الجمهورية الجزائرية الديمقراطية الشعبية

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

وزارة التعليم العالي والبحث العلمي

MINISTRYOF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

جامعة الاخوة منتوري كلية علوم الطبيعة و الحياة

**Brothers Mentouri, Constantine1 University - Faculty of Life and Natural Sciences**

قسم الكيمياء الحيوية والبيولوجيا الخلوية والجزيْئية

**Department of Biochemistry, Molecular and Cellular Biology**

**Thesis submitted for Master Degree in Natural and Life sciences**

**Feild: Biochemistry**

**Titled:**

# Phytochemical study and biological applications of *Opuntia ficus-indica* as an anti-aging agent.

**Presented by:** **On: / / 2021**

*GHARSELLAH Khaoula*

*BOUKAOUS Racha El Bahaa*

**Examination board:**

**President:** *Moussawi Samira* (MCB - UFM Constantine 1).

**Supervisor:** *MOUAS T. Nardjes* (MCA - UFM Constantine 1).

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**2020/2021**

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# ACKNOWLEDGMENTS:

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We cannot forget to have a glance at all our teachers from kindergarten whose priceless teachings lead us to this day.

# DEDICATION:

*I want to dedicate this humble work to those I love, my dear. Family , my father Elarbi and my mother Boufrioua Roukaia who were always supporting me to give the best I can , without forgetting my sisters Lynda , Assia , Kamillia ,Leila and my brother Mouhammed , those who I love and I appreciate their offering of positive energies .*

*I dedicate this work and give special thanks to my best friend Khaoula.*

*A special wink to my beloved nephew Mounib also Rouaa, Layen and Miral.*

*Racha* ♥

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*I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my lovely parents, Houda and Saleh whose words of encouragement and push for tenacity ring in my ears.*

*My sisters Sarah, Raitha, my brother Amine have never left my side and they are very special.*

*I also dedicate this dissertation to all my friends who have supported me throughout the process especially Racha.*

*A special thanks to my cousins Mohammed and Ranim* ♥

*Khaoula* ♥

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## LIST OF ABREVEIATIONS:

| | |
|---|---|
| **A05** | Absorbance at 0,5 µg/mL |
| **ABTS** | 2,2-azinobis 3-ethyl benzothriazolin -6-sulfonic acid |
| **Abs** | Absorbance. |
| **CUPRAC** | Cuprac ion reducing antioxidant capacity |
| **DF** | Dietary fibers |
| **DPPH** | 1,1-Diphenyl-2-pic-ryl-hydrazyl (DPPH) |
| **DE** | Dry Extract. |
| **GAE** | Gallic Acid Equivalent |
| **HPLC** | High Performance Liquid Chromatography |
| **IC50** | The half maximal inhibitory concentration |
| ***In vivo*** | Within a living organism |
| **NLCs** | Nanostructured Lipid Carriers |
| **SLNs** | Solid lipid nanoparticles |
| **OFHE** | *Opuntia ficus* hexane extracts |
| ***OFI*** | *Opuntia ficus indica* |
| **PP** | Prickly Pears |
| **NO** | Nitric oxide |
| **ROS** | Reactive oxygen spices |
| **TPC** | Total Phenolic Content |

# General introduction

**Introduction:**

Nobody can escape from aging, although “Wrinkled, sagging skin is not the inevitable result of getting older. It’s a disease, and you can fight it” Nicholas Perriconea prominent American dermatologist **(Gerald.E et al)**, we can fight the process of aging by natural cosmetic products based on natural ingredients from medicinal plants as prickly pears instead of chemical compounds like synthetic antioxidants (BHA , BHT…) which could be harmful on the skin in long term.

The world of cosmetics is characterized by a constant search for new compounds which are able of satisfy customer demands and high expectations. The worldwide economic success of cosmetic argan oil has encouraged the search for other oil seeds, which present sufficiently similar chemical profile as prickly pear oil seeds, which is currently receiving a lot of attention and cactus oil has only recently entered the cosmetic market and in the future cactus seeds can be a substitute for argan oil as an ingredient in cosmetic products of antiaging (**Ciriminna et al., 2017; Guillaume et al., 2015).** Economically, cactus seeds that have been simply considered as waste for years, now contain the "most expensive oil in the world" with market prices possibly reaching 500 €/L vs 120 €/L for argan oil, which previously held this title. In addition, prickly pear plant is ubiquitous on the contrary; argan is specific in Morocco **(S. Gharby et al, .2021)**.

Currently, prickly pears have long neglected, now it is the subject of many scientific research around the world. Apart from oil, which is the most expensive in the world, due to the richness of trace elements, amino acids, therefore cell regeneration, anti-aging, Nopal have many pharmacological properties that are still being exploited and studied **(Benattia, 2017).** *Opuntia ficus-indica* communally known as prickly or cactus pear, belong to the cactaceae family are originated from Central America (Mexico); it is a tropical and subtropical plant that grows in arid and semi-arid climates. The different part of this plant are rich in bioactive compounds especially antioxidant ones that are effective in many fields such as cosmetic, medicine , industry (pigments) and nutrition (**El-Mostafa et al, 2014).**

The demand for natural antioxidants is increasing day by day, and consumers becoming more conscious; so they demand more natural sources in cosmetics and food **(Aditya S et al, 2020).**

In the frame of valorization of *OFI*, there is important benefits in different parts of it:

This study aims to valorize different parts of *OFI* as natural sources in different fields and demonstrate their biological activities.

**The first part** divided in three chapters, the first one is about:

**Chapter one:** generalities about skin and aging, dermocosmetic based on natural extracts of OFI.

**Chapter two:** generalities, taxonomy, origin and biological activities of *OFI.*

**Chapter three:** biochemical analysis principals

**The second part** represents the experimental part contains two chapters:

**Chapter one:** describe the experimental and all technics and methods used for biochemical study which includes extraction, colometric assay and antioxidants activities.

**Chapter two:** a phytochemical study, which discuss results of biological activities.

The third part represents the conclusion and perspectives about valorization of *OFI*.

# Chapter 1:

# Dermocosmetic and Opuntia ficus-indica

## I.1 Human skin:

Human skin is the largest epithelial surface with a weight and surface in the order, of about 4kg and 1.8 m², 15% of the total body weight in adult humans. It is the widest organ in the organism. The constitution is approximately the same on the whole body, but still exist variations especially concerning the thickness, components and the way, variety of the implantation of appendices, theses varieties provides a perfect adaptation **(Khavkin, J et al., 2011, Barel, A. O., 2014).**

Skin is organized into 3 layers: the epidermis, thedermis, and the hypodermis (Fig. 1).

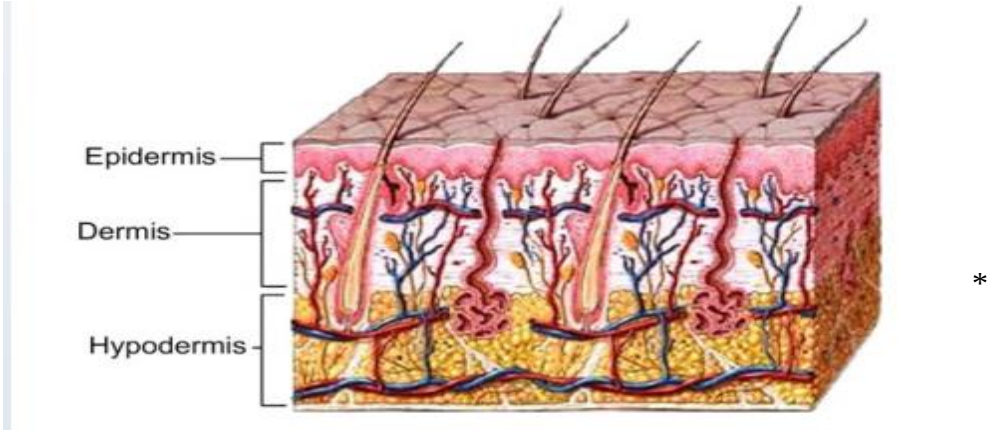


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**Figure 1:** The3 layers of the skin: epidermis, dermis, and hypodermis **(Khavkin, J et al., 2011).**

### I.1.1 Classification skin types:

There are different classifications have been proposed, they all privilege specific criteria, which leads to four mains types: normal skin, dry skin, oily skin and mixed skin, this criteria is based on the feeling not the causes **(Barel, A. O., 2014).**

- **Normal skin:**

There is no definition to normal skin contrary to the other types, simply it is not dry or even oily or mixed and no more pathological skin. There is a very thin protective epithelium that constitutes the epidermis. It plays the main part in protecting the organism against external aggressions assured by the cohesion between epithelium cells and keratinocytes. The latters migrate from the dermo epidermal junction to the skin surface caused by desmosomes. At

cosmological level, we must be content with a less strong definition and considernormal skin as a young skin, structurally and functionally balanced and requiring no careapart from those necessary for its cleaning**(Barel, A. O., 2014).**.

### I.1.2 Skin aging and causes:

All living organisms are programmed for aging, nobody can escape from it.
In human begins, aging is a physiological process corresponding to progressive homeostatic capacity of the body systems. However, it is evident that the process develop differently among individuals of the same age. In any given subject, senescence is heterogeneous among organs and among their constitutive tissues, cells, and subcellular structures. In addition, the process is different between individuals and even between organs, cells and tissues.
Many in vitro studies showed that the age of any tissue depends on the behavior of skin-derived cells and aging problem is even more complex and sever in cases where the skin has lost its protective mechanical roles **(Barel, A. O., 2014).**

There are two main processes that induce skin aging: extrinsic and intrinsic caused by extrinsic and intrinsic factors. Extrinsic factors are: sun which is responsible of photo aging also the air, these two factors are related to the environment, unhealthy food and smoking accelerate the process of aging. Intrinsic factors are related to genetics, and biological modifications: physiological, histological and biochemical ones. Mechanisms for aging skin include the actions of reactive oxygen species (ROS), mtDNA mutations, and telomere shortening, as well as hormonal changes **(Tobin, D. J.,2017).**

## I.2 Definition of dermocosmetics:

Dermocosmetics is a science which is now a branch of dermatology using cosmetics of different skin disorders to preserve the aesthic appearance of the skin by using them for ameliorate photo protection dry or aged skin, inflammatory skin, illnesses like acne, rosacea, dermatitis nail or hair developed regulating in ways comparable to those of medicinal products and now these products are important for the dermatologists **(dreno., 2014).**

## I.3 Development of new techniques in cosmetic research:

The cosmetics industry developed many techniques used in research of cosmetics for understanding the normal physiology of skin , hair and nail after that lot of these techniques become basic methods used for inventing and evaluate new products as an example the principal of photostable sunscreens which was logged to dermatology by cosmetics research

and worldwide, mandatory testing now exists with decided standards for assessing the photostability of UVA and UVB sunscreens along with their safety and efficacy .

Most of dermocosmetics ingredients are passed in vitro for measuring their specific effect on protein and gene expression .then finished products are assessed through non-invasive in vivo techniques.

**I.4 Development of dermocosmetic formulation:**

The goal of developing a cosmetic formulation from solid pestes to emulations moreover aqueous lotions is to guarantee that its active ingredient are bioavaible and stable on skin of the user without messing the role of maintain the required physical chemical also microbial quality standards when stocked under appropriate conditions .

For achieving the appropriate equilibrium between the skin delivery and bioavaibility of active ingredients we use the identification also the final formulation of product should ensure the long-term stability up to three years.

**I.5 The use of *Opuntia ficus-indica* in dermocosmetic:**

**I.5.1 Production of Moisturizers and hydration products:**

The plant of OFI is too famous and has a big scienceeconomic importance to the semiarid region because of its Cladodes which contain (80-90%) of water and carbohydrates (3-7%) are galacturonic acid glucose rhamnoose, arabinose, more over phenolic components like kaempherol and quercetin.

The cosmetic industry made those corposants as moisturizers also as anti-aging products. These moisturizers are not curative but preventive cosmetic products against xerosis and delaying of premature ageing without missing their dermatological role in wide variety of skin disorders.

Furthermore, ruin evaporation of skin moisture by made an epiculaneous lipoic film that stopped the water loss.

