub>4</sub>OH solution

NH <sub>4</sub> OH	10 g
distilled water	100 ml

- Measure 10 g of NH<sub>4</sub>OH using a chemical balance and transfer it to a watch glass.
- Prepare a setup of a volumetric flask (One that's able to measure 100ml) with a funnel.
- Wash the powder into the flask
- Fill the flask with distilled water up to the 100ml mark (The meniscus of the solution should be at 100ml).
- Mix the solution in the flask thoroughly. And then the 10% NH<sub>4</sub>OH solution is ready.

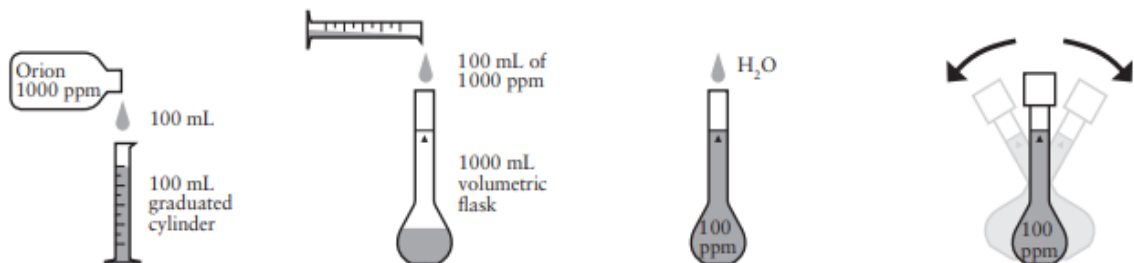
#### 5. Ferric chloride solution.

Ferric chloride FeCl<sub>3</sub> is conventionally prepared in an anhydrous form by allowing a chlorine gas to affect glowing iron. As a solution it is in turn obtained by dissolving an iron oxide or carbonate or a metallic iron in a hydrochloric acid or nitrohydrochloric acid.

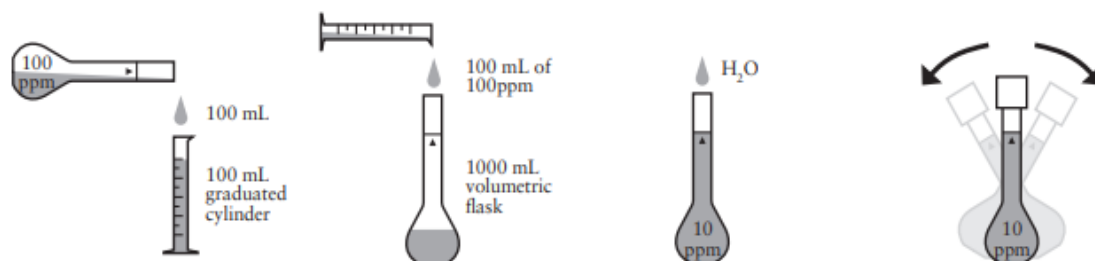
#### 6. 10% ammonia

Using a graduated cylinder

- a) Prepare a 100 ppm ammonia standard by measuring 100 mL of the 1000 ppm ammonia as nitrogen (N) standard (Orion 951007) using a graduated cylinder. Add the 100 mL measured to a 1000 mL volumetric flask. Add 900 mL of distilled/deionized (DI) water, diluting to the mark indicated on the flask. Mix the solution well. **Water Analysis Instruments, Thermo Fisher Scientific**



- b) Prepare a 10 ppm ammonia standard by measuring 100 mL of the 100 ppm ammonia standard from Step 1 using a graduated cylinder. Add the 100 mL measured into a 1000 mL volumetric flask. Add 900 mL DI water, diluting to the mark indicated on the flask. Mix the solution well.



## 7. 0.5M sodium bicarbonate solution

<b>NaHCO<sub>3</sub></b>	<b>42 g</b>
<b>NaOH</b>	<b>0.72 g</b>
<b>de-ionised water</b>	<b>900 ml</b>
<b>NaOH or concentrated H<sub>2</sub>SO<sub>4</sub></b>	<b>/</b>

0.5M sodium bicarbonate solution: Dissolve 42 g sodium bicarbonate (NaHCO<sub>3</sub>) and 0.72 g sodium hydroxide (NaOH) pellets in 900 ml of de-ionised water. Adjust the pH to 8.5 using a saturated solution of NaOH or concentrated H<sub>2</sub>SO<sub>4</sub> and make up to 1 litre with de-ionised water.

## APPENDIX 2:

- Determination of Total phenolic content (TPC) by Folin–Ciocalteu’s assay.** The total phenolic content was expressed as gallic acid equivalents in mg per g of dry extract (GAE/gDE), obtained from the standard curve based on gallic acid. Where: A blank= is the absorbance of the control reaction at t=0 (containing all reagents except the test compound), and A sample: is the absorbance of the test compound after 60 min.

The TPC was calculated by the following formula:  $C=c \times V/m$

where C is the concentration of gallic acid, determined from the calibration curve (mg/mL);

V is the volume of sample solution (ml);

m is the weight of sample in test (g).

Gallic acid solutions with concentration ranging from 0–400 mg·L<sup>-1</sup> were used to obtain the calibration curve.

## 2. Determination of Flavonoids contents

Dissolved 1 mg extract in 1 ml MeOH.

Total flavonoid content of extracts was expressed as equivalent of quercetin (QE) in mg per g extract in dry weight basis.

