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Dedication

Not all letters can find the right words
All words cannot express gratitude, love, respect,
recognition, it is simply that I dedicate this memoir

To ***my perfect parents:***

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Chaima

Abstract

Our study focused on the research of the antioxidant and antimicrobial activity effect, as well as the evaluation of the cytotoxic effect of ethanolic extracts from the aerial part of *Cynodon dactylon* L (*Poaceae* family) extracted using two methods maceration assisted extraction (MAE) and ultrasound assisted extraction (UAE).

The phytochemical tests realized have highlighted various secondary metabolites in the aerial part of the plant including saponosides, terpenes, alkaloids, reducing sugars, cardiac glycosides, flavonoids and polyphenols. The yields of crude extracts were 9.40% for the MAE extract and 12.52% for the UAE extract.

The results of the estimation of the phenolic compounds showed that the extract obtained by MAE contains a high level of polyphenols and total flavonoids estimated by (42.14 ± 0.75 mg EAG / gE and 23.57 ± 0.78 Mg EQ / gE), compared to the extract obtained by the UAE, which estimated by (29.93 ± 0.14 mg EAG / gE and 13.53 ± 0.33 Mg EQ / gE). In contrast, the content of condensed tannins in the extract of UAE (19.34 ± 0.48 Mg EC/gE) is higher than the extract of MAE (15.99 ± 0.63 Mg EC/gE).

The evaluation of the antioxidant activity, carried out by two methods (Trapping of the free radical DPPH and ferric reducing antioxidant power FRAP) revealed a considerable antioxidant response. the DPPH test revealed that the MAE extract represents the most active extract, with an IC_{50} =7.52 ± 0.037 mg / ml and followed by the UAE extract with an IC_{50} = 8.83 ± 0.032 mg / ml. The same for the FRAP test, with value of 15.83 ± 0.37 mg EAA / g for MAE extract, followed by the UAE extract with a value of 15.38 ± 0.12 mg EAA / g.

The antibacterial activity was evaluated against four strains: *Pseudomonas aeruginosa, Salmonella Typhimurium, Staphylococcus aureus* and *Bacillus cereus.* Also the antifungal activity against two strains *Aspergillus carbonarius* and *Aspergillus parasiticus*. the results that we have reached show that all the strains targeted have high susceptibility to the two ethanolic extracts of *Cynodon dactylon* L.

The evaluation of cytotoxicity against RBCs was carried, the results shown the non-toxic effect of *C. dactylon* extracts. Thus making it suitable for the preparation of drugs involved in the treatment of various diseases.

Keywords: *Cynodon dactylon*, phenolic compounds, antioxidant activity, antimicrobial activity, cytotoxicity.

المـــخلص

ركزت دراســتنا على البحث عن تأثير النشــاط المضــاد للأكســدة والمضــاد للميكروبات، و تقييم تأثير الســمية الخلوية للمســتخلصــات الايثانولية للجزء الهوائي من نبات النجم .*Cynodon dactylon* L (العائلة النجيلية) المستخرجة باستخدام طريقتين، الاستخراج بواسطة النقع والاستخراج بواسطة الموجات فوق الصوتية .

وقد ســلطت الاختبارات الكيميائية النباتية المحققة الضــوء على العديد من المســتقلبات الثانوية في الجزء الهوائي من النبات بما في ذلك التربينات، الصــابونوزيدات، القلويدات، الســكريات المرجعة، الجليكوســيدات القلبية، الفلافونيدات والبوليفينول. حيث بلغ مردود المستخلصات الخام نسبة 9.40٪ للمستخلص المستخرج بواسطة النقع و نسبة 12.52٪ للمستخلص المستخرج بواسطة الموجات فوق الصوتية.

أظهرت نتائج تقدير المركبات الفينولية أن المســتخلص الذي تم الحصــول عليه بواســطة النقع يحتوي على مستوى عال من البوليفينول الإجمالي والفلافونويدات المقدرة بـ (42.14 ± 0.75 مغ مكافئ من حمض الغاليك/غ من المادة الجافة و 23.57 ± 0.78 مغ مكافئ من الكرسيتين/غ من المادة الجافة)، مقارنة بالمستخلص الذي تم الحصول عليه بواســطة الموجات فوق الصــوتية، والتي قدرت بـــ (29.93 ± 0.14 مغ مكافئ من حمض الغاليك/غ من المادة الجافة و 13.53 ± 0.33 مغ مكافئ من الكرســيتين/غ من المادة الجافة). في المقابل، محتوى التانينات المكثفة في المستخلص المستخرج بواسطة الموجات فوق الصوتية (19.34 ± 0.48 مغ مكافئ من الكاتيشين/غ من المادة الجافة) أعلى من المستخلص المستخرج بواسطة النقع (15.99 ± 0.63 مغ مكافئ من الكاتيشين/غ من المادة الجافة).

كشف تقييم النشاط المضاد للأكسدة، الذي تم إجراؤه بطريقتين (قوة احتجاز الجذر الحر DPPH• والقدرة الارجاعية للحديد FRAP) عن اســتجابة كبيرة مضــادة للأكســدة. كشــف اختبار DPPH أن المســتخلص المســتخرج بواسطة النقع يمثل أكثر المستخلصات نشاطًا ، بقيمة (0.037 ± 7.52=IC_{50} مغ / مل) ويليها المستخلص المستخرج بواســطة الموجات فوق الصــوتية بقيمة (0.032 ± 8.83= IC_{50} مغ / مل). نفس الشــيء بالنســبة لاختبار FRAP ، بقيمة (15.83 ± 0.37 مغ مكافئ من حمض الاســكوربيك /غ من المادة الجافة) للمســتخلص المســتخرج بواســطة النقع، يليه المســتخلص المســتخرج بواســطة الموجات فوق الصــوتية بقيمة تســاوي (15.38 ± 0.12 مغ مكافئ من حمض الاسكوربيك /غ من المادة الجافة).

تم تقييم النشـــاط المضـــاد للبكتيريا على أربع ســـلالات: *Pseudomonas aeruginosa*، *Salmonella Typhimurium*، *Staphylococcus aureus* و *Bacillus cereus*. أيضــا النشــاط المضــاد للفطريات على ســلالتين *Aspergillus carbonarius* و *Aspergillus parasiticus* . أظهرت النتائج التي توصــلنا إليها أن جميع الســلالات المستهدفة لها حساسية عالية للمستخلصين الإيثانوليين لـ .*Cynodon dactylon* L

تم إجراء تقييم الســمية الخلوية ضــد كريات الدم الحمراء، حيث أظهرت النتائج التأثير غير الســام لمستخلصات *C. dactylon* مما يجعلها مناسبة لإعداد الأدوية المشاركة في علاج الأمراض المختلفة.

الكلمات المفتاحية: *Cynodon dactylon*، المركبات الفينولية، النشـــاط المضـــاد للأكســـدة، النشـــاط المضـــاد للميكروبات، السمية الخلوية.

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Abbreviation list

A	
APX	Ascorbate Peroxidase
ARP	Anti-Radical Power
ATCC	American Type Culture Collection
B	
BDE	Bond Dissociation Energy
C	
CAT	Catalase
CHI	Chalcone Isomerase
CHS	Chalcone Synthase
CoA	Coenzyme A
D	
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	Diphenyl Picryl Hydrazyl
E	
EGCG	Epigallocatechin Gallate
EtOH	Ethanol
F	
FRAP	Ferric Reducing Antioxidant Power
G	
GEN	Gentamicin
GPX	Glutathione Peroxidase
GR	Glutathione Reductase
H	
H_2O_2	Hydrogen Peroxide
HDL	High Density Lipoprotein
HRBCs	Human Red Blood Cells
I	
IC_{50}	Inhibitory Concentration of 50%.
IP	Ionization Potential
L	
LCMS	Liquid Chromatography-Mass Spectrometry

LDL	Low Density Lipoprotein
M	
MAE	MAE Maceration Assisted Extraction
MIC	MIC Minimum Inhibitory Concentration
O	
O.D.	Optical Density
P	
P	Penicillin
PAL	Phenylalanine Ammonialyase
PBS	Sodium Phosphate Buffer
Pcs	Phenolic Compounds
PH	Potential Hydrogen
POD	Peroxidase
R	
R•	Free Radical
RBCs	Red Blood Cells
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
S	
SC	Supercritical
SCFE	Supercritical Fluid Extraction
SDS	Sodium Dodecyl Sulfate
SOD	Superoxide Dismutase
SPE	Solid Phase Extraction
T	
TCA	Trichloroacetic Acid
U	
UAE	Ultrasonic-Assisted Extraction
UV	Ultraviolet
W	
WHO	World Health Organization

Introduction

While globalization has generated many benefits for society, it has also raised new challenges, particularly with regard to health. In recent years, there have been notable increases in the occurrence of many diseases which affected the human health around the world **(Sherman, 2010)**.

One of the health challenges, are infectious diseases. There are many pathogenic microorganisms who cause infections which treatment with the usual antibiotics is not effective, that is to say, the pathogenic bacteria have developed resistance to the antibiotic drugs **(Álvarez-Martínez et al., 2020)**. In the present scenario, this multiple drug resistance necessitates a search for new antimicrobial substances from natural sources **(Li et al., 2013)**.

Moreover, oxidative stress has actually been described as a crucial etiological factor involved in various chronic human diseases such as cancer, cardiovascular and neurodegenerative diseases, inflammation and diabetes mellitus **(Ghedadba et al., 2015)**. The interplay between oxidants and antioxidants is important in maintaining health, owing to the fact that the generation of free radicals is balanced by the body's endogenous antioxidant systems and the ingestion of exogenous antioxidants. The oxidative stress occurs, if the generation of these radicals exceeds the protective effects of antioxidants **(Tan et al., 2018)**. Synthetic antioxidants have been assumed to cause negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with antioxidants occurring naturally including the bioactive compounds of plants **(Poojary et al., 2016)**.

Plants are widely used as a potent source for isolation of several drugs and formulations in treatment of many diseases. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies **(Balasundari & Boominathan, 2018)**. Besides, plant extracts have been shown to be clinically effective and relatively less toxic than existing drugs **(Al-Rifai et al., 2017)**.

Algeria is one of the richest countries with plants because of its vast area and climate diversity, but still there are many species of plants that have a few studies are caring out about them, so in this study, we chose one of these plants, which is *Cynodon dactylon* (L.) Pers. This plant is a perennial herb with some traditional properties such as anti-inflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent and other diseases **(Badri & Solanki, 2011)**. But at the same time, especially in our region –ElOued- it is

considered as a weed that has harmful effects on neighboring crops and is discarded **(Hillis, 2005)**.

The main objective of our study was designed to evaluate this medicinal herbs and to figure out the biological activities of the aerial part of *C. dactylo*n extracts. Therefore, we will present a fundamental research on the *Cynodon dactylon* plant which is based on a phytochemical screening of our plant allowing initially a selection of bioactive molecules. Thereafter, the most active molecules are subjected to an extraction by two methods Maceration Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE) allowing the obtaining of two crude ethanolic extracts from our plant, then we will determine the total polyphenols, flavonoids and condensed tannins contents of the crude extracts, also we will estimate the antioxidant and antimicrobial activity of the ethanolic extracts and finally we will assess the toxicity of our crude extract by a cytotoxicity model which is the hemolysis test. So this manuscript is divided into two parts:

The first part is devoted to a bibliographical synthesis, which consists of two chapters, one on the presentation of the plant studied (*Cynodon dactylon* L. Family: Poaceae). and the other on phenolic compounds.

The second part concerns the experimental part, which has two chapters, one on the materials and methods of our study; The second chapter brings together all the results which will be followed by a discussion. Finally, we ended our work with a conclusion which is a set of reflections completes this work and perspectives.

First part

Bibliographic Part

Chapter One

Cynodon dactylon L. Pers

I.1. Poaceae family

Grasses (Poaceae or Gramineae) are the fifth most diverse family among the flowering plants or Angiosperms and the second most diverse family among the Monocotyledons. Poaceae comprises about 10,000 species in approximately 700 genera **(Finot et al., 2011)**.

There is a great diversity in Poaceae and have vital role in the survival of all organism especially human and animals. The most abundant plant on earth are grasses of Poaceae that are found on all continents including Antarctica because grasses have the unique capability to adopt to all types of habitats on earth **(Saadullah & Ashfaq., 2016)**.

I.2. *Cynodon dactylon* (L.) Pers

Cynodon dactylon (Bermuda grass) is perennial grass rich in metabolites, including proteins, carbohydrates, minerals, flavonoids, carotenoids, alkaloids, glycosides and triterpenoids. Distributed throughout the world, especially in warm temperate and tropical regions (Figure 01) **(Kaliyaperumal et al., 2013)**.

Figure 01 : *Cynodon Dactylon* (L.) Pers **(Original photo., 2019)**

It is tested to have antidiabetic, diuretic, anti-oxidant, anti-ulcerative, allergic activity, its anticarcinogenic potential and its protective action against right heart failure **(Singh et al., 2007; Albert-Baskar & Ignacimuthu, 2010)**.

I.2.1. Common names around the world

Algerian: Nedjem **(Chehma, 2006)**.

Arabic: Thaiel, Najeel, Echrish, Tohma **(Al-snafi, 2016)**.

India: Dhub, Doob **(Al-snafi, 2016)**.

Chinese: Gou ya gen **(Al-snafi, 2016)**.

French: Chiendent dactyle, Chiendent pied-de-poule **(Paul et al., 2012)**.

English: Bermuda grass, Devil's grass, Dogtooth grass, Quick grass **(Paul et al., 2012)**.

Spanish: Came de niño, Chepica brave, Grama rastrera, **(Paul et al., 2012)**.

I.2.2. Description

It is a perennial plant carrying many long-sheathed leaves surrounding the stem and flattened limb 2.5-20cm in length. The inflorescence consists of 2 to 6 toothed spikes, 50mm in length at most, straight, purplish or green, arranged in two rows close. It is a plant with a long crawling rhizome on the surface of the soil about 20m in length, it is very ramified (Figure 02) **(Benzahi, 2017)**.

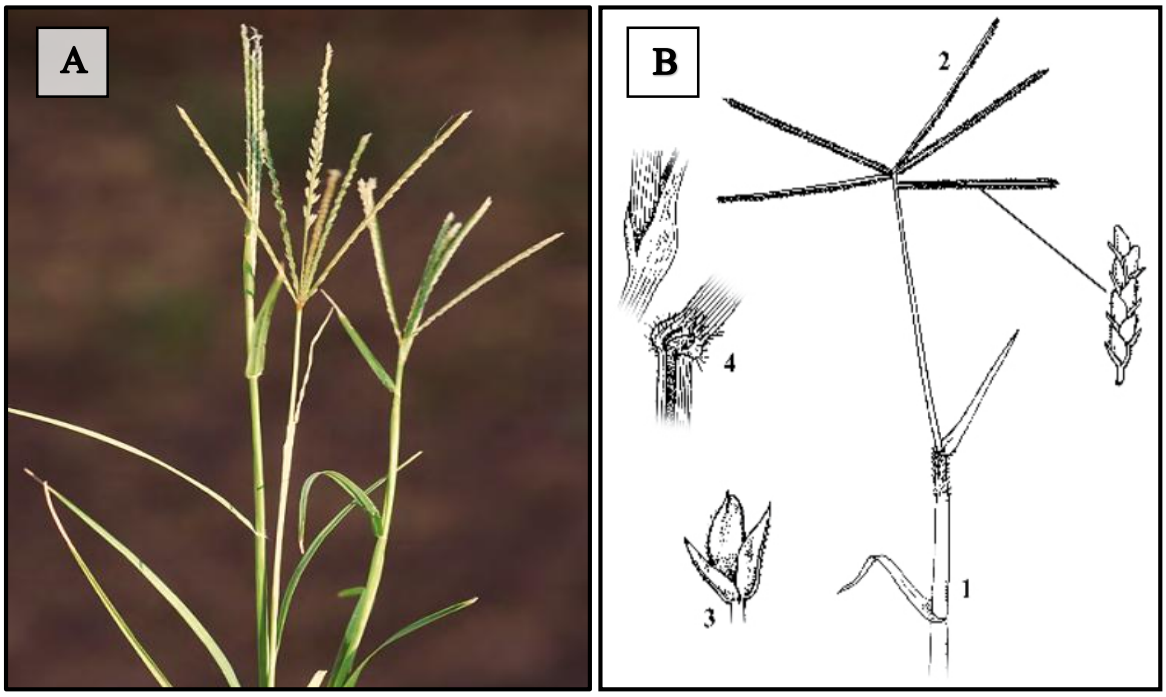


Figure 02: Morphology of *Cynodon dactylon*

A: real photo **(José et al., 2018),** B: schematic photo (**Hillis, 2005)**.

I.2.3. The general life cycle

- In the spring when the temperature begins to increase new stolons elongate and aerial shoots sprout.
- The characteristic prostrate growth of Bermuda grass lasts for one to several months, early in the season, before flowering culms develop.
- Most of the lateral growth, produced in concentric circles from the original rhizome, occurs throughout the summer **(Dara, 1992)**.

I.2.4. Ecology

It can survive in wide range of soil type and with a wide range (5.0-8.0) of pH values. however, alkaline soils are tolerated more than acidic ones, tolerates long periods of flooding, but little to no growth occurs without adequate soil aeration. Optimal temperature for growth is 24°C **(Paul et al., 2012)**.

I.2.5. Taxonomical classification

The taxonomy of *Cynodon dactylon* according to **(Al-snafi, 2016)**

Kingdom **:** Plantae
Subkingdom **:** Tracheobionta
Super division **:** Spermatophyta
Division **:** Magneliophyta
Class **:** Liliopsida
Subclass **:** Commelinidae
Order **:** Cyperales
Family **:** Poaceae
Genus **:** Cynodon
Species **:** *Cynodon dactylon*

I.2.6. Traditional uses

Cynodon dactylon is a weed and has been regarded to possess varied medicinal properties. The aqueous fluid extract of some parts is used as anti- inflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent. Since, high potential of hypoglycemic، hypolipidemic and antioxidant activities. It used also as a folk remedy for cancer, convulsions, cystitis, diarrhea, headache, hemorrhage, hypertension. It is also useful against pains, inflammations, toothache and grippe in children and is applied to bleeding cuts and wounds to stop bleeding **(Badri & Solanki, 2011; Singh et al., 2009)**.

- For epistaxis, a mixture of the plant with honey.
- For oral administration, a juice of the plant with honey 2-3 times a day.
- For local application, a paste of the plant extract.
- For problem of urine retention, a decoction of *Cynodon dactylon* mixed with sugar. **(Badri & Solanki, 2011)**.

I.2.7. Previous studies realized on *Cynodon dactylon*

To achieve an improvement in traditional medicine, phytochemical investigations must be made, in order to provide a scientific justification for the traditional use of natural substances.

According to the literature consulted, there are extensive studies and different tests carried out on *Cynodon dactylon* have shown several biological and biochemical effects. Among the most important:

I.2.7.1. Antioxidant studies

Recent studies have been conducted on Ethyl acetate, Methanol and aqueous extract of some parts of *Cynodon dactylon* exhibited significant *in vitro* antioxidant activity by DPPH method, hydroxyl radical scavenging activity, reducing power and lipid peroxide free radical scavenging assay. Also in vivo antioxidant activity increased glutathione peroxidase (GPX), Superoxide dismutase (SOD) and catalase antioxidant enzyme levels **(Albert-Baskar &Ignacimuthu, 2010; Tuhin et al., 2016; Kumar et al., 2018**).

Another kind of Antioxidant studies was realized by **Shuduan et al., (2010)**, on the physiological responses of anti-oxidative enzymes and carbohydrate contents of Bermuda grass. The results suggest that The activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) in roots increased with the increase of the durations and depths of submergence, so Bermuda grass balancing between the formation and detoxification of activated oxygen species.

I.2.7.2. Antifungal studies

There are two studies on antifungal potential of *Cynodon dactylon*, the first was evaluated against *Botrytis cinerea* Pers. Different organic fractions were isolated from *C. dactylon* methanol extract viz. n-hexane, chloroform, ethyl acetate and n-butanol. Minimum inhibitory concentration (MIC) of these isolated fractions and synthetic fungicide was recorded. Chloroform, ethyl acetate and synthetic fungicide were found most effectual in retarding conidial germination of test fungus. This study concluded that methanolic extract of *C. dactylon* contains antifungal agents against *B. cinerea* **(Faiza et al., 2015)**.

The second was realized by **Abdelaali &Lakhdar, (2016)** on antifungal activity of *C. dactylon* ethanol Solid Phase Extraction (SPE) extract against *Ganoderma boninense* was investigated. Based on Liquid Chromatography-Mass Spectrometry (LCMS) analysis, some possible antifungal compounds against *G. boninense* were identified as Tokoronin,

Ophiopogonin C and Cyclopassiflosides (Saponins), Elemicin (Phenolics), 5-oxo-7octenoic acid, Stearidonic acid and 17-Hydroxylinolenic acid (Fatty acid), Neocnidilide (carboxylic acid), Gingerglycolipid B and Apiole.

I.2.7.3. Antibacterial studies

Many studies effected on the antibacterial activity of *Cynodon dactylon*. Different organic solvents such as n-butanol, petroleum ether, methanol, ethyl acetate and chloroform were used to extract the bioactive compounds from *Cynodon dactylon* to screen the antibacterial activity against infectious disease causing bacterial pathogens such as *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus Coagulasse* (ATCC 5118), *Staphylococcus aureus* ATCC 25923, *Klebcsiella pneumonie*, *Enterococcus faecalis*, *Proteus mirabillis* and *Pseudomonas aeruginosa* by paper disc method. In general, leads to deduce that *C. dactylon* extracts showed stronger antibacterial activity against the bacterial pathogens **(Suresh et al., 2008; Yogesh et al., 2011; Abdullah et al., 2011; Abdullah et al., 2012; Shafaque, 2014; Syahriel et al., 2014; Abdelaali & Lakhdar, 2016)**.

I.2.7.4. Anti-hyperlipidemia studies

In the recent past, an increasing research evidence are getting accumulated, which clearly indicate the positive role of *C. dactylon* extracts on hypolipidemic. The results of this studies showed a significantly decrease ($p<0.05$) in the lipid profile (cholesterol, triglyceride, and HDL) except LDL **(Mahesh & Brahatheeswaran, 2007; Singh et al., 2007; Singh et al., 2008; Edeh et al., 2014)**.

I.2.7.5. Anti-hyperglycemic studies

Diabetes has been treated with natural products with high antidiabetic potential and lesser or no side effects. Therefore, there is an increasing demand of several medicinal plants or their extracts. The hypoglycemic and antidiabetic effect of administration of the aqueous extract of *Cynodon dactylon* were tested in hyperglycemic rats. The researchers conclude that aqueous extract of *C. dactylon* is able to reduce the fasting blood sugar and the urine sugar level in diabetic rats **(Singh et al., 2007; Jarald et al., 2008; Alam et al., 2018)**.

For the ethanolic extract of *C. dactylon* showed in addition to the fasting blood glucose_ a significant increase in the liver glycogen and a significant decrease in the glycosylated hemoglobin levels. **(Mahesh & Brahatheeswaran, 2007; Singh et al., 2008)**.

I.2.7.6. Another studies

Cynodon dactylon showed curative effect against streptozotocin induced hepatic injury in diabetic rats. Also decreases the cardiovascular biomarkers (cardiac troponin I, homocysteine) and adenosine deaminase activity **(Singh et al., 2009; Azimzadeh & Digale, 2017)**.

Chapter two

Phenolic Compounds

II.1. Polyphenolic compounds

Many medicinal plants contain various secondary metabolites, such as polyphenolic compounds. These bioactive compounds, representing the most desirable phytochemicals due to their potential to be used as additives in food industry, cosmetics, medicine and others fields **(Bencheikh, 2012; Bujor et al., 2015).**

The phenolic compounds are responsible for pigmentation and plants defense of pathogens agents. These compounds are different from plant family to other، from organ to other or at certain stages of development **(Bujor et al., 2015; Patricia, 2017)**.

A large group of chemical compounds that included in the polyphenolic compounds, which classified according to the number of carbon atoms present in four representative subgroups (Figure 03): flavonoids, phenolic acids, tannins and stilbenes **(Patricia, 2017)**.

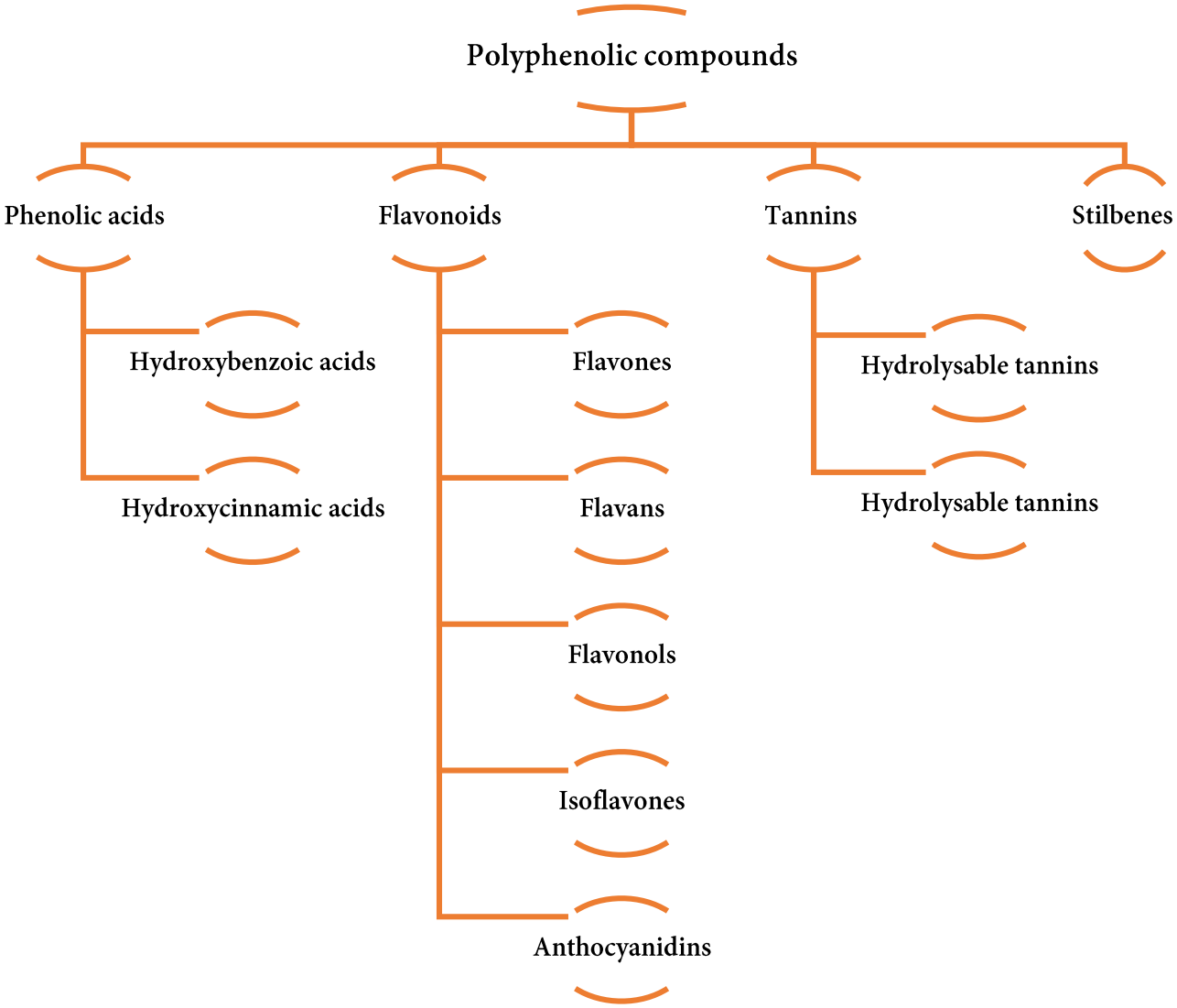


Figure 03: Main classes of polyphenolic compounds **(Ozcan et al., 2014)**.

II.2. Main classes of polyphenolic compounds

II.2.1. Phenolic acids

Phenolic acids are aromatic secondary plant metabolites widely distributed throughout the plant kingdom **(Bencheikh, 2012)**. These compounds containing carboxyl group with one or more hydroxyl groups grafted onto a benzene nucleus **(Bujor et al., 2015)**. Based on position of the hydroxyl group, phenolic acids can be divided into two main types, benzoic acid and cinnamic acid derivatives **(Tsao, 2010)**.

II.2.1.1. Hydroxybenzoic acids

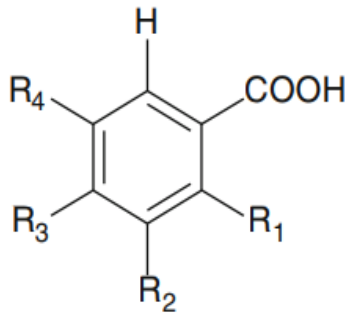
The basic skeleton in common have the (C6–C1) structure, this subclass includes gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids **(Ozcan et al., 2014)**.

II.2.1.2. Hydroxycinnamic acids

These acids are aromatic compounds with a three-carbon side chain (C6–C3), with caffeic, ferulic, p-coumaric and sinapic acids being the most common (Table 01) **(Ozcan et al., 2014)**.

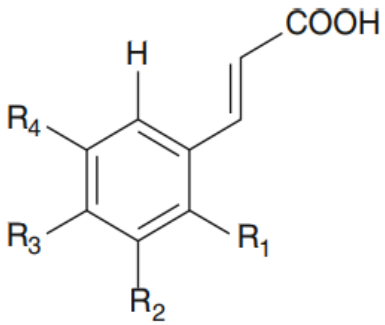
Table 01: Phenolic acids: hydroxybenzoic and hydroxycinnamic acids **(Goleniowski et al., 2013)**.

Hydroxybenzoic acids



Name	R1	R2	R3	R4
Benzoic acid	H	H	H	H
p-Hydroxybenzoic acid	H	H	OH	H
Vanillic acid	H	OCH3	OH	H
Gallic acid	H	OH	OH	OH
Protocatechuic acid	H	OH	OH	H
Syringic acid	H	OCH3	OH	OCH3
Gentisic acid	OH	H	H	OH
Veratric acid	H	OCH3	OCH3	H
Salicylic acid	OH	H	H	H

Hydroxycinnamic acids



Name	R1	R2	R3	R4
Cinnamic acid	H	H	H	H
o-Coumaric acid	OH	H	H	H
m-Coumaric acid	H	OH	H	H
p-Coumaric acid	H	H	OH	H
Ferulic acid	H	OCH3	OH	H
Sinapic acid	H	OCH3	OH	OCH3
Caffeic acid	H	OH	OH	H

II.2.1.3. Biosynthesis of phenolic acids

As show the Figure 04, that all phenolic compounds like the phenolic acids, gallic acid and cinnamic acid are considered to be metabolites of the shikimate pathway **(Goleniowski et al., 2013)**.

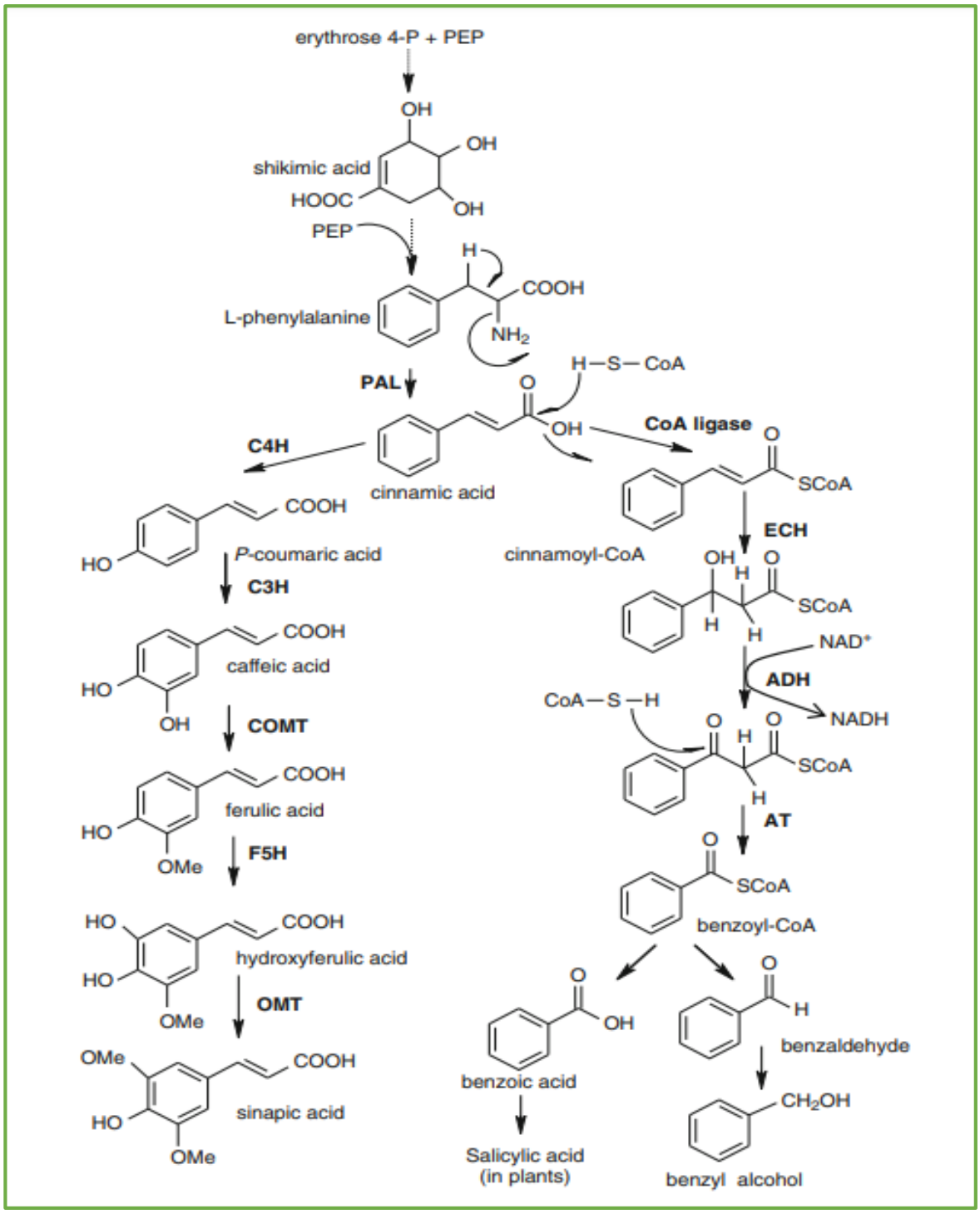


Figure 04: Pathway of the shikimic acid in the biosynthesis of phenolic acids **(Goleniowski et al., 2013)**.

II.2.2. Flavonoids

II.2.2.1. Generalities

Flavonoids are the most abundant PCs in fruits and vegetables, they account for nearly two-thirds of dietary PCs; and, as a group, they are the most bioactive **(Laura et al., 2019)**. They are C_{15} compounds (Figure 05) all of which have the structure (C_6-C_3-C_6) which are two benzene rings (A and B) linked together by a group of three carbons **(Vermerris & Nicholson, 2008)**. The C_3 moiety forms an aliphatic chain or a six-membered heterocyclic ring (ring C) attached to ring A **(Verma et al., 2020)**.

Flavonoids can be divided into six groups or families, according to differences in the pyran ring. In each family, individual compounds differ in their pattern of hydroxylation and methylation of rings A and B **(Laura et al., 2019)**.

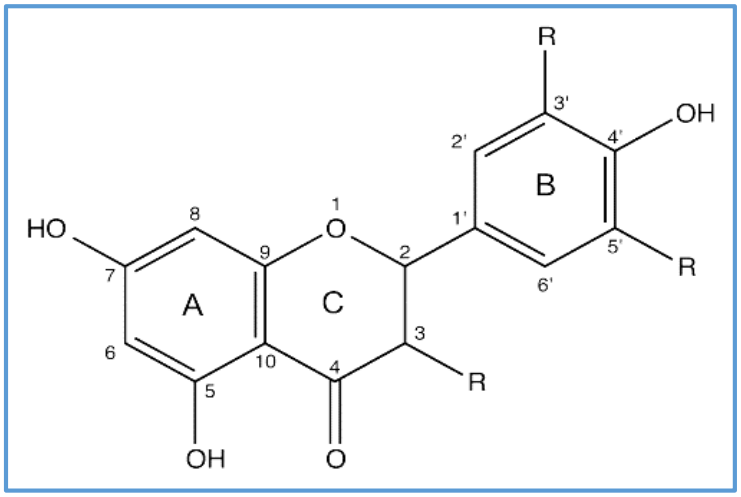


Figure 05: Basic chemical structure and numbering pattern of flavonoids **(Yáñez et al., 2013)**.

II.2.2.2. Synthesis of Flavonoids

Flavonoids are synthesized via the phenylpropanoid pathway . The phenylalanine structure from phenolic compounds is transformed to cinnamate by the enzyme phenylalanine ammonialyase (PAL). The cinnamate 4-hydroxylase (CH_4) converts cinnamate to p-coumarate (Figure 06), and then an acetyl-CoA group is added by the CoA ligase enzyme to yield cinnamoyl-CoA. Lastly, this product is transformed by chalcone synthase (CHS) to yield a general chalcone structure .The chalcone structure is further metabolized by the chalcone isomerase (CHI) to the general chiral flavanone structure. From the general chiral flavanone structure, the other derivatives, namely, dihydroflavonols, flavonols, flavones, flavan-3-ols, flavan-3,4-diols, isoflavonoids, and neoflavonoids, are

further metabolized by a well-characterized enzymatically derived process **(Yáñez et al., 2013)**.

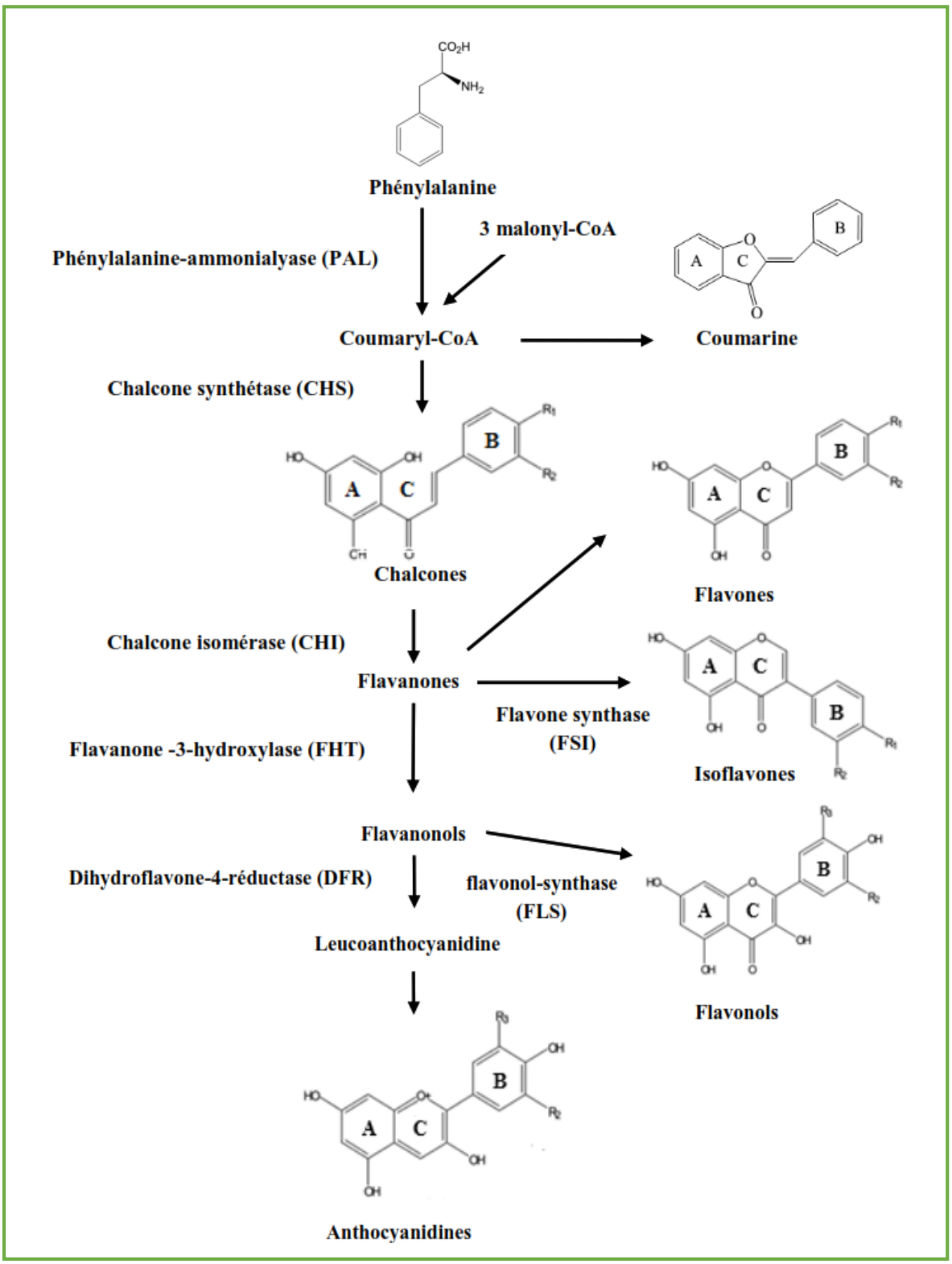


Figure 06: Main stages of biosynthesis of the different classes of flavonoids **(Saffidine, 2015)**

II.2.3. Tannins

The tannins are complex phenolic substances, amorphous, rarely crystalline substances, soluble in water and alcohol, having an astringent and bitter taste **(Mukherjee et al., 2019)**.

Chemically, tannin is not a simple substance, but an aggregation of a complex organic compound. Tannin is classified in terms of its chemical property into:

II.2.3.1. Hydrozable tannin, that is hydrolyzed by heating with a dilute acid to generate gallic acid, ellagic acid, and the like **(Tahir et al., 2019)**. These compounds are consist of gallic acid and its dimeric condensation product, hexahydroxydiphenic acid, esterified to a polyol, which is mainly glucose. hese metabolites can oxidatively condense to other galloyl or hexahydroxydiphenic molecules and form high-molecular-weight polymers. As their name indicates **(Bravo, 1998)**.

II.2.3.2. Condensed tannin or proanthocyanidins, that is polymerized to generate phlobaphene that is insoluble in water **(Tahir et al., 2019)**. Condensed tannins are high-molecular-weight polymers. The monomeric unit is a flavan-3-01 (catechin, epicatechin, etc.), with a flavan-3,4-diol or leucoanthocyanidin molecule as its precursor. Oxidative condensation occurs between carbon C-4 of the heterocycle and carbons C-6 or C-8 of adjacent units **(Bravo, 1998)**.

II.2.4. Stilbenes

Stilbenes are another class of compounds that are part of polyphenols with 1,2-diphenylethylene as basic structure **(Bujor, 2016)**.

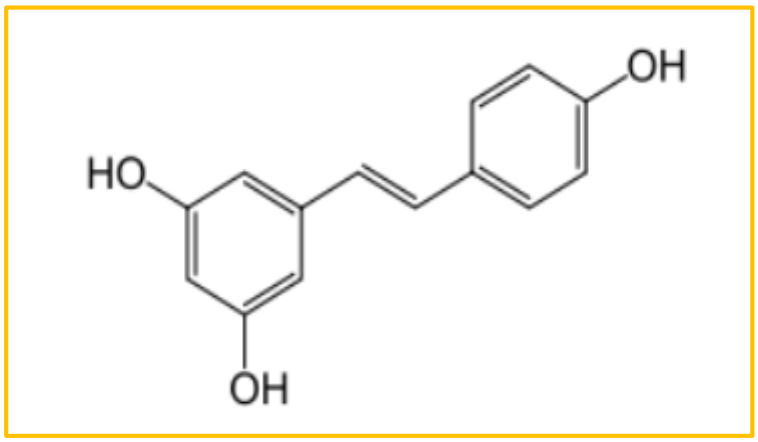


Figure 07: Chemical structure of stilbene (resveratrol) **(Bujor, 2016)**.

Resveratrol (Figure 07) is the main representative of this group of phenolic compounds. This compound exists in two stereoisoforms with configuration cis- or trans-,

the latter being the most widely studied, although cis-resveratrol may also possess health promoting properties **(Giovinazzo et al., 2012)**. High interest in this antioxidant compound is linked to its use in the treatment of cardiovascular diseases. However, resveratrol also demonstrates significant neuroprotective activity in vitro and in vivo **(Kelsey et al., 2010)**.

II.3. phenolic compounds roles in plant physiological processes

Researches carried in the recent years have shown that natural compounds with aromatic structure, such as phenolic compounds have extremely complex roles in plant physiological processes. These compounds help to preserve the integrity of plant with continuous exposure to environmental stressors, including relatively high temperatures **(Bujor, 2016)**, defensive agents (antifungal, insecticidal, and allelopathic), signal substances between autotrophs and heterotrophs (symbiosis, pathogenesis, feeding, and ovipositing) and ultraviolet absorbing **(Tahara, 2007)**.

The phenolic compounds as bioregulators have an important role in the growth and development of different parts of plants: roots, stems, buds, leaves **(Tanase et al., 2013)**. The functional diversity of flavonoids is due to their structural diversity and their general properties as polyphenols. Due to the great numbers of known flavonoids estimated to be about 10,000 **(Tahara, 2007)**. Besides being radical scavengers, flavonoids, for example are able to function as chelators for metals, depending on the molecular structure **(Stingu et al., 2012)**.

II.4. Extraction of phenolic compounds

Several techniques are used to extract phenolic compounds. These techniques are either conventional, such as maceration, soxhlet extraction, or new, such as microwave assisted extraction, ultrasound, supercritical fluid or subcritical.

II.4.1. Conventional Extraction Techniques

II.4.1.1. Maceration

It is very famous and inexpensive technique to isolate the desired components. Its operation is quite simple **(Hafiz & Colin, 2020).** In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing **(Handa et al., 2008)**.

II.4.1.2. Soxhlet extraction

Soxhlet extraction (Figure 08) is a most commonly used conventional technique to extract different compounds from the plant materials. It is an ordinary case of a comprehensive solid–fluid extraction. This procedure relies on the exchange of the target compound(s) from the sample (solid) to appropriate organic solvent(s). It is guaranteed that the extraction solvent (fluid) remains reliably in contact with the sample during this thermal extraction process **(Hafiz & Colin, 2020).**

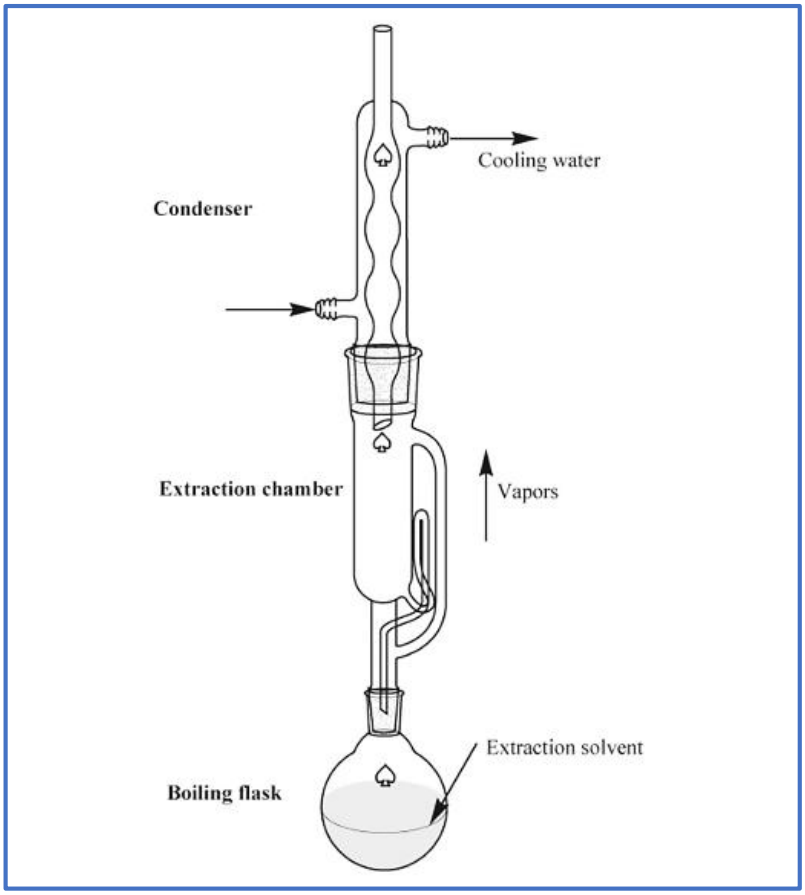


Figure 08: Soxhlet extractor **(Hafiz &Colin, 2020)**.

II.4.2. Novel Extraction Techniques

II.4.2.1. Microwave-assisted extraction

In recent years has observed an increasing attention for new extraction techniques such as Microwave-assisted extraction enabling accelerating and shortening extraction times, efficient extraction, automation, and reduction of organic solvent consumption **(Spingo & Faveri, 2009)**.

Microwaves heat up the molecules by dual mechanism of ionic conduction and dipole rotation. Non-ionizing electromagnetic waves positioned between the X-ray and infrared rays in the electromagnetic spectrum with frequency between 300 MHz to 300 GHz are called microwaves. The two types of oscillating perpendicular fields that generate microwaves are the electric field and magnetic field. Both The ionic conduction and dipole rotation are responsible for heating of substances **(Tatke & Jaiswal, 2011)**.

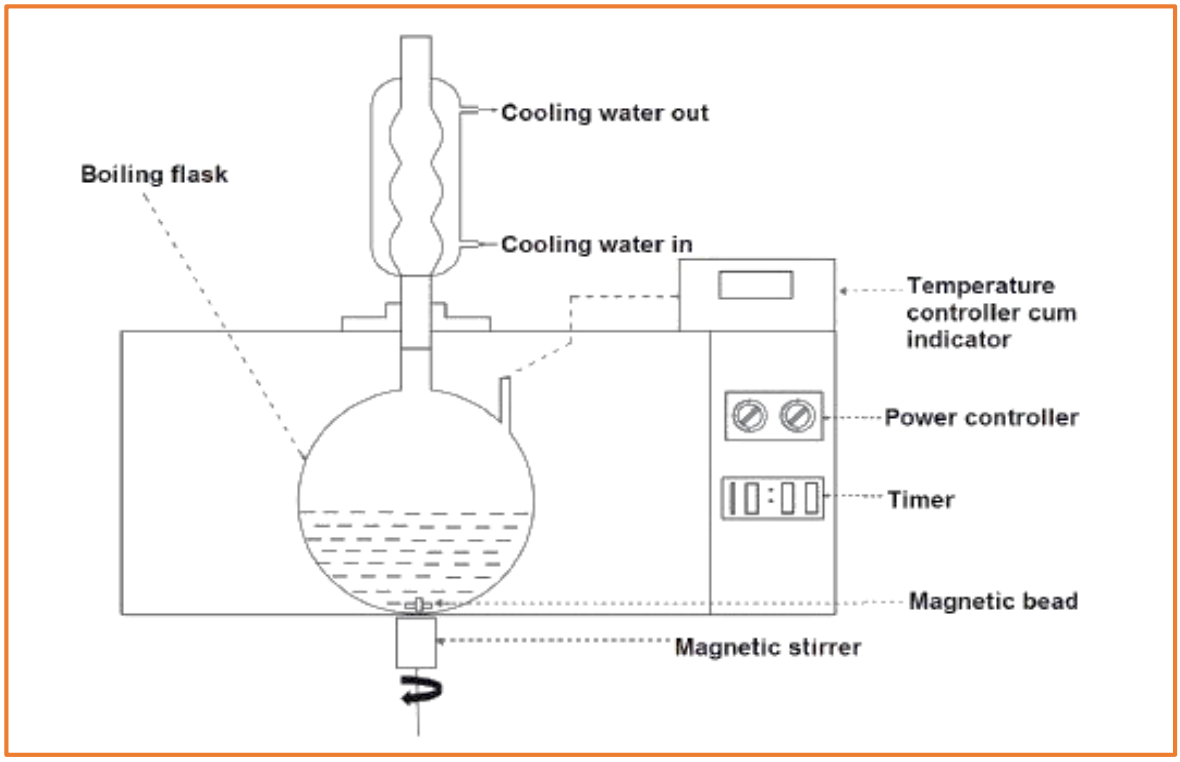


Figure 09: Experimental set for microwave-assisted extraction **(Krishnan & Rajan, 2017).**

The application of microwaves montage is clear in Figure 09. For extracting phenolic compounds present in plants requires considerable optimization of the operational parameters such as temperature, time and microwave power **(Azevedo & Rezende, 2016)**.

II.4.2.2. Ultrasound-Assisted Extraction

Ultrasound- or ultrasonic-assisted extraction (UAE) is used for bioactive compounds including the polyphenols from various plant samples. In UAE, ultrasound is used to disrupt the sample matrix that helps in improved penetration of the solvent into the cell and enhances mass transfer. UAE is employed in numerous extraction methods in order to achieve high extraction efficiencies in short times at low temperatures, with a small amount of sample and extraction solvents **(Saini & Keum, 2016)**.

Sound waves are mechanic vibrations applied to the solid, liquid, or gas with frequencies higher than 20 kHz **(Meireles, 2009)**. Like any sound wave ultrasound is propagated via a series of compression and rarefaction waves induced in the molecules of the medium through which it passes. At sufficiently high power the rarefaction cycle may exceed the attractive forces of the molecules of the liquid and cavitation bubbles will form **(Vinatoru et al., 2017).** These bubbles (Figure 10) will grow during the rarefaction phases and decrease during the compression phases **(Michel, 2011).** Such bubbles grow by a process known as rectified diffusion **(Vinatoru et al., 2017).**

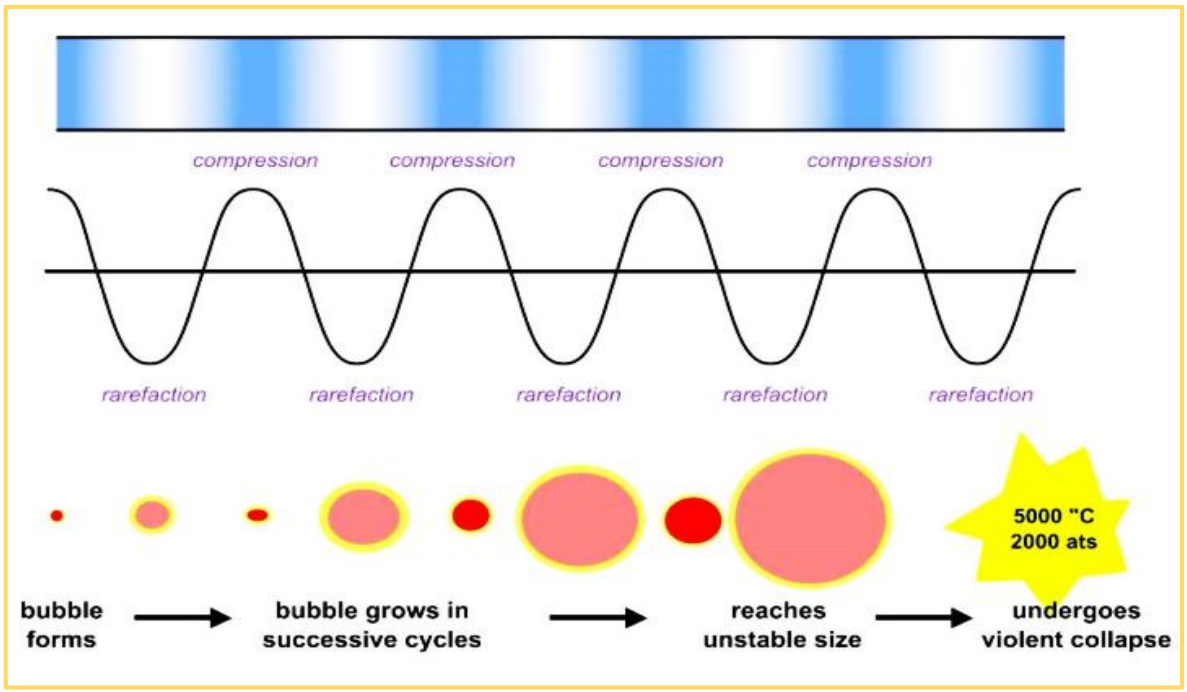


Figure 10: Creation of cavitation bubbles **(Vinatoru et al., 2017)**.

When the cavitation bubbles are formed near a solid surface (Figure 11) they become asymmetrical, and the resulting implosion produces jets of liquid projected at very high speed towards the surface of the solid, as well as " a local increase in temperature and pressure. In the case of a plant matrix, these jets of liquids will pierce the plant walls and thus allow the release of molecules in the liquid medium **(Michel, 2011)**.

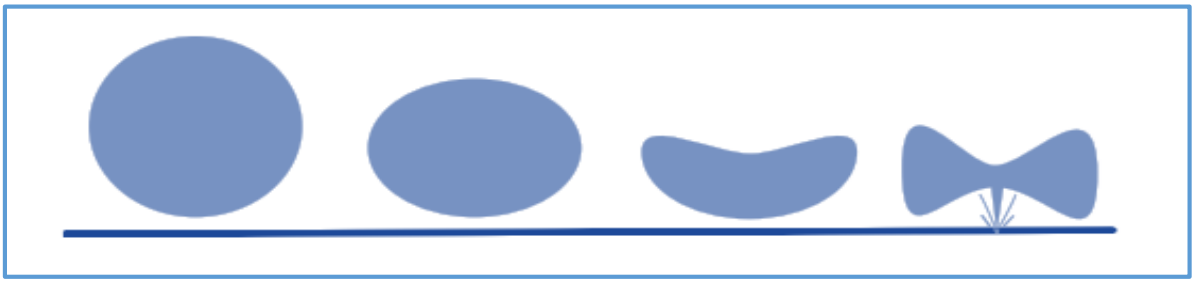


Figure 11: Evolution of a cavitation bubble near a solid surface **(Michel, 2011)**.

II.4.2.3. Supercritical Fluid Extraction

Supercritical (SC) fluid extraction (SCFE) is the widely used method for the extraction of lipids, flavors, and bioactive compounds, removal of alcohol from wine and beer, and encapsulation of liquids for engineering solid products **(Brunner, 2005)**.

SCFE use carbon dioxide (CO_2) as the solvent that can be recovered without damaging the substrate and extract. The use of CO_2 is also having several other advantages as it is non-corrosive in the presence of water, non-toxic, non-flammable, and can be obtained at low cost **(Brunner, 2005)**. SC extraction with CO_2 operate near critical temperature and pressure (Tc = 304 K, Pc = 7.4 MPa). SC-CO_2 extraction prevents the thermal damage to labile compounds, as it operates near-environmental temperature **(Imsanguan et al., 2008)**.

II.5. Biological properties of polyphenols

II.5.1. Anticancer activity

Purification of phenolic compounds from natural sources to homogeneity is a challenging task. Therefore, several studies have tested the ability of either crude extracts rich in phenolic compounds or the fractions containing a mixture of phenolic compounds for inhibiting cancers in vitro and in vivo. For example, the extracts of *Pandanus amaryllifolius* containing gallic acid, cinnamic acid and ferulic acid are reported to inhibit breast cancer cell lines in vitro **(Ghasemzadeh et al., 2013).** Similarly, the extracts of *Baccharis trimera*, containing gallic acid, pyrogallol, syringic acid and caffeic acid were shown to suppress the formation of tumor cell colonies and proliferation of Siha cells in a dose dependent manner **(Oliveira et al., 2013).**

II.5.2. Antioxidant activity

Phenols are potent antioxidants, they are compounds that prevent biomolecules (proteins، nucleic acids, polyunsaturated lipids, and sugars) from undergoing oxidative damage through free radical-mediated reactions **(Heleno et al., 2015)** and their beneficial effects include antiinflammatory, antidiabetic, cardioprotective, neuroprotective, antitumor, and antiaging properties **(Zhang et al., 2017)**.

There are two main mechanisms by which antioxidants perform these properties: free radical inactivation and electron transfer **(Valko et al., 2007)**. In the first mechanism the free radical (R•) can remove a hydrogen atom from the antioxidant (ArOH), which becomes radical. The lower the bond dissociation energy (BDE) of the O–H bonds, the easier the reaction of the inactivation of the free radical and therefore the greater the antioxidant action.

$$R\bullet + ArOH \rightarrow RH + ARO\bullet$$

In the second mechanism, the antioxidant can donate an electron to the free radical, which becomes a cation radical. In this mechanism, the lower the potential ionization (IP), the easier the electron abstraction, meaning greater antioxidant activity.

$$R\bullet + ArOH \rightarrow RH^{-} + ARO\bullet^{+}$$

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups and may contribute directly to antioxidative action **(Bendary et al., 2013)**.

II.5.3. Antimicrobial activity

Polyphenols are endowed with important and diverse antimicrobial activity, probably due to their structural diversities **(Cowan, 1999)**. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms **(Das et al., 2010)**. The number and sites of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity **(Pandey & Kumar, 2013).** It has also been reported that the more phenolic compounds are oxidized more they are inhibitors of microorganisms **(Scalbert, 1991).**

Plant-derived phenolic, such as phenolic acids, flavonoids, stilbenes and tannins, can inhibit the growth and activity of many microorganisms, including food-related pathogens as well as clinically important bacteria, fungi and protozoa **(Takó et al., 2020).** The antimicrobial activity of phenolic compounds from several plant species has been shown to inhibit *S. aureus* in chicken soup **(Hadžifejzović et al., 2013; Yuste & Fung, 2002).** Moreover, the antibacterial activities of polyphenolic compounds specially the flavonoids use different mechanisms for the antimicrobial effects, such as permeabilization and destabilization of the plasma membrane or inhibition of extracellular enzymes **(Górniak et al., 2019).**

II.5.4. Anti-inflammatory activity

Inflammation is a pathological process that develops in a cascade of cause and effect processes that the elements of damage and defense are always present. Pharmacological regulation is one of the most difficult problems. Inflammation therapy focuses on eliminating or limiting the lesion and its pathogens **(Aleksandrova, 2020)**.

Polyphenols were previously reported to have anti-inflammatory effects by modulating this inflammatory cascade **(Abdelouhab, 2019).** A study done showed that the ability of cocoa flavonoids to reduce the inflammation effects in type 2 diabetics and olive oil phenolic compounds in hypertensive women **(Suen et al., 2016)**. Other study provide show that an anti-inflammatory effect that is due to intakes of anthocyanins and flavonols may contribute to the reduction in risk of certain chronic diseases **(Cassidy et al., 2015)**.

II.5.5. Antidiabetic Activity

Recently, oxidative stress and ROS have been accepted as important environmental risks for different types of chronic disorders such as cancer, age-related pathologies, cardiovascular diseases, arteriosclerosis and diabetes **(Gulcin et al., 2019)**.

Plants include many biological active phytochemicals such as phenols and polyphenols that possess structural features that scavenge ROS and eliminate oxidative damage. Their biological effects including the antioxidant ability of the phenols from medicinal plants make them crucial products for their protective effects against some degenerative disorders including diabetes **(Gulcin et al., 2019)**.

In vivo and *in vitro* investigations suggest a significant function of polyphenols in the prevention and management of Type 2 diabetes through the insulin dependent approaches, for instance, protection of pancreatic islet β-cell, attenuation of oxidative stress and stimulation of pancreas to secrete insulin, as well as the insulin independent approaches including inhibition of glucose absorption, inhibition of digestive enzymes, modification of inflammation response, and so on **(Sun et al., 2020)**.

The inhibitory activities of phenolic compounds against α-amylase and α-glucosidase have been evaluated to show their potential as good sources of new antidiabetic drugs to improve insulin resistance and increase glucose uptake **(Odeyemi & Dewar, 2020)**.

II.6. Side effects and toxicity of polyphenols

Current scientific literature provides a great deal of information on the potential health benefits of polyphenols. These reports emphasize the role of phenolic compounds in the prevention and treatment of several chronic pathological conditions. However, numerous reports highlight the side effects and toxicity associated with phenolic compounds.

II.6.1. Carcinogenic activity

Even though flavonoids are often considered to be safe as they are of "plant origin", consumption of flavonoids should be done cautiously as some flavonoids show the ability

of direct interaction with DNA and/or enhance carcinogenic activation into DNA modifying agents **(Hodek et al., 2006)**.

Some flavonoids, such as quercetin, have mutagenic pro-oxidant effects which may interfere with essential biochemical pathways **(Rietjens et al., 2005).** Enhanced expression of cytochrome p450 by flavonoids in colon tissue might be responsible for increasing incidence of colorectal carcinoma in humans **(Hodek et al., 2006)**.

II.6.2. Genotoxic activity

Simple phenolic compounds which are ingested daily n our food at the molecular level can induce double strand DNA breaks *in vitro* **(Yamada et al., 1985)**. The green tea catechin especially (−)-epigallocatechin gallate (EGCG) was shown to induce hydrogen peroxide (H_2O_2) generation and cause subsequent oxidative damage to isolated and cellular DNA in the presence of transition metal ions **(Furukawa et al., 2003)**.

II.6.3. Cytotoxic activity

Mostly the citrus flavanones, like hesperetin and naringenin, were reported to have inhibitory activity on a number of protein kinases **(So et al., 1996; Huang et al., 1999; Fischer et al., 2000)**. This inhibition is mediated via binding of the phenolic compounds to the ATP binding site, presumably causing three-dimensional structural changes in the kinase leading to its inactivity. Usually the cellular effects of flavonoids depend on the interaction with the membrane or uptake by the cytosol **(Zuzana, 2011)**.

Flavonoids may also interact with mitochondria, interfere with pathways of intermediary metabolism and/or downregulate the expression of adhesion molecules **(Panes et al., 1996, Soriani et al., 1998)**.

Second part

Experimental Part

Chapter One:

Material & Methods

I.1. Material

I.1.1. Biological material

I.1.1.1. Plant material

The plant material consists of the aerial part (stems, leaves) of the plant *Cynodon dactylon* L., it was harvested in September in the region of TAGHZOUT (El-Oued).

This plant has been dried out of light and moisture at room temperature for two to three weeks. After drying, it was grinding and reduced to powder then stored carefully in a closed glass bottle in a dry place, for later use (Figure 12).

Figure 12: *Cynodon dactylon* L. from El-Oued region

(Original photo, 2019)

a. Botanical identification

The botanical identification of the plant studied was made by Dr. HILLIS Youssef; Lecturer and researcher at the Research Center for Arid and Semi-Arid Zones (CRSTRA) in Touggourt.

b. Presentation of the harvest site

The plant samples were taken from a site in the TAGHZOUT region exactly the BAGOUZA region, located at 33 ° 29'09.8 "North 6 ° 43'21.6" East. The site is part of the Wilaya of El Oued, located in the south-east of Algeria (Figure 13).

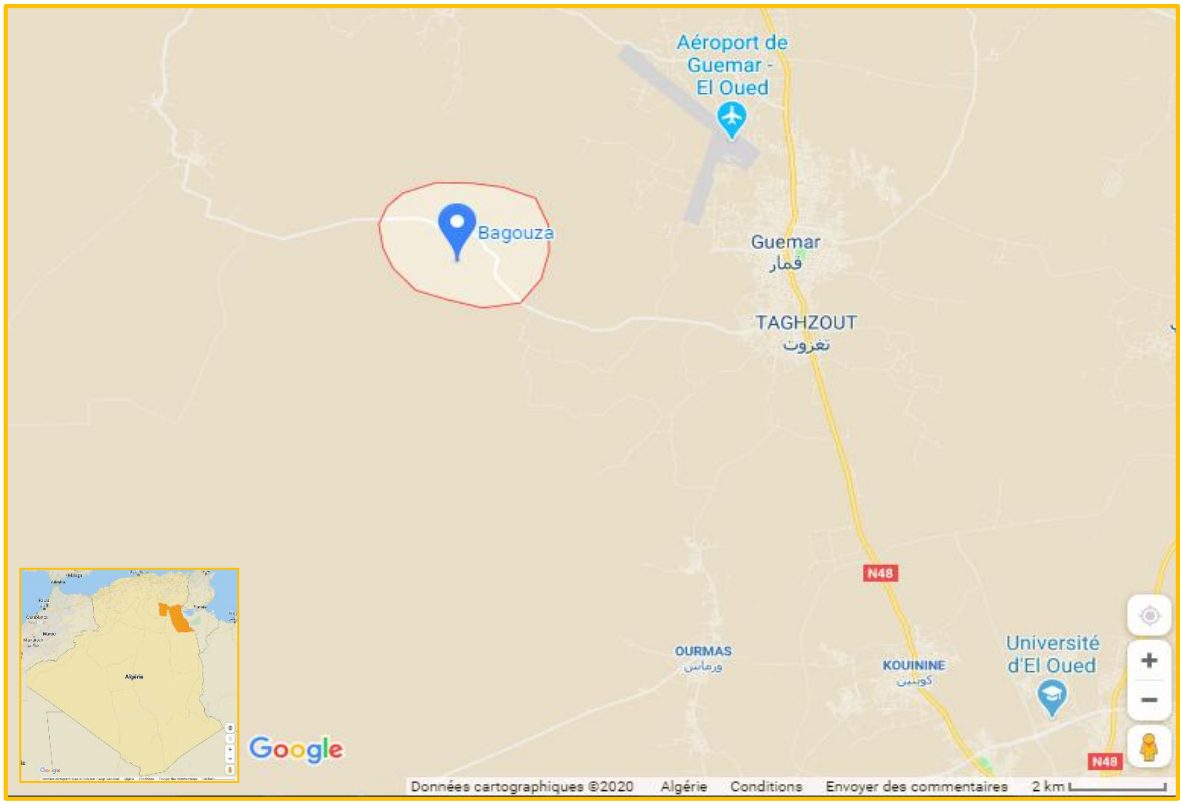


Figure 13: Geographical location of the BAGOUZA - TAGHZOUT study area (Wilaya of El Oued) **(Google maps, 2020).**

I.1.1.2. Microorganisms

The four bacterial strains ATCC **Gram-** (*Pseudomonas aeruginosa* ATCC 9027, Salmonella enterica subsp. *Enterica serovar Typhimurium* ATCC 14028) and **Gram+** (*Staphylococcus aureus* ATCC 44300, *Bacillus cereus* ATCC 10876) were provided to us free of charge by the SAIDAL Laboratory.

The two fungi strains (*Aspergillus carbonarius* M333, *Aspergillus parasiticus CBS* 100926) were provided to us by the laboratory SAIDAL.

I.1.1.3. Human blood

For the evaluation of hemolytic activity, we used a universal model of animal cells; fresh human blood is obtained by donation from healthy, non-smoking single donor who have not received medical treatment for at least a fortnight.

I.1.2. Chemicals and biochemical products

For the evaluation of *C. dactylon* activities, we used many chemicals and biochemical products from the Laboratory of biochemistry- University of El-Oued.

Table 02: The list of chemicals and biochemical products used in our study

Product	Form	chemical formula	molar mass (g/mol)
2,2-diphenyl-1-picrylhydrazyl (DPPH)	Powder	$C_{18}H_{12}N_5O_6$	394,32
Aluminum trichloride	Powder	$AlCl_3$	133,34
Ammonium hydroxide	Powder	NH_4OH	35,04
Ascorbic acid	Powder	$C_6H_8O_6$	176,12
Catechin	Powder	$C_{15}H_{14}O_6$	290,26
Chloroform	Liquid	$CHCl_3$	119,38
Dimethyl sulfoxide (DMSO)	Liquid	C_2H_6OS	78,13
Dragendorff reagent	Liquid	/	/
Ethanol	Liquid	C_2H_5OH	46,07
Fehling liquor	Liquid	/	/
Folin-Ciocalteu	Liquid	/	/
Gallic acid	Powder	$C_7H_6O_5$	170,12
Hydrochloric acid	Liquid	HCl	36,46
Iron trichloride	Liquid	$FeCl_3$	162,2
Magnesium chips	/	Mg	24.31
Magnesium chloride hexahydrate	Powder	$MgCl_2.6H_2O$	203.3
Mayer reagent	Liquid	/	/
Methanol	Liquid	CH_3OH	32,04
Potassium ferricyanide	Liquid	$K_3Fe(CN)_6$	329,24
Quercetin	Powder	$C_{15}H_{10}O_7$	302,236
Sodium carbonate	Liquid	Na_2CO_3	105,9888
Sodium chloride	Powder	NaCl	58,44
Sodium dodecyl sulfate (SDS)	Powder	$NaC_{12}H_{25}SO_4$	288,372
Sodium hydroxide	Liquid	NaOH	39,997
Sodium phosphate buffer (PBS)	Liquid	/	/
Sulfuric acid	Liquid	H_2SO_4	98,079
Trichloroacetic acid (TCA)	Liquid	$C_2HCl_3O_2$	163,38
Vanillin	Powder	$C_8H_8O_3$	152.15

I.2. Methods

The experimental stages in the present work is a investigation to test the biological activities of the polyphenols of *Cynodon dactylon* L, which is a spontaneous plant with medicinal nature harvested in Algerian Sahara.

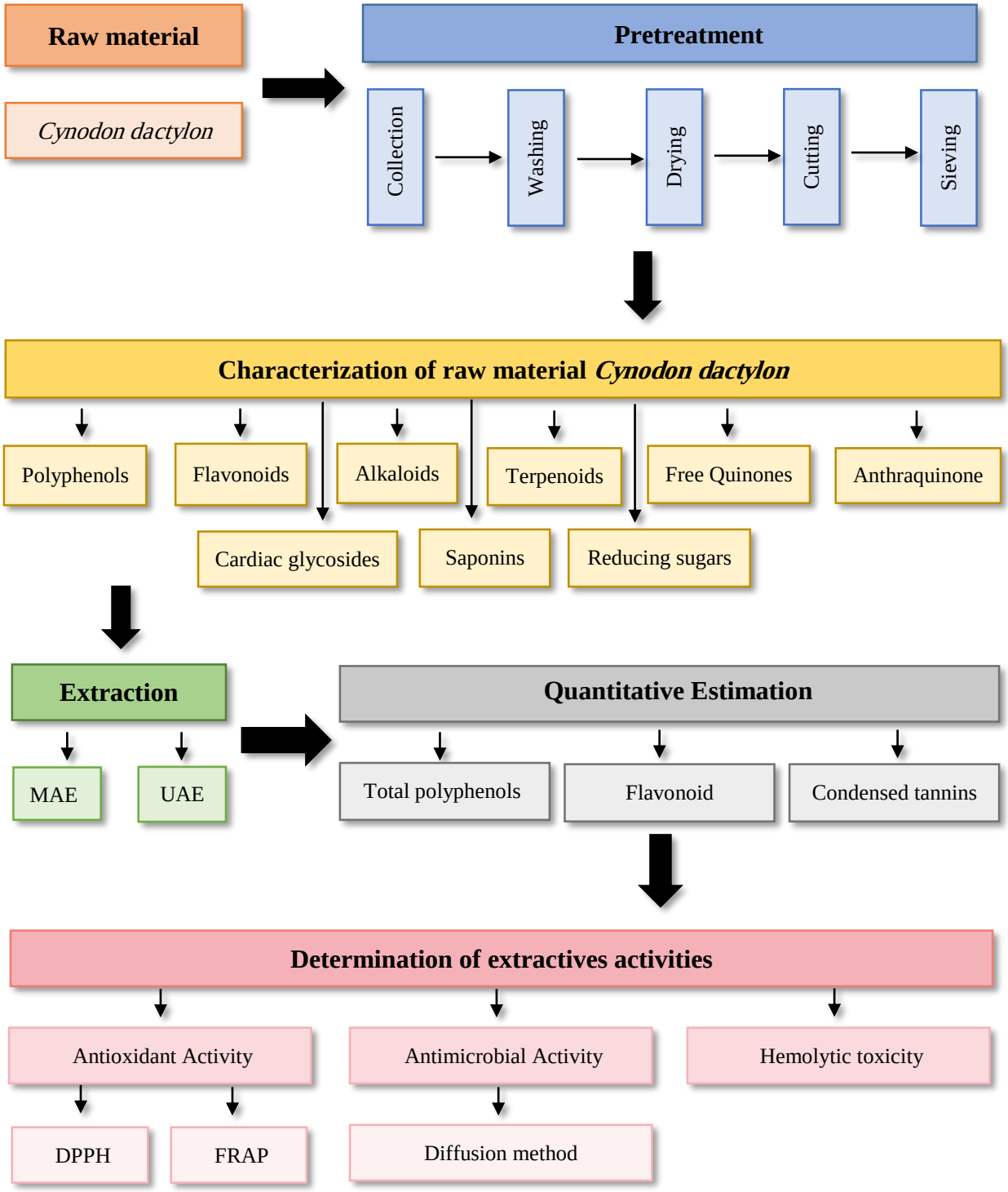


Figure 14: Global diagram of the different experimental stages.

The study concerns the extraction of polyphenols from the plant using two methods, one is conventional and the second is modern, which are respectively Maceration Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE). Thus, the most important objectives are:

On the one hand, to study the biological properties (Figure 14), including the antioxidant and antimicrobial activities of the two polyphenol extracts of *Cynodon dactylon*.

On the other hand, it is necessary to study the toxicity of this polyphenols by the hemolytic activity of the tow extracts against the human red cells.

I.2.1. Phytochemical tests

The solution used for the chemical group detection tests was conducted on the aqueous extracts of the vegetable powders obtained by decoction, according to **Kaneria et al., (2012)**, 5 g of dried powder of *Cynodon dactylon* L was boiled with 100 ml of distilled water for 30 min in a water bath. Then the extracts are filtered using Whatman N°01 paper. This preparation has been preferred to be as close as possible to the conditions of use of *Cynodon dactylon* in traditional medicine. We used the analytical techniques described in the work of **N'guessan et al., (2009); Tlili, (2015); Hamid et al., (2018); Fettah, (2019)**.

I.2.1.1. Polyphenols

The reaction with iron trichloride ($FeCl_3$) made it possible to characterize the polyphenols. To 2 ml of the aqueous extract, we added a drop of 2% iron trichloride alcoholic solution. The appearance of a blue-blackish or green color more or less dark was the sign of the presence of polyphenols **(N'Guessan et al., 2009)**.

I.2.1.2. Flavonoids (Shibata reaction)

To 5 ml of the aqueous extract is added 5 ml of hydrochloric alcohol (4 ml EtOH and 1 ml of concentrated HCl), about 0.5 g of magnesium chips, the appearance of a pink, orange or purplish red coloring is produced when there are flavonoids (flavonols, flavones, flavonones) **(Tlili, 2015)**.

I.2.1.3. Alkaloids

To acidify the medium, 5 ml of 1% HCl are added to 1 ml of the aqueous extract. The mixture is heated in a water bath and divided into two equal volumes. The two volumes are treated separately by the reagents of:

- In the first tube, add a few drops of Mayer reagent (yellowish color).
- In the second tube, add a few drops of Dragendorff reagent (orange color) **(Tlili, 2015; Fettah, 2019)**.

I.2.1.4. Terpenoids

To 5 ml of extract are added 2 ml of chloroform and 3 ml of Sulfuric acid (H_2SO_4). The positive test is indicated by the appearance of two phases and a brown color at the interface **(Tlili, 2015)**.

I.2.1.5. Saponins

In a test tube, introduce 10 ml of the extract to be analyzed, shake for 15 seconds and let the mixture sit for 15 minutes. The appearance of persistent moss indicates the presence of saponosides **(Fettah, 2019)**.

I.2.1.6. Free quinones

A few drops of sodium hydroxide (1% NaOH) are added to 5 ml of extract. Turning color to yellow, red or purple indicates the presence of free quinones **(Tlili, 2015)**.

I.2.1.7. Anthraquinones

A volume of 5 ml of Ammonium hydroxide 10% (NH_4OH) is mixed with 10 ml of extract, maintaining stirring. The violet color indicates the presence of anthraquinones **(Tlili, 2015)**.

I.2.1.8. Cardiac glycosides

Two ml of chloroform is added to 1 ml of the extract, the appearance of a brown-reddish color after the addition of H_2SO_4 indicates the presence of cardiac glycosides. **(Hamid et al., 2018)**.

I.2.1.9. Reducing sugars

1 ml of Fehling liquor is added to 5 ml of extract and the tubes containing the mixtures are heated in a water bath at 40°C. A positive test is indicated by the appearance of a brick-red color **(Fettah, 2019)**.

I.2.2. Extraction of phenolic compounds

The extraction of natural products specially the phenolic compounds is generally of the solid-liquid type by diffusion from the solid matrix (plant tissue or fine vegetable powder) to the liquid matrix (solvent) **(Michel, 2011)**.

In our study the phenolic compounds were extracted from our plant by two different methods (Figure 16): Maceration Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE)

I.2.2.1. Maceration Assisted Extraction (MAE)

Maceration is also called cold extraction. The process of maceration was used to separate the different constituents and ingredients of the plants in the form of crude extract

(Irshad et al., 2012). It consists in contacting the plant material with the solvent without or with agitation. This procedure, despite the long extraction times and the use of a considerable amount of solvents, is relatively inexpensive. In addition, it takes place at room temperature, which is very positive to maintain the integrity of polyphenolic molecules **(Fettah, 2019)**.

In our study, the extraction is carried out according to the methods of **Diouf et al., (2009); Tuhin et al., (2016)** with some modification. 20 g of *Cynodon dactylon* was extracted with 400 ml 80% (ethanol: water =80:20) in a glass Erlenmeyer flask at room temperature for 24 h. Erlenmeyer flasks were completely covered with aluminum foil to prevent degradation of molecules photosensitive. This maceration is repeated 3 times with solvent renewal. The hydro-alcoholic extract is recovered after filtration using an N°01 filter paper, the ethanol is removed from the filtrate by evaporation under reduced pressure in a Rota-vapor and then oven-dried for at least 48 hours at temperature does not exceed 40°C, and kept until use.

I.2.2.2. Ultrasound Assisted Extraction (UAE)

UAE is an extraction technique that can be used with any type of solvent and is easy to install. Indeed, the extraction can be carried out in a very simple manner using an ultrasonic bath (Figure 15) **(Michel, 2011)**.

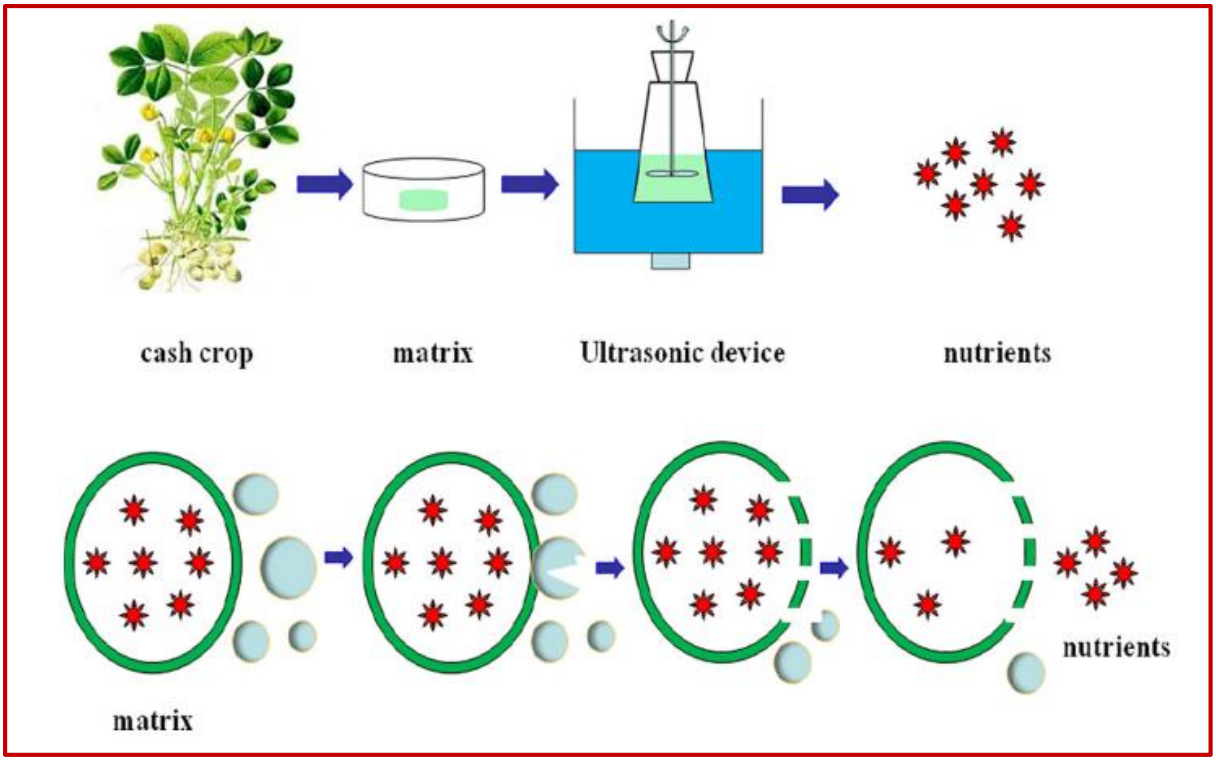


Figure 15: Schematic representation of the major processes during ultrasonic extraction from cash crops **(Wen et al., 2018)**.

Ultrasound in extraction can also disrupt cell walls, making it easier to release their contents **(Mason et al., 1996)**, and increasing the extraction efficiency and extraction kinetics **(Michel, 2011)**. The main benefits are a shorter and more efficient extraction, moderate working temperatures, thus allowing a reduction in energy consumption and having an advantage for the compounds of interest sensitive to heat **(Pradal, 2016)**.

In our study, the ratio of vegetable powders to ethanol for assisted ultrasonic extraction is the same as for maceration, to facilitate comparison between the two methods. The drugs were placed here in a 500 ml Erlenmeyer flask, itself placed in a bath thermostatically controlled at 30 ° C to compensate for the temperature increases caused by the high molecular agitation generated by the ultrasound (POWERSONIC university of El Oued), for 30 min. The resulting mixture was subsequently treated in exactly the same way as the mixture obtained by maceration **(Stpierre, 2012)**.

I.2.2.3. Calculation of yields of dry extracts

The extraction yield corresponds to the percentage of the active ingredient dissolved in the organic solvent used for the extraction. Determined based on the weight of the dry extract in relation to the weight of dry plant material made into powder used for the extraction **(Abe et al., 2010)**.

The yield is expressed as a percentage by mass in relation to the quantity of material dry according to the formula:

$$R\ (\%) = [M_1 / M_0] \times 100$$

R: yield of extracts expressed in g / 100 g of dry matter،

M_1: quantity of recovered extract expressed in g،

M_0: quantity of the vegetable powder used for extraction expressed in g.

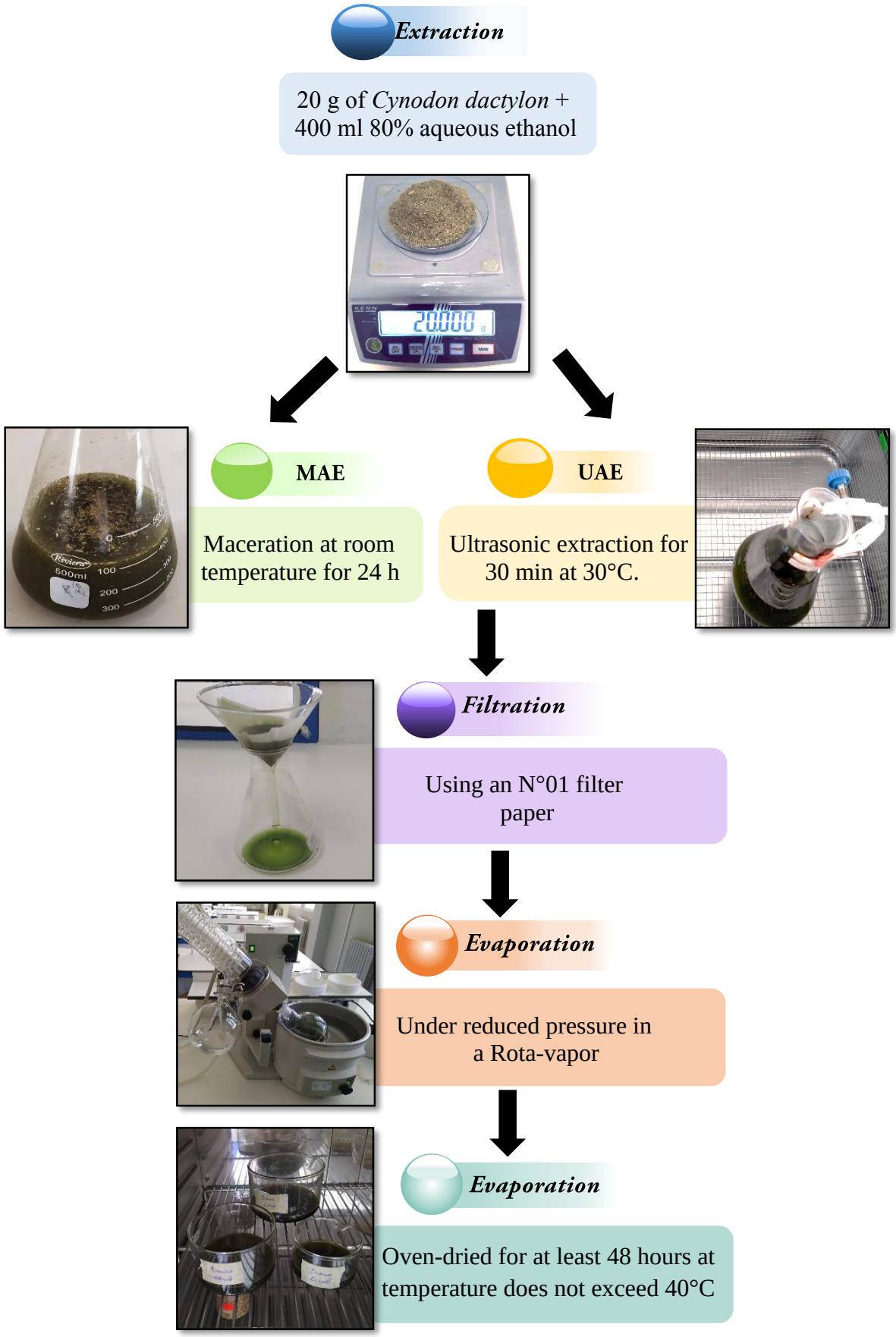


Figure 16: Protocol for obtaining Ultrasound Assisted Extraction (UAE) and Maceration Assisted Extraction (MAE) extracts from *Cynodon dactylon*

I.2.3. Quantitative Estimation

I.2.3.1. Estimation of total polyphenols contents

The determination of the total phenols of the various extracts is carried out according to the method described by **Boizot & Charpentier, (2006)**, via the Folin-Ciocalteu reagent, which is based on the reduction in the basic medium of the phosphotungestic acid mixture H3P (W_3O_{10}) and phosphomolybdic acid $H_3PMO_{12}O_4$ by the oxidizable groups of the phenolic compounds present in the sample. The blue metal tungsten (W_8O_{23}) and molybdenum (Mo_8O_3) metal oxide reduction products have a maximum absorption at about 750 nm whose intensity is proportional to the level of the phenolic compounds present in the sample **(Hadjadj, 2017).**

The assay protocol is carried out as follows: 200 µl of each extract dissolved in distilled or point of range water is added to 1 ml of Folin Ciocalteu reagent (diluted 10 times in distilled water). After 4 min of incubation at room temperature, 800 µl of Na_2Co_3 (7.5%) also diluted in distilled water, are added to the mixture. The whole, previously shaken, is incubated in the dark for 2 hours. The absorbance is then read at 765 mm by a UV / visible spectrophotometer.

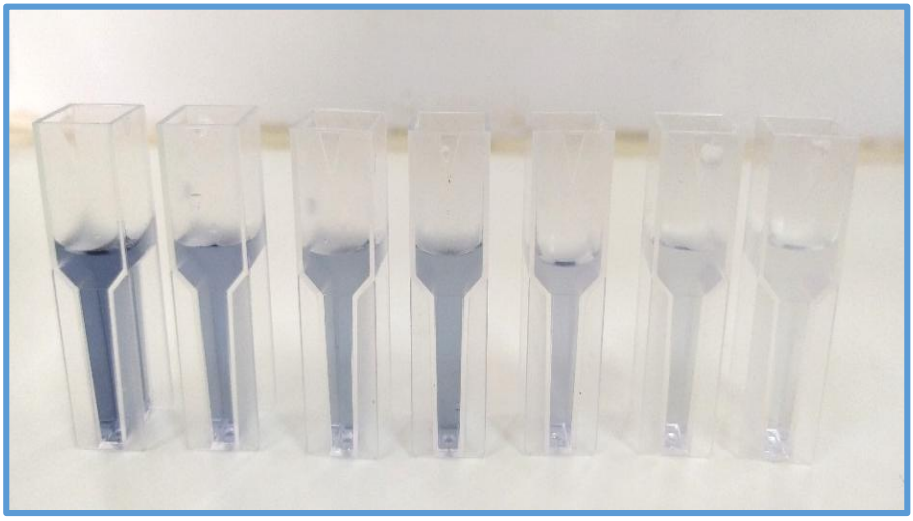


Figure 17: The calibration of gallic acid for the polyphenols estimation **(Original photo, 2020)**.

The concentration of total polyphenols for each sample is calculated from the regression equation for a calibration range in aqueous medium (0 to 200 µg / ml), established with gallic acid as a control under the same operating conditions as the extracts (Figure 17). With Excel we convert these results into a quantity (mg) of gallic acid equivalent per g of

dry vegetable material extracted (Mg EAG/g E). Triplicate measurements were taken and mean values calculated.

I.2.3.2. Estimation of Flavonoid contents

The method of **Quettier-Deleu et al., (2000)** is used to determine the flavonoid contents of our samples using aluminum trichloride as reagent. The method is based on the oxidation of flavonoids by this reagent, resulting in the formation of the flavonoid-stable aluminum complex of yellowish color, detectable in the visible at 430 nm.

A 1 ml intake of extract or standard (prepared in 80% methanol) is added to 1 ml of a freshly prepared solution of $AlCl_3$ (2% in methanol). After 10 min of reaction, the absorbance is read with a spectrophotometer at 430 nm. The results are expressed in mg equivalent quercetin / g of dry vegetable material with reference to the calibration curve of the quercetin.

I.2.3.3. Estimation of condensed tannins contents

The determination of condensed tannins in extracts of *C. dactylon* is carried out according to the method of **Schofield et al., (2001).**

This assay is based on the attachment of the vanillin aldehyde moiety to the catechin A-ring carbon 6 to form a red chromophore complex that absorbs light at 500 nm. To 400 µl of each sample or standard, 3 ml of a solution of vanillin (4% in methanol) and 1.5 ml of concentrated hydrochloric acid are added. The mixture is incubated for 15 minutes and the absorbance is read at 500 nm. The concentrations of condensed tannins are deduced from the calibration ranges established with catechin (0-0.5 mg / ml), and are expressed in milligram of catechin equivalent per gram of extract (Mg EC / g E).

I.2.4. Evaluation of biological activities

The biological activities to be carried out are the antibacterial activity, the antioxidant activity and the hemolytic activity of the crude polyphenol extracts of the two extracts (MAE and UAE) of *Cynodon dactylon*.

I.2.4.1. Antioxidant activity

I.2.4.1.1. DPPH radical trapping test

The hydrogen atom or electron donating abilities of the resultant compounds and some compounds was measured from the bleaching of the purple-colored methanol solution (Figure 18) of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical, DPPH as a reagent **(Sharma et al., 2007).**

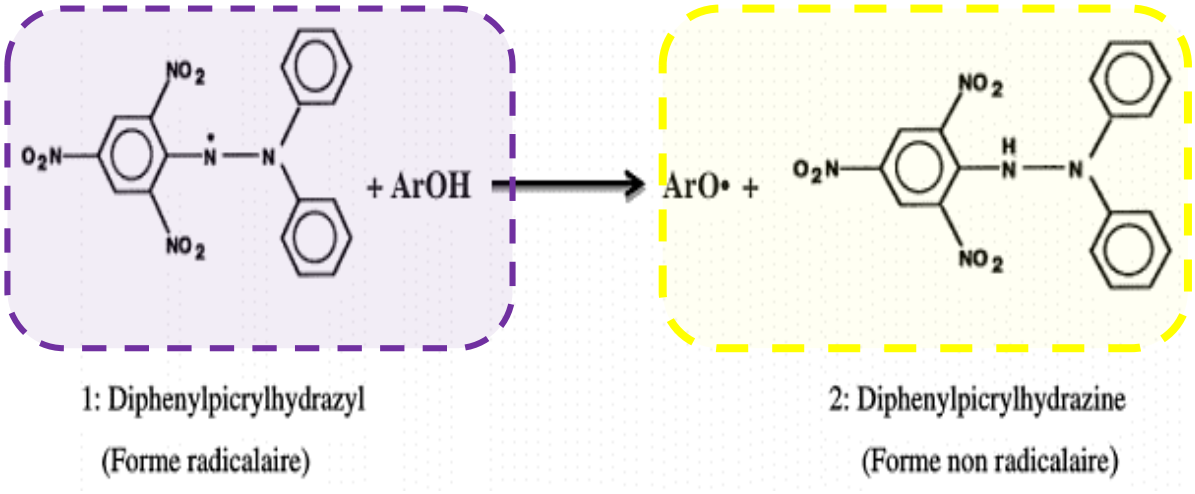


Figure 18: DPPH• radical reduction mechanism **(Molyneux., 2004)**

The test was performed by mixing 50 µl of extract or standard with 1.95 ml of DPPH dissolved in methanol (4 %). After shaking, the reaction was placed safe from light during 30 min and the absorbance was read at 517 nm. Inhibition of free radical DPPH in percent (I %) was calculated in following way:

$$I\ \% = (A_{blank} \_ A_{sample}/A_{blank}) \times 100;$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate **(Saffidine et al., 2015)**.

I.2.4.1.2. Ferric reducing antioxidant power (FRAP)

Reducing power of extracts of *C. dactylon* was measured by method of **Do et al., (2014).** According to this method the reduction of Fe^{3+} to Fe^{2+} was determined by measuring absorbance of the Perl's Prussian blue complex. This method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants.

For this purpose, 1mg of each extract was diluted with 1ml of distilled water. 0.5 ml of diluted extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution in a test tube, followed by incubating in a water bath at 50 °C for 30 minutes. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1% iron

trichloride freshly prepared was added, absorbance of these mixtures were measured at 700 nm using a UV spectrophotometer.

A calibration curve with different concentrations (2.5 µg / ml to 500 µg / ml) is carried out using ascorbic acid with the same experimental procedure. The results are expressed in milligrams (mg) equivalent of ascorbic acid per gram of extract (mg EAA / g E) (Figure 19).

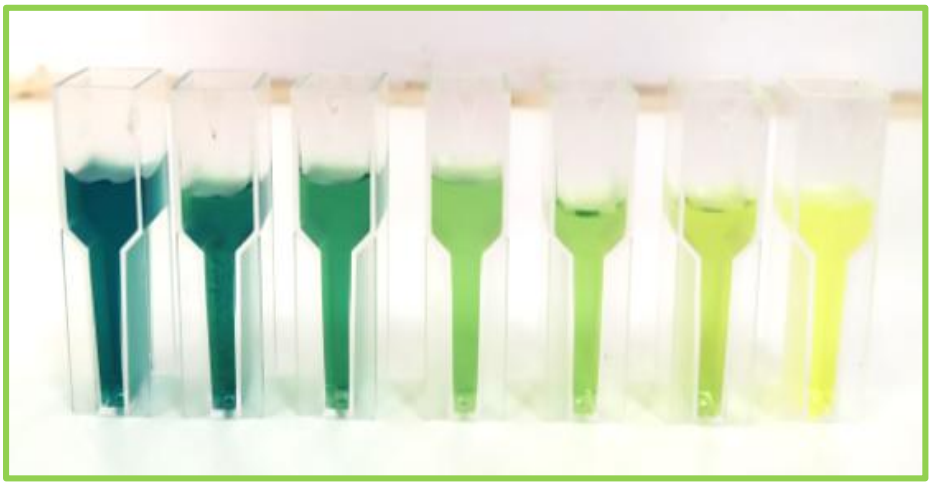


Figure 19: The calibration of ascorbic acid for the measure of reducing power of extracts of *C. dactylon* **(Original photo, 2020)**.

I.2.4.2. Antimicrobial Activity

Susceptibility of the microbial strains to the polyphenolics compounds and ethanol extract were investigated by using the agar diffusion method described in the work of **Rahal, (2005)**. This method used to research the antimicrobial activity of *C. dactylon* extracts studied in the Microbiology laboratory at Echahid Hamma Lakhder University of El Oued.

I.2.4.2.1. Inoculum preparation

The different microbial strains were sub-cultured by the streak method, and then incubated at 37 ° C for 18 to 24 h in order to obtain a young culture and well-isolated colonies which were subsequently used to prepare the inoculum by placing them in tubes containing 10 ml of sterile physiological water for each strain.

I.2.4.2.2. Antibiotic application of different extracts

After adjusting the turbidity of the suspension used as an inoculum, a swab is deceived in the suspension and its content is spread over the entire surface of the Mueller-Hinton agar (for bacteria) and the entire surface of the Sabouraud agar (for fungi) three times. After each application, the box was rotated by approximately 60 ° in order to ensure a homogeneous distribution of the inoculum.

Sterile Wattman N°03 paper discs, 6 mm in diameter, each impregnated with 10 µl of each extract of *C. dactylon* with different concentrations (2.5 mg/ml, 5 mg/ml 7.5 mg/ml, 10 mg/ml) dissolved in dimethyl sulfoxide (DMSO), are deposited using forceps on the surface of the agar medium. They should be perfectly applied flat without slipping by pressing slightly above the surface of the discs.

The Petri dishes are first left for 30 min at room temperature for a pre-diffusion of the substances. After the plates were incubated at 37 °C during 24 h for bacteria and at 25°C during 48 h for fungi, the diameters of the distinctly clear zones were measured in millimeters. All the tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition diameters produced **(Chirane et al., 2019)**.

Negative controls (disks impregnated with DMSO) and positive controls (antibiotics) were tested. All tests are repeated three times.

Gentamicin (GEN ^{10}) and Penicillin (P ^{10}) are the antibiotic chosen because of their fairly broad spectrum of action and their frequent use in hospitals for the treatment of infections caused by most of the germs in our study (Figure 20).

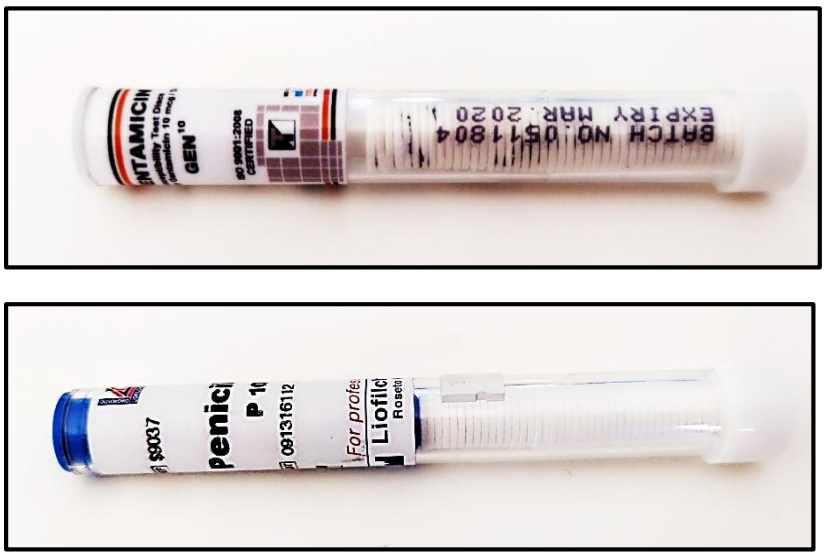


Figure 20: Antibiotics Gentamicin (GEN ^{10}) and Penicillin (P ^{10})

(Original photo, 2020).

I.2.4.3. Evaluation of hemolytic activity of the extracts against human red blood cells

I.2.4.3.1. Preparation of the erythrocyte suspension

This hemolytic effect test of the plant studied is carried out according to the method of **Weiss et al., (2012); Bentabet, (2015); Elalaoui, (2015)**.

Freshly drawn blood on a heparinized tube is centrifuged at 4000 rpm for 5 minutes. After removal of the supernatant, the pellet is washed twice with 10 mM sodium phosphate

phosphate buffer (PBS), pH 7.4 containing 150 mM NaCl, then suspended again in this same buffer **(Bentabet, 2015)**.

I.2.4.3.2. Preparation of extracts and sodium dodecyl sulfate (SDS)

Different concentrations of plant extracts (20mg/ml, 10mg/ml, 5mg/ml, 1mg/ml and 0.1 mg/mL) were dissolved in the PBS and sodium dodecyl sulfate (SDS) with the same concentrations **(Weiss et al., 2012)**.

I.2.4.3.3. Measurement of hemoglobin leakage

The human red blood cells are suspended in the 10 mM PBS buffer pH 7.4 (0.5 ml are brought into contact with 9.5 ml of sodium phosphate phosphate buffer (PBS) 10 mM, pH 7.4) **(Bentabet, 2015)**.

The samples were added 1:1 (v/v) to the RBC suspension **(Weiss et al., 2012).** The tubes are mixed gently and the erythrocyte suspension is incubated at 37 ° C for 60 minutes. As soon as the extract, which corresponds to the reaction time zero, samples of 500 µL from the reaction solution are taken at regular intervals (each 15 min), to which we have added 2 ml of an ice-cold washing solution (NaCl 150 mM, $MgCl_2$ 2 mM). After centrifugation at 4000 rpm for 5 minutes, we recovered the supernatant on which we measured the hemoglobin leakage by reading the optical density at a wavelength of 548 nm **(Bentabet, 2015)**.

A negative control tube is prepared in the same experimental procedures. It is composed of the erythrocyte suspension and the PBS buffer solution, in the absence of extract. A tube of total hemolysis contains the erythrocyte suspension and 2% aqueous sodium dodecyl sulfate solution. **(Weiss et al., 2012)**.

The hemolysis rate of the different extracts is calculated as a percentage (%) relative to the total hemolysis according to the following formula (**Elalaoui, 2015)**:

$$\textbf{Hemolysis rate (\%)} = [(\textbf{Abs from extract} - \textbf{Abs from negative control}) / \textbf{Abs Total hemolysis}] \times 100$$

I.3. Statistical analyzes

In this study, we used the statistical test using the EXCEL software (version 2016). All the experiments were carried out in triplicate; the results are given in the form of means with its standard deviation.

Chapter two

Results & Discussion

II.1. Results

II.1.1. Phytochemical Screening

The phytochemical screening allowed us to highlight the presence of secondary metabolites in the plant tissues of our plant and to have a good idea about its pharmacological activities. These metabolites confer protection against bacteria, fungi and pesticide attacks and are therefore responsible for antimicrobial activity against certain microorganisms **(Marjorie, 1999)**.

The detection of these chemical compounds is based on component solubility tests, precipitation reactions and color change. The results obtained by the phytochemical tests carried out on the aqueous extract of the *C. dactylon* plant are shown in the table 03.

Table 03: Results of phytochemical tests of the aerial part of *Cynodon dactylon*

Test of	Reagent	Results
Polyphenols	**$FeCl_3$**	**+**
Flavonoids	**Mg^{++}**	**+**
Alkaloids	**Mayer**	**+**
	Dragendorff	**-**
Terpenoids	**H_2SO_4**	**+**
Saponins	**Foam test**	**+**
Free Quinones	**NaOH**	**+**
Anthraquinone	**NH4OH**	**-**
Cardiac glycosides	**H_2SO_4**	**+**
Reducing sugars	**Fehling**	**+**

(+): Presence, (-): Absence

The phytochemical tests realized have highlighted various secondary metabolites in the aerial part of the plant including free quinones, saponosides, terpenes, alkaloids, reducing sugars, cardiac glycosides, flavonoids and polyphenols. Thus the absence of certain compounds such as Anthraquinones.

II.1.2. Yields of dry extracts

Raw extracts from *Cynodon dactylon* recovered after evaporation were weighed to determine the dry weight obtained. The results were expressed as a percentage. Table 04 summarizes the characteristics as well as the yield of each extract.

Table 04: Yields, aspects and colors of raw extracts of *C. dactylon*

Extraction	Yield (%)	Aspects	Colors
Maceration Assisted Extraction (MAE)	9.40	Paste	Brown
Ultrasound Assisted Extraction (UAE)	12.52	Paste	Brown

The extraction yields obtained depend both on the plant studied and on the extraction method. The results obtained show that there is a slight difference between the yields of the two extractions, and are respectively 9.40% for the maceration extract and 12.52% for the ultrasound extract.

II.1.3. Quantitative Estimation

II.1.3.1. Estimation of total polyphenols contents

The hydroalcoholic extracts were analyzed quantitatively by spectrophotometer, for their contents of total polyphenols using the Folin-Ciocalteu method. The results obtained are expressed in mg equivalent of gallic acid per gram of the extract (mg EAG/gE). This content is calculated using the equation of the linear regression of the calibration curve drawn with gallic acid (Figure 21)

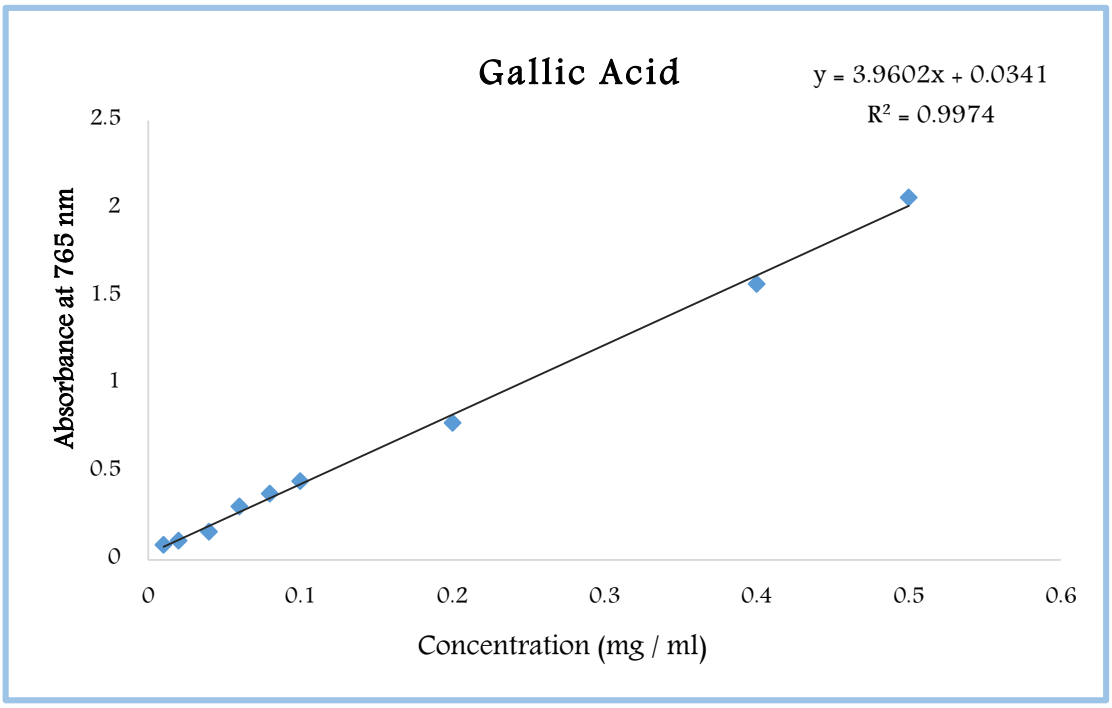


Figure 21: Gallic acid Calibration curve for the determination of total phenols.

The results show that the contents of phenolic compounds vary considerably between the two extracts of the plant (Figure 22). We note that the extract obtained by MAE contains a high level of total polyphenols (42.14 ± 0.75 mg EAG / gE), compared to the extract obtained by UAE, where the contents are of the order of 29 .93 ± 0.14 mg EAG / gE.

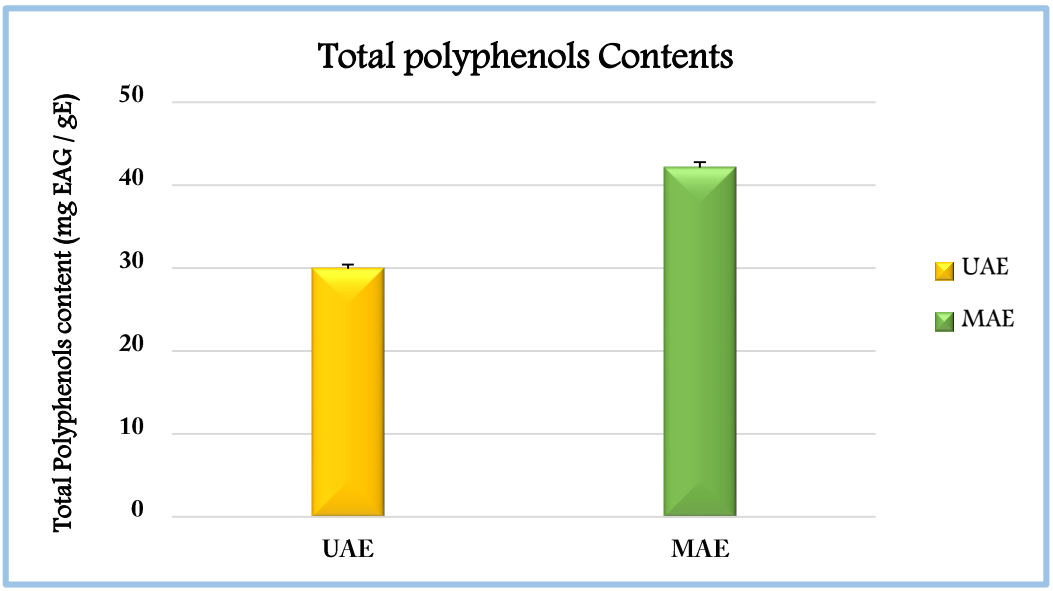


Figure 22: Evaluation of the total polyphenols of the two extracts of *Cynodon dactylon*.

II.1.3.3.Estimation of Flavonoid contents

The flavonoids were estimated using the aluminum chloride ($AlCl_3$) colorimetric method. The results obtained are expressed in mg Quercetin equivalent per gram of the extract (mg EQ /g E) using the equation of the linear regression of the standard curve of Quercetin (Figure 23).

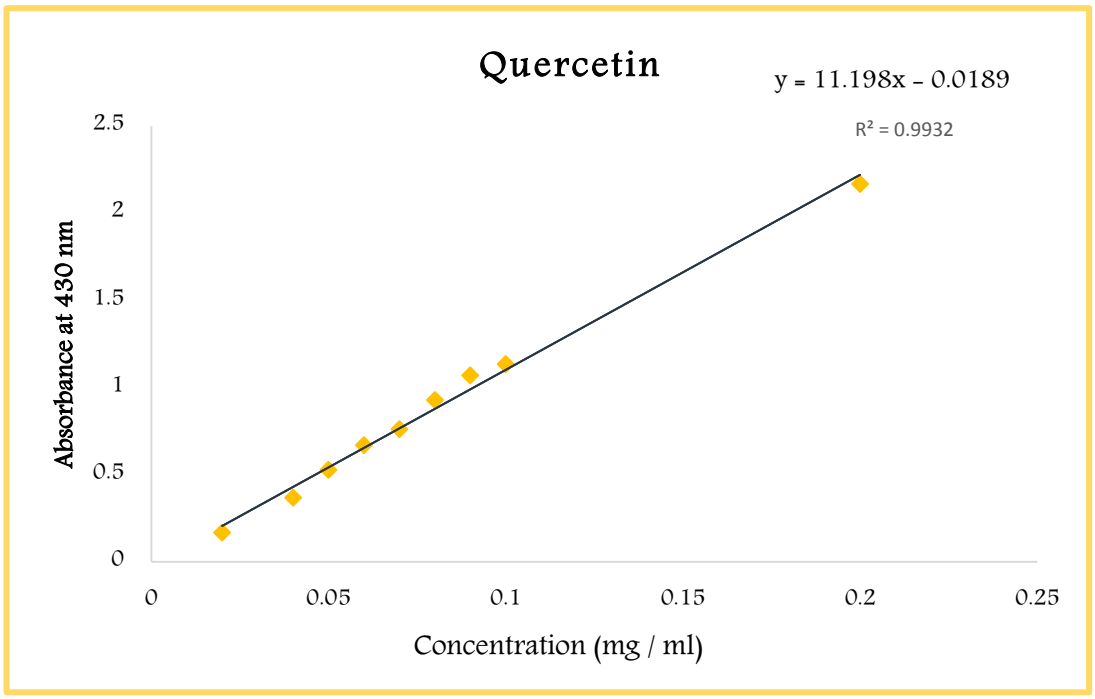


Figure 23: Quercetin calibration curve for the determination of flavonoids

The results of the flavonoid content (Figure 24) of the aerial part of *Cynodon dactylon* show that, similar to polyphenols, the flavonoids determined in the extract of MAE are more important than those determined in the extract of UAE with averages of 23.57 ± 0.78 Mg EQ / g E and 13.53 ± 0.33 Mg EQ / g E respectively.

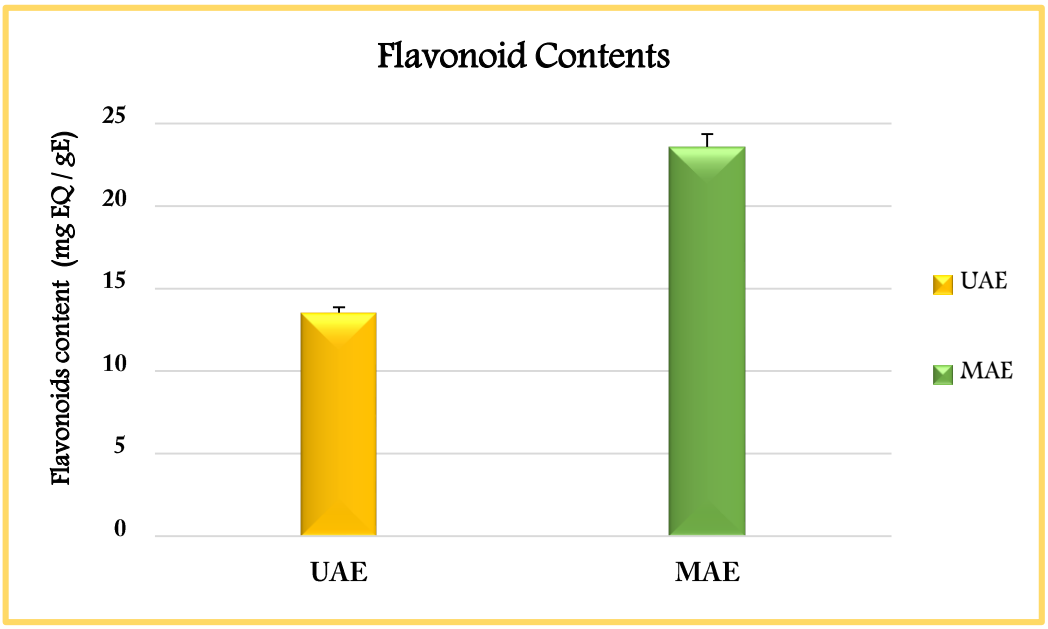


Figure 24: Evaluation of the Flavonoid contents of the two extracts of *Cynodon dactylon* .

III.1.3.3. Estimation of condensed tannins contents

The results obtained for the determination of condensed tannins are expressed in mg catechin equivalent per gram of the extract (mg EC/g E) using the equation of the linear regression of the calibration curve plotted for catechin (Figure 25) .

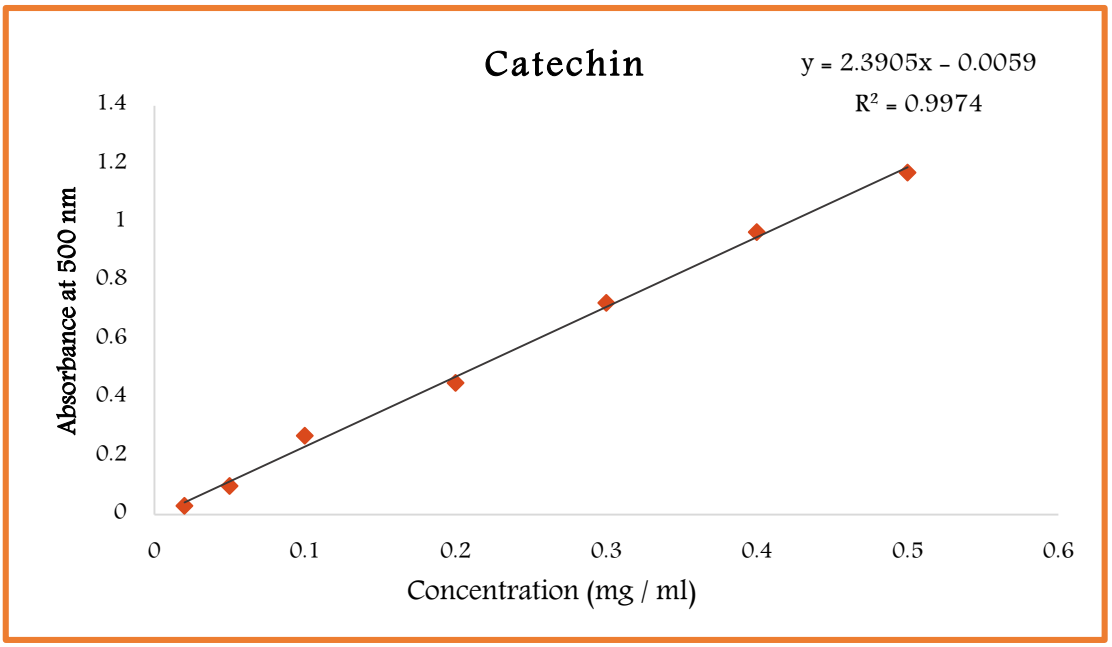


Figure 25: Catechin calibration curve for the determination of condensed tannins.

In contrast to polyphenols and flavonoids, the results presented in (Figure 26) show that the content of condensed tannins in the extract of UAE 19.34 ± 0.48 Mg EC/gE is higher than the extract of MAE 15.99 ± 0.63 Mg EC/gE.

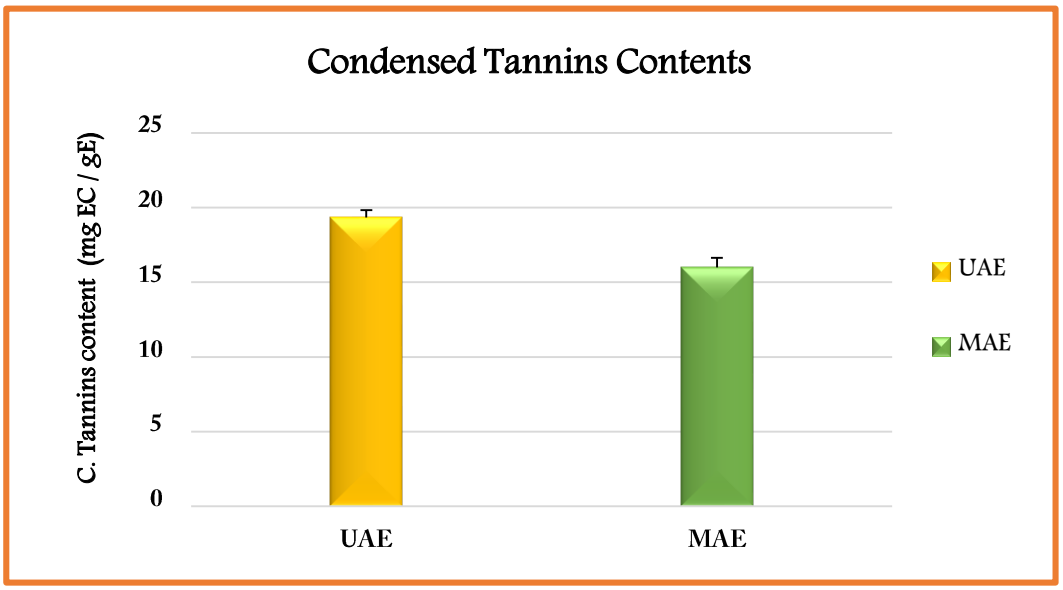


Figure 26: Evaluation of the Condensed Tannins contents of the two extracts of *Cynodon dactylon*

II.1.4. Evaluation of biological activities

II.1.4.1.Antioxidant activity

II.1.4.1.1. DPPH radical trapping test

The DPPH • radical is generally one of the most widely used compounds for the rapid and direct evaluation of antioxidant activity due to its stability in radical form and the simplicity of the analysis **(Bozin et al., 2008)**. The results obtained are expressed as a percentage of 50 % inhibition of the free radical DPPH• and compared to the reference molecules (ascorbic and gallic acid)

For reasons of clarity, we will speak in another parameter expressing the anti-free radical power (Table 05) was calculated from the first parameter noted: "ARP" (anti-radical power, equal to 1 / IC_{50}). More these values move away from zero, more the antioxidant power increases. **(Brand-Williams et al., 1995).**

Table 05: Results of the antioxidant activity evaluated by the DPPH test

Sample	IC_{50} (mg / mL)	ARP ($1/IC_{50}$)
MAE	7.52 ± 0.037	0,132
UAE	8.83 ± 0.032	0,113
Gallic Acid	0.05 ± 0.014	20,00
Ascorbic Acid	0.08 ± 0.006	12,50

For comparative purposes, two standard antioxidants are used, gallic acid and ascorbic acid (Figure 27), they showed a powerful anti-free radical activity with IC_{50} of the order of 0.05 ± 0.014 mg / mL and 0.08 ± 0.006 mg / mL and ARPs of around 20.00 and 12.50 respectively. The lower the IC_{50} value, the more the extract is considered a powerful antioxidant.

For *Cynodon dactylon* extracts, the MAE extract represents the most active extract, with an IC_{50} of the order of 7.52 ± 0.037 mg / ml and an ARP: 0.132 followed by the UAE extract with an IC_{50} 8.83 ± 0.032 mg / mL and a ARP: 0.113.

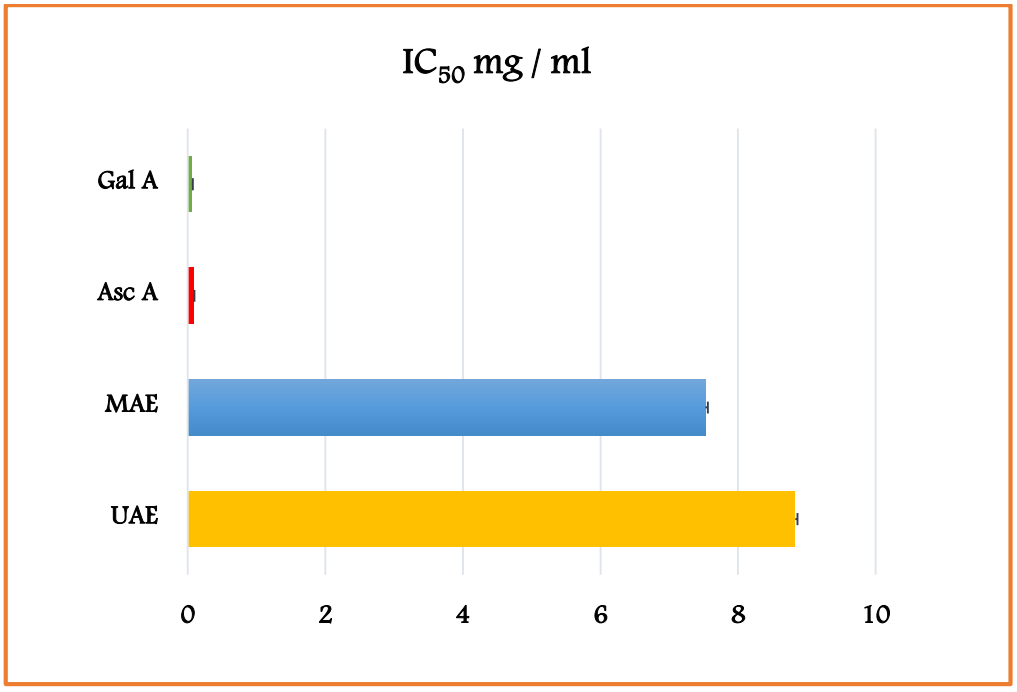


Figure 27: Histogram of the results of 50% inhibitory concentrations of DPPH.

II.1.4.1.2. Ferric reducing antioxidant power (FRAP)

In our study, we opted to test the different ethanolic extracts (UAE and MAE). A calibration curve (Figure 28) is performed using ascorbic acid. The results are expressed in milligrams (mg) equivalent of ascorbic acid per gram of extract (mg EAA / g).

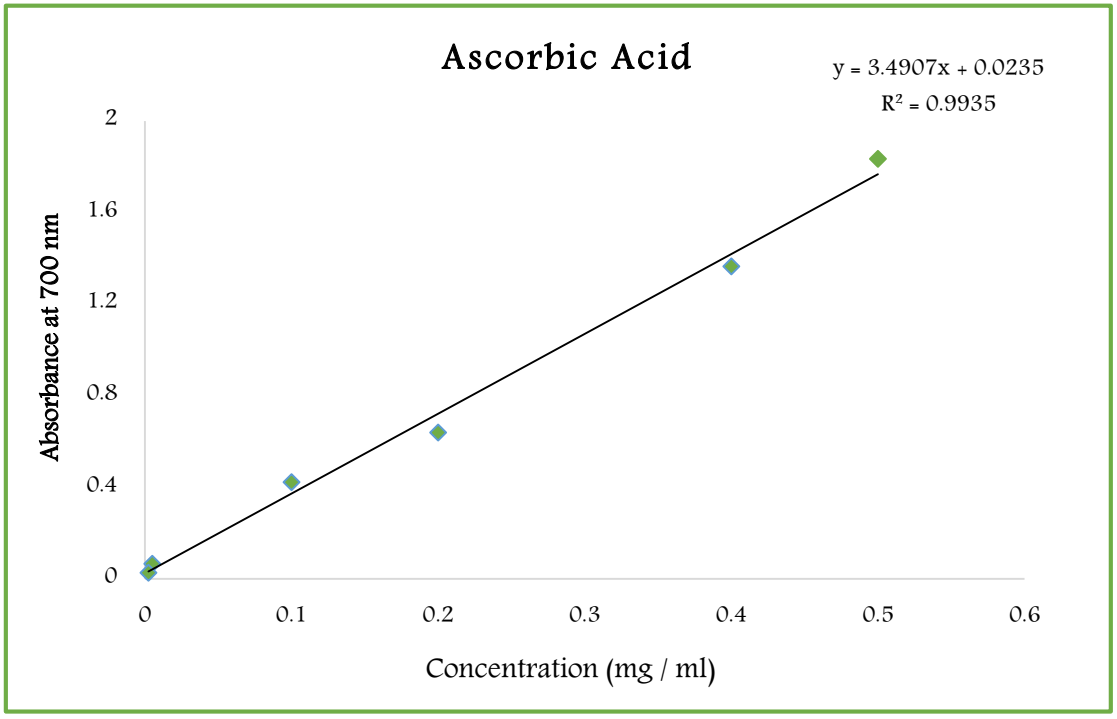


Figure 28: Ascorbic acid calibration curve for the FRAP test

The results of the FRAP test mentioned in Table 06 indicate that the hydro-ethanolic extract of the aerial part of *Cynodon dactylon* by maceration has the most significant antioxidant effect of the order of 15.83 ± 0.37 mg EAA / g, followed by the ultrasound extract with a value equal to 15.38 ± 0.12 mg EAA / g.

Table 06: Results of the antioxidant activity evaluated by the FRAP test

Extracts	Antioxidant activity (mg EAA/g)
MAE	15.83 ± 0.37
UAE	15.38 ± 0.12

II.1.4.2. Antimicrobial activity

We studied *in vitro* the antimicrobial power of isolated extracts of *Cynodon dactylon* by the method of diffusion of the discs on a solid agar medium, Mueller-Hinton for bacteria and Sabouraud for fungi.

The antimicrobial activity of the extracts was estimated in terms of the diameter of the inhibition zone around the discs containing the extracts to be tested against six pathogenic germs, which are *S. Typhimurium P. aeruginosa, S. aureus, B. cereus, A. carbonarius* and *A. parasiticus.*

DMSO has been tested as a solvent, the results show that the solvent is suitable and has no effect on the normal growth of microbial strains.

Table 07: Antimicrobial activity *in vitro* determined as the diameter of the zone of inhibition tested as a function of the different concentrations of extracts Ultrasound Assisted Extract (UAE) and Maceration Assisted Extract (MAE) extracts from *Cynodon dactylon*

Extracts	Dose (mg/ml)	Inhibition diameters (mm)					
		P. aeruginosa	*S. Typhimurium*	*S. aureus*	*B. cereus*	*A. carbonarius*	*A. parasiticus*
MAE	**2,5**	11.66±0.57	08.66±1.15	12.33±1.15	10.00±0.00	07.33±0.57	06.00±0.00
	05	11.66±0.57	09.00±0.00	13.33±0.57	11.00±1.15	08.00±0.57	06.00±0.00
	7,5	12.00±0.57	09.33±0.57	13.33±0.57	10.00±0.00	12.16±0.28	07.00±0.00
	10	13.00±0.57	10.00±0.57	14.00±1,00	10.00±0.57	10.00±1.15	13.16±1.04
UAE	**2,5**	07.66±1.15	07.66±1.52	09.00±1,00	07.00±1.73	06.00±0.57	06.00±0.00
	05	10.00±0.00	08.00±1.73	09.66±0.57	08.00±1.73	06.50±0.50	06.00±0.00
	7,5	09.00±1,00	09.00±1.52	10.66±0.57	09.00±1.00	09.66±0.57	06.00±0.00
	10	10.00±1.52	09.00±1.52	11.33±0.57	10.00±1.15	09.00±0.57	06.00±0.00
GEN[10]		31.00±0.00	26.00 ± 1.73	28.66±1.52	27.33±2.51	29.66 ± 4.61	06.00±0.00
PEN		06.00±0.00	06.66 ± 1.15	10.33±0.57	06.66±1.15	06.00 ± 0.00	06.00±0.00
DMSO		06.00±0.00	06.00±0.00	06.00±0.00	06.00±0.00	06.00±0.00	06.00±0.00

The antibacterial effect of polyphenols from *C. dactylon* L according to our results (Table 07) we note that, all the bacterial strains tested are sensitive to the two crude extracts of polyphenols (MAE and UAE) from *C. dactylon* L, to the Exception of the fungal strain *A. parasiticus* does not show any sensitivity against the UAE extract.

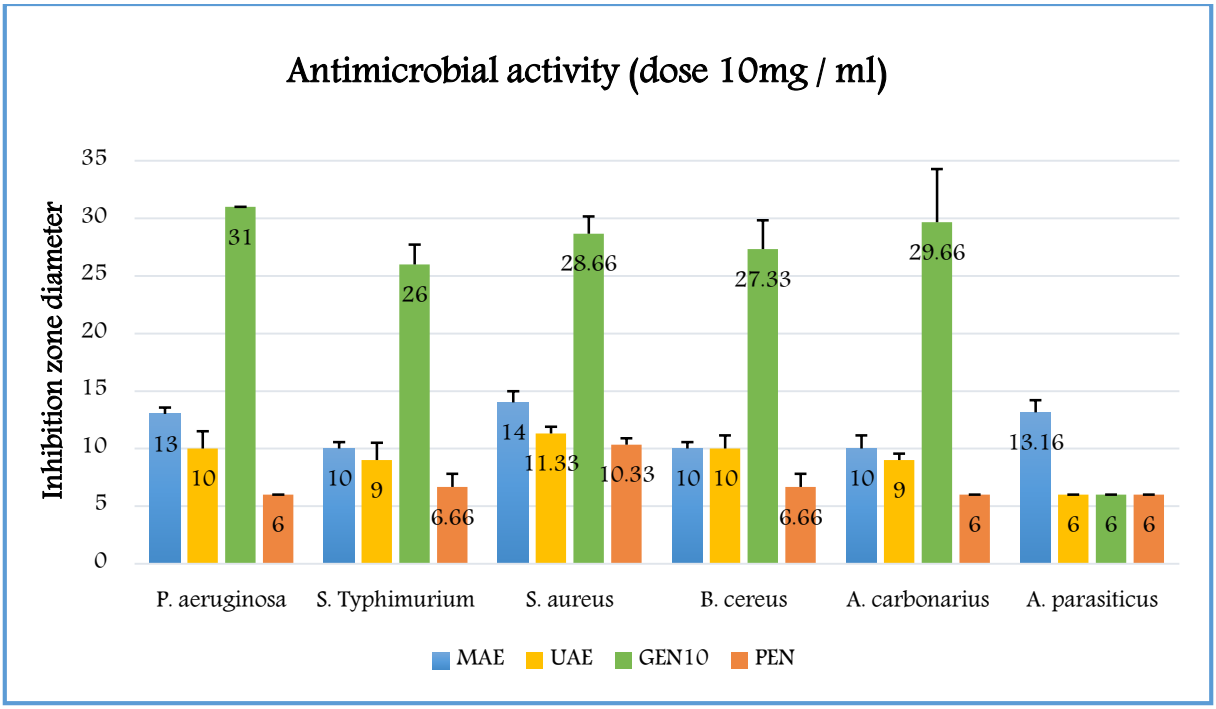


Figure 29: Comparison of the antimicrobial power of the different ethanolic extracts Ultrasound Assisted Extraction (UAE) and Maceration Assisted Extraction (MAE) on the strains tested at a dose of 10 mg / ml.

From the results in Figure 29, it is clear that the MAE extract is the most active compared to the UAE extract. This extract recorded the largest zones of inhibition against the *S. aureus* and *A. parasiticus* strains of the order of 14.00 ± 1.00 mm and 13.16 ± 1.04 mm respectively. This inhibition activity is greater than that of the reference antibiotic penicillin (10.33 ± 0.57 mm) and less than that of Gentamicin (28.66 ± 1.52 mm). The UAE extract also showed an interesting inhibitory activity with respect to the *S. aureus* strain with a diameter of 11.33 ± 0.57 mm and remains superior to that of penicillin.

II.1.4.3. Evaluation of hemolytic activity of the extracts against the hRBCs

The hemolysis test has been evaluated because, even if a plant has antioxidant or antibacterial power, its use in traditional medicine and in pharmacological preparations will be impossible in the presence of their hemolytic effect, which is an indicator of cytotoxicity. The hemolytic toxicity of the extracts was evaluated against erythrocytes from blood taken from non-smoking single donor.

The results show the evolution of the hemolytic effect as a function of time, Hemolysis rate (%), for 60 min, in a PBS buffer medium (pH = 7.4) containing an erythrocyte suspension, incubated at 37 ° C, and in the presence of the concentrations of the different extracts of *C. dactylon*. The concentrations tested as a positive control are 0.1mg / ml to

20mg / ml, and a tube of total hemolysis caused by 20 mg/ml of SDS (Figure 30-B). Also we note that PBS has no effect on human red blood cells us the (Figure 30-A) show.

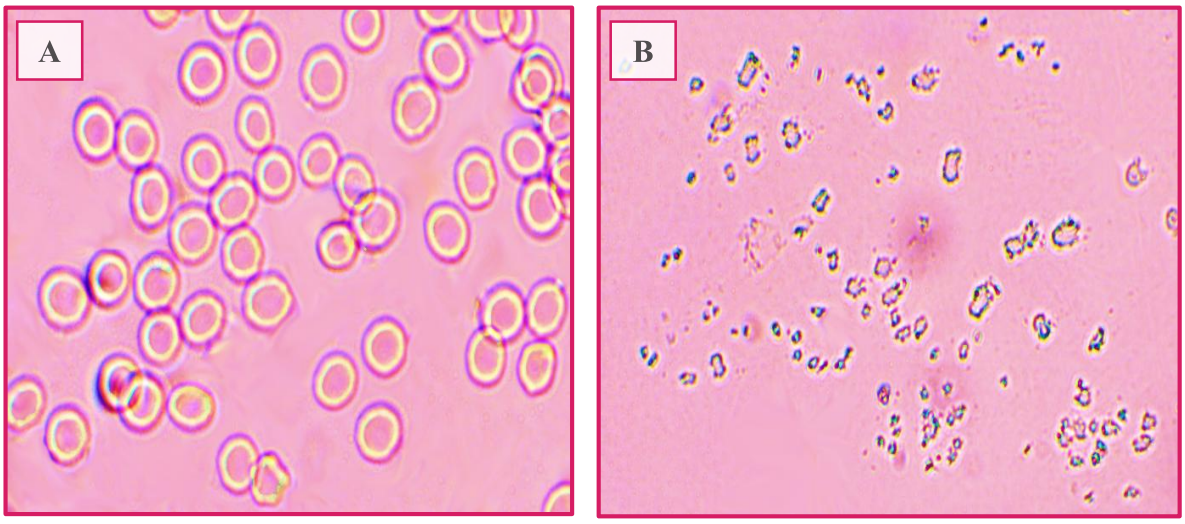


Figure 30: Microscopic observation of human red blood cells at 40X magnification
A: hRBCs with the negative control (PBS), **B:** hRBCs with the positive control (SDS)

II.1.4.3.1. The hemolytic activity of the Maceration Assisted Extract (MAE)

The results relating to the rates of hemolysis induced by the different concentrations of the MAE extract of the aerial part of *Cynodon dactylon* ranging from 0.1 to 20 mg / ml with respect to red blood cells are presented in Figure 31.

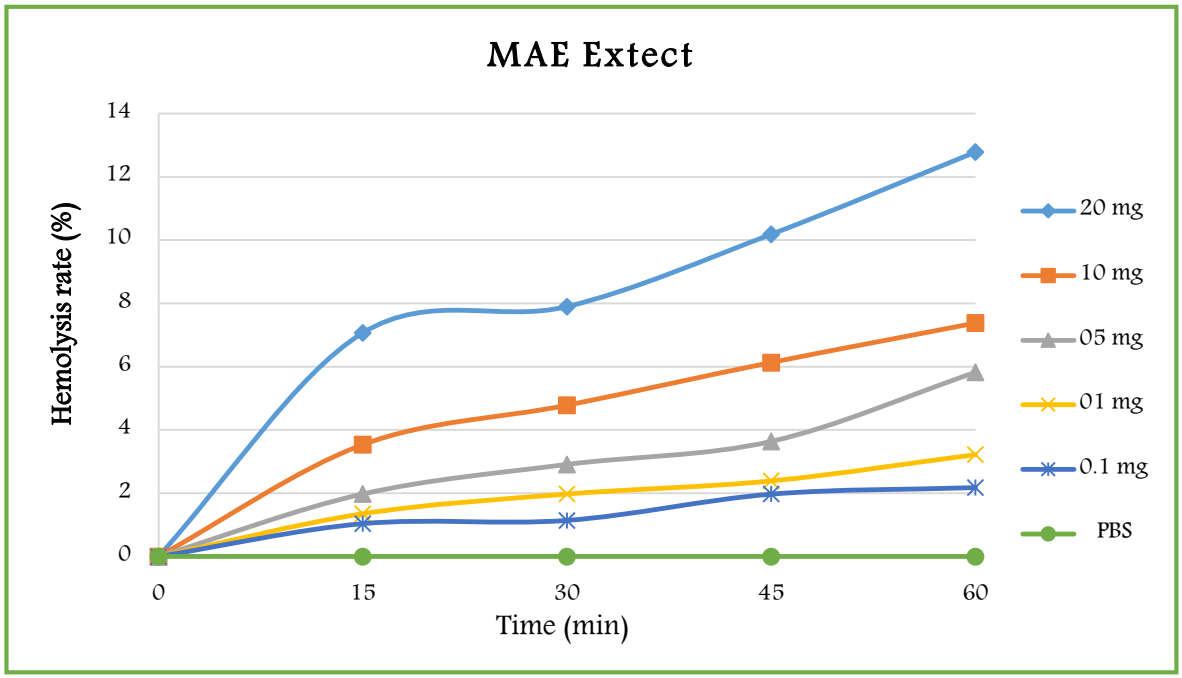


Figure 31: Effects of MAE extract solubilized in PBS on the leakage of intracellular hemoglobin in human red blood cells.

We find that the leakage of intracellular hemoglobin depends on the final concentration of the tested extract. Indeed, the maximum hemolysis rate estimated at 12, 78 % is reached with a final concentration of 20 mg / ml of the UAE extract. On the other hand, the minimum hemolysis rate estimated at 02, 18 % is reached with a final concentration of 0.1 mg / ml up to 60 minutes of incubation. It is important to note that PBS has no effect on human red blood cells.

II.1.4.3.2. The hemolytic activity of the Ultrasound Assisted Extract (UAE)

With regard to the effect of the extract UAE of *Cynodon dactylon* on the leakage of hemoglobin from the red blood cells, the results are presented in Figure 32.

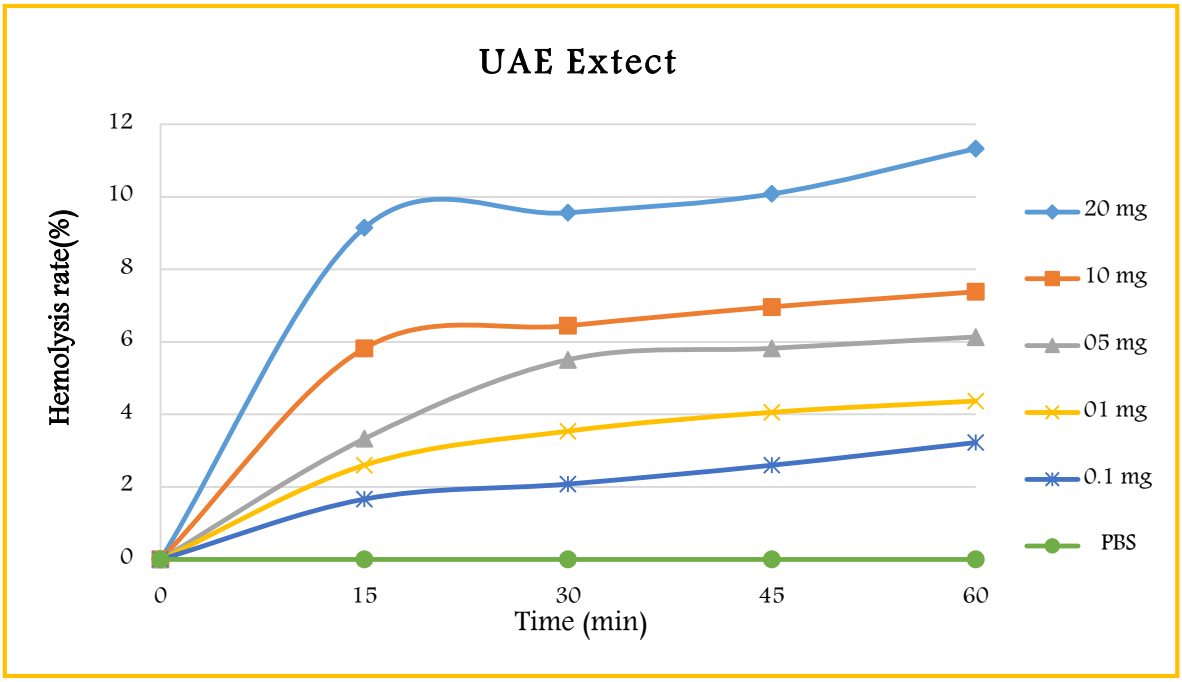


Figure 32: Effects of UAE extract solubilized in PBS on the leakage of intracellular hemoglobin in human red blood cells.

We note that this extract is more less toxic than the previous extract. At a low concentration of 0.1 mg / ml, we recorded very low rates of hemolysis estimated at 3.22%. On the other hand, at a concentration of 20 mg / ml, the absorbance arrived at 11.33% after 60 min.

II.1.4.3.3. The hemolytic activity of the positive control sodium dodecyl sulfate (SDS)

Figure 33 show the rates of hemolysis induced by the different concentrations of the SDS positive control ranging from 0.1 to 20 mg / ml.

Indeed, the final concentration of 20 mg / ml of sodium dodecyl sulfate (SDS) causes total hemolysis. For the other concentrations, the minimum hemolysis rate (0.1 mg / ml)

estimated at 50.51% is reached with a final concentration of 0.1 mg / ml up to 60 minutes of incubation. Also we note that PBS has no effect on human red blood cells.

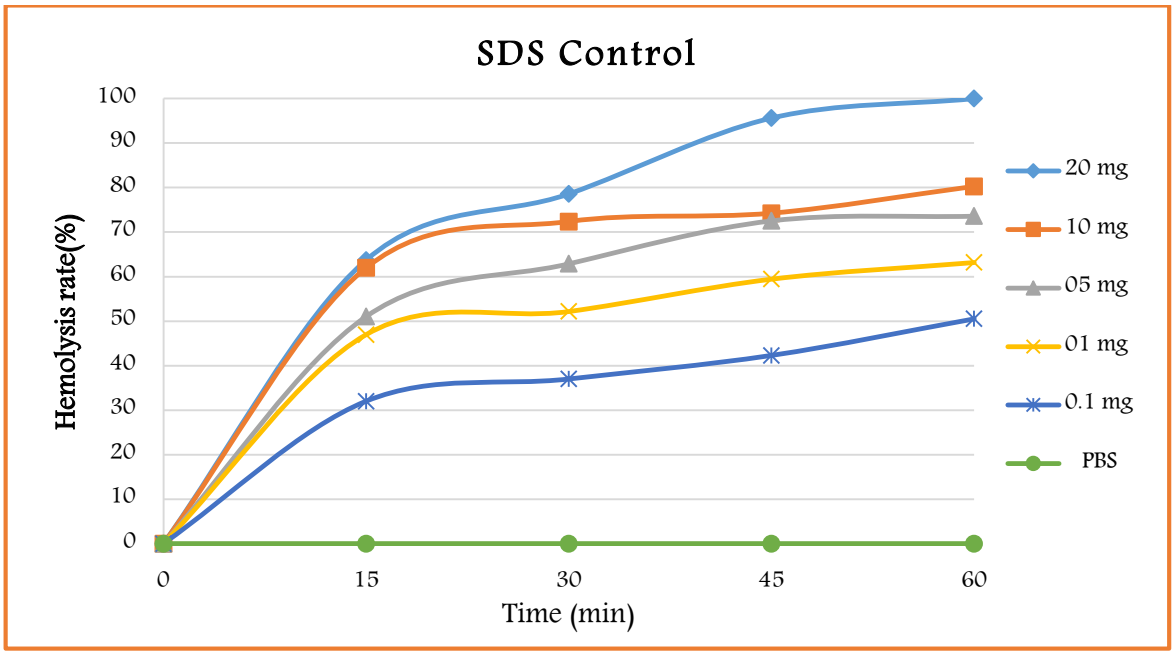


Figure 33: Effects of sodium dodecyl sulfate (SDS) on the leakage of intracellular hemoglobin in human red blood cells.

II.1.4.3.4. Comparison of the hemolytic activity of the different ethanolic extracts

The results obtained show that the percentages of hemolytic effect are directly proportional to the increase in concentrations and hydro-ethanolic extracts (MAE and UAE) and in contact time with human red blood cells (Figure 34).

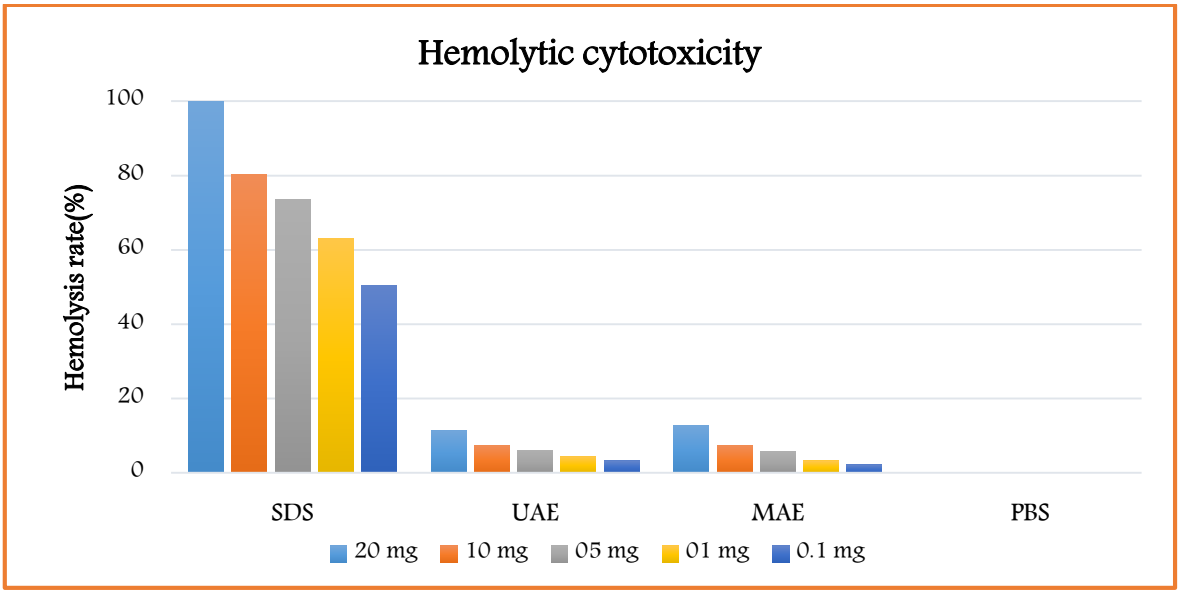


Figure 34: Comparison of the hemolytic cytotoxicity of the different ethanolic extracts Ultrasound Assisted Extract (UAE) and Maceration Assisted Extract (MAE) on the human red blood cells tested at different doses after 60 minutes of incubation.

After 60 minutes, the percentages of hemolysis of the UAE extract tested, are less than the hemolysis of the MAE extract. Therefore, at a concentration of 20 mg / ml, after 60 minutes of contact with human erythrocytes, maximum hemolysis (12.78%) is obtained with the MAE extract followed by the UAE extract (11.33%) of *C. dactylon*. But by comparing these two results with the cytotoxicity of SDS, it becomes clear to us that SDS is toxic more than eight times the toxicity of the extracts.

Therefore, we can say that these two extracts may be slightly hemolytic at high concentrations and after one hour of contact with hRBCs.

II.2. Discussion

Medicinal plants according to World Health Organization (WHO) are defined as plants which used to extract active compounds and bearing the curative medicinal properties **(Siddiqui & Prasad, 2017).** Studies on these active compounds have been increasing over the last 16 years and these compounds have been seriously considered for their capacity to improve human health **(Valdés et al., 2015).** *Cynodon dactylon* (Bermuda grass) is a perennial plant traditionally used as an herbal medicine in many countries **(Pashaie et al., 2017)**.

II.2.1. Test phytochimique

In the present study, the results obtained by the phytochemical tests carried out have made it possible to highlight various secondary metabolites in the aqueous extract of the aerial part of *C. dactylon* including free quinones, saponosides, terpenes, alkaloids, reducing sugars, cardiac glycosides, flavonoids and polyphenols.

We note that these results are similar to those obtained in the **Syahriel et al., (2012)** study on the aqueous extract of the same species collected in Kota Kinabalu (Malaysia), also similar to **Madhan et al., (2018)** study on our species which collected in Tamilnadu (India) and **Kumar et al., (2014)** study in the same country. Our results are very near to the results which cited in the study of **Karim et al., (2015)** on the methanolic extract of *C. dactylon* harvested from Lahore (Pakistan).

These molecules can show many properties. In fact, polyphenols have been associated with a reduced risk of a number of chronic diseases, including cancer, diabetes, cardiovascular disease and neurodegenerative disorders. **(Handore & Khandelwal, 2017; Rasouli et al., 2017).** Polyphenols protects organisms against chronic pathologies by modulating numerous physiological processes, such as cellular redox potential, enzymatic activity, cell proliferation and signaling transduction pathways **(Luca et al., 2019).** It has also been shown that the flavonoids are endowed with antioxidant and anti-inflammatory properties **(Wang & Mazza, 2002).**

Alkaloids also have therapeutic properties, being anti-inflammatory, antinociceptive, antitumoural, antioxidant and antimicrobial **(Rosales et al., 2020).** Terpenoids represent an interesting pharmacological approach for the treatment of inflammatory diseases **(Hortelano et al., 2020).**

It has also been shown that the saponins have many biological activities such as the treatment of diabetes, obesity and osteoporosis; also have a hemolytic property **(Kregiel et al., 2017).**

II.2.2. Extraction yield:

The yield varies according to the plant species, the organ used, the drying conditions, the content of each plant species in metabolites and the nature of the solvent used in the extraction **(Isidore et al., 2019).** Moreover, in addition to the above, UAE depended the micro-environmental extraction parameters such as time, frequency, power, temperature, and solvent/material ratio influence the treatment outcomes and efficiency **(Dzah et al., 2020).**

The percent yield extracts through maceration and ultrasound-assisted extraction was assessed by dividing the weight of the extracts with the samples weight and multiplying by 100 **(Safdar et al., 2016).** The extract of the aerial part *Cynodon dactylon* obtained by the UAE method is gave a high yield (12.52%), This yield is much higher than that found by **Savadi et al., (2020)**, i.e. (3.29%) of a polyphenolic extract from rhizomes of *C. dactylon* collected from Mashhad (Iran).

Contrariwise, the extract obtained by the MAU method also gave a good yield (09.40%), this yield is lower than that found on the study of **Mahesh & Brahatheeswaran, (2007)**, That was valued with 15.60% for the hydro-ethanolic extract of *C. dactylon* collected in Malaysia. On the other hand, it is higher than that observed by **Syahriel et al., (2012)**, that estimated by (7.065%) on the extract of the same plant harvested in India.

This result can be explained by the combined use of water and organic solvent to facilitate the extraction of chemicals which are soluble in water and / or in the organic solvent **(Do et al., 2014).** Beside, alcoholic solvents are capable of increasing the permeability of cell walls **(Seidel, 2005).** In addition, extraction at room temperature can make it possible to extract the maximum of bioactive compounds and to prevent their degradation. A certain degree of temperature can inactivate bioactive compounds and reduce their extraction yield in the solvent used **(Lahmar et al., 2017).**

From a comparative point of view, the results of extraction yield of our plant, show that the extract of the UAE method has a yield higher than the extract of MAE method. With a few explanations, the cavitation and implosion generated by the ultrasonic radiation causes ruptures in the cells walls and numerous microscopic channels in the tissues, this sponge effect facilitates the penetration of the solvent into the solid matrix and increases the mass transfer for the extraction (**Fu et al., 2019).** So, it is important to emphasize that the use of

ultrasonic radiation greatly improved the extraction of polyphenols **(Quiroz-Reyes et al., 2013).**

II.2.3. Quantitative Estimation

II.2.3.1. Estimation of total polyphenols contents

Phenolic compounds (PCs) form a wide group of compounds originating from the secondary metabolism of plants found in different natural sources such as fruits, vegetables, tea and honey **(Lima et al., 2019).** These compounds possess antioxidant, antimicrobial, anti-cancer, anti-inflammatory properties and inhibit lipid peroxidation **(Salehi et al., 2019).** This is the reason why, the estimation of the total phenols of our plant was carried out in our study.

Before the discussion of polyphenols content, it's important to note that the estimation of total polyphenols was carried by the Folin-Ciocalteu test, which implies all the reducing molecules, such as reducing sugars or vitamin C, are dosed, which therefore makes this non-selective dosage compared to polyphenols **(Fukushima et al., 2009).**

After extrapolation of the O.D. results on the calibration curve, the content of total phenolic compounds in our sample is estimated at 42.14 ± 0.75 mg EAG / g E for MAE extract, and 29 .93 ± 0.14 mg EAG / g E for UAE extract. These results may be due of the solvent type, i.e. the polarity of the solvent used in the extraction. The high solubility of phenols in polar solvents like the hydro-ethanolic solvents gives a high concentration of these compounds **(Ghedadba et al., 2015).**

Our results are near from **Mangathayaru et al., (2009)** results on the shoots of *Cynodon dactylon* collected in the region of Chennai (India) who find a polyphenol content of value 47 ± 0.33 EAG / g E. On the other hand, the results obtained by **Biswas et al., (2016)** on the same plant in the region of Kolkata (India) are less important of the order (23.97 ± 0.16 EAG / g E) for polyphenols content.

These differences in the phenolic compounds of the extracts essentially depend on: their origin **(Ebrahimzadeh et al., 2008)**, the geographic location where they grow, the maturity phase of the plant, the variety of the plant, the season when they harvest and the diseases that can affect the plant **(Park & Cha, 2003)**.

The distribution of secondary metabolites may change during the development of the plant. This may be due to the harsh climatic conditions of the places where they grow (high temperature, high exposure to the sun, drought and salinity) which modifies the biosynthesis of secondary metabolites such as polyphenols (**Falleh et al., 2008)**.

Actually, the total content of phenolic is controlled by many parameters and conditions, some of them are the extraction techniques, solvents used **(Turkmen et al., 2006; Do et al., 2014; Altemimi et al., 2016).** also the microenvironment conditions of extraction influence on biochemical properties of extracts such as temperature, solvent to material ratio, and time **(Zhong & Wang, 2010; Li et al., 2016; Maran et al., 2017).** For UAE, additional conditions such as frequency, intensity, and power of the ultrasound **(Wen et al., 2018).**

According to the above results, we note that the extract obtained by MAE contains a high level of total polyphenols, compared to the extract obtained by the UAE. And here it should be noted that a high extraction yield for an extract does not necessarily mean a high content of active compounds. As **Savadi et al., (2020)** cited, despite the higher yield of one extraction method than another, the content of the active compounds extracted such as phenolic compounds may be lower in the extract with a higher yield.

From an explicative point of view, the low polyphenols content in the UAE raw extract may be due to the cavitation effects in bioactive compounds induced by the ultrasonic microenvironment, which could cause changes in the end products. This change phenomenon includes the molecular structure, conformation and biological activity of the compounds **(Wen et al., 2018).** Additionally, the extended time can damage extracted natural antioxidants **(Jovanović et al., 2017).** Several researchers have demonstrated that the long extraction time increases the possibility of the oxidation of phenolic compounds, which can lead to very low levels **(Nazck & Shahidi, 2004; Nazck & Shahidi, 2006; Chirinos et al., 2007; Drużyńska et al., 2007; Yap et al., 2009).**

Many studies have also been conducted to compare different extraction methods and their content of active ingredients. These studies indicate that there are insignificant differences between the extraction methods. For example, maceration, ultrasound and microwaves methods have been used by **Savadi et al., (2020)** to extract the Rhizome of *C. dactylon*. The results showed that the phenolic content of the extract obtained by maceration extraction was higher.

II.2.3.2. Estimation of Flavonoid contents

The determination of the flavonoids content of the crude extracts MAE and UAE were carried out by means of a spectrophotometric assay according to the aluminum trichloride method.

We note, from our results, that it appears clearly that the highest level of flavonoids was detected in the MAE extract (23.57 ± 0.78 Mg EQ / g E), on the other hand the quantity recorded for the extract UAE (13.53 ± 0.33 Mg EQ / g E) remains the lowest.

The low contents of flavonoids compared to phenolic compounds are logical because flavonoids represent a large part of the polyphenols **(Boussahel, 2011).** So, as we mentioned earlier, flavonoids also follow the same trend as polyphenols.

In general way, the flavonoid content of *C. dactylon* is higher than the hydro-ethanolic extract of the same plant of the region Kolkata (India) with a rate equal to 14.84 ± 0.17 mg EQ / g E which is found by **Biswas et al., (2016).**

II.2.3.3. Estimation of condensed tannins contents

The quantitative estimation of *Cynodon dactylon* L. suggested the presence of a considerable content of condensed tannins in the two extracts, but in the extract MAE is higher than the extract EAU.

Chemically, condensed tannins are defined as polymeric flavonoids **(Hagerman, 1988; Asadi et al., 2019).** However, they can appear as oligomers as well, when they are composed of two to ten monomeric units **(Haslam, 2007; Asadi et al., 2019).** In the form of polymeric flavonoids, they have limited to no solubility in water, whereas in oligomeric form, they are water soluble **(Bennick, 2002; Asadi et al., 2019).**

May be we can explain the lower tannins level of the MAE as cited in the work of **Hoyos-Martinez et al., (2019)**, which mentioned that there is an equilibrium was reached between the tannins concentration in the plant matrix and the solvent (Fick's law of diffusion), resulting in the slowdown of the extraction.

Numerous studies have observed that the temperature increases the extraction yields and the tannin content by increasing the mass transfer coefficient **(Kemppainen et al., 2014).** Moreover, the increment of the temperature results in the improvement of the solute solubility and diffusion coefficient and also decreases of solvent viscosity, which promotes the extraction **(Al-Farsi & Lee, 2008).**

In another work, regarding the extraction times, the increment of this parameter (almost 1 hour) can lead to higher extraction yields and amount of tannin in the extract **(Ivanovic et al., 2014).** The extended exposure time to sound radiations increased the temperature of the water bath **(Dalzell & Kerven, 1998).** These results can be explained the higher condensed tannins in the UAE extracts.

II.2.4. Evaluation of biological activities

II.2.4.1. Antioxidant activity

As the phenolic substances make one of the major groups of compounds acting as primary antioxidants or free radical scavengers, it was reasonable to determine their antioxidants activity in the selected plant extracts **(Li et al., 2008).**

There is not an universal method by which antioxidant activity can be measured quantitatively in a very precise way. Most often it is necessary to combine the answers of different and complementary tests to have an indication on the antioxidant capacity of the sample to be tested **(Tabart et al., 2009).**

In this study, the antioxidant power of *C. dactylon* extracts has been demonstrated by two techniques: trapping the free radical diphényl-picrylhydrazyle(DPPH•) and ferric reducing antioxidant power (FRAP).

II.2.4.1.1. DPPH radical trapping test

DPPH• It is a stable free radical dissolves in either ethanol or methanol, which DPPH• free radical reduction is determined by the decrease in its absorption at 517 nm and the color of the DPPH• assay solution changes from purple to light yellow **(Safdar et al., 2016).**

The MAE extract had a higher inhibitory activity against the DPPH• radical compared to the UAE extract. These two results comparing to both standard ascorbic and gallic acids, the activity of removing DPPH• radicals from extracts of *C. dactylon* are very lower.

According to our results, the different extraction methods had different levels of total polyphenols and flavonoids. So, for that raison the MAE extract showed the highest antioxidant power. The extraction method can be explained that the compositions and the content of phenolic compounds and respectively the antioxidant activity are different **(Kahkonen et al., 1999).**

Evidences show that there is a positive correlation between the level of phenolic compounds and antioxidant activity of plants **(Savadi et al., 2020; Deghima et al., 2020).** The antioxidant mechanism of phenolic compounds can be summarized as a transfer based on hydrogen atoms or a single electron transfer through protons **(Yan et al., 2020).** Besides, the antioxidant potential of the phenolic compounds in plant extracts depends on their type and concentration as well as the number and position of hydroxyl groups in the aromatic ring **(Bendary et al., 2013; Pereira et al., 2009).** There is a strong relation between the antioxidant activity of phenolic acids and the number / position of hydroxyl groups in the molecule. The antioxidant efficiency of mono-phenols is strongly enhanced by the introduction of a second hydroxyl group at the ortho-or para- positions **(Pereira et al., 2009).**

Based on the results obtained, DPPH• free radical scavenging activity of the two extracts of *C. dactylon* were concentration dependent. DPPH• assay support that our extracts contains compounds that are capable of donating hydrogen to a free radical to remove odd electron which is responsible for radical's reactivity.

The comparison of the antioxidant power of the extracts of our plant in polyphenols with other works, showed that our plant has a high antioxidant power, for example, the results that **Melinda et al., (2010)** on the methanolic extract of leaves of *C. dactylon* obtained by maceration, found that the maximum percentage of DPPH inhibition • is less than 50% for a high concentration (20 mg / ml). They are significantly lower than our results.

On the other hand, other studies have reported a large variation in the antioxidant activity of *C. dactylon* polyphenols, the results of **Soraya et al., (2015); Madhan et al., (2018)** and **Savadi et al., (2020)** have shown great antioxidant power with a percentage of DPPH• inhibition about 50% for a concentration near to 1 mg / ml of extracts of *C. dactylon*. These values are very high. Moreover, **Biswas et al., (2016)** also showed great antioxidant power by trapping DPPH with an IC_{50} (2.29 ± 0.13 mg / ml).

II.2.4.1.2. Ferric reducing antioxidant power (FRAP)

Ferric reducing power is linked with the antioxidant potential of a compound so it may serve as a good indicator of antioxidant activity. In this assay, reducing power assay is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidant compounds visible in changing the yellow color of the test solution to various shades of green and blue, depending on the reducing power of antioxidants. Increased absorbance at 700 nm indicates high ferric reductive ability **(Basu & Maier, 2016).**

In general, the MAE and UAE extracts showed important ferric reducing power with increasing concentrations. Although, there is slight different between the reducing power of the two extracts, this is due to the different contents of phenolic compounds.

Some earlier studies and compared to our results, found that the polyphenols of the studied species, *Cynodon dactylon* L, have a strong reducing power according to **Madhan et al., (2018)** and **Melinda et al., (2010).** In contrast, our results remain higher than that shown in the work of **Biswas et al., (2016)** with value 12.96 ± 0.68 mg EAA / g E.

The reducing power property of plant extracts indicates that the antioxidant compounds are electron donors **(Yen & Chen, 1995; Huang et al., 2005)**. This can be interpreted by the richness of these extracts in phenolic compounds, it can therefore be deduced from this test that the polyphenols in particular the flavonoids play a very important role in the chelation of the transition metals **(Ghedadba et al., 2015).**

Certain specific flavonoids are known to chelate metals thanks to interactions between the reduced forms of many transition metals, mainly by iron which is causal factor for the development of radicals **(Cherrak, 2017).** These later, released in the Fenton reaction (formation of hydroxyl radicals resulting of the reaction of iron with hydrogen peroxide) **(Ghedadba et al., 2015).**

Flavonoids can chelate metal ions preventing them in the participation to form free radicals, which is one of the mechanisms of their antioxidant activity by the transformation of Fe^{3+} into its reduced form Fe^{2+} and form insoluble metal complexes (Figure 35) **(Ferrali et al., 1997).** Thus, the overall antioxidant flavonoids seem to be a combination of direct reaction with free radicals and chelating properties responsible for the production of reactive oxygen species **(Symonowicz & Kolanek, 2012).**

II.2.4.2. Antimicrobial activity

Nowadays there exist more than 250 types of infections caused by bacteria **(Balasundari & Boominathan, 2018).** Among these microorganisms, we find the *Pseudomonas aeruginosa, Staphylococcus aureus* **(Riley, 2009)** and *Salmonella Typhimurium* that are known to be one of the main elements of human physiological flora **(Zhang & Fowler, 2013).**

The discovery of antibiotics has enhanced people's health and prolonged human life **(He et al., 2020).** However, bacterial resistance to antimicrobials is a rapidly growing problem with potentially devastating consequences **(Sanchez & Doron, 2017).** Many studies have focused on active substances from widely sourced plants with strong antioxidant and antibacterial activities **(Ćavar et al., 2012; Baczek et al., 2017; Ceylan et al., 2019).**

The antibacterial activity of hydro-ethanolic extracts which we obtained by two methods (EAM, UAE) of *Cynodon dactylon* L were screened in this study against bacteria and fungi by the technique of diffusion on disc **(Rios & Recio, 2005).** The antibacterial activity was determined by measuring the diameter of zone of inhibition recorded.

The results obtained show that all the extracts of *Cynodon dactylon* L have a significant inhibitory activity against bacterial and fungal growth with a different degrees linked to the content of the extracts on polyphenols. These confirms the broad spectrum of antimicrobial activity of *C. dactylon* extracts.

In the present study, the two hydro-ethanolic extracts of *C. dactylon* was found to be active against the tested bacterial strains of Gram-positive *Staphylococcus aureus* and *Bacillus cereus*, which shows significant antibacterial activity with an inhibition zone

diameter of 12.33 ± 1.15 mm and 10.00 ± 0.00 mm respectively for MAE extract, also 09.00±1,00 mm and 07.00±1.73 respectively for UAE extract at a minimum concentration 2.5 mg / ml.

Our results are superior by comparing with the results found by **Syahriel et al., (2012)** on the ethanolic extract obtained from whole part of the same plant, they found an inhibition zone diameter of 9.0 ± 0.00 against *S. aureus* and *B. cereus*. Another study by **Abdullah et al., (2014)** gave the same observation, which confirms the moderate effect of the ethanolic extract of the *Cynodon dactylon* plant on the same strains.

In a previous study, **Atmani & Sekhri, (2016)** cited that the hydro-ethanolic extract of *C. dactylon* has been shown a 16 mm inhibitory activity at the concentration 1 mg / ml vis-à-vis the strain *Staphylococcus aureus*, which is a high result. On the other hand, according to the results obtained by **Chaudhari et al., (2011)** who did not detect any inhibitory effect for the methanolic extract on the same strain.

The study of **Suresh, (2008)** revealed no inhibitory activity of the aqueous extract of *C. dactylon* against the *Staphylococcus aureus* strain, but it has an inhibitory effect with zone of inhibition of 09.00 mm by the chloroform extract.

Our extracts of *C. dactylon* was found to be active also against the tested bacterial strains of Gram-negative *Pseudomonas aeruginosa* and *Salmonella typhimurium*, which shows significant antibacterial activity with an inhibition zone diameter of 11.66±0.57 mm and 08.66±1.15 mm respectively for MAE extract at a minimum concentration 2.5 mg / ml. These results increased with the increase of concentration. So, in the high concentration 10 mg/ ml, we obtained 13.00±0.57 mm and 10.00±0.57 mm for *P. aeruginosa* and *S. Typhimurium*. For the UAE extract the antibacterial activity are less Important than the MAE extract, with 10.00±1.52 mm and 09.00±1.52 mm respectively for *P. aeruginosa* and *S. Typhimurium* strains.

According to **Syahriel et al., (2012)** and **Abdullah et al., (2014)**, the antibacterial power of the ethanolic extract of *C. dactylon* against the *P. aeruginosa* strain reveals an inhibitory activity of the order 08.00 ± 0.00 mm at a very high concentration. Other studies by **Chaudhari et al., (2011)** and **Atmani & Sekhri, (2016)** gave the same observation, which confirms the moderate effect of *C. dactylon* extract on the same strains with zone of inhibition of 07.00 mm. these results are inferior to our results.

The study of **Suresh, (2008)** revealed no inhibitory activity of the aqueous extract of *C. dactylon* against the *Staphylococcus aureus* strain, but it has an inhibitory effect with zone of inhibition of 08.60 mm by the chloroform extract.

Generally, gram-negative bacteria were more resistant to antibiotics than gram positive bacteria. The resistance is due to the differences in their cell wall composition. In gram-negative bacteria the outer membrane acts as a great barrier to many environmental substances including antibiotics. Presence of thick murine layer in the cell wall prevents the entry of the entry of the inhibitors **(Syahriel et al., 2012)**. In the present study revealed that there is no significance between gram-negative and gram positive bacteria in term of susceptibility to the ethanolic extracts of our plant.

The zone of inhibition increases considerably with the concentration of the extracts **(Dordevic et al., 2007).** This obtained in our results with the exception of two cases, one resulting from the extract of MAE (state of the strain *B. Cereus* C: 05mg / ml; zone of inhibition = 11mm); The other results from UAE (state of C: 05 mg / ml; zone of inhibition = 10 mm). This can refer to the extract which may be more absorbed by the disc, which can give erroneous results.

These antimicrobial effect of *C. dactylon* extract can be explained by the presence of The polyphenols, flavanols, flavonols and phenolic acids, which possess the highest antibacterial activity thanks to many effects, some of them the ability to inhibit bacterial virulence factors such as enzymes and toxins, or interact with cytoplasmic membrane **(Quideau et al., 2011)**.

In summary, the presence of phenolic hydroxyl groups, which have high affinity for proteins, and microbial enzyme-inhibition may enhance antibacterial effects of flavonoids through another mechanism. What is more, an increase in the hydrophobicity of the flavonoids by long aliphatic chains substitution facilitates interactions with the bacterial cytoplasmic membrane, thus increases antibacterial activity of these compounds **(Pereira et al., 2009; Miklasińska-Majdanik et al., 2018)**.

The mechanisms of antibacterial action of polyphenols are not yet fully deciphered but these compounds are known to involve many sites of action at the cellular level. Several authors explained this activity by the modification in permeability of cell membranes **(Sikkema et al., 1995)**. Some specific polyphenols such as ellagitannins, catechins or nor-lignans have demonstrated high affinity for bacterial membranes and great disruption capacity **(Álvarez-Martínez et al., 2020)**. The polyphenols change the polarity of the membrane compounds and consequently their locations in the bilayer. The accumulation of these phenolic compounds in the hydrophobic part of the membrane will cause a change in the fluidity of the membrane **(Sikkema et al., 1995)**.

Moreover, it can interact and penetrate eventually the membranes **(Álvarez-Martínez et al., 2020)**. Thus, phenolic compounds can change various intracellular functions induced by hydrogen binding of the phenolic compounds to enzymes or by the modification of the cell wall rigidity with integrity losses due to different interactions with the cell membrane **(Ikigai et al., 1993; Cushnie & Lamb, 2011; Taguri et al., 2006).** The capacity of tannins to create complexes with proteins by hydrogen bonds, hydrophobic bonds or covalent bonds, then allows them to deactivate microbial, enzymatic adhesions and the cellular envelopes transporting proteins of microorganisms **(Cowan, 1999)**.

In addition, polyphenols have many potential mechanisms preventing their antibacterial activity such as inhibition of extracellular microbial enzymes. For example, condensed phenylpropanoids-tannins may induce damages at the cell membrane and even inactivate the metabolism by binding to enzymes **(Chung et al., 1998)**. Beside, phenolic compounds can follow another mechanism which have a direct action on microbial metabolism or deprivation of the substrates necessary for microbial growth, in particular essential mineral micronutrients such as iron and zinc (via the chelation of proanthocyanidin with metals), the depletion of which can severely limit bacterial growth **(Daglia et al., 2012)**.

Flavonoids also may act through inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses **(Haraguchi et al., 1998)**. Recent studies have shown that flavonoids activate the gene modulation of bacteria, suggesting that flavonoids are closely related to gene activation and expression **(Boudet, 1999)**.

Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The aim of this work was to evaluate in vitro the potential antifungal activity of *Cynodon dactylon* extracts which obtained with MAE and UAE against *Aspergillus carbonarius* and *Aspergillus parasiticus*.

The two fungal strains: *Aspergillus carbonarius* and *Aspergillus parasiticus* are evaluated in relation to our extracts (MAE and UAE), the results show that the extract of MAE has a high sensitivity for the strain of *A. parasiticus* by an inhibition zone of 13.16 ± 1.04 mm.

On the other hand, this strain is completely resistant to the extract of UAE. Contrariwise, the *Aspergillus carbonarius* strain showed an inhibition zone of 10.00 ± 1.15 mm and 09.00 ± 0.57 mm respectively for MAE and UAE. This is due to the nature of the wall of fungal strains composed of a complex network of proteins and polysaccharides and whose composition varies according to fungal species **(Yen & Chang, 2008)**.

In previous study on *Cynodon dactylon*, the data on the antifungal activity on our plant are scarce. Therefore, there is a study of **Balasundari & Boominathan, (2018)** which confirm the fungicide effect of hydro-methanolic extracts of *C. dactylon* against *Aspergillus flavus* and *Aspergillus niger*.

For MAE the preferable effect that UAE, despite the extract due to ultrasound contains an appreciable quantity of compounds (the yield); This observed difference can be attributed to several factors such as extraction methods **(Athamena, 2009)**.

In addition, the antifungal activity of polyphenolic extracts could also be due to the presence of certain polyphenolic compounds that can bind to proteins of microorganisms, which blocks their enzymatic activities **(Thirumurugan, 2010)**. This activity is mainly due to the ability of these molecules to inhibit the expression of DNA and the synthesis of certain enzymes and membrane proteins of microorganisms **(Ulanowska et al., 2006).**

Among the phenolic groups, tannins are having high toxic against fungus, is a well-established fact either by reducing the growth of filamentous fungi or by slowing the germination of spores, or by biochemical approaches by action on mold respiration **(Djabali, 2012)**.

II.2.4.3. Hemolytic activity of the extracts against the human red blood cells

These results obtained by this test can help more to understand and to develop antimicrobial and antioxidant compounds. A good antibiotic must be low toxic to human or animals **(Li et al., 2013).** Considering the use of this compound in medicine, the study of their hemolytic property must be carried out, taking into account the sensitivity of human beings to the hemolysis induced by certain substances **(Devecioglu et al., 2001)**.

The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood, moreover, its membrane has similarities with other cell membrane. Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. This hemolysis relates to concentration and potency of extract. Furthermore, the hemolytic activity of each extract is related to their chemical composition (**Mohammedi & Atik, 2014)**.

Secondary metabolites which induce direct damage of membrane integrity causing the cell lysis are considered as compounds with possible cytotoxic effects **(Mouffouka et al., 2020)**. thus, the hemolysis activity of *Cynodon dactylon* extracts has been explored in this study.

These results showed that the cytotoxicity assay was used to characterize the probability of *C. dactylon* induced cell death and the release of hemoglobin was used to quantify the membrane damaging properties. It is observed that the both of extracts (MAE and UAE) of our plant are much lower than sodium dodecyl sulfate (SDS), thus indicating its low toxicity.

In the present study, we observed slight difference between the two extracts, where the percentages of hemolysis of the UAE concentrations tested, are less than the hemolysis of the MAE concentrations, with maximum hemolysis of 12.78% and 11.33% for MAE and UAE respectively. As we discuss the previous results, this is referring to the extraction method used in our study and content of phenolic compounds.

However, the mechanism of the toxicity of these compounds has been documented relatively poorly. The available data show that the presence of some compounds like phenols or catechol could also induce hemolysis. **(Mouffouka et al., 2020).** These mean that the phenolic compounds probably exert their toxic effects at the level of the membrane, this result is supported by observations that phenol changes membrane functioning and influences protein-to-lipid ratios in the membrane. The accumulation of lipophilic compounds into lipid bilayers may enhance their availability to the cell but may also cause toxicity problems **(Sikkema et al., 1995)**.

Red blood cells (RBCs) are anucleated cellular architecture with a plasma membrane envelope where the glycoproteins are oriented along the lipid bilayer. Membrane proteins can be separated by using strong detergents like Sodium dodecyl sulphate **(John et al., 2018)**. SDS bind to proteins by predominantly hydrophobic interactions causing unfolding of the tertiary structure **(Bhuyan, 2010)**.

Detergents have both hydrophilic and hydrophobic properties which help to separate membrane proteins leading to disruption of cell membrane. Surfactant concentration plays an important role in the solubilization of membrane. At low concentration, some changes can occur in the membrane permeability whereas at higher concentration there might be drastic effects like cell lysis **(John et al., 2018)**.

In the present study also we observed the total lysis of the red blood cells with high concentration 20 mg/ ml, and we confirmed the total hemolysis of SDS with a microscopic observation of human red blood cells at 40X magnification with and without Sodium dodecyl sulphate.

The data on the patterns of hemolysis of polyphenols are scarce, and they are not evaluated on *Cynodon dactylon*. Therefore, this study provides new knowledge about total effect of hydro-ethanolic crude extract of *Cynodon dactylon*.

Therefore, and based on an article of **Álvarez-Martínez et al., 2020**, which mentioned that polyphenols are well tolerated by the human body, our results suggested the non-toxic effect of the extract thus making it suitable for the preparation of drugs involved in the treatment of various diseases.

Conclusion

"*Traditional medicine brings together all the knowledge, skills and practices based on the theories, beliefs and experiences which different cultures use to maintain health as well as to prevent, diagnose, relieve or treat physical and mental illnesses*" **(WHO, 2009)**. Thus, our study came to contribute to the construction of traditional medicine by adding knowledge on our plant by carrying out this work in order to enhance the *Cynodon dactylon* which grows in our local environment, region of 'Oued-souf (southeast from Algeria).

In our study, the phytochemical tests realized have highlighted various secondary metabolites in the aerial part of the plant including saponosides, terpenes, alkaloids, reducing sugars, cardiac glycosides, flavonoids and polyphenols.

The aerial part of *Cynodon dactylon* were subjected to two methods of extraction of phenolic compounds, one using the Maceration Assisted Extraction and the other using Ultrasound Assisted Extraction, accordingly we could estimate the extraction yield, where the highest value was recorded by the UAE crude extract.

The results of the estimation of the phenolic compounds showed that the extract obtained by MAE contains a high level of polyphenols and total flavonoids estimated by (42.14 ± 0.75 mg EAG / gE and 23.57 ± 0.78 Mg EQ / gE), compared to the extract obtained by the UAE, which estimated by (29.93 ± 0.14 mg EAG / gE and 13.53 ± 0.33 Mg EQ / gE). The results show that MAE is preferable for the extraction of polyphenols and flavonoids. In contrast, the content of condensed tannins in the extract of UAE (19.34 ± 0.48 Mg EC/gE) is higher than the extract of MAE (15.99 ± 0.63 Mg EC/gE). The results show that UAE is preferable for the extraction of condensed tannins.

The results of the DPPH and FRAP tests have shown that, compared to the phenolic compounds content, the extracts of the aerial part of *C. dactylon* have a moderate antioxidant capacity. It also reported that the MAE extract is more active than the UAE extract.

The antibacterial activity was evaluated against four strains: *Pseudomonas aeruginosa, Salmonella Typhimurium*, *Staphylococcus aureus* and *Bacillus cereus.* Also the antifungal activity against two strains *Aspergillus carbonarius* and *Aspergillus parasiticus*. the results that we have reached show that all the strains targeted have high susceptibility to the two ethanolic extracts of *Cynodon dactylon* L, with favoring for the MAE extract.

The evaluation of cytotoxicity against RBCs was carried, the results shown the non-toxic effect of *C. dactylon* extracts with a very low hemolysis at very high concentration, with slight difference refer to the phenolic compounds content of our extracts.

From these results, it can be concluded that the study of the biological activity of the ethanolic extracts of *C. dactylon* L. suggests that this plant represents a promising source of natural compounds which have very important biological activities, this activity being different from one extract to another according to the extraction method which influence on the nature of the compounds present in the extracts and the efficiency of the biological activities of these compounds. Moreover, this preliminary study indicates the non-toxic effect of the compounds of our extracts which making it suitable for the preparation of drugs involved in the treatment of various diseases.

Perspectives

One of the main limits of our study is linked to the time allotted for the laboratory preparation, the conceptual approach as well as the technical methods to develop it. We still need time to explore the different axes of our research which will allow us to study in depth and precise the evaluation of the compounds of *Cynodon dactylon* and its activities and to better explain it. These works open up a certain number of perspectives in order to be able to deepen the evaluation of our plant. These perspectives are therefore numerous and relate to the following points:

- Optimization of ultrasonic assisted extraction uses for optimal yields of phenolic compounds.
- The isolation, characterization, and identification of secondary metabolites of *Cynodon dactylon* L. using high-performance identification techniques (high-performance liquid chromatography or chromatography coupled with spectroscopic techniques such as mass and NMR).
- The determination of minimum inhibitory concentrations (MIC), bactericides (CMB) and fungicide (CMF), extracts from this plant.
- The evaluation of other biological activities of the extracts studied, in order to better enhance it, and explore other medicinal plants and test them *in vivo*.

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Annexes

Annex 01:

The study of the antioxidant activity of polyphenolic extracts is carried out with the realization of these curves:

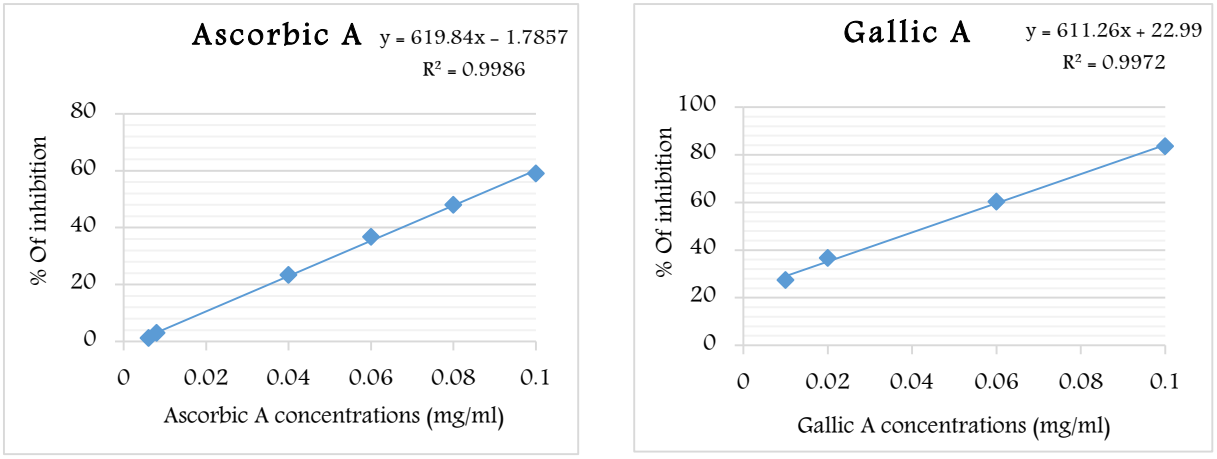


Figure 01: Percentage inhibition of the DPPH radical • by ascorbic acid and gallic acid

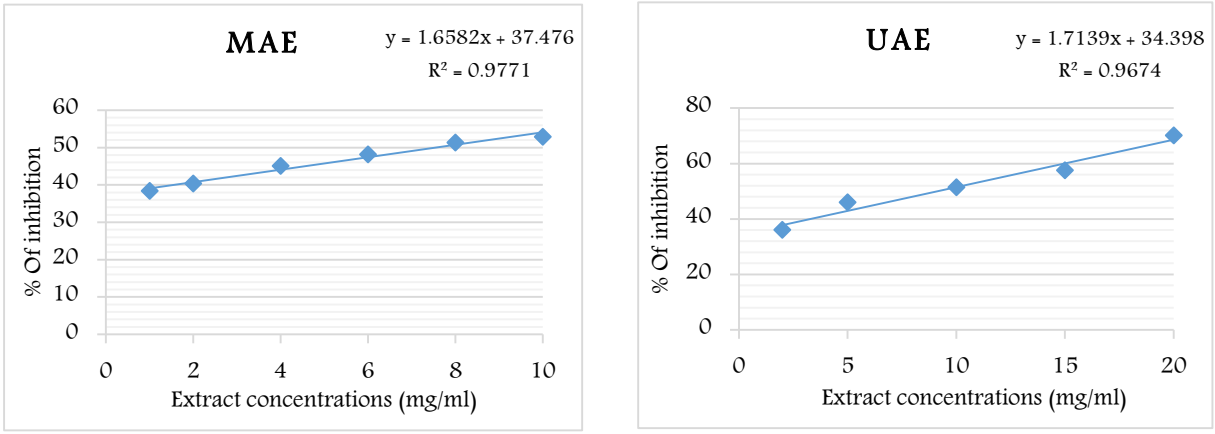
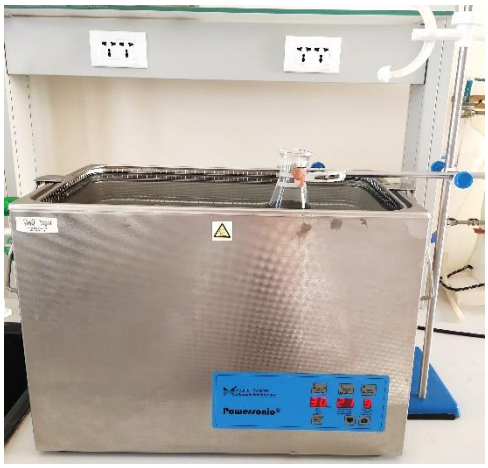


Figure 02: Percentage inhibition of the DPPH radical • by Maceration Assisted Extraction extract and Ultrasound Assisted Extraction extracts from *Cynodon dactylon*.

Annex 02

The types of devices used during the experiment



Ultrasonic bath

Rota-vapor

Precision scale

Scale

Optical microscope

Spectrophotometer