Moisturizers has also occlusive mechanism ,the activation of hydration can be also made by moisturizing that’s happen if re-hydratate the stratum corneun and forming aquaporin which are transmembrane proteins form water channels and facilitate water flux through the cell plasma membrane **(Deters,2012).**

Polysaccharide fraction in Cladodes powder have the ability of the mucilage fraction to fix the dermal tissues with the made of physical barrier on the skin and deep hydration**, (Missaoui, 2020).**

### I.5.2 Cosmetic Nanoemulsions:

Nanoemulsions are attractive systems for using in the cosmetics, pharmaceutical, food and other industries up to their low amount of surfactant, higher stability against coalescence, lack of toxicity or irritant characteristics, low viscosity, good appearance, moreover versatility of formulation as foams, creams, liquids and sprays. Nanoemulsions are particularly useful systems for cosmetics because the small droplet size ensures a closer contact with the stratum corneum (SC), rising the amount of active compound reaching the desired site of action also, nanoemulsions can carry actives into the skin improving the skin layer penetration, and enhancing efficacy.

Dry skin is a common problem in the population. Its main characteristic is a rough appearance and peeling, we called that phenomenon xerosis. The barrier function and maintenance of the water content are dependent on the SC that provides mechanical protection in addition act against water loss and the passage of external solutes. Changes in the stacking of corneocytes and delipidation are causes of cutaneous dryness leading to decreased skin flexibility and harming the skin's protective barrier.

These systems can be made by high or low emulsification energy methods.

High-energy approaches utilize mechanical devices (microfluidizers, high pressure, Homogenizers or ultrasonic methods) which generate intense forces capable of making very fine oil droplets.

**Table 1:**Nanoemulsion compositions **(Rendo cezar de Azevedo Ribeiro, 2015).**

| Components | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | FX | FXE |
|---|---|---|---|---|---|---|---|---|---|---|
| **BHT** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Caprylic/Capric triglyceride** | 2.0 | 4.0 | 8.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 |
| **Ethylhexyl palmitate** | 2.0 | 4.0 | 8.0 | 1.0 | 1.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 |
| **C12-C15 Alkyl benzoate** | 1.0 | 2.0 | 4.0 | 0.5 | 0.5 | 1.0 | 1.5 | 0.5 | 1.0 | 1.0 |
| **Paraffinum liquidium** | 5.0 | 10.0 | 20.0 | 2.5 | 2.5 | 5.0 | 7.5 | 2.5 | 5.0 | 5.0 |
| **Phenoxyethanol and caprylic Glycol** | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| **Sorbitan Oleate** | 4.7 | 4.7 | 4.7 | 4.7 | 2.4 | 2.4 | 2.4 | 1.4 | 4.7 | 4.7 |
| **Polysorbate 80** | 5.3 | 5.3 | 5.3 | 5.3 | 2.6 | 2.6 | 2.16 | 1.6 | 5.3 | 5.3 |
| **EDTA** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Xanthan Gum** | .. | .. | .. | .. | .. | .. | .. | .. | 0.5 | 0.5 |
| **OFI (L) extract** | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1.0 |
| **Distilled Water** | 78.8 | 68.8 | 48.8 | 83.8 | 88.8 | 83.8 | 78.8 | 90.8 | 78.3 | 77.3 |

Notes: FX=sample added to 0.5% Xanthan Gum: FXE =sample added to 0.5% xanthan Gum and 1% *Opuntia ficus-indica* extract.

This study aimed to produce and characterize an oil in water (O/W) Nanoemulsion containing *Opuntia ficus-indica* (L.) Mill hydro glycolic extract, as well as evaluate its preliminary and accelerated thermal stability and moisturizing efficacy **(Rendo cezar de Azevedo Ribeiro, 2015).**

### I.5.3 Incorporation of prickly pear seed oil in the encapsulation of vitamin A:

A study demonstrates the advantage of using prickly pears (pp) seed oil to develop Nanostructured lipid carriers NLCs incorporating vitamin A, in comparison with SLNs Solid lipid nanoparticles free from *Opuntia* seed oil.

NLCs formulations containing vit A pp seeds oil incorporation have shown superior actions than SLNs carries that are without pp seeds oil, this was correlated with the availability of pp seeds oil, also it keeps the perfect state of the crystalline form state which provide more flexibility and exposition , well drug loading proved by many techniques such as ex vivo, this latter encourage the use of pp seed oil in cosmeceutical applications **(AlZahabi et al,.2019).**

# Chapter 2:

# Generalities about Opuntia ficus-indica.

## II.1 Morphology of *Opuntia ficus-indic*a:

The genus *Opuntia* contains about 300 species many of them produce very tender and edible stems and fruits **(Hegwood, 1994**).

Among these species, we select here *Opuntia* ficus-indica.it is used as fodder for livestock. It is interesting because of the environmental conditions in which it is develops and its resistance to extreme climatic conditions **(Hernández-Urbiola and al, 2011).**

**Figure 2:** *Opuntia ficus-indica* plant

### II.1.1 Stem:

Long stems measuring up 3 to 5 m high, matte green color, a languor of 30 to 50 cm and a width of 15 to 30 cm, (figure 5) **(Halmi,.2014).**

### II.1.2 Cladodes:

Snowshoes, called cladodes, are 30 to 40 centimeters long, 15 to 25 cm wide and 1.5 to 3 cm thick. Green in color, they unite with each other, by forming kinds of branches.

**Figure 3**: *Opuntia ficus-indica* cladodes.

They are covered with a waxy cuticle (the cutin) that limits the transpiration of the plant and protects it while ensuring the chlorophyllic instead of leaves.

### II.1.3 Flower:

They are marginal on the summit of cladodes from 5 to 8 cm.

They are hermaphrodites; the prickly pear gives flowers and fruits in abundance. The flowers appear on the top of the rackets, about 4 to 10 cm their colors yellow, orange or red. These flowers are edible, like the fruit, which they give birth; yellowish-green skin is also adorned with small thorns.

In the temperate climates, flowering takes place in April, May. Cladodes can bear up to thirty flowers. **(Amale Boutakiout,.2015).**

**Figure 4**: *Opuntia ficus-indica* flower**.**

### II.1.4 Fruit (skin, seeds, juice):

Unilocular ovoid or piriformis provided thorns, they are inside greenish or yellow when they ripe **(Boutakiout, 2015)**.

**Figure 5**: *Opuntia ficus-indica* fruit**.**

*Opuntia ficus-indica* commonly called prickly pear or Nopal cactus belongs to the dicotyledonous angiosperm *Cactaceae* family, a family that includes about 1500 species of cactus. Most Cactus pears are widely distributed in Europe, Southwestern United States, Northern Mexico, much of Latin America, South Africa, and the Mediterranean countries.

Cactus pear (*OFI*) is commonly known as "prickly pear" and grouped under the Cactaceae family. (**El-Mostafa et al, 2014)**.

In some arid regions and hot, the plant can give fruit twice a year. Called prickly pear, this fruit has a flesh of a color varying from light yellow to purplish red and whose taste is reveals delicious and subtle.

The table below shows how the pulp of fruit rich of active compounds.

**Table 2**: Antioxidant and phytochemicals cactus pear pulp (**Tesoriere et al.2004).**

| Compound | Value for 100g pulp |
|---|---|
| **Vitamin C (mg)** | 29± 2 |
| **α- tochopherol (µg)** | 80 ±5 |
| **B – carotene (µg)** | 1.5 ±0.2 |
| **Betanine (mg)** | 1.21 ±0.15 |
| **Indicaxanthin (mg)** | 9.3 ±0.68 |
| **Polyphenols** | ND |

Note: ± Standard deviation of 5 determination performed in duplicate on 5 lots of fruits and, not detectable.

The fruit of *OFI* rich also of sterols showed in the following table.

**Table 3:** Distribution and contents of sterols (g/kg) in *Opuntia ficus-indica* fruit including pulp and skin **(Ramadan and Morsel 2003a, 2003b).**

| Main component identified | Pulp | Skin |
|---|---|---|
| **Campsterol** | 8.74 | 8.76 |
| **Stigmasterol** | 0.73 | 2.12 |
| **Lanosterol** | 0.76 | 1.66 |
| **β- Sitosterol** | 11.2 | 21.1 |
| **⌂ 5-avenasterol** | 1.43 | 2.71 |
| **Ergosterol** | - | 0.68 |

The fruits are picked at the end of July to September, as soon as they get a little soft **(Boutakiout ,. 2015).**

### II.1.5 Seeds:

The seeds of the fruit are rich in vitamins especially vitamin E and trace elements, confer many properties and it is from these seeds that one obtains a highly sought after oil **(Nature and Health Review, 2011).**

**Figure 6**: wet seeds in fruit of *Opuntia ficus-indica*

**Table 4**: Distribution and contents of sterols (g/kg) in *Opuntia ficus-indica* fruit including seed **(Ramadan and Morsel 2003a, 2003b).**

| Main component identified | Seed |
|---|---|
| **Campsterol** | 1.66 |
| **Stigmasterol** | 0.30 |
| **Lanosterol** | 0.28 |
| **β- Sitosterol** | 6.75 |
| **⌂ 5-avenasterol** | 0.29 |
| **⌂ 7-avenasterol** | 0.05 |

## II.2 Origin and Geographical Distribution :

The genus Opuntia, native to Mexico, appears on the emblem of the Mexican flag. Its geographical distribution is very wide: Mexico, Sicily, Chile, Brazil, Turkey, Korea, Argentina and North Africa **(figure 7**). It was introduced first in Spain and later in the 16th century to North and South Africa. It spread rapidly in the Mediterranean basin and became naturalized there to the point of becoming a characteristic element of the landscape.

It is essentially developed on the western part of the Mediterranean: southern Spain, Portugal, and North Africa (Tunisia, Algeria and Morocco). In some countries such as Italy, Spain or Mexico; cactus cultivation is practiced in an intensive and modern way with research and development programs for the production of the fruit or fodder and even for industrial uses.

On the other hand, in Australia and South Africa this plant, in particular the angiosperm variety is considered as a weed because of the ease with which it spreads **(Benattia, 2017).**

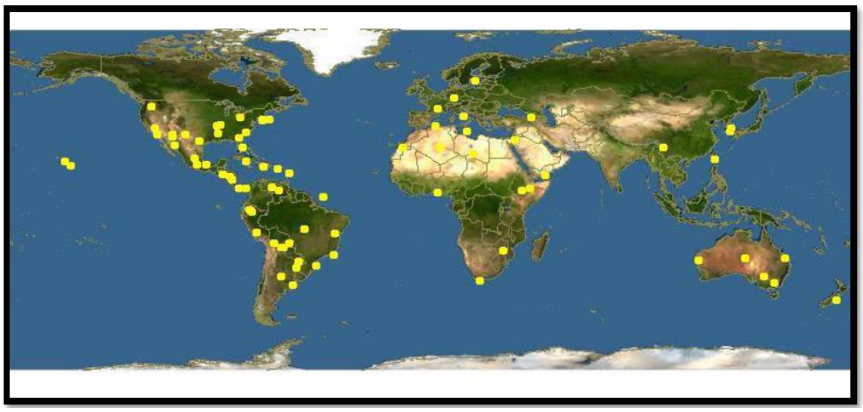


**Figure 7:** World distribution of *Opuntia ficus-indica* **(Benattia, 2017).**

## II.3 Taxonomic classification of *Opuntia ficus-indica*:

**Kingdom:** Plantae

**Subkingdom:** Tracheobionta

**Class:** Magnoliopsida

**Subclass:** Caryophyllidae

**Order:** Opuntiales

**Family:** Cactaceae

**Subfamily:** Opuntioideae

**Genus:** *Opuntia*

**Species:** *Opuntia ficus indica (L)* **(Wallace et Gileson, 2002).**

**II.4 Terminology and nomenclature of Opuntia ficus-indica:**

Prickly pears are known as different nouns in the world:

**In Mexico:** Nopal, the Mexican name of the plant, comes from the word Nochtliin Nahuatl, a classical language of the Aztecs.

**In Spain:** besides Nopal, Nopallito, it is colloquially called Nopalcito, Tuna, Ensada, Higos de Pala,and Higos of Mauro**(Schweizer, 1997).**

In Brazil, Palma de gado.

**In Andalusia:** Chumbera or Higuera Chumbra. **(Schweizer, 1997).**

**In countries Francophones of the Mediterranean Sea**: where it is very popular, the Opuntia is a namecomes from the Latin Opuntius, from Oponte**(Schweizer, 1997).**

**In Italy**: fico de India **(Schweizer, 1997).**

**In England:**Barbary fig, Devils tongue, Prickly pear **(Schweizer, 1997).**

**In Germany:**Feigenkaktus, Feigendistel, Indische Feige, Opuntie.

**In Holland:** Gewconevyg, Indiannschewyg.

**In Portugal:** Opuncia.

**En Egypt :** El-tin-el-Choki.

**In Morocco:** distinguish three varieties of O. ficus indica; he first, with prickly cladodes, is called “Christians’ nopal”,the second, with inermis cladodes, is “Muslims’ nopal” and serves as a green fodder for cattle. The last variety, with large inermis cladodes, is referred to as “Moses’ nopal”, grows essentially in the south of Morocco (Ifni region) and produces a big pear (**El-Mostafa et al, 2014)**. It is also called “Zaaboul”, “Aknari”in the south of Morocco and “hendia” or “hendi” in the north.**(Benattia, 2017).**

**In Algeria and Tunisia: "**hendia" or "hendi".**(Benattia, 2017).**

### II.5 Cactus in nutrition and prevention of diseases:

Cactus pears are rich in ascorbic acid, vitamin E, carotenoids, fibers, amino acids and high amount of glucose and fructose. This fruit is also rich in flavonoids, betaxanthins and betacyanins. These compounds shown interesting biological activities: anti-inflammatory, antioxidant, antimicrobial, anti-diabetic and neuroprotective effect (**El-Mostafa et al, 2014)**.

#### II.5.1 Source of natural colorants:

Beside the nutritional value of prickly pears (PP) fruit, it contains a high amount of natural pigments, which can be a good alternative for synthetic colorants. It provides different colors based on betalains, this last one is nitrogen containing plants pigments, they provide the yellow betaxanthins, and the red-purple betacyanins **(figure1) (J.A. FERNÁNDEZ-LÓPEZ *et al,* 2002).**

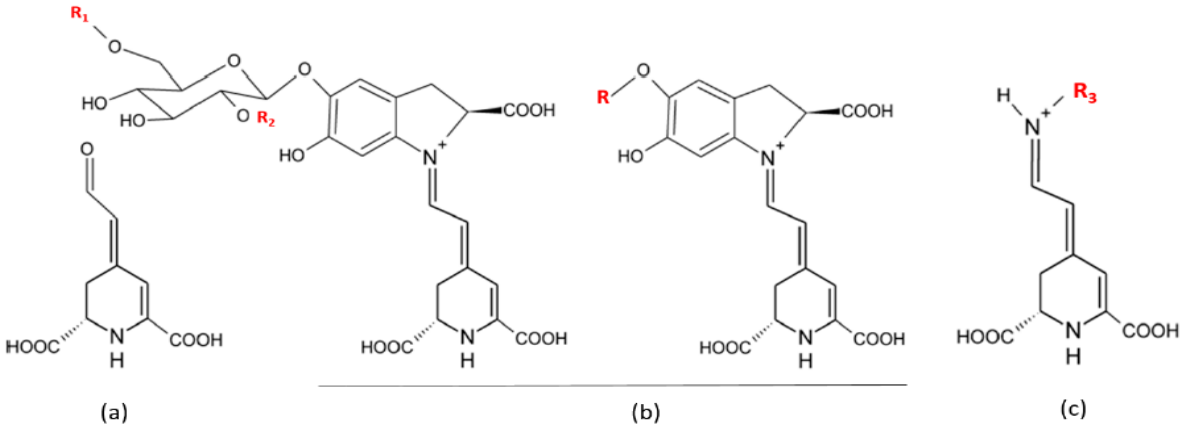



**Figure 8:** General structure of betalamic acid (**a**), betacyanins (**b**) and betaxanthins (**c**) (**El-Mostafa et al, 2014)**.

Betalains are extracted after different processes like selection, classification, washing and conditioning. The extract must be concentrated under rotary evaporation at 35˚C, then pressed to eliminate water.

They are recognized as natural food colorants, in contrast to other natural pigments, their appearance is maintained over a wide pH range (from 4 to 7). This property makes them ideal for coloring low acid foodstuff. **(J.A. FERNÁNDEZ-LÓPEZ et al, 2012).**

The antioxidant properties of these betalains pigments represent an additional argument in favor of the development of their use in nutrition, health and prevention from diseases caused by synthetic colorants **(El-Mostafa et al, 2014)**.

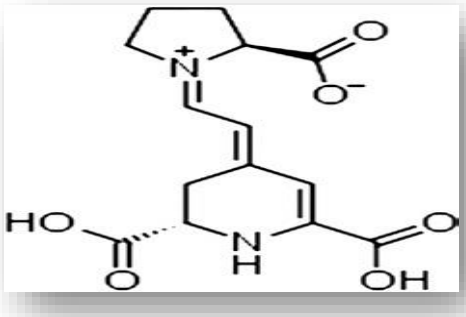



**Figure 9:** Chemical structure of *OFI* xanthin pigment determined by HPLC extracted from *Opuntia* fruits **(J.A. FERNÁNDEZ-LÓPEZ et al, 2012).**

### II.5.2 Flower source of nutritional compounds:

The study of the OFHE showed that the flowers are an excellent source of food supplements like fiber which is used for prevention of illnesses as diabetes, treatment of gastro intestinal disorders, illnesses associated with low dietary fiber intake, also the flowers are a good source for minerals as zinc and potassium **(Enouri, 2014)**.

### II.5.3 Cladodes source of dietary fibers:

Currently, consumers are interested in healthy food, and they prefer a diet low in calories, cholesterol and fat, but also they want a food with higher fibers content.

Dietary fibers (DF) are parts of fruits, vegetables, nuts and legumes that can't be digested by humans. It's recommended to take 25-38g/day of dietary fibers; the consumption of adequate quantities of DF reduces the risk of degenerative diseases, including diabetes, obesity, coronary heart disease, bowl cancer and gallstones.

In Mediterranean countries, PP plant grows spontaneously and consumed as fresh fruits, cladodes are used as complements to feed animals, the rest go for waste , so many studies are interested in studying different uses of cladodes. In addition to the nutritional value, DF has technological proprieties that can be used in the production of food, can change texture and stability of the food during production and storage, used to replace wheat flour in the preparation of bakery products, they also can be used to replace wheat flour in the preparation of bakery products **(M.A. Ayadi et al, 2009).**

### II.5.4 Medical relevance of cactus compounds:

Cactus pears have been used for a while in traditional medicines in treatment of burns, wounds, edema, hyperlipidemia, obesity and catarrhal gastritis. Recently many studies are occupied in studying therapeutic actions of its compounds and extracts. These last ones shown many pharmacological effects (antiviral, anti-inflammatory and antioxidant...) in vivo and in vitro studies applied on animals and cell models respectively (**El-Mostafa et al, 2014)**

### II.5.5 Healing experimentally induced gastric mucosa ulcer:

Gastric ulcer is a gastro duodenal disease that result a painful lesion of gastric mucosa, this disease may be as dangerous as causing a perforation of the stomach and internal hemorrhage, that can lead to death if it is not treated immediately.

This pathology is a result of an unbalance secretion of gastric hydrochloric acid and other gastric secretions as pepsins, the treatment is based on the reduction of factors induces gastric mucosal damage, although these drugs are risky for human health. That is why several studies are interested in developing alternative treatment methods natural potential of bioactive compounds in plants that would clearly be less aggressive than conventional drugs.

A study shown that *OFI* oil has significantly decreased the mucosal damages like lesions, ulcers, bleeding and necrosis observed in several areas in the gastric wall of fast rats then treated orally with absolute ethanol, a major protection with dose 2 (7ml/kg/bw) than dose 1 (3.5ml/kg/bw) which gives sufficient amount of bioactive compounds **(IkramKhémiri et Lotfi Bitri,2019).**

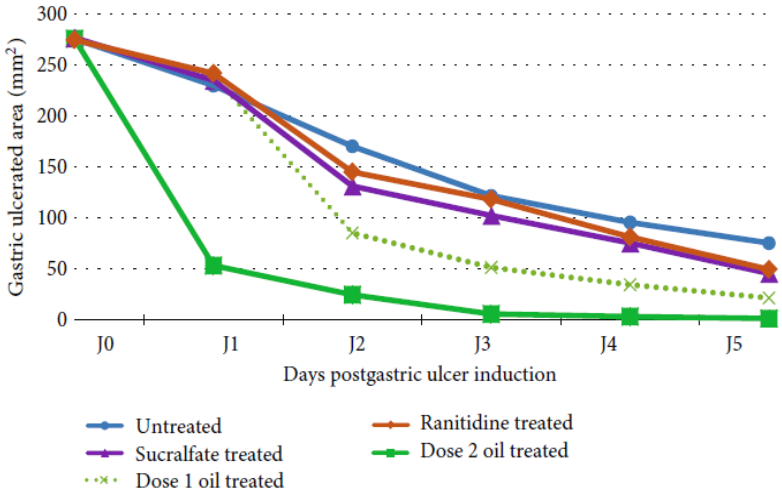



**Figure 10:** Healing rate evolution of the ulcerated areas during the five days postulcer induction in the different experimental groups.

Image represents the evolution of the healing rate of the ulcerated surface of gastric mucosa over five days postulcer induction. Comparison between the different groups indicates that OFI oil dose 2 is the most efficient treatment to speed up the healing process compared to OFI oil dose 1, ranitidine, and sucralfate **(Khémiri, I., & Bitri, L., 2019).**

# Chapter 3:

# Biochemical analysis principle

### I.1 Quantification of the total polyphenols content:

The total phenolic contents in the above four extracts were measured using the Folin–Ciocalteu method (Singleton and Rossi, 1965). The Folin–Ciocalteu is a yellow reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) that reacts with phenols and non-phenolic reducing substances to form chromogens. The latter can be detected spectrophotometrically, since in alkaline conditions the oxoyungstate and oxomolybdate formed in this redox reaction display a blue coloration proportional to the concentration of polyphenols **(Apak et al., 2018).**

### I.2 DPPH assay:

1,1-Diphenyl-2-pic-ryl-hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge **(Eklund et al.,2005).**The free radical can be reduced by the hydrogen transfer from different antioxidants in the medium The reduction of the free radical DPPH (2,2'-diphenyl-1-picryl hydrazyl) was monitored by UV-visible spectrometry, measuring the decrease in absorbance at 517 nm caused by the antioxidants. In the presence of free radical scavengers, the DPPH (2.2 Diphenyl 1 picryl hydrazyl) of violet color is reduced to 2.2 Diphenyl 1 picryl hydrazine yellow.

DPPH• purple DPPH• yellow

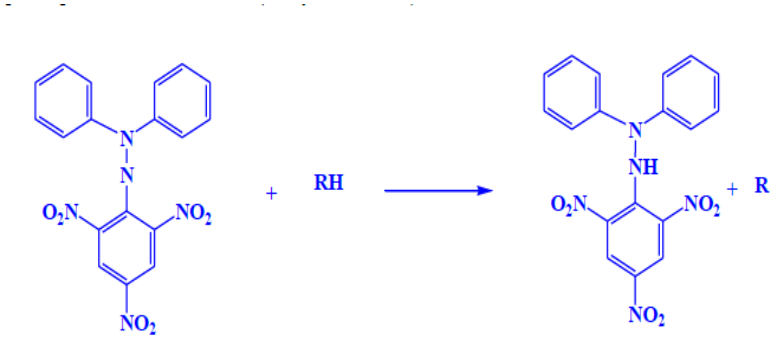



**Figure 11:** DPPH• radical transformation into DPPH.

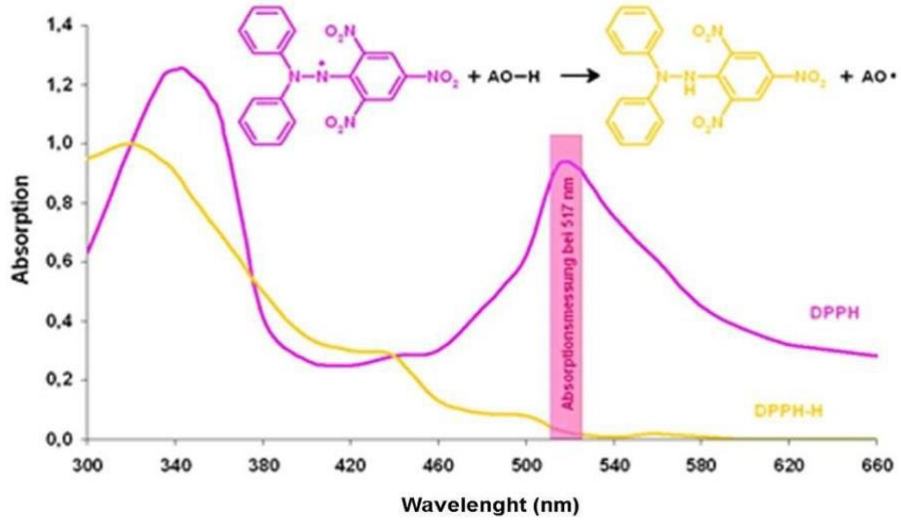



**Figure 12:** Curve show the transformation color of purple DPPH into yellow **(Benatttia., 2017).**

**I.3 ABTS assay:**

The ABTS+ cation radical (2,2-azinobis (3-ethyl benzothriazolin -6-sulfonic acid ) is stable in its free form adding an antioxidant to a solution of this cation radical leads to its reduction and a decrease in the absorbance .

This decrease depends on the quantity of antioxidants present in the medium **(mansour et al , 2011)**.

The activity molecule total antioxidant is inferred from its ability to inhibit the ABTS+ radical:

*

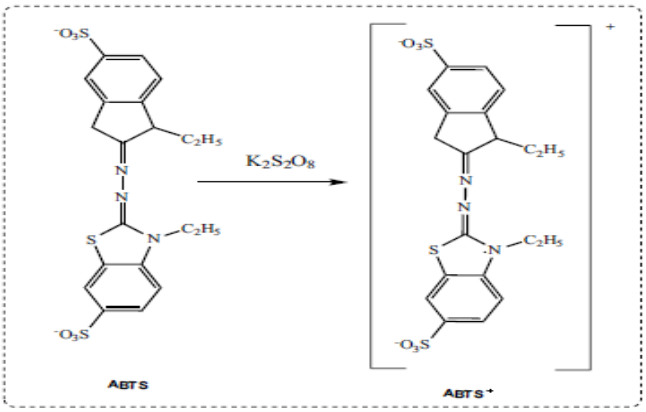



**Figure 13**: Formation and trapping of the ABTS°+ radical by an H° donor antioxidant.

## I.4 Antioxidant capacity test by copper reduction (CUPRAC):

The CUPRAC (Cuprac ion reducing antioxidant capacity ) method is based on monitoring the decrease in the increased absorbance of the neocuproene (NC) ,copper (cu2+) Nc 2-Cu +2 complex indeed in the presence of an antioxidant ,the copper-neocuproene complex is reduced and this reaction is quantified in spectrophotometrically at a wavelength of 450 nm **(Apak,R, et al 2004).**

The principal of this test is based on the conversion of phenolic hydroxyl into quinones through the reduction of the Cu+2-Nc complex thus producing a chromogenic complex of Cu+2-Nc which absorbs at 450nm.

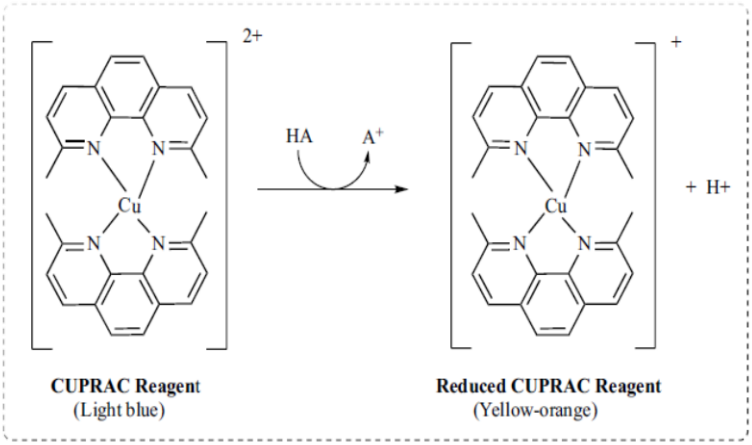


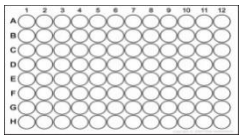

**Figure 14**: Reduction of the chromogenic complex of Cu + 2-Nc.

# Experimental part

This study was concerned to the extraction of different parts of the prickly pears plant OFI, the quantification of total phenolic content on the one hand and evaluate *in vitro* their antioxidant activities by ABTS, trapping the free radical DPPH and CUPRAC to suppose a correlation between both of them and a possible structure activity relationship.

These activities were measured in laboratory: LOST.

# Materials and methods



## I.1. Materials:

### I.1.1. Plant materials:

The plant used in the present study is prickly pear cactus or nopal, well adapted to arid and Semi-arid climates like that of Algeria that produces sweet nutritionally rich edible fruits, and it has been used in traditional folk medicine, that was harvested during the summer of 2020 in el kheroub, Constantine. Different parts of the plant were investigated to obtain four extracts namely:

➢ E1: Dry cladodes.

➢ E2: Dry peels.

➢ E3: Juice.

➢ E4: Seeds powder.

### I.1.2. Laboratory equipment:

All used chemicals are of analytical quality and listed in the appendix.

Other laboratory equipment used is:

➢ UV spectrophotometer.

➢ Precision balance.

➢ Laboratory oven

➢Round-bottom flasks.

➢ Magnetic stirrer.

➢ Rotary evaporator.

➢ Beakers.

➢ Flasks.

➢ Funnel.

➢ Test tubes.

➢ Test tube rack.

➢ Filter paper.

➢ Pipettes.

## I.2 Methods of study:

As mentioned above, four different parts of the prickly pears plant are the subject of our practical part. Experimental is divided into three stages as shown in figure.

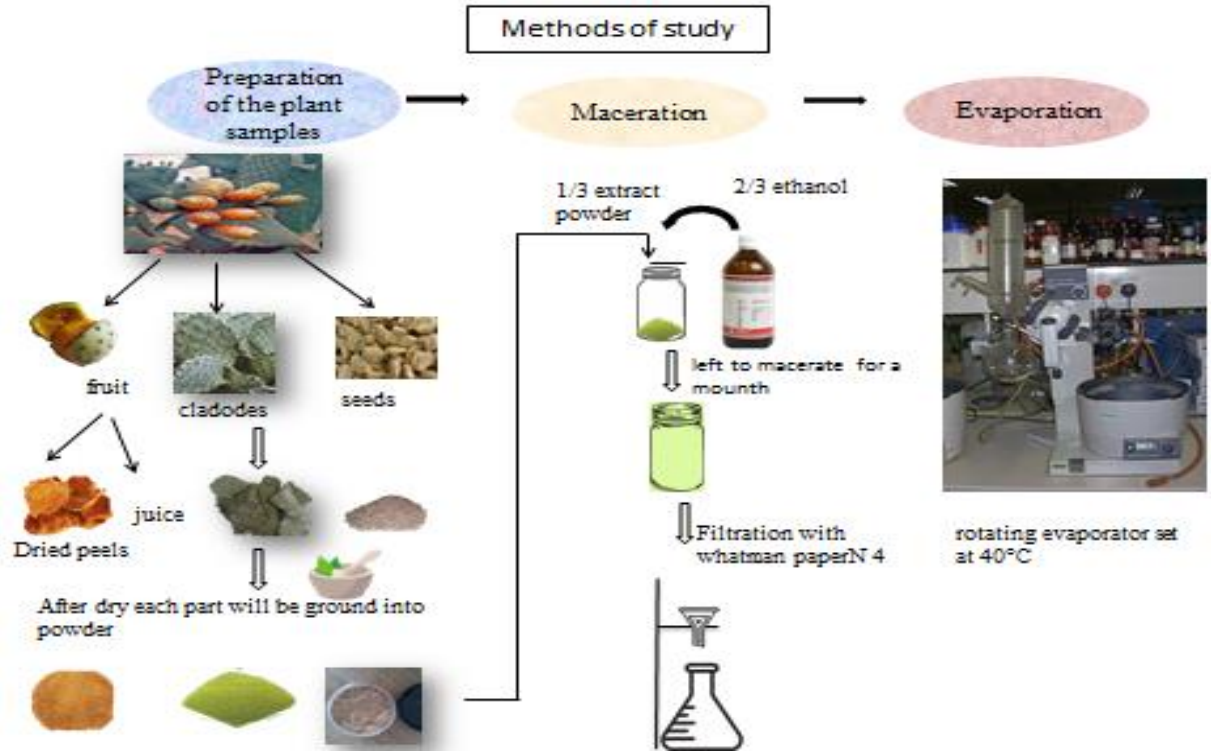



**Figure 15:** Methods of study protocol.

### I.2.1 Preparation of the plant samples:

The fruits were purchased from local market, brushed with running water many times to remove the glochides and the impurities, peeled by the hand, juice pulp is separated from seeds, the later abundantly washed several times with distilled water.

### I.2.2 Maceration of the plant samples

Maceration is an inexpensive homemade technique for preparing tonic. Moreover, this technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration technique has multiple steps in extraction.

This process is done in a closed vessel where a fitting solvent (ethanol) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known

as marc to regain an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and detached from unwanted materials by filtration. Continual agitation during maceration facilitates extraction by two processes: (1) promotes diffusion, (2) separates concentrated solution from the sample surface by adding new solvent to the ethanol for increasing the extraction yield.

### I.2.3 Evaporation (concentration):

The reinforced extract putted into a wiped film evaporator it will be concentrated there under vacuum in order to produce a thick concentrated extract. The concentrated extract is further putted into a vacuum chamber dryer to product a solid mass out of solvent. The solvent regain from the wiped film evaporator and vacuum chamber desiccative is recycled back to the percolator or extractor for the next batch of plant material. The solid mass consequently obtained is pulverized and used directly for the desired pharmaceutical formulations or more processed for isolation of its phyto constituents , we got in our experience four extracts , an orange gum which is E3 , the green one was it E1 but E2 and E4 were green brownish .

### I.2.4 Dilution samples:

A range of seven dilutions (1/2, 1/4, 1/8, 1/16, 1/32, 1/64) was established for the four extracts, starting from the mother solution (2mg/2ml methanol), to obtain the concentrations [200, 100, 50, 25, 12.5, 6.25, and 3.125] µg /ml, as shown in the following figure:

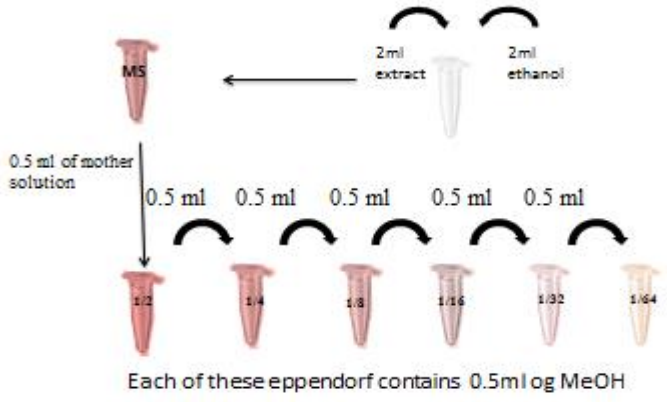



**Figure 16:** Preparation of the mother solution & the dilution range.

## I.3 Quantification of the total polyphenols content:

### ❖ Protocol:

0.4 ml of each sample was firstly added 1.5 ml of Sodium carbonate (Na2CO3) (7,5%) then 2 ml of diluted Folin–Ciocalteu reagent (1/10 H2O), then mixed by VORTEX. After that, the reaction mixtures are further incubated for 2 hours at room temperature in the dark, and finally, the absorbed optical density is recorded at the wavelength of 765 nm.

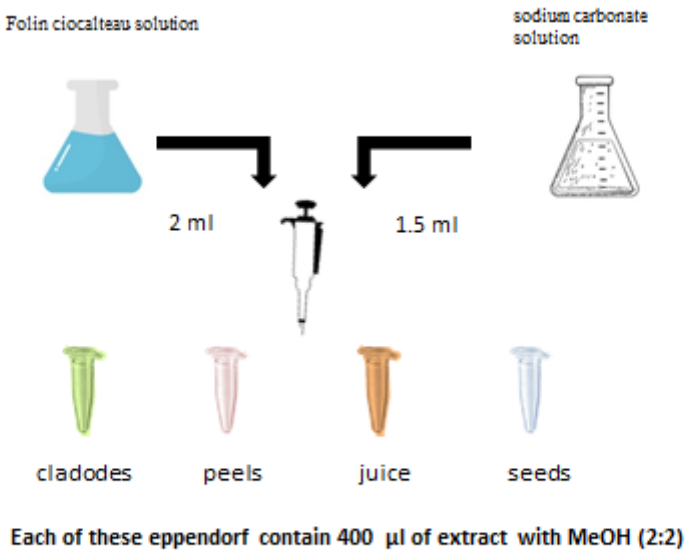



**Figure 17:** Total phenolic Content protocol.

## I.4 Biological activities analysis:

### I.4.1 DPPH assay:

### ❖ Protocol:

A volume of 160µl of DPPH methanolic solution added to 40 µl of extracts in 96 well plate, the later will be incubated in the dark at room temperature for 30 min; the absorbance was measured at 517 nm **figure 18**.

- A negative control prepared with 5 mL DPPH with 40-µL methanol.
- Results of antioxidant assay were compared to BHA at different concentration.

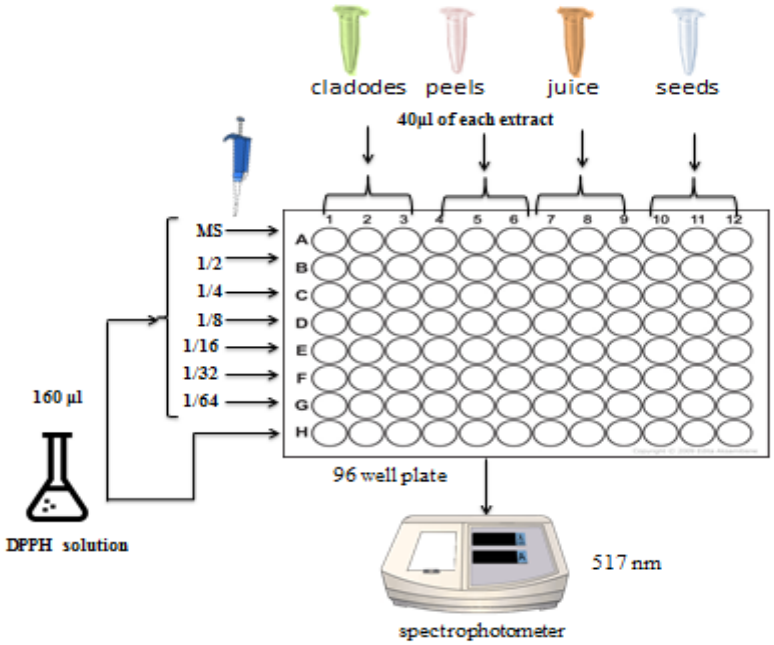



**Figure 18**: DPPH experimental protocol.

### I.4.2 ABTS trapping assay :

The spectrophotometric analysis of the trapping activity of ABTS° + was determined according to the method of Re et al. (1999), with slight modifications.

❖ **protocol :**

**160 µl** of the ABTS°+ solution was added to **40 µl** of the extract solution at different concentrations, the microplate was then placed in the dark and at room temperature 10 minutes of incubation. The absorbance of the reaction medium was measured at a wavelength of 734 nm.

At the same time, a negative control was prepared by mixing **40 µl** of methanol with **160 µl** of the ABTS°+ solution.

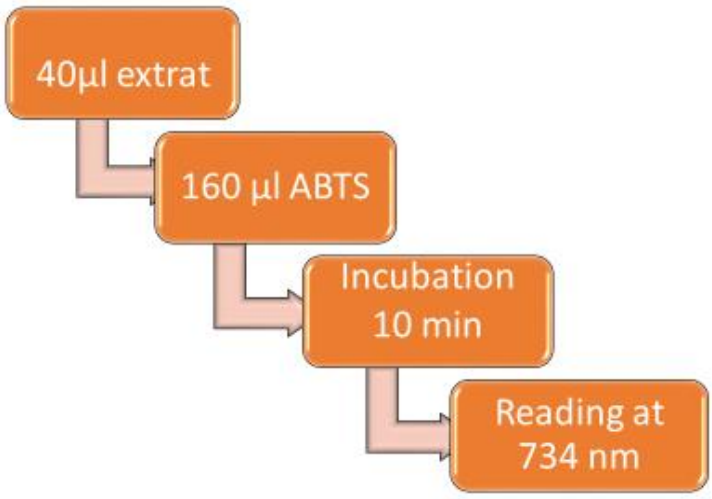



**Figure 19**: Diagram of **the ABTS** °+ trapping activity protocol **(Re et al., 1999)**

The trapping capacity of ABTS°+ was expressed as a percentage inhibition and calculated using the same equation used for the DPPH method.

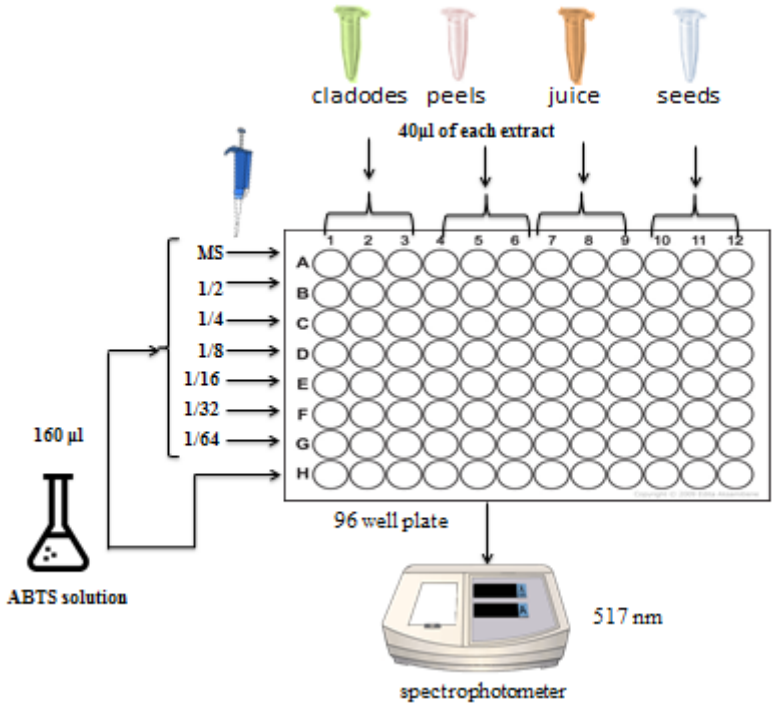



**Figure 20:** ABTS experimental protocol.

$$\%Inhibition = \frac{control\ Abs - Abs\ extract}{Abs\ control} \times 100$$

- Such as :
  - ✓ Abs control is the absorbance of the reaction containing only the reagents.
  - ✓ Abs Extract is the absorbance of the reaction containing the reagents and the extract.

### I.4.3 The antioxidant capacity assay by copper reduction (CUPRAC) :

The reduction of copper was determined by the CUPRAC method described by Apak et al. (2004).

❖ **protocol :**

A solution (S1) at PH = 7.0 was prepared from **1.927g** of ammonium acetate (ACNH4) and **25 m**l of H2O, a second solution (S2) was made with **0.039g** of Neocupronin dissolved in **25 ml** of MeOH, and a third solution (S3) was prepared with **0.042625g** of (Cu Cl2, 2H2O) dissolved in **25 ml** of H2O.

Then **60 µl** of (S1), **50 µ**l of (S2) and **50 µ**l of (S3) were added respectively to **40 µl** of different concentrations of extracts.

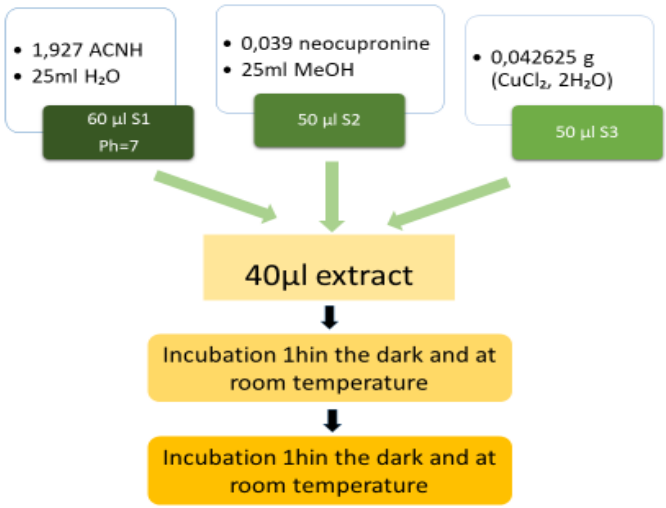
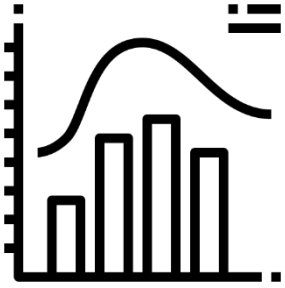



**Figure 21**: CUPRAC Test protocol diagram.

The microplate was protected from light, and after 1 hour of incubation, the absorbance was measured at 450 nm.

- ✓ The negative control was carried out by replacing the extracts with methanol.
- ✓ BHA was taken as antioxidant standards for this test.

# Result and discussion



## II.1 Quantitative analysis:

The values of the total polyphenolic compound content of the four extract E1, E2, E3, E4 respectively are expressed in µg equivalent of Gallic acid per mg of dry extract (µg GAE/ mg DE) using the linear regression equation of its calibration curve (figure. )

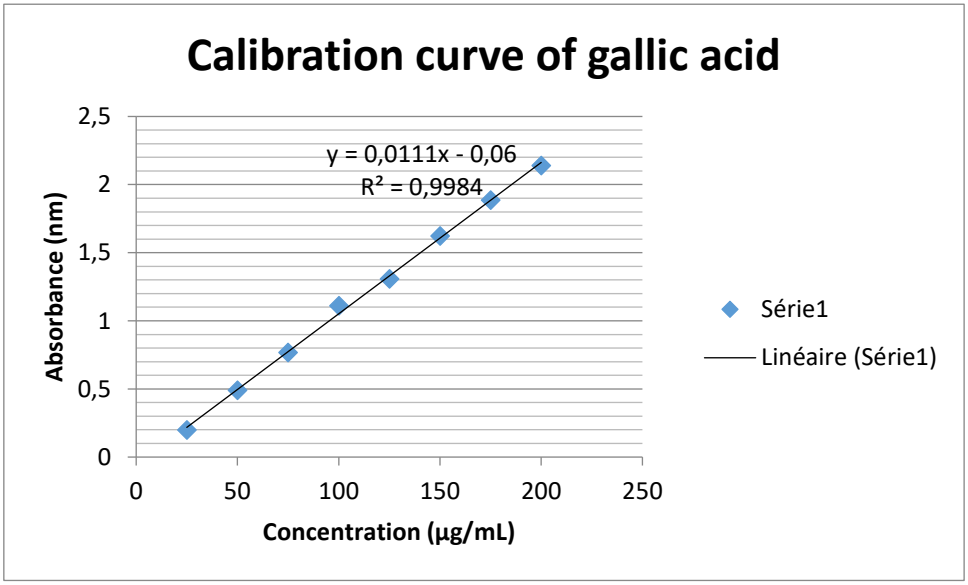



**Figure 22:** Calibration curve of Gallic acid (µg GAE/mg DE)

According to the following formula:

$$TPC = C*V/M$$

With:

**C:** [a.g] µg/ ml.

**V:** Extract volume (ml).

**M:** Extract weight (mg).

## II.2 Total phenolic compound content and discussion:

The results of the four extracts: dried Cladodes (E1), dried peels (E2), juice (E3) and dried seeds (E4) shown in the table and illustrated in the histogram below:

**Table 5**: Total phenolic content (µg GAE/mg DE) in the four extracts

| TPC µg GAE/ mg of extract | Extract |
|---|---|
| 36.29 | **E1 (Cladodes)** |
| 39.328 | **E2 (peels)** |
| 35.568 | **E3 (juice concentrate)** |
| 42.672 | **E4 (seeds)** |

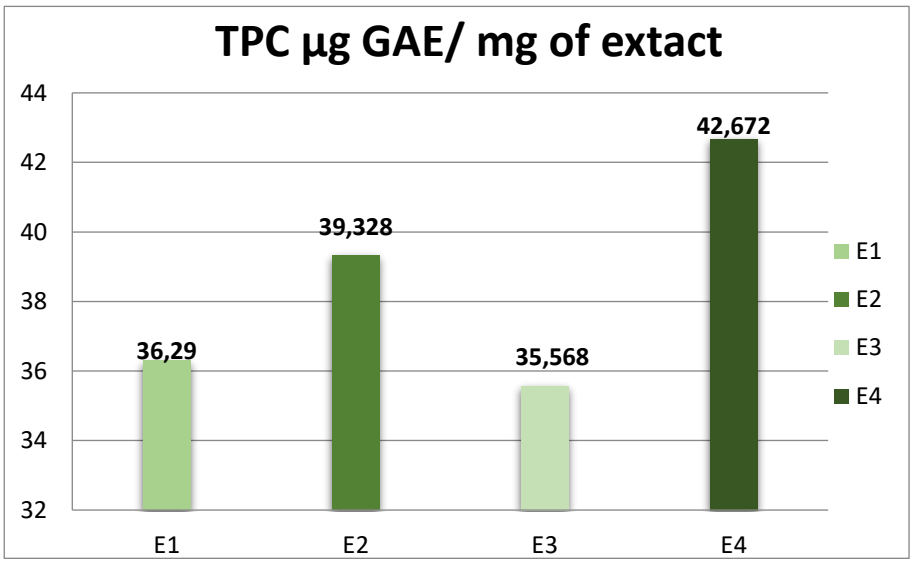



**Figure 23:** Total phenolic compounds content expressed in µg GAE/mg of content (DE).

- The total phenolic content in Fig shows the highest concentration in polyphenols in extracts E4 (seeds) with a value of 42.67) followed by relatively low value of 39.328 of E2 (peels), 36.29 of E1 (cladodes). As we can see in the fig the extract that contains the concentration equal to 35.568 µg GAE/ mg of extract is E3.

**Table 6:** TPC preceding results of OFI.

| Previous work | The value of TPC (µg GAE/ mg of extract) | TPC of this study (µg GAE/ mg of extract) | Extracts |
|---|---|---|---|
| **Halmi, 2015**<br>**Gallegos-Infanteet al 2009** | 15.4-318<br>180 | 36.29 | **E1 Cladodes** |
| **Bourhia et al ,2020**<br>**Faten M et al, 2014** | 1739.92- 2409.66<br>221-1501. | 39.328 | **E2 Dried Peels** |
| **Belviranl et al, 2020**<br>**Temagoult A et al, 2017** | 218.8<br>45,70 | 35.568 | **E3 Juice fruit** |
| **Benattia, 2017** | 68-144.50 | 42.672 | **E4 Dried seeds** |

From the previous table:

The result of E1 (15.4-318 mg/g) **(Halmi., 2015)** is very close to the result (36,29 µg/mg) In addition, E3 (juice fruit) values of total phenolic content are very close to the mentioned value in **(Temagoult A et al, 2017)**.

On the contrary, the results are lower in comparison with precedent results of other extracts E1, E3, E4 with a huge difference.

This interpretation makes it possible to deduce that the concentration of the polyphenols varies as a function of the nature of the solvent and the extraction method used. A study demonstrated that the total phenol content in the dried samples was lower than that in fresh cladodes **(JOSE.A et al 2009).**

In addition, it can be due to the geographical origin, the cultivar, the variety and especially the degree of maturity (seeds) and the duration of storage which has been proved by the work of **(Hid Sadok T et al. 2013)**, carried out on Opuntia cladodes at different stages of development.

The low specificity of the Folin-Ciocalteu reagent is the main drawback of the colorimetric assay. The reagent is extremely sensitive to the reduction of all hydroxyl groups

not only of phenolic compounds, but also of certain sugars and proteins. **(Vuorela, 2005; Gomez-Caravaca et *al.*, 2006).**

Compared to other studies, the results of the seeds extract are consistent with those mentioned in **(El Mustafaet al,.2014)**fruit seeds contain high amounts of phenolic compounds ranging from 48 to 89 mg.

### II.3 Evaluation of biological activities:

The antioxidant activity of different parts of the plant is evaluated by the following methods: ABTS, trapping the free radical DPPH and CUPRAC.

### II.3.1 DPPH free radical scavenging assay result and discussion:

It is a method, which uses DPPH as a relatively stable free radical, purple in solution and exhibiting maximum absorption characteristic at 517 nm. The protocol applied based on the disappearance of this maximum when a compound reduces the DPPH with property anti-free radical, causing discoloration from purple to yellow. The scavenging activity was measured according to the formula:

$$\%\ Inhibition\ = \frac{\text{Abs control } - \text{Abs sample}}{Abs\ control} \times 100$$

With:

**Abs control**: is the absorbance of DPPH + methanol.

**Abs sample**: is the absorbance of DPPH radical + sample.

The results reported in the table below as absorbance:

**Table 7:** Antioxidant activity by the DPPH scavenging assay.

| Extracts | % Inhibition in DPPH assay | | | | | | | | BHA equivalent |
|---|---|---|---|---|---|---|---|---|---|
| Extract Concentrations (µg/ml) | 3.125 | 6.25 | 12.5 | 25 | 50 | 100 | 200 | IC50 | $IC_{50\ BHA}$/ $IC_{50\ inhibitor}$ |
| E1 | NA | 0,26±2,65 | 1.5625 | 3,21±3,82 | 4,31±2,89 | 10,43±3,10 | 23,18±5,08 | 431.4 | 0.10 |
| E2 | NA | 0.06±2,03 | 1,77±2,64 | 2,13±3,24 | 2,68±1,22 | 3,40±6,52 | 7,37±1,63 | 1350,85 | 0.03 |
| E3 | NA | NA | 0.77±2.10 | 2,72±6,89 | 3,08±1,13 | 4,59±2,56 | 5,78±3,89 | 1730,1 | 0.025 |
| E4 | 6,06±5,62 | 12,02±2,44 | 13,72±6,67 | 34,02±3,24 | 44,71±7,43 | 68,10±5,16 | 77,12±2,11 | 55,91 | 0.80 |
| | 0.78125 | 1.5625 | 3.125 | 6.25 | 12.5 | 20 | 50 | | |
| BHA | NA | NA | 0.46±11,69 | 15.57±1,32 | 55.67±1,80 | 69.86±2,04 | 74,35±1,52 | 44.9 | 1 |

The results of the previous table represented using comparative curves:

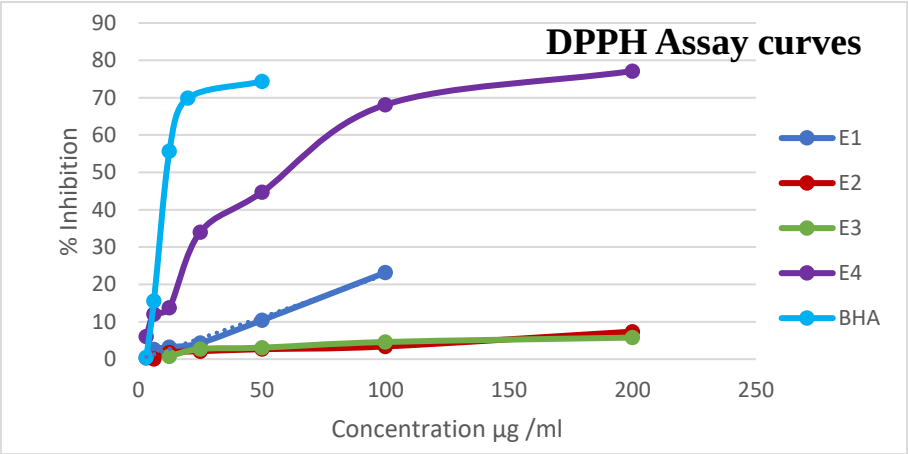



**Figure 24:** Absorbance of the DPPH activity for the four extracts and the BHA.

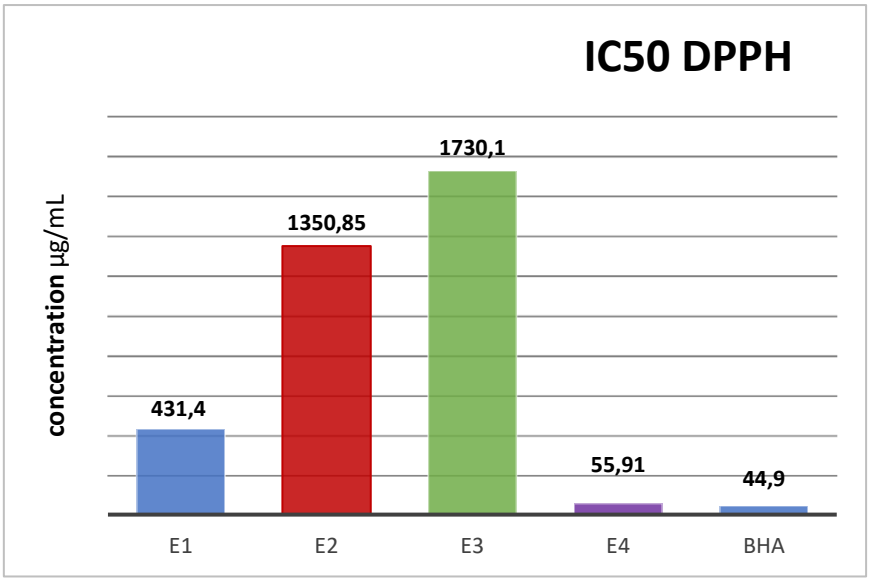



**Figure 25:** IC50 values of the four extracts and BHA for DPPH assay.

The values shown in the histogram below in order to facilitate the comparison of the IC50 values of the four extracts with the standard (BHA) followed by their discussion:

There is a huge variance in the IC50 of the four extracts and this may depends on the variety of the total phenolic content in each fraction.

The extract of the seeds E4 showed the lowest IC50 (55.91 µg/ml) and the closest to the one of BHA (44.9 µg/ml). This gives it the highest antioxidant potential among the other

extracts that express higher values of IC50, and thus have a lower antioxidant potential as followed: Cladodes extract E1 with (431.4 µg/ml), come peels extract E2 (1360.85 µg/ml) and next juice E3 (1730.1 µg/ml), which are close to each other and present the lowest antioxidant activity in the order.

The extracts shown the highest antioxidant activity by DPPH has free radical scavengers and able to react with the DPPH radical, which might be attributed to their electron donating ability. The reaction is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals.

The IC50 And the antioxidant activity of the tested extract are inversely proportional.

This assay is convenient in the application and thus most popular, nevertheless, it is limited as they use non-physiological radical.

### I.3.1.1 Comparison with previous work:

**Table 8:** IC50 DPPH comparison preceding results of OFI.

| **Previous work** | **IC50 DPPH in previous work (µg/ml)** | **IC50 DPPH of this study (µg/ml)** | **Extracts** |
|---|---|---|---|
| **Missaoui,2020** | 20 – 740 | 431.4 | **E1** |
| | 500 | 55.91 | **E4** |
| **Faten M et al, 2014** | 1.20 | 1350.85 | **E2** |
| **Aditya S et al 2020** | 13.22 | 1730.1 | **E3** |

From the table we remark the IC50 DPPH of E1 cladodes (14.22 µg/ml) is close to the first previous work mentioned in (**Missaoui,.2020) (**20µg/ml). Although the other results E1 of are lower than the other results mentioned in the same work.

The result obtained in the research **(Faten M et al, 2014)** of E2 (peels) (1.20) are compared to BHT (0.78) used instead of BHA. **(Galati EM,.2003)**.

A study that the antioxidants molecules containing in all extracts of *Opuntia ficus-indica* as ascorbic acid, tocopherols, flavonoids are responsible of the reducing and discoloration of DPPH by transferring an electron **(De Pooter HL et Schamp N,.1986).**

The polyphenols contained in the extracts of *Opuntia ficus-indica* are probably responsible for the antioxidant activity of these extracts. This is in accordance with the previous work on extracts from *Satureja montana*, a species rich in phenolic compounds that are responsible for many activities biological including antioxidant and antimicrobial activity**(Ćetković GS,.2007)**.

Another study proves that the fruit juice of the Sicilian prickly pear *Opuntia ficus-indica* has the ability to reduce DPPH, and this antioxidant activity due to phenolic compounds, which are effective radical scavengers.
This leads to suggest that the antioxidant effect of different extracts of our plant may be due to a synergism between polyphenols and other components.

Day by day, the demand for the natural antioxidants is growing, so as a suggestion a substitute for synthetic antioxidants as BHA in foods, medical fields, because they are synthetic compounds, raising concerns about their safety related to their metabolism and possibility absorption and accumulation in cells, tissues, organs and body (**Kulawik et al. 2013; Anraku et al. 2018)** Also, due to toxicological concerns of synthetic antioxidants **(Nakatani N,.2000).**

Legislative restrictions on BHA and BHT have been imposed due to concerns about their toxicity and carcinogenic potential **(Wichi 1988; Sherwin 1990).**

Besides the antioxidant and anticancer effects of BHA and BHT, a review mentioned that BHA induces in animals tumors of the forestomach, which are dose dependent, whereas BHT induces liver tumors in long-term experiments **(Saito. M et al,. 2003).**

OFI stem cells are from all parts of the plant has cosmetic effects as increasing the flexibility of the epidermis, antioxidant, anti-wrinkle, anti-aging, UV protective.

The four extracts shown an antioxidant activity and each of them could be used as a source in different domains:

The fruit extract can be effective as an ingredient in night cream against dark spots damage caused by oxidation also assist skin's moisture levels by using DPPH assay **(Aditya .S et al,.2020**).

The oil contained in the seeds of *OFI* shows anti-inflammatory properties, which offer significant potential as functional ingredient of nutraceutical and food supplement products.

### II.3.1.2 Correlation between DPPH assay and TPC:

The DPPH radical scavenging activity increases as the concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increase and with it antioxidant activity. The redox characteristics of phenolic compounds are primarily responsible for their antioxidant activity. Which can be useful for adsorbing and neutralizing free radicals, as well as quenching singlet and triplet oxygen and degrading peroxides.

### II.3.2 The ABTS activity assay results and discussion:

The results of ABTS assay are presented as percentage of inhibition and not as absorbance:

**Table 9:** Antioxidant activity measured by the ABTS assay

| Extracts | % Inhibition in ABTS assay | | | | | | | | BHA equivalent |
|---|---|---|---|---|---|---|---|---|---|
| Extract Concentrations (µg/ml) | 3.125 | 6.25 | 12.5 | 25 | 50 | 100 | 200 | IC50 | $IC_{50\ BHA}$/ $IC_{50\ inhibitor}$ |
| E1 | 7.07±3,16 | 11.65±3,16 | NA | NA | NA | NA | NA | 26,82 | 8.08 |
| E2 | NA | NA | 0.53±9,18 | 3.83±1,63 | 9.17±2,02 | NA | NA | 272.62 | 0,79 |
| E3 | 4,36±4,07 | 8,80±0,94 | 9,17±3,39 | 9,62±1,38 | 9,85±2,26 | 13,31±0,47 | 15,26±6,10 | 655,3 | 0.33 |
| E4 | 9,25±2,87 | NA | NA | NA | NA | NA | 76,99±6,99 | 129,88 | 1.66 |
| BHA concentration | 0.78125 | 1.5625 | 3.125 | 6.25 | 12.5 | 20 | 50 | | |
| BHA | NA | NA | NA | NA | 42.01±1,58 | 42.47±5,48 | 46,12±1,58 | 54.20 | 1 |

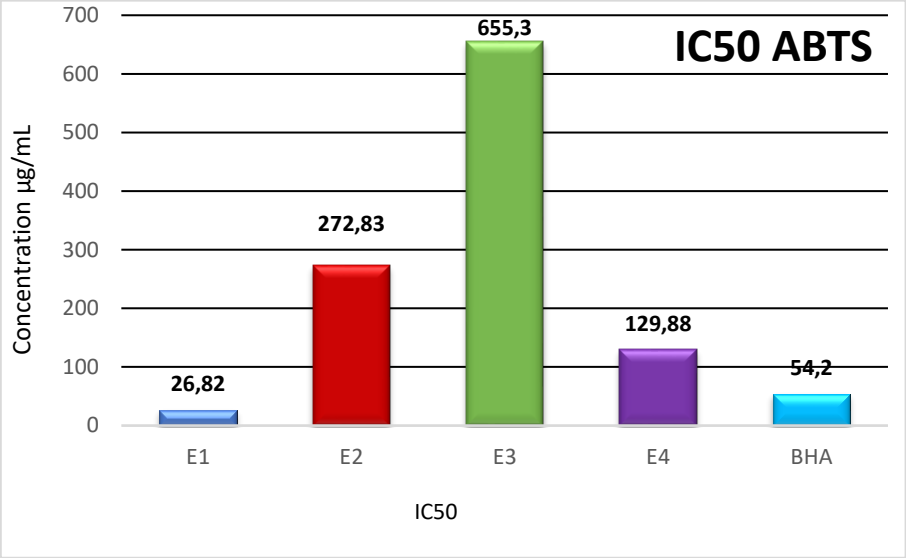



**Figure 26**: IC50 values of the four extracts and the BHA in ABTS activity

The different part of *OFI* have near ability of ABTS species with E1 Cladodes with IC50 = 26.82 µg/ml, E2 the skin with 272.83 µg/ml , E4 the Seeds 129.88µg/ml ,then E3 Jus with 655.3µg/ml , respectively , which are generally close to the one observed in the BHA IC50=54.2 µg/ml except the jus E3 .

This assay dedicated for both lipophilic and hydrophilic compounds, ABTS is oxidized by oxidants to its radical cation ABTS+ with extreme color, moreover the capacity of antioxidant's measured as an ability of test compounds to reduce the color reacting immediately with the ABTS radical.

This process based on the activation of netmyoglobin , acting as peroxidase with H2O2 to generate ferryhnyoglobin radical which then reacted with ABTS to form the ABTS+ radical cation (Miller et al ,1993) .

The K2S2O8 as oxidant shows that the presence of perodisulphate increases the rate of ABTS+, the scission of the K2S2O8 may take place after the electron transfer.

$S_2O_8^{2-} + ABTS \rightarrow SO_4^{2-} + SO_4^{\cdot -} + ABTS^{\cdot +}$

$SO_4^{\cdot -} + 2ABTS \rightarrow SO_4^{2-} + 2ABTS^{\cdot +}$

$S_2O_8^{2-} + 3ABTS \rightarrow 2SO_4^{2-} + 3ABTS^{\cdot +}$.

In the presence of excess ABTS, the sulfate radical will react according to leading to overall reaction represented by ABTS+ radicals one more reactive than DPPH radicals that

invobe HAT the reactions with ABTS+ radicals generally invobe SET **(Kaviarasan et al ,2007) ,(Koksal et al ,2009).**

Moreover, ABTS+ is soluble in aqueous and organic solvents and isn't affected by ionic power, so it can be determined both hydrophilic and lipophilic antioxidant capacities **(Awika et al, 2003).**

### II.3.2.1 Comparison with previous work:

**Table 10:** ABTS values in different scientific articles

| **Previous work** | **IC50 DPPH in previous work (µg/ml)** | **IC50 DPPH of our extracts (µg/ml)** | **Extracts** |
|---|---|---|---|
| **Santiago et al** | 4500 | 26.82 | **E1 cladodes** |
| **Cruz ,2015** | 115.68 | 655.3 | **E3 Juice** |
| **Zafra rojas, 2013** | 26.3 | | |

The values of our experience comparing them with other experiences, we notice a huge difference in the results, and that is may up to many causes one like the verity of the fruit and the plant worked on also the water used for the watering plant and fruit and the soil quality and its compositions.

### II.3.3 The copper reduction (CUPRAC) activity assay results and discussion:

The results of the hydroxyl radical scavenging are reported in the table below as absorbance.

**Table 11:** Antioxidant activity measured by the CUPRAC assay.

| **Extracts** | **% Inhibition in CUPRAC assay** | | | | | | | | **BHA equivalent** |
|---|---|---|---|---|---|---|---|---|---|
| **Extract Concentrations (µg/ml)** | **3.125** | **6.25** | **12.5** | **25** | **50** | **100** | **200** | **A05** | **$A_{0.5\ BHA}$/ $IC_{0.5inhibitor}$** |
| **E1** | **0,20±0,01** | **0,22±0,02** | **0,28±0,06** | **0,35±0,00** | **0,45±0,05** | **0,58±0,41** | **0,59±0,05** | **86.2** | **0,29** |
| **E2** | **0,17±0,01** | **0,19±0,03** | **0,24±0,12** | **0,24±0,04** | **0,26±0,14** | **0,26±0,00** | **0,36±0,01** | **277** | **0.09** |
| **E3** | **0,16±0,01** | **0,16±0,02** | **0,16±0,04** | **0,18±0,07** | **0,18±0,01** | **0,19±0,03** | **0,19±0,03** | **526.31** | **0,04** |
| **E4** | **0,22±0,04** | **0,24±0,11** | **0,32±0,04** | **0,37±0,03** | **0,55±0,02** | **0,85±0,05** | **1,56±0,05** | **45.45** | **0.55** |
| **BHA concentrations** | **0.78125** | **1.5625** | **3.125** | **6.25** | **12.5** | **20** | **50** | | |
| **BHA** | **0,17±0,02** | **0,23±0,02** | **0,32±0,01** | **0,50±0,01** | **0,83±0,02** | **1,43±0,02** | **2,23±0,07** | **25** | **1** |

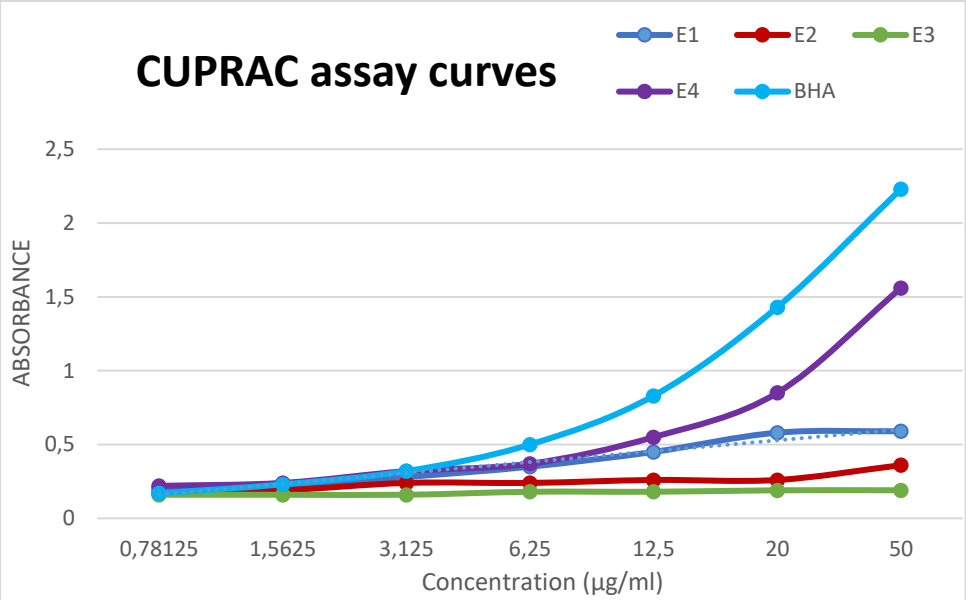



**Figure 28:** Absorbance of the CUPRAC assay for the four extracts and the BHA.

The values showed in the histogram below in order to make the comparison of the A 0.5 values of four extracts with BHA (stander) simple but significant.

Followed by the discussion:

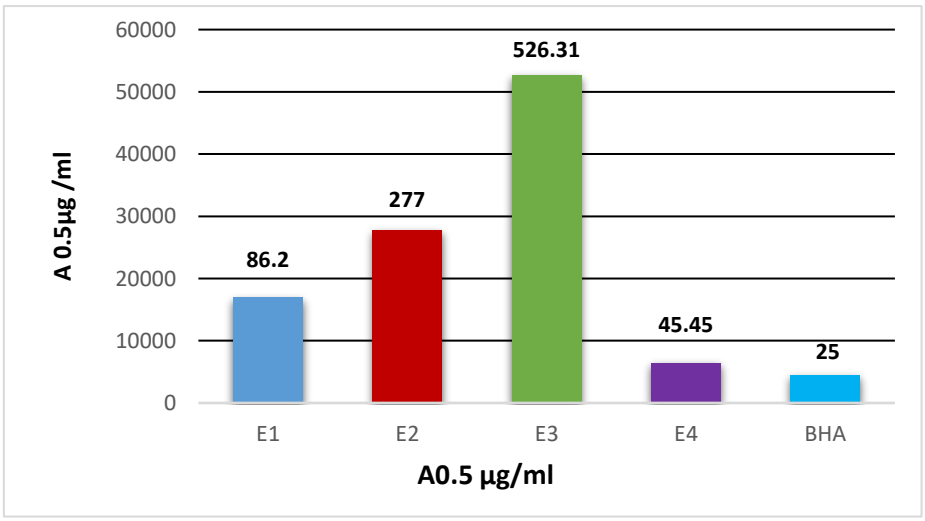



**Figure 29**: A 0.5 values of the four extracts and the BHA for the CUPRAC activity.

The CUPRAC assay established on the reduction of cu2+ to cu+ by the combined action of all antioxidants decreasing in aqueousethanolic medium ph=7 in the presence of neocuproine

( 2.9 –dimethyl-1,10-phenanthroline ) by polyphenols, yielding cu+ complexes with maximum absorption peak at 450nm.

In the CUPRAC assay E4 = 45.45µg /ml of seeds part showed the lowest and the closer one to the BHA= 25µg /ml ,this means it has the highest antioxidant potential comparing with the other extracts those expressed higher values of A 0.5 but having a lower antioxidant potential : the cladodes E1= 86.2µg /ml then the skin E2= 277µg /ml and finally the juice E3 = 526.31µg /ml , this makes jus the lowest antioxidant potential but the highest in A 0.5.

This process is also in the same time cost effective, fast, stable and selective moreover suitable for a different of antioxidants regardless of chemical type or hydrophobicity **(Gulçin and Dastan ,2007) , (Karman et al ,2009).**

The CUPRAC reagent is selective, because it has lower redox potential than those of Folin, and near to the one of ABTS +/ABTS redox couple **(Apak et al ,2005).**

## I. Comparison between the antioxidant activities: ABTS , DPPH and CUPRAC:

After comparing the results of the three antioxidant activities ABTS , DPPH and CUPRAC ,the results of IC50 showed that ABTS has the closer values to IC50 BHA= 5.27µg/ml between ( E1= 10 ,37.E2= 14,57. E3= 30,37. E4= 4,609 )µg/ml , which means that this activity is the effective one and the ones given the best results in scavenging assays.

# Conclusion and Perspectives:

OFI known as “prickly pears” belongs to the cactaceae family, originated from Mexico cultivated in different regions in the world as southern Europe, Mediterranean region, Middle East and southern Africa, cactus pear plant grows spontaneously.

The present study showed that cactus pears contains polyphenols in all analysis parts: cladodes, peels, fruit and seeds which are supposedly with the presence of other compounds as vitamins, carotenoids provides an antioxidant activity could be effective against aging.

In Algeria, prickly pears are consumed exclusively as fresh fruit and cladodes as an animal feed complement. Only a small quantity is being used for processing, that is why there is need to create a far better outlet for seasonally surplus production, which used in animal feeds or otherwise attend waste. Despite the fact that several countries have invested, such as Morocco, the first exporter of seeds oil, the most expensive oil, also Tunisia and Italy, and their choice didn’t happened by chance rather than intention.

Nopal could be a great source of bio-molecule such as dietary fiber, mineral and natural antioxidant compounds, and can be used in a way such as good natural colorants alternatives for synthetic ones.

This shrub is miraculous, nothing is thrown away, and everything is useful. Modern scientific research is interested by biologically active compounds isolated from plant extracts, which are considered benefic chemical plants from which it is necessary to make the maximum profit because of several pathologies appear cause of the intensive use of synthetic products, *OFI* could be a good source for prevention from diseases.

This study showed that generally seeds contains the highest amount of TPC correlated with the highest anti-oxidant activity in the three essays: DPPH, ABTS and CUPRAC, followed by peels extract, cladodes and juice in the order.

Further the result of the present study demonstrated that Nopal is one of the *Opuntia* species that merits more investigation and research ,and as perspectives :

- Exploit seeds fruit of cactus pears in production of oil directed to be used in cosmetics.
- Use cladodes powder as natural source of dietary fibers.
- Work on the production of natural colorants from peels, cladodes, fruit and flower.

- Produce dermocosmetics products from cladodes powder and frost as moisturizers.
- Trade on peels and cladodes to be destined for animal food (forage)
- Reserve more cultivable land for this plant to avoid erosion.
- Create a far better outlet for seasonally surplus production that save environment from excessive residues.
- Improve the scientific studies in the way of exploring new bioactive molecules.
- Make aware Algerians about the value of this plant by

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# Appendices

**Appendix 1:**

## Laboratory equipment:

**Rotary evaporator**: is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation, have been used after the maceration of the four extracts.

**Spectrophotometer**: can measure the intensity of a light beam at different wavelengths. Although spectrophotometry is most commonly applied to ultraviolet, visible, and infrared radiation, has been used to measure absorbance of the extract to quantify the polyphenols content

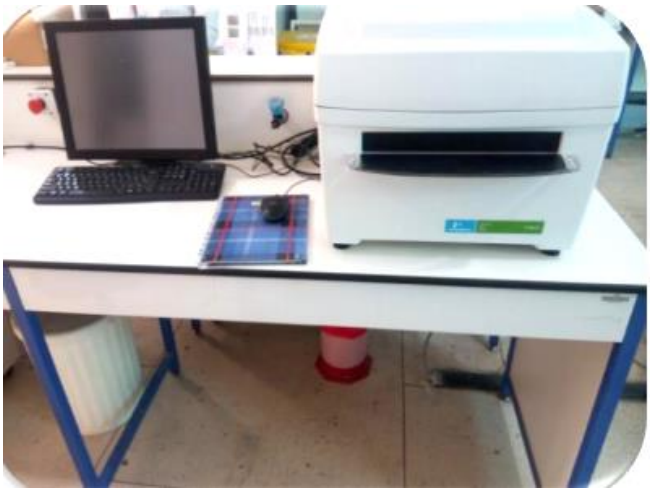


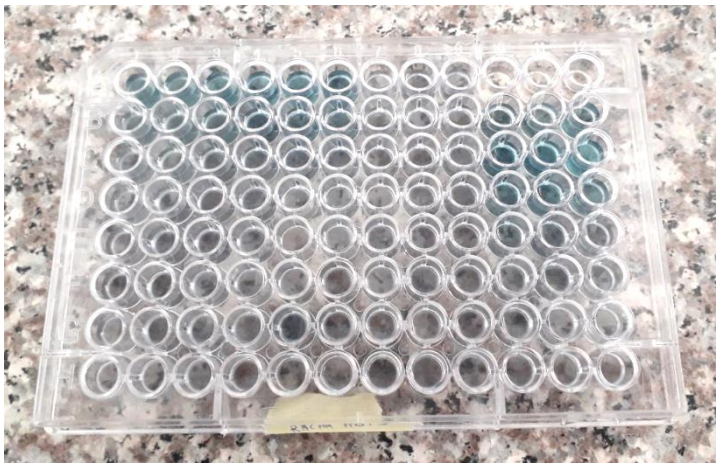
**Plate reader**: instrument used to detect biological, chemical or physical events of samples in microtiter plates, has been used to measure antioxidant assays.

**Oven laboratory**

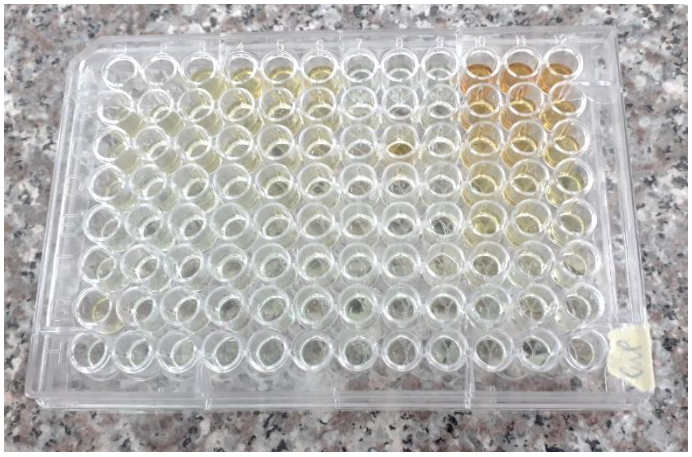
**Precision balance**

**Appendix 2: Result of biological activities.**

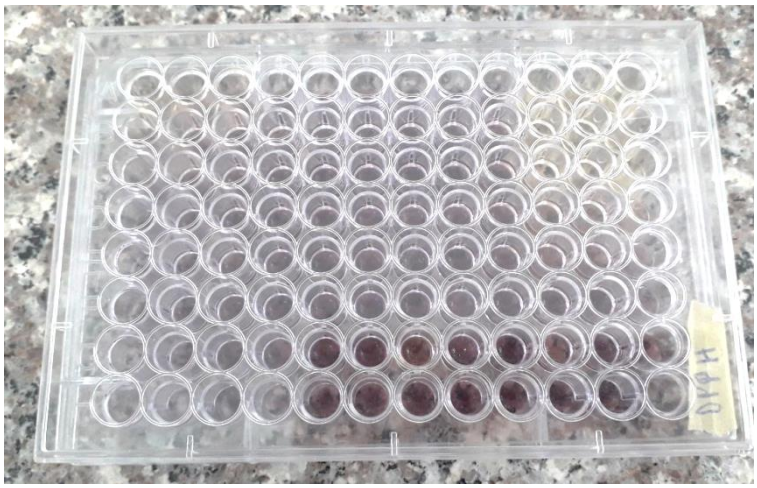
**CUPRAC assay.**



**DPPH assay.**



**ABTS assay.**



# Abstracts

## Abstract

In the framework of valorizing medicinal Algerian plants, *Opuntia ficus-indica* (OFI) a plant grows spontaneously and has been neglected for a while, it is widely known for its beneficial properties. The aim of the present work is to focus on biological activities of Nopal plant especially the antioxidant one, and their uses in dermocosmetic natural products, the valorization of different parts of Nopal plant, also interest through emphasis on some of their uses in many fields.

This study aimed to focus on the antioxidant activities of its different parts: juice, cladodes, dried seeds and peels, by quantifying total phenolic content and three antioxidant assays: DPPH, ABTS scavenging assays and reducing antioxidant power by CUPRAC, comparing the obtained results and supposing correlation between antioxidants methods and TPC.

The increasing interest in preventive medicine encourages use of natural products against senescence of the skin and skin cancer, also the use of nutraceuticals bioactive compounds of vegetable origin, OFI with important nutritional values can be source of dietary fibers and natural pigments.


**Key words:** *Opuntia ficus-indica*, antioxidant activity, polyphenols, Dermocosmetics.

## Résumé

Dans le cadre de la valorisation des plantes médicinales Algériennes, *Opuntia ficus-indica* (OFI) est une plante spontanée, connue par ces propriétés bénéfiques mais cependant pas assez exploitée. Le présent travail vise à investir ses activités biologiques spécifiquement les activités antioxydantes, et son utilisation comme actif et aditif dans les dermocosmétiques à base de produits naturels, mais aussi la valorisation des différentes parties du figuier de barbarie et leur utilisation dans différents domaines.

L'activité antioxydante des différentes parties : jus, raquettes, graines sèches et épluchures a été mise en évidence par la quantification des polyphénols totaux et trois méthodes pour mesurer l'activité antioxydante : le piégeage du radical libre DPPH, le piégeage du radical ABTS et la réduction du cuivre par la méthode CUPRAC, aussi la comparaison des résultats en supposant une corrélation entre les polyphénols et ces méthodes.

L'intérêt croissant pour la médecine préventive encourage l'utilisation des produits naturels pour la prévention de vieillissement de la peau et du cancer de la peau, aussi de composés biologiques d'origine végétale, *OFI* avec ses valeurs nutritionnelles peut être également une source de fibres et de colorants naturels.


**Mots clés :** *Opuntia ficus-indica*, Activité antioxydantes, polyphénols, dermacosmetiques.

## ملخص


في إطار تثمين النباتات الطبية الجزائرية ،ينمو نبات التين الشوكي تلقائيا لكن تم إهماله لفترة من الوقت و هو معروف على نطاق واسع بخصائصه المفيدة .

الهدف من العمل الحالي هو التركيز على الأنشطة البيولوجية لنبات التين الشوكي و خاصة مضادات الأكسدة و استخداماتها في المنتجات الطبيعية الجلدية و التجميلية ،وتثمين أجزاء نبات التين الشوكي و الاهتمام بها من خلال التركيز على استخداماتها في العديد من المجالات .

نحن قمنا بدراسة مختلف أنشطة مضادات الأكسدة لأجزاء نبات التين الشوكي: العصير، الاوراق، البذور الجافة و قشور عن طريق قياس المحتوى الفينولي الكلي و ثلاث فحوصات لمضادات الأكسدة ، ABTS، DPPH،CUPRAC.

وقد تمت مقارنة النتائج التي تم الحصول عليها وافتراض الارتباط بين أساليب مضادات الأكسدة والمحتوى الفينولي الكلي.

يشجع الاهتمام المتزايد بالطب الوقائي على استخدام المركبات النشطة بيولوجيا حيث يمكن ان تستخدم للوقاية ضد شيخوخة البشرة وضد سرطان البشرة، ويمكن أن يكون نبات التين الشوكي بقيم غذائية محددة مصدرا للألياف الغذائية والأصباغ الطبيعية.


**الكلمات المفتاحية:** نبات التين الشوكي، نشاط مضاد للأكسدة، بوليفينول، مستحضرات التجميل الجلدية.

**Academic year :**2020/2021

**Presented by :** *GHARSELLAH Khaoula*

*BOUKAOUS Racha El Bahaa*

# Phytochemical study and biological applications of *Opuntia ficus-indica* as an anti-aging agent.

End of cycle dissertation for obtaining a master degree in Biochemistry.

## Abstract

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The increasing interest in preventive medicine encourages use of natural products against senescence of the skin and skin cancer, also the use of nutraceuticals bioactive compounds of vegetable origin, *OFI* with important nutritional values can be source of dietary fibers and natural pigments.

**Mots clés :** *Opuntia ficus indica*, antioxidant activity, polyphenols, dermocosmetics.

**Research laboratory:** Laboratory for obtaining Therapeutic Substances LOST.

## Evaluation jury:

**Jury president :***MOUSSAWI Samira* (MCB –UFM Constantine 1)

**Supervisor:** *MOUAS Toma Nardjes* (MCA –UFM Constantine 1)

**Examiner :** *LEMOUI Radouane* (MCB –UFM Constantine 1)

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