Total flavonoid content is calculated by using the formula:  $C=cV/m$  where,

C=total flavonoid content mg QE/g dry extract,

c=concentration of quercetin obtained from calibration curve in mg/mL,

V=volume of extract in mL,

m=mass of extract in gram.

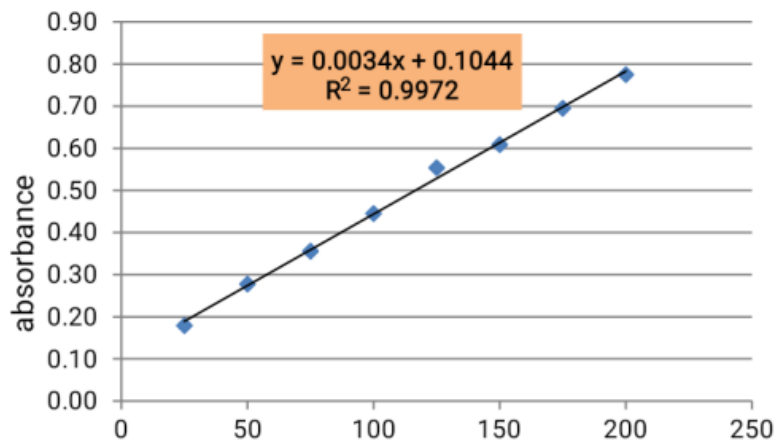
## 3. Free radical scavenging activity (DPPH assay) of extracts

The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation: % inhibition=  $100 (A(\text{blank})-A(\text{sample})) / A(\text{blank})$ .

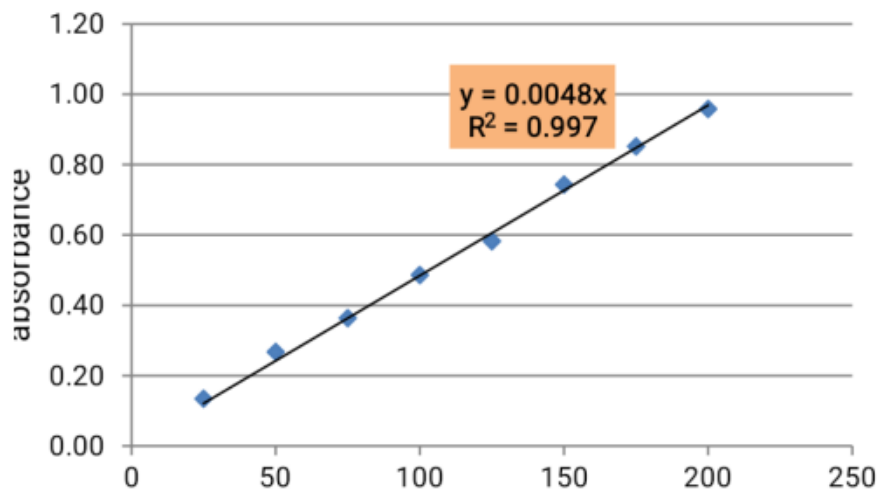
Antioxidant activity of the samples was expressed as IC<sub>50</sub>, defined as the concentration of the test material required to cause a 50 % decrease in initial DPPH concentration. Results were expressed in mg Trolox equivalents (TE)/g of samples.

### APPENDIX 3:

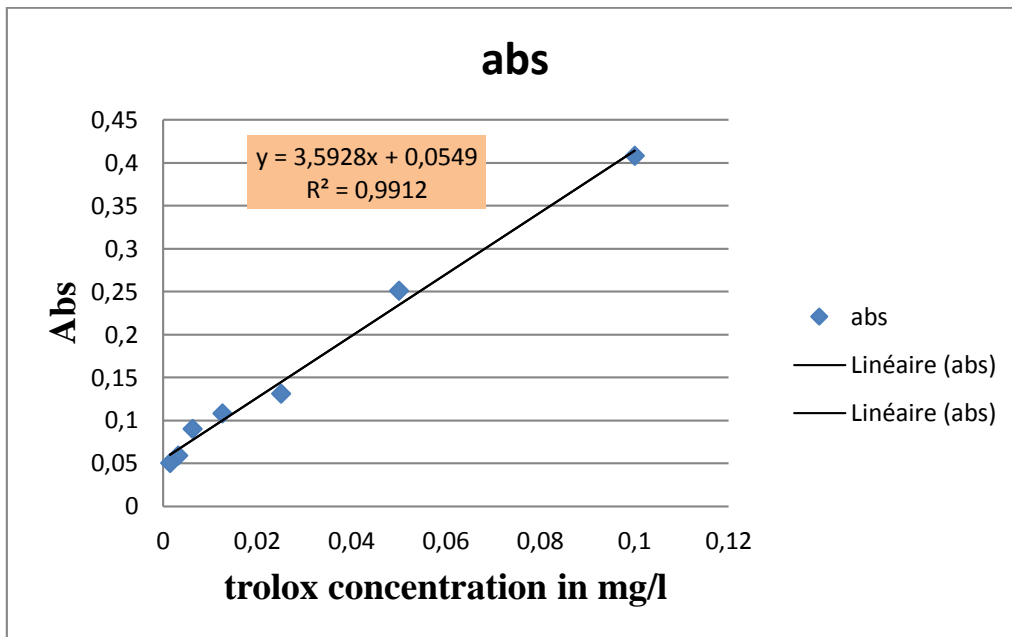
- **Galic acid calibration curve:**



- **Quercetin calibration curve:**



- Trolox calibration curve:



- Inhibition % calibration curve:

