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**Phytochemical study and biological activity of
different extract from flowers of parasitic plant**

***Cistanche tinctoria* (Desf.) Beck.**

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Dedication

إلى أمي . . ليلية العينين . . تلك التي أهدتني عمرا وأكثر . .

إلى أبي . . من لم تمنحني الحياة فتات ذكرى معه أسد بها جوع الحنين . .

إليهما . . من تحتاج أحري في اعتناق أجدية أخرى كي تصف امتناني وحيي

لهما . .

مرحمكما الله

شنتقل عواطف



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CHENGUEL AOUATEF

Abstract:

In order to evaluate the natural products of the desert plants, we conducted this work which is based on study the differences in amount of phenolic compounds and estimate the antioxidant activity of the flowers of the parasitic plant *Cistanche tinctoria* (Desf.) Beck (Orobanchaceae) growing in Oued-Souf region.

After the extraction processes, the yield of the three extracts (Crude, Flavonoids phase Ethyl acetate and Tannins) was estimated with a variance in its values, the highest value recorded in the crude extract (11.26 %), however the tannin extract got the lowest value (0.505%). The results of the estimation of phenolic compounds showed that polyphenols and flavonoids were estimated by 167.74 ± 24.36 mg EAG/g Extract and 26.76 ± 2.69 mg EQu/g Extract, respectively.

The antioxidant activity was performed by three tests, the results of the first one which is the DPPH' test, showed that tannin extract had the best inertial capacity than the other extracts ($IC_{50} = 8.12$ μ g/ml), however the results of Hemolysis test showed that the Flavonoid extract got the lowest disintegration (22.47%) between the extracts. The third test was the Reducing power assay and its results showed that the tannin extract had the highest reducing power between the extracts of *Cistanche tinctoria* (Desf). Beck. The three tests were applied using the Ascorbic acid as a standard compound.

The qualitative analysis of the extract using High Performance Liquid Chromatography (HPLC) showed the differences of concentrations and types of some phenolic compounds in the crude extract of *Cistanche tinctoria* (Desf). Beck, such as: Chlorogenic acid (1.152 μ g /mg Extract) and Naringin (27.456 μ g /mg Extract).

Key words: *Cistanche tinctoria* (Desf). Beck., Crude Extract, Tannins, Polyphenols, Flavonoids, Antioxidant activity, DPPH test, Hemolysis test, Reducing power assay, HPLC.

المخلص:

قصد تثمين المنتجات الطبيعية في النباتات البرية الصحراوية، أُجريت هذه الدراسة والتي تهدف الى التقدير الكمي للمركبات الفينولية وكذا دراسة النشاطية المضادة للأكسدة لأزهار نبات الترتوث *Cistanche tinctoria* (Desf). Beck والنامي بمنطقة وادي سوف. بينت النتائج أن هناك اختلاف في قيم المرودود بين المستخلصات: إذ أن أعلى قيمة للمرودود سجلت عند المستخلص الخام (11.29%)، بينما سجل مستخلص التانينات أدنى قيمة (0.505%). كما أظهرت نتائج التقدير الكمي لعديدات الفينول والفلافونويدات للمستخلص الخام اختلافًا بينهما وقدرت النتائج بـ: 167.74 ± 24.36 mg EAG/g Extract و 26.76 ± 2.69 mg Equ/g Extract على التوالي.

من خلال دراسة النشاطية المضادة للأكسدة للمستخلصات المختلفة، أبدت نتائج اختبار DPPH تفوق مستخلص التانينات على باقي المستخلصات بقدرة تثبيطية قدرت بـ $IC_{50} = 8.12$ µg/ml) أما في اختبار انحلال كريات الدم الحمراء (Hemolysis) فقد تميز مستخلص الفلافونويدات بأقل نسبة انحلال قدرت بـ (22.47%)، في حين أن نتائج اختبار القدرة الارجاعية (Reducing power assay) قد أظهرت تميز مستخلص التانينات بأكبر قدرة ارجاعية من بين المستخلصات الثلاث.

أما التحليل النوعي للمستخلص الخام لأزهار نبات الترتوث باستعمال الكروماتوغرافيا السائلة عالية الأداء HPLC والتي أبدت نتائج اختلافات في نوع وتركيز المركبات الفينولية، على غرار حمض الكلوروجينيك (1.152 ميكروغرام/ملغ من المستخلص) والنارجينين (27.456 ميكروغرام/ملغ من المستخلص).

الكلمات المفتاحية: نبات الترتوث *Cistanche tinctoria* (Desf). Beck، المستخلص الخام، التانينات، المركبات الفينولية، الفلافونويدات، النشاطية المضادة للأكسدة، اختبار DPPH، اختبار Hemolysis، القدرة الارجاعية، HPLC.

Résumé:

Afin de valoriser notre ressources végétales naturelles, ce travail a été menée pour faire une étude quantitative des composés phénoliques et étudiés l'activité antioxydante des extraits des fleurs *Cistanche tinctoria* (Desf). Beck. qu'il est développe dans la zone d'Oued-Souf.

Les résultats ont montré une différence dans les valeurs de rendement entre les extraits: la valeur la plus élevée a été enregistrée à l'extrait brut (11.29%), et l'extrait de tanin était le plus bas (0.505%). Aussi les résultats de l'estimation quantitative des polyphénols et flavonoïdes pour l'extrait brut était différente et ils ont été estimées par: 167.74 ± 24.36 mg EAG/g Extrait et 26.76 ± 2.69 mg EQu/g Extrait, respectivement.

L'activité antioxydante a été évaluée par trois tests. la premier test est le test DPPH• et les résultats ont montré que l'extrait de tanins était supérieur au reste des extraits avec une capacité d'inhibition ($IC_{50} = 8.12$ µg/ml), mais à test d'hémolyse, l'extrait de flavonoïdes qui a donné la meilleure protection de érythrocytes (22.47% d'érythrocytes dissous). La troisième test était le test de Pouvoir Réducteur, les résultats de ce test ont montré que l'extrait de tanins a un bon pouvoir réducteur plus élevé que les autres extraits.

L'analyse qualitative des extraits par Chromatographie Liquide Haute Performance (HPLC) a montré les différences de concentration et de qualité de composés phénoliques, par exemple chez l'acide Chlorogénique (1.152 µg /mg Extrait) et Naringine (27.456 µg /mg Extrait).

Mots-clés: *Cistanche tinctoria* (Desf). Beck., Extrait brut, Tanins, Composés phénoliques, Flavonoïdes, L'activité antioxydante, Test DPPH, Test d'hémolyse, Pouvoir Réducteur, HPLC.

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Abbreviation list

- AA:** Ascorbic acid.
- AlCl₃:** Aluminum trichloride.
- BHA:** Butylhydroxyanisol.
- BHT:** Butylhydroxytoluene.
- DNA:** Deoxyribonucleic acid.
- DPPH:** 1,1-diphenyl-2-picrylhydrazyl.
- FeCl₃:** Ferric chloride.
- FR:** Free radical.
- GST:** Glutathione S-transferase.
- GP_x:** Glutathione peroxidases.
- GR_x:** Glutathione reductases.
- HIV-1:** Human immunodeficiency virus 1.
- HO:** Heme oxygen.
- H₂O₂:** Hydrogen peroxide.
- HPLC:** High performance liquid chromatography.
- I%:** Percentage of inhibition.
- IC₅₀:** Concentration of inhibition 50% of DPPH radical.
- LDL:** Low density lipoprotein.
- LMWA:** Low molecular weight antioxidant.
- Mg GAE/g EX:** Mg of Gallic acid equivalents in 1 g of the extract.
- Mg QAE/g EX:** Mg of Quercetin equivalent in 1 g of the extract.
- NADPH:** Nicotinamide adenine dinucleotide phosphate.
- O₂:** Oxygen.
- PG:** Propyl gallate.
- RNS:** Reactive nitrogen species.
- ROS:** Reactive oxygen species.
- SH:** Sulfhydryl.
- SOD:** Superoxide dismutase.
- TPC:** Total phenolic contents.
- TPHQ:** Tertiary butylhydroquinone.
- UV:** Ultraviolet.

Introduction

Introduction

Plants are the GOD's special gift to this planet, indeed every plant on the planet has the potential and capability of serving one purpose or another. They are so important for our lives because they used for many things such as: beautification, medicines and of course as a food. That's why they have been the subject of man's curiosity since time immemorial, but still the man do not fully exploit them, especially since the compounds of some unstudied plants may serve the purpose of healing some diseases.

Plants have many active compounds, they are the richest bio-resource of drugs of traditional and modern medicine (Hammar et al., 1999), this medicinal value is based on some chemical substances that produce a definite physiological action on the human body and the most bioactive compounds of plants are flavonoids and tannins (Edeoga et al., 2005).

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 *Cistanche tinctoria* (Desf.) Beck. This plant is a rare traditional medicinal plant, it used as a treatment for abdominal pains, muscle contractions and other diseases. So, in this work we will try to figure out the biological activity of different extracts from the flowers of *Cistanche tinctoria*, and how we determine their antioxidant activity? And could we really classify it as a medicinal plant?

In order to find answers to those questions, we will study the parasitic plant *Cistanche tinctoria* (Desf.) Beck. (flowers), which belongs to the Orobanchaceae family and grows in Oued-souf region (south-east of Algeria), this will be based on preparing the extracts using the Maceration method, then we will determine the total phenolic and flavonoid contents of the crude extract, also we will estimate the antioxidant capacity using three different assays: DPPH radical scavenging assay, Hemolysis assay and Reducing power assay, and in order to figure out the phenolic compounds of the methanolic extract, we will use the HPLC technique.

Introduction

The research was divided into two parts:

- ❖ **Bibliographic synthesis:** includes three chapters, the first one focuses on the study of the phenolic compounds, the second one was about oxidative stress and antioxidants and the third one was about the plant *Cistanche tinctoria* (Desf.) Beck, and its family.
- ❖ **Experimental part:** this part includes two chapters, the first chapter was about the materials and methods used during the whole study, and the second one was about the results of this research and their discussion.

First part

Bibliographic synthesis

Chapter I

Phenolic compounds

Chapter I: Phenolic compounds

1- Preface:

Secondary metabolites (Figure 01) are highly numerous in number and chemically diverse in nature (Li et al., 2014), they are defined as natural products (Bellebcir, 2008) which differ in structure, function and quantity (Maksym, 2014) according to the type of plant (Ewane, 2012) or in the same plant (Loic, 2011). These products are actually not important for plant growth but they could be used for development under optimal growth conditions (Maksym, 2014). They can be classified in many ways, based on their chemical structure, composition, their solubility in various solvents, or pathway by which they are synthesized, but the simple classification includes three groups, one of them is: Phenolic compounds (Mudanda, 2010; Bellebcir, 2008; Carocho & Ferreira, 2013).

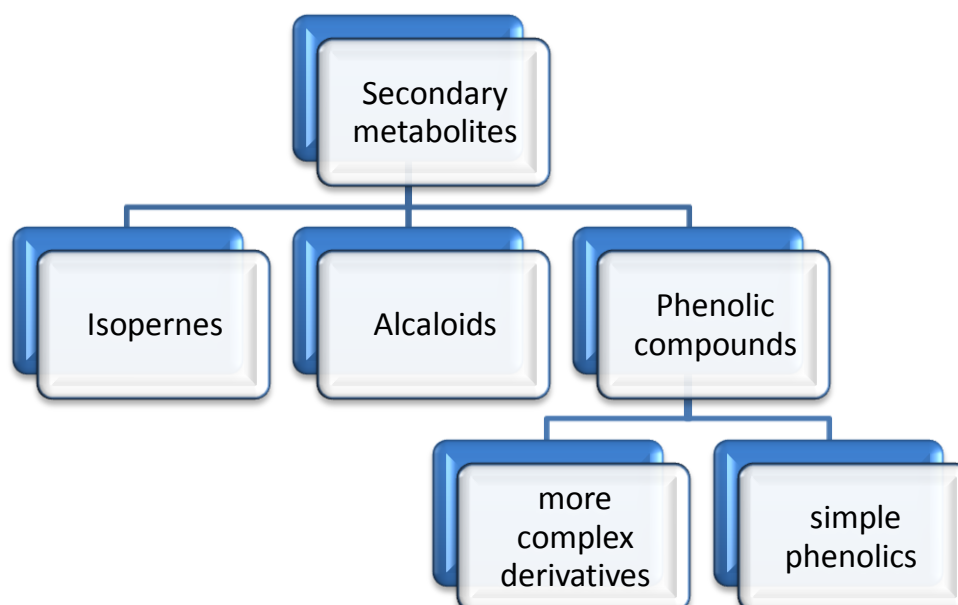


Figure (01): The three groups of secondary metabolites.

1- Phenolic compounds:

2-1- Definition:

Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom and their chemical structure is based on at least one aromatic ring bonded to one or more hydroxyl groups (Loic, 2011; Chanforan, 2010; بن سلامة, 2012), they are mainly synthesized from the amino acid phenylalanine (Cooper &

Chapter I: Phenolic compounds

Nicola, 2015) which is converted to cinnamic acid, despite the structural diversity, the groups of compounds are often referred to as "Polyphenols"(Abedini, 2014).

These molecules represent a variety of functions in plant growth and development, they take a part in the regulation of seed germination, also taking part in defense responses during excessive sun exposure, injuries, infection and heavy metal stress (Renata et al., 2006; Kanoun, 2010; 2010، عمر)

They are derived from pentose phosphate, shikimate (Ncube & Staden, 2015), and phenylpropanoid pathways (Aberoumand & Deokule, 2008) in plants. These molecules are usually divided into two groups: simple phenols and more complex derivatives. Each one of these groups includes others ones (Boumendjel, 2007).

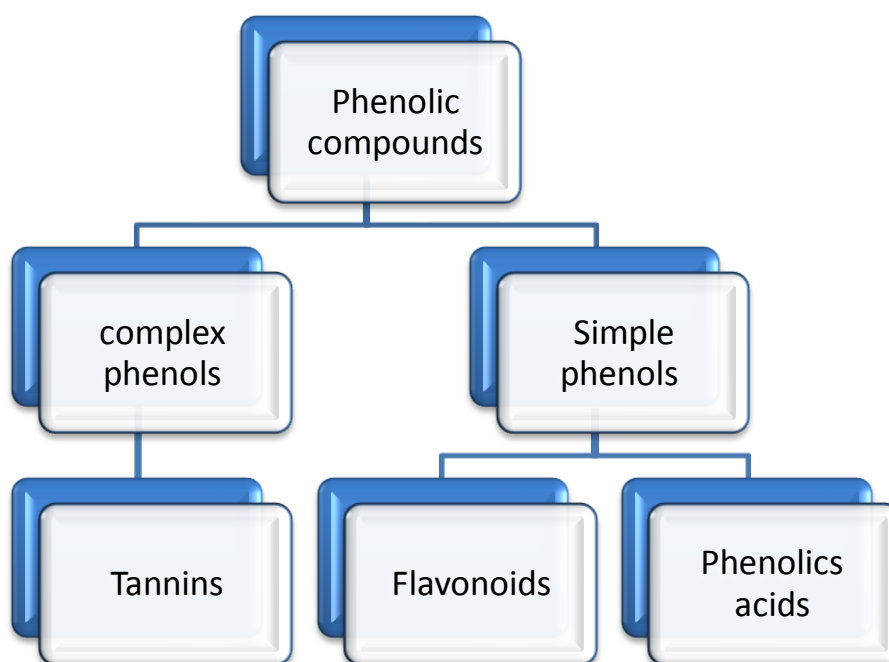


Figure (02): The classification of phenolic compounds.

2-2- Sources:

Phenolic compounds are very widespread in plant foods, especially in fruits, vegetables, grains, seeds, cereals, legumes and beverages such as coffee and fruit juice. (Duthie & Kyle, 2000; Benhammou, 2012).

Chapter I: Phenolic compounds

2-3- Classification:

As we said before, phenolics possess an aromatic ring bearing one or more hydroxyl groups and this ring or rings are synthesized in the shikimate acid pathway from amino acid phenylalanine (بن خنائة، 2004). That is why phenolic compounds can be classified in a number of ways. According to Chanforan (2010), they are classified into many groups based on the number of carbons in the molecule. (Table 01).

Table (01): The most important classes of phenolic compounds (Chanforan, 2010)

Structure	Class	Examples
C_6	Simple phenolics	Catechol, Hydroquinone
C_6-C_1	Hydroxybenzoic acids	P-hydroxybenzoic
C_6-C_3	<ul style="list-style-type: none">Hydroxycinnamic acidsCoumarins	* caffeic acid * scopolein
C_6-C_4	Naphthoquinones	Juglone, plumbagin
$C_6-C_2-C_6$	Stilbenoids	Resveratrol
$C_6-C_3-C_6$	Flavonoids <ul style="list-style-type: none">FlavonolsFlavanolsIsoflavonoids	<ul style="list-style-type: none">QuercetinCatechinDaidzein
$(C_6-C_3)_2$	Lignans	Pinoresinol
$(C_6-C_3)_n$	Lignins	/
$(C_{15})_n$	Tannins	/

2-4- Role and importance of phenolic compounds:

There is a lot of studies that have reported the advantages of phenolics, such as anti-aging, anti-inflammatory, antioxidant (Benhammou, 2012; Harkat, 2008). In addition to the above, there are relevant antioxidant enzymes to counter oxidants. Polyphenols, especially flavonoids, phenolic acids and tannins, have the important property of inhibiting α -

Chapter I: Phenolic compounds

glucosidase and α -amylase, which are key enzymes and responsible for the digestion of dietary carbohydrates to glucose. The high antioxidant capacity makes polyphenols as an important key factor which is involved in the chemical defense of plants and predators and in plant-plant interferences (Dixon and Pavia, 1995). Another valuable property of phenolic compounds is in their ability to chelate heavy metal ions such as iron and copper ions (Renata et al., 2006) due to the presence of suitable functional groups: hydroxyl and carboxyl (Michalaka, 2006).

Also phenolics are responsible for the color (such as yellow, red, blue pigments and orange), taste and flavor (such as eugenol and vanillin) of foods and protect the plant against insects, fungi, bacteria and viruses (Kanoun, 2010; Harkat, 2008; 2010، عمر).

2-5- Phenolics groups:

2-5-1- Simple phenols:

2-5-1-1- Phenolic acids:

Phenolic acids are simple molecules represented the basic unit of phenolic compounds, they consist 2 subgroups: the hydroxybenzoic and hydroxycinnamic acids (Sexena et al., 2012; 2012، بن سلامة; Kanoun, 2010). The phenolic acids have an interesting biological properties, and according to many studies, they have diverse functions including photosynthesis, enzyme activity, protein synthesis and structural compounds. A large number of phenolic acids present in vegetable foods, such as nuts and fruits (Boukri, 2014).

2-5-1-2- Flavonoids:

a. Definition:

The term "flavonoid" is generally used to describe a broad collection of natural products with low molecular weight and it is from the Latin word "Flavus" which means yellow (Harborne, 1973). Flavonoids were originally described as Vitamin "P" by Szent et al in 1936 (2012، بن مرعاش).

Flavonoids are one of the largest groups of plant secondary metabolites. The general structure of a molecule is based on 15-carbon skeleton (connected by three-carbon bridge (C6-C3-C6) (Speisky, 1994; 2015، بكة وحفيان), which consists of two aromatic rings A and B linked via a heterocyclic benzopyran ring, where the 2-phenylchromane considered as the basic unit for it (Jiri et al., 2013; Athamena, 2009; 2012، بن سلامة).

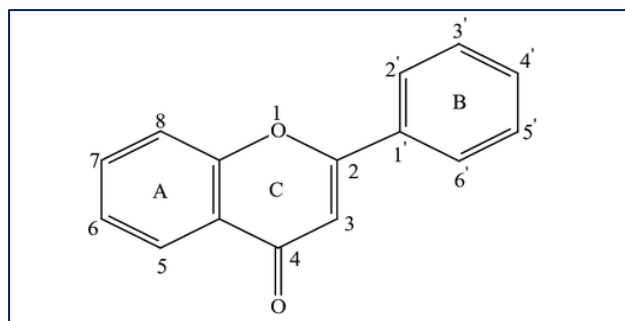


Figure (03): The basic unit of flavonoids (بكرة وحفيان، 2015)

b. Distribution of flavonoids:

Flavonoids are one of the most widespread groups of plant substances, they occur in the root, leaf, flowers, bark, fruits, vegetables, seeds and pollen also they exist in cacao, coffee and tea (Marfak, 2003). These compounds could be found as glycosides dissolved in water (حوقة ومرغن، 2016). About 2% of the total carbon photosynthesized are converted to Flavonoids which in turn contribute to the myriad bright colors in plant tissues (Markham, 1982).

c. Classification:

Flavonoids have been classified into a variety of classes: Chalcones, flavones, flavonols, flavandiols, flavanols, isoflavonoids, proanthocyanidins and their derivatives anthocyanidins. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the ring C (Cindy, 2013) while individual compounds within a class differ in the pattern of substitution of the A and B rings (Bruneton, 1999; حوقة ومرغني، 2016) , their general structures are shown in the following figure (04).

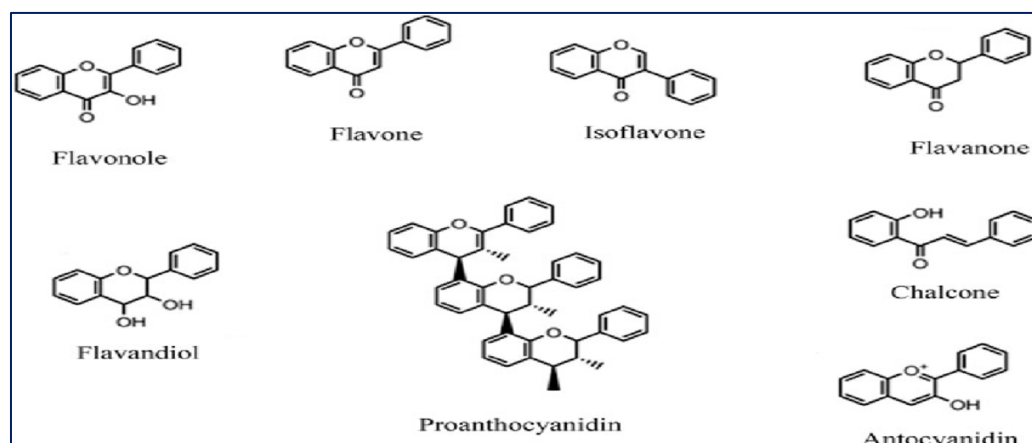


Figure (04): The different structures of flavonoids classes (Zolghadri *et al.*, 2019)

Chapter I: Phenolic compounds

d. Biological activity of flavonoids:

✓ **Antioxidant activity:**

Flavonoids possess many biochemical properties, but the best described property of almost every group of flavonoids is their capacity to act as antioxidants. The antioxidant activity of flavonoids depends upon the arrangement of functional groups on the nuclear structure (Pandey et al., 2012), the configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability (Nijveldt et al., 2001). This activity is due to a combination of the flavonoids's iron chelating and free radical scavenger properties, also the inhibition of oxidase enzymes such as lipoxygenase, NADPH oxidase and xanthine oxidase (Kumar & Pandey, 2013).

✓ **Anticancer activity:**

Fruits and vegetables are having an enormous amount of flavonoids, which have been used as cancer chemopreventive agents (Mishra et al., 2013). Flavonol quercetin is contained in dietary fruits and vegetables, especially onions and apples. Quercetin flavonol is inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast (Brisselmans et al., 2005). Many biological properties of flavonoids are sometimes proved to be cancer chemopreventive, for example: the natural resources of Anthocyanidins have the potential to fight against colorectal cancer, the sources of flavones (leek, kale, parsley, broccoli, pepper..) used to treat breast, thyroid, stomach, lung, oral and colon cancer.

Also the sources of isoflavonoids such as soy flour and soy milk fight against prostate, breast and kidney cancer, The mechanism of action of flavonoids in the molecular study is downregulation of mutant p53 protein, cell cycle arrest, tyrosine kinase inhibition, inhibition of heat-shock proteins, estrogen receptor bonding capacity and inhibition of expression of Ras proteins (Duthie et al., 2000).

✓ **Anti-inflammatory activity:**

Inflammation is the complex biological of vascular tissues to harmful stimuli, such as pathogens, damaged cells and chemical irritation. It is initiated by migration of immune cells from blood vessels and release of mediators at the site of damage. Many types of cells involved with the immune system have been shown to alter their behavior in the presence

Chapter I: Phenolic compounds

of flavonoids (Rathee et al., 2009). A number of flavonoids such as hesperdin, apigenin, luteolin and quercetin are reported to possess anti-inflammatory and analgesic effects. Flavonoids may affect specifically the function of enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases (Nishizuka et al., 1988).

✓ **Antiviral activity:**

In the last decade, many studies have been developed to figure out the antiviral activity of flavonoids, actually the most of these studies were concentrated about showing up the effect of flavonoids against human immunodeficiency virus (HIV). Most of the work related to antiviral compounds revolves around the inhibition of various enzymes associated with the life cycle of the virus. The studies show the ability of flavonoids to inhibit the multiplication of HIV virus by inhibiting HIV-1 reverse transcriptase (Harbone and Williams, 2000). In addition the flavonoids chrysin, acacetin and apigenin prevent HIV- activation via a novel mechanism that probably involves inhibition of viral transcription (Critchfield et al., 1996).

✓ **Antibacterial activity:**

A lot of studies have realized the antibacterial activity of flavonoids, especially the ones with hydrophobic substituents such as prenyl groups, this activity depends on structure, namely on the substitution on the aromatic rings (Wenzel, 2013). Several flavonoids have been shown to possess potent antibacterial activity such as: flavones, flavanones, flavanols, chalcones, isoflavonoids, bioflavonoids and galangin. The antibacterial mechanisms of flavonoids are summed as follows: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin of the cell membrane, alteration of the membrane permeability and attenuation of the pathogenicity (Xie et al., 2015).

e. Importance of flavonoids in plants:

According to a lot of studies, flavonoids carry out functional roles of amazing significations in plant-environment interaction, one of these roles is protecting plants from germs and parasites also their ability to protect cell tissues from UV damage (Muhammad et al., 2011). In addition, flavonoids have been used as a way of classification based on the

Chapter I: Phenolic compounds

colors they give for fruits and flowers (Clugston *et al.*, 2002), and they could regulate auxin movement and catabolism because of their ability to create auxin gradients translates into phenotypes with different morphoanatomical features, so they have a role as a growth regulator (Taylor and Grotewold, 2005).

2-5-2- Complex phenols (tannins):

2-5-2-1- Definition of tannins:

The name "tannin" is derived from the French "Tanin" (tanning substance) and it is used for a range of natural polyphenols (Karamali and Teunis, 2001). According to Bate-Smith and Swain tannins are water-soluble phenolic compounds with a molar mass between 500 and 3000 (Haslam, 1989). They may be subdivided into:

- ✓ Hydrolysable tannins are derivatives of Gallic acid (3, 4, 5-trihydroxyl benzoic acid) (Vargas, 2009)
- ✓ Condensed tannins are usually referred to as Proanthocyanidins (Frutos *et al.*, 2004),

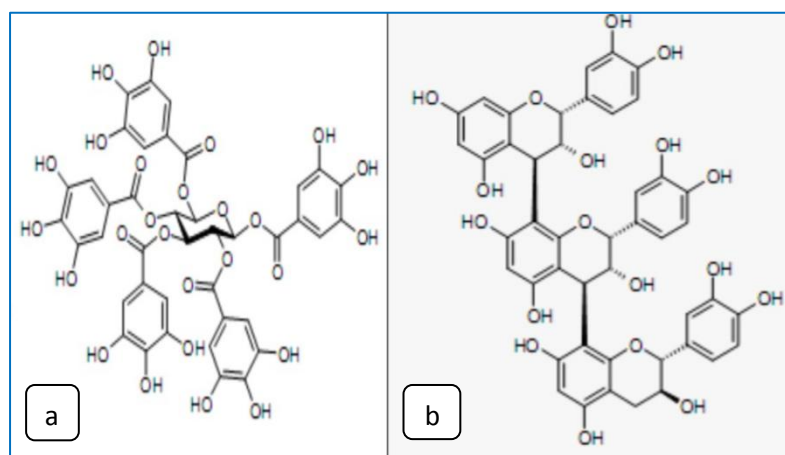


Figure (05): The basic structure of Tannis:

a- Hydrolysable tannins, b- condensed tannins (Frutos *et al.*, 2004).

2-5-2-2- Sources of tannins:

The main sources of tannins are: grape, muscadine grape, peach, blackberry, apple juice, raspberries, pomegranate, walnuts, olive, plum, haricot bean, chick-pea, lentils, tea, cocoa, coffee, chocolate and immature fruits (Balasundram *et al.*, 2006).

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2-5-2-3- Properties of tannins:

- They are widely distributed in plants and occur in especially high amounts in the bark of certain trees and in galls (Pradeep et al., 2006)
- Tannins can complex with proteins, starch, vitamins, and minerals at moderate pH and dissociate at lower pH (Juan Maria et al., 2017), and this ability of making complexes caused the leather tanning' property (Özeker, 1999).
- Because of their high chemical reactivity, tannins have played an important role in thermosetting systems for several decades, such as tannin-based adhesives and tannin-based foam material (Jingjing et al., 2019).
- They cause the bitter taste in immature fruits (Benhammou., 2012).

Chapter II

Oxidative stress
&
Antioxidants

Chapter II: Oxidative stress & Antioxidants

1- Oxidative stress:

The imbalance between pro-oxidant and antioxidants, called oxidative stress (Binghua et *al.*, 2019). This is caused by lack of antioxidants or by the accumulation of free radicals, primarily the reactive oxygen species (ROS) and the reactive nitrogen species (RNS), after the stimulation of the endogenous and external environment, oxidative stress could lead to cell death and the dysfunction of physiology, which could ascribe to DNA damage, inflammation (Xiaole et *al.*, 2019).

2- Free radicals:

2-1- Definition of free radicals:

A free radical is defined as any atom, molecule or a fragment of atoms and molecules with one or more unpaired electrons, capable of short independent existence (Tvrđá et *al.*, 2017), they are uncharged, very reactive, and short-lived molecules of less than 10^{-6} s in biological systems. Some oxygen species known as reactive oxygen species, in fact they are non-reactive but they could generate free radicals (Godwill, 2018). Many studies show that free radicals cause a lot of oxidative damage to various molecules in living organisms such as: proteins, lipids and nucleic acids and these are involved in the interaction phases of many diseases such as: respiratory ailments, cancer, heart diseases, atherosclerosis and even neuronal death (Chun-Yung et *al.*, 2018).

2-2- Types of free radicals:

They are generally classified into two major categories of compounds:

- Reactive oxygen species (ROS).
- Reactive nitrogen species (RNS).

Which includes the free radicals and the non-free radicals, where the first one are more reactive and less stable than the second one because they contain at least one unpaired electron in the shells around the aromatic nucleus (Godwill, 2018).

Chapter II: Oxidative stress & Antioxidants

Table (02): Some examples of reactive oxygen and nitrogen species (Tvrdá et al., 2017).

Reactive oxygen species (ROS)			
Radicals	Symbol	Non-radicals	Symbol
Superoxide	$O_2^{\bullet-}$	Hydrogen peroxide	H_2O_2
Hydroxyl radical	OH^{\bullet}	Hypochlorous acid	$HOCl$
Peroxyl radical	ROO^{\bullet}	Hypobromous acid	$HOBr$
Alkoxy radical	RO^{\bullet}	Ozone	O_3
Hydroperoxyl radical	HO_2^{\bullet}	Singlet oxygen	$^1\Delta_g$
Lipid peroxyl radical	LOO^{\bullet}	Lipid peroxide	$LOOH$
Reactive nitrogen species (RNS)			
Radicals	Symbol	Non-radicals	Symbol
Nitric oxide	NO^{\bullet}	Nitrous acid	HNO_2
Nitrogen dioxide	NO_2^{\bullet}	Nitrosyl cation	NO^+
		Nitroxyl anion	NO^-
		Dinitrogen tetroxide	N_2O_4
		Dinitrogen trioxide	N_2O_3
		Peroxynitrite	$ONOO^-$

2-3- Sources of free radicals:

Free radicals are produced either from normal cell metabolism in situ, which means internal sources or from external sources (Sultan, 2014):

2-3-1- Internal sources:

a- Mitochondria:

They are known as "powerhouse of a cell", because they are responsible for 90% of the energy production in cells (Ozcan and Ogun, 2014). In addition, mitochondria are the main source of reactive oxygen species (Tsolaki et al., 2017), because there are about 90% to 95% of cellular oxygen is used up in oxidative phosphorylation and 3% from that pool can be converted to superoxide. Also, mitochondria produce H_2O_2 and OH^{\bullet} by Fenton reaction and dismutation of O_2 (Ozcan and Ogun, 2014).

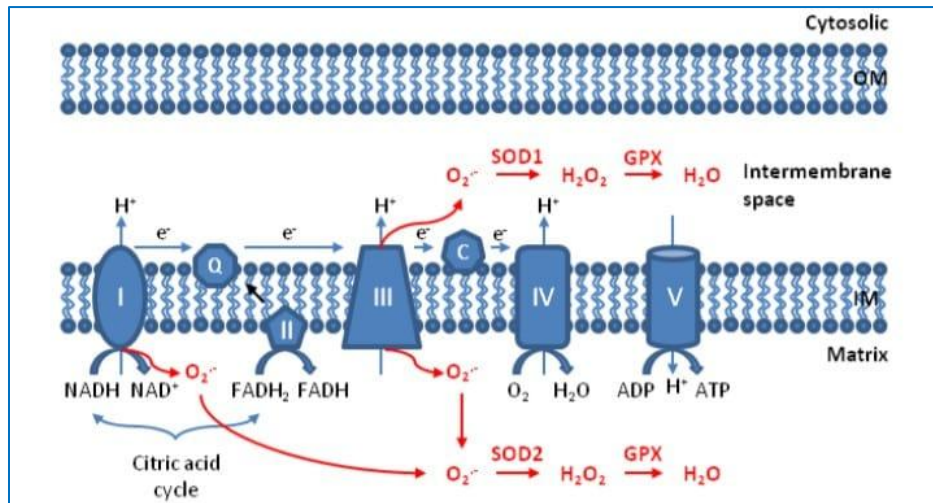


Figure (06): Production and disposal of mitochondria ROS (Li et al., 2013)

b- NADPH oxidase:

The NADPH oxidase is the major enzymatic source of ROS generation in cells (Ozcan and Ogun, 2014). Using the NADPH as the electron donor, the NADPH oxidase catalyzes the production of superoxide by the reduction of oxygen by the following reaction:

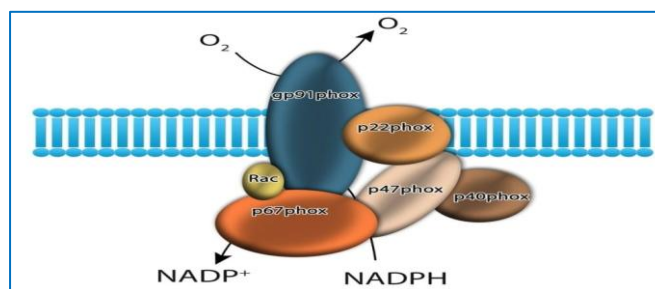
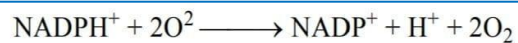


Figure (07): Schematic diagram of the structure of the active NADPH oxidase complex (Ozcan and Ogun, 2014).

c- Lipoxygenases:

This enzyme is one of ROS sources in blood vessel walls (Koushishi, 2009), also, it intervenes in the production of some kinds of ROS by stimulating the LBs and this is proved by some studies in the last few years (Werz et al., 2000), which suggest that LTB4 treatment of fibroblasts and neutrophils results in ROS generation (Ozcan and Ogun, 2014).

Chapter II: Oxidative stress & Antioxidants

2-3-2- External sources:

According to Sultan (2014), these are some of the external sources of free radicals:

- Environmental pollutants
- Cigarette smoke.
- Radiations.
- Certain drugs.
- Industrial solvents.
- Alcohol.
- Ultraviolet light.
- Ozone.
- Pesticides.
- Anesthetic solvents.

2-4- Damages of free radicals:

In fact, free radicals with a little concentration have a very important physiological roles, for example expansion of blood vessels (Faivaier, 2003), but the continual influx and generation of ROS either from internal or external sources would cause an oxidative damage of cellular components and may impair many cellular functions (Godwill, 2018), this damage could be in:

2-4-1- Lipids:

As we know cellular membranes are highly rich in unsaturated fatty acid, that is why they are vulnerable to oxidative damage this damage has three stages and in general is known as lipid peroxidation (Meral et *al.*, 2000).

2-4-2- Proteins:

Proteins are the most targets for attack by ROS predominantly by the OH^\bullet , RO^\bullet and nitrogen-reactive radicals causing damage. Hydrogen peroxide and superoxide radicals have weak effects on proteins except for proteins containing SH groups. Following the oxidation, proteins are susceptible to many changes in their function which include inactivation, chemical fragmentation (Godwill, 2018).

Chapter II: Oxidative stress & Antioxidants

2-4-3- DNA:

DNA is a very sensitive molecule for ROS, which can cause several types of damages for it, such as: modification of DNA bases, loss of purines (apurinic sites), DNA-protein cross-linkage, damage to the deoxyribose sugar and damage to DNA repair system (Godwill, 2018).

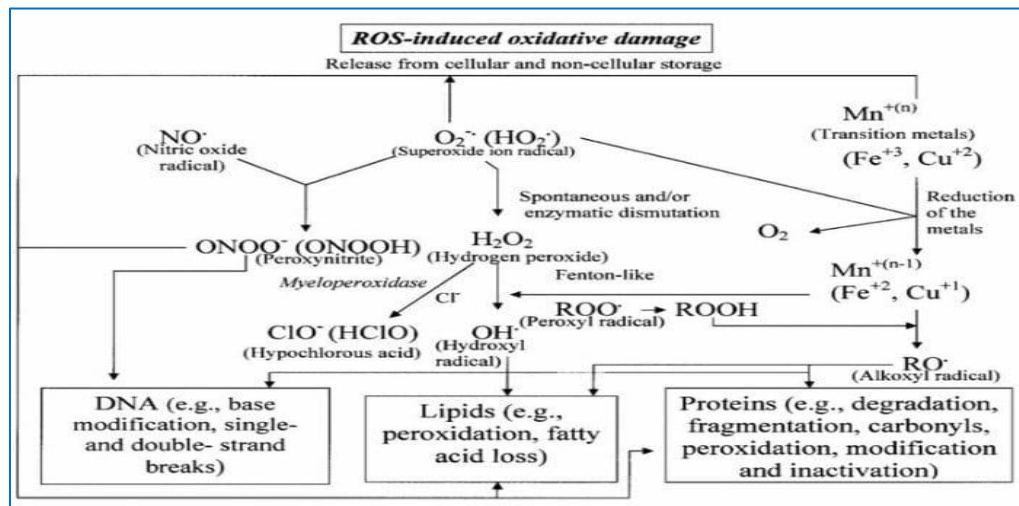


Figure (08): Reactive oxygen species (ROS)-induced oxidative damage (Kohen and Nyska, 2002)

3- Antioxidants:

3-1- Definition of antioxidants:

Antioxidants are defined as molecules that dispose, scavenge and inhibit the formation of ROS or oppose their actions (Tvrdá *et al.*, 2017). In other words, antioxidants are chemicals that bind with free radicals and nullify their effect from causing damage to biological molecules, they bind with them by giving up their own electrons. These results in the termination of oxidative chain reactions, and the free radicals are no longer able to attack the cell. Antioxidant attains free radicals state after donating its electron. It can accommodate the change in electrons without becoming reactive, and that is why they are not harmful (Rizwan, 2018).

3-2- Classification of antioxidants:

Antioxidants can be divided into two dominant categories based on their sources:

Natural antioxidants & Synthetic antioxidants.

Chapter II: Oxidative stress & Antioxidants

3-2-1- Natural antioxidants:

According to Godwill (2018), this category is the most important because they directly act on free radicals either by decomposing, scavenging or converting free radicals to less reactive forms. This defense mechanism involves 2 groups:

3-2-1-1- Enzymatic antioxidants:

The enzymatic antioxidants include superoxide dismutase (SOD), catalase, glutathione reductase (GRx) and glutathione peroxidase (GPx).

a- Superoxide dismutase (SOD):

Superoxide dismutases are metal-containing enzymes that catalyze the conversion of two superoxides into oxygen and hydrogen peroxide, which is less toxic than superoxide (Tvrdá et al., 2017).

In other words, SOD are proteins which stimulate the dismutation of superoxide to hydrogen peroxide according to the following reaction formula (Serrano and Klann, 2004):



They exist in three forms in mammalian tissues and tissue distribution (Godwill, 2018):

- Copper zinc superoxide dismutase (CuZnSOD).
- Manganese superoxide dismutase (MnSOD).
- Extracellular superoxide dismutase (ECSOD).

b- Catalase (CAT):

Catalase catalyzes the decomposition of hydrogen peroxide to molecular oxygen and water, thereby completing the detoxifying reaction started by SOD (Tvrdá et al., 2017).

Actually, catalase was the first antioxidant enzyme to be characterized (Shalini and Sivakumar, 2015). It is located mostly within the peroxisomes of cells which contain most of the enzymes capable of generating hydrogen peroxide. It consists of four protein subunits, each containing a heme group and molecule of NADPH (Godwill, 2018; Shalini and Sivakumar, 2015).

Chapter II: Oxidative stress & Antioxidants

It may be found in bone marrow, heart, kidney, liver, blood and mucous membranes (Januel, 2003), also in mitochondria and endoplasmic reticulum (Tvrdá et al., 2017).

c- Glutathione reductases (GRx):

GRx is a flavine nucleotide dependent enzyme (Shalini and Sivakumar, 2015) and it has a similar tissue distribution to glutathione peroxide (Godwill, 2018). It has been found in the prostate gland, seminal vesicles, sertoli cells (Tvrdá et al., 2017).

The role of GRx is to generate GSH from GSSG using NADPH in order to increase the ratio of reduced to oxidized glutathione (Godwill, 2018; Shilina, 2009).

d- Glutathione peroxidases (GPx):

They are a family of selenium-containing enzymes, which catalyze the reduction of H_2O_2 and organic peroxides, including phospholipid peroxides (Tvrdá et al., 2017).

It has been found in liver, kidney and highly found in almost all tissues. Its subcellular location is usually the cytosol and mitochondria, it catalyzes the oxidation of reduced glutathione (GSH) decomposing hydrogen peroxide or another species such as: hydrogen peroxide and lipid hydroperoxide, acts as substrates for these enzymes (Godwill, 2018; Shalini and Sivakumar, 2015).

e- Other enzymatic antioxidants:

Superoxide dismutase (SOD), catalase, glutathione reductase (GRx) and glutathione peroxidase (GPx) were the most known enzymatic antioxidants, but there are other enzymes may also participate in the enzymatic control of oxygen radicals and their products. Tvrdá et al (2017) have given an examples about them such as: Glutathione S-transferase (GST), Ceruloplasmin, Transferring and Heme oxygenase (HO).

3-2-1-2- Non-enzymatic antioxidants:

They are also known as synthetic antioxidants or dietary supplements (Tvrdá et al., 2017), they are usually low-molecular-weight antioxidant (LMWA) compounds capable of preventing oxidative damage either by directly interacting with ROS or indirectly by chelating metals (Godwill, 2018).

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They include:

a- Glutathione (GSH):

It is an endogenous tripeptide that protects the cells against free radicals by donating either a hydrogen atom or an electron (Shalini and Sivakumar, 2015). It is the most abundant thiol protein in mammalian cells, this molecule has three precursors: cysteine, glutamine acid and glycine (Tvrdá et al., 2017). Glutathione exists as GSH in its reduced form and 2 of GSH molecules can be joined via oxidation at their SH groups of the cysteine residue into a disulfide bridge to form GSSG which is the oxidized form (Godwill, 2018).

b- Vitamin C:

This vitamin is considered one of the organism's most powerful antioxidant agent due to its capacity to donate two electrons from its double link at positions two and three, in such as way that it interacts with FR, blocking their harmful effect (Morals-Gonzlães et al., 2012). It is a water-soluble vitamin, and it is one of the important molecules which human body need (Can and Frie, 1999) it is widely distributed in various tissues (Zaho et al., 2019). This molecule acts by scavenging the superoxide radical anion, hydrogen peroxide, hydroxyl radical, singlet oxygen and reactive nitrogen oxide. Vitamin C has been found in citrus fruits, peppers, strawberries, tomatoes, broccoli, Brussels sprouts and other leafy vegetables (Tvrdá et al., 2017).

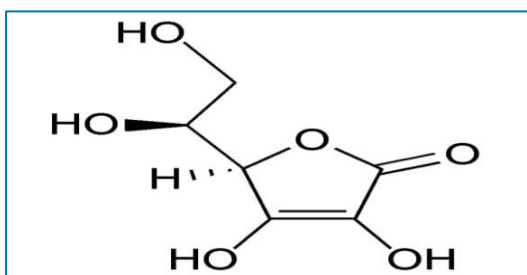


Figure (09): Chemical structure of Vitamin C (Brakat et al., 2012).

c- α -tocopherol (Vitamin E):

It is a fat-soluble vitamin with high antioxidant properties (Zaho et al., 2019). Vitamin E involves 2 subgroups: tocopherols and tocotrienols (Mozos e al., 2017; Tvrdá et al., 2017), both of these subgroups can further be divided into four lipophilic molecules which means there are eight isoforms of Vitamin E (Zaho et al., 2019; Shalini and

Chapter II: Oxidative stress & Antioxidants

Sivakumar, 2015). The major difference between tocopherols and tocotrienols is the side chain, the first one has a saturated phytyl tail, while the second one poses an unsaturated side chain (Zaho et al., 2019).

Vitamin E plays a function as an efficient "chain breaker" during lipid peroxidation in cell membranes and various lipid particles including low-density lipoprotein (LDL). Its role is to scavenge lipid peroxy radicals and to terminate the lipid peroxidation chain reactions (Godwill, 2018). This vitamin has been found in high concentrations in palm oil, rice bran and oily plants (Mozos et al., 2017).

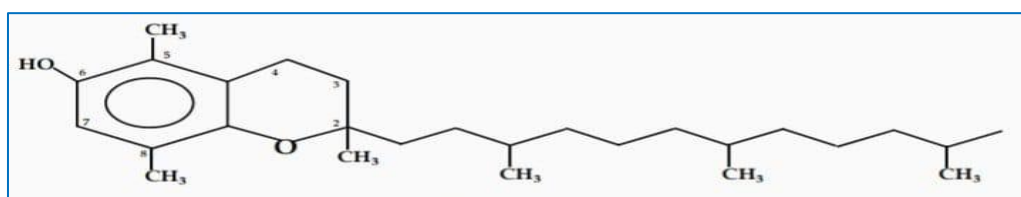


Figure (10): Chemical structure of Vitamin E (α -tocopherol)

(Morals-Gonzl aes et al., 2012).

d- Retinol (Vitamin A):

Vitamin A, also called trans-retinol, is an isoprenoid alcohol that performs several important functions in the organism. (Morals-Gonzl aes et al., 2012). It is produced as a result of the breakdown of β -carotene and is a carotenoid produced in the liver (Shalini and Sivakumar, 2015). As such, it is considered as a vital antioxidant that prevents human LDL against copper stimulated oxidation, eliminates FR and protects the DNA in its mutagenic action (Godwill, 2018; Morales-Gonzl aes et al., 2012).

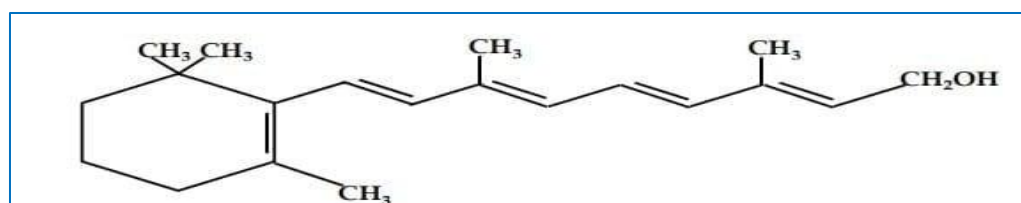


Figure (11): Chemical structure of Vitamin A (Retinol) (Morals-Gonzl aes et al., 2012).

e- Other non-enzymatic antioxidants:

There are other substances which may contribute to the maintenance of oxidative homeostasis such as: bioflavonoids, flavonoids, carotenoids, lycopene,

Chapter II: Oxidative stress & Antioxidants

hydroxyciannamates, catnitine, taurine, selenium, albumin, uric acid, lipoic acid,(Godwill, 2018; Tvrda *et al.*, 2017).

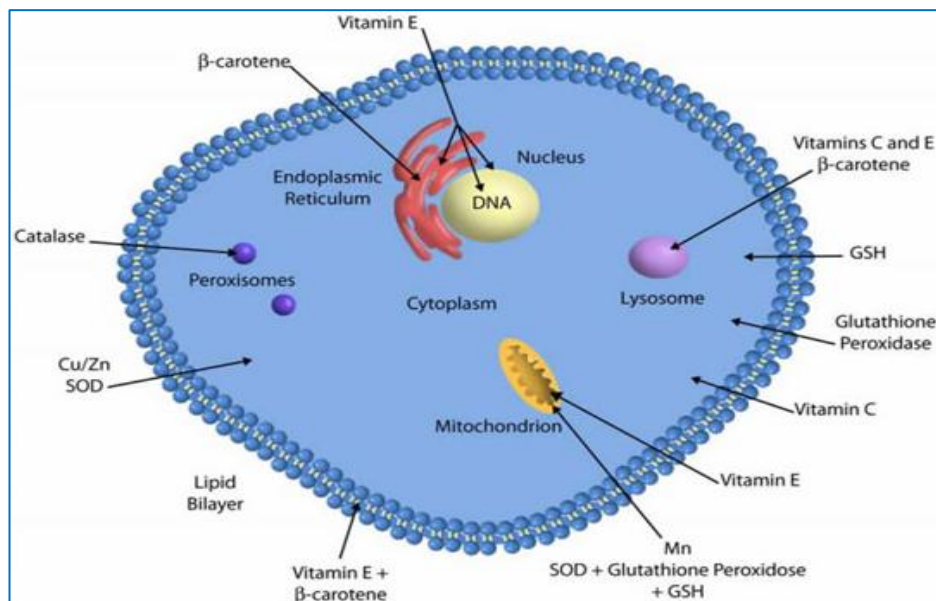


Figure (12): Diagrammatic representation of the site of enzymatic and non- enzymatic antioxidants (Shalini and Sivakumar, 2015).

3-2-2- Synthetic antioxidants:

They are chemically synthesizing compounds since they do not occur in nature (Atta *et al.*, 2017). Butylhydroxyanisol (BHA) , Butylhydroxytoluene (BHT), Propyl gallate (PG) and Tertiary butyl hydroquinone (TBHQ) are types of synthetic antioxidants, which used as additives in foods to prevent oxidation of lipid (Gul *et al.*, 2013).

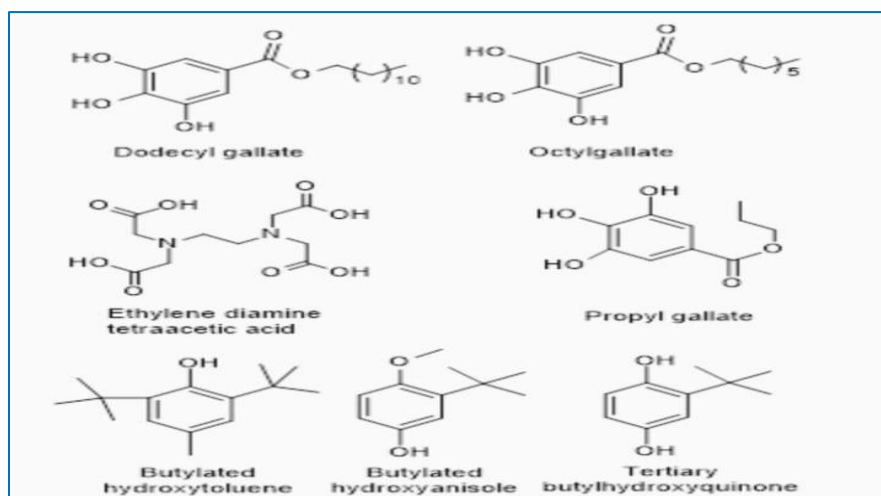


Figure (13): Structures of some synthetic antioxidants (Atta *et al.*, 2017).

Chapter III

*A taxonomic study of
Cistanche tinctoria (Desf.) Beck*

Chapter III: A taxonomic study of *Cistanche tinctoria* (Desf). Beck

1- Presentation of the Orobanchaceae family:

The Orobanchaceae family (in English: Broomrape) is one of only a dozen families of angiosperms in which the parasitic habit is known (parasitic plants), it is the largest family of haloparasitic flowering plant. This family comprises about 2060 species in 90 genera. The family has a cosmopolitan distribution, but the main places are the Mediterranean, western and central Asia, northern Africa and North America (Piwowarczyk and Madeja, 2014; Thieret, 1971).

The Orobanchaceae are plants whose calyx are campanulate (4-5 meters), the corolla more or less labiate, the stamens didynamous, the glandular ovary in its inner base, the capsule is unilocular with 2 free valves, each of them carrying two longitudinal placentas loaded with seeds. The members of this family are achlorophyllous, consisting of a fleshy body with spike or raceme inflorescence. They are all root-parasitic plants and obtain water and nutrients from hosts via their invading haustoria (Chen and Hsiao, 2011; Quezel and Santa, 1963).

1- Presentation of the genus *Cistanche*:

The genus *Cistanche* which belongs to the family Orobanchaceae includes 16 species. They form an attractive group of phanerogamic root parasites. The occurrence of the genus is restricted to certain arid and semi arid regions of Africa, Asia and the Mediterranean area, including parts of Southern Europe (Ramadam et al., 2010).



Figure (14): External morphology of the genus *Cistanche* (Ozenda et Capdepon, 1977).

Chapter III: A taxonomic study of *Cistanche tinctoria* (Desf). Beck

2- Presentation of the plant *Cistanche tinctoria*:

3-1- Definition:

Cistanche tinctoria (figure 15) is a parasitic plant like all members of the Orobanchaceae family, it is chlorophyll-free, obligate parasitic (Ozenda, 1977). The flowers are bright yellow and distributed throughout the plant in brown tones, the leaves are lanceolated, reduced to brownish scales, This plant is gathered from the wild and used locally in foods (2007، حليس), stem is thick with height 30 to 120 cm (Ozenda, 1977).

The vernacular Arab name is EL-tarthouth (2007، حليس) and this plant quite common in high plateaus and in northern Sahara and it is rarer in western, central and southern Sahara (Ozenda, 1977).



Figure (15): *Cistanche tinctoria* (original photos).

Chapter III: A taxonomic study of *Cistanche tinctoria* (Desf). Beck

3-2- Classification:

According to Ould Saadi (2016) , the classification of *Cistanche tinctoria* is:

Kingdom:	Plantae.
Subkingdom:	Tracheophyta.
Division:	Angiosperms.
Class:	Dicotyledons
Order:	Lamials.
Family:	Orobanchaceae.
Genus:	<i>Cistanche</i> .
Specie:	<i>tinctoria</i> .

3-3- Distribution of *Cistanche tinctoria*:

Local:	In high plateaus and in northern Sahara and it is rarer in western, central and southern Sahara (Ozenda, 1977).
Regional:	North Africa.
In the whole world:	North Africa (Ould Saadi., 2016).

3-4- Traditional uses:

As a rare traditional medicinal plant, the dried whole plant is used for the treatment of abdominal pains, diarrhoea, muscle contractions and bruises (Bouzitouna et al., 2015). Also the underground part of young shoots of the plant is useful against intestinal problems and diabetes (Lakhdari et al., 2016). In Tissint region (Marroco), the local people applied the powder of the plant for injuries and in Egypt the dry powder mixed with camel's milk and used for bruising. (Bouzitouna et al., 2015).

In addition to the medicinal virtues of this plant, its shots and stems had been used for food application. Nomades in southern Morocco and Algeria eat the lower part of the plant, they mixed it with cereals to make a kind of oatmeal or bread (Bouzitouna et al., 2015; 2007، حليس). Also, it is used for tanning and dyeing leathers (Bouzitouna et al., 2015).

Second part

Experimental part

Chapter I

Materials & Methods

Chapter I: Materials & Methods

1- Materials:

1-1- Plant materials (*Cistanche tinctoria*):

In this study, we used the flowers of *C. tinctoria* (Desf). Beck, which have been collected during the flowering period (29th March. 2018), from Taleb Larbi region (figure 16), (33°42'27.8"N) and (07°18'57.1" E) that affiliated regionally to El-oued state and it's located about 60 km toward the Tebessa road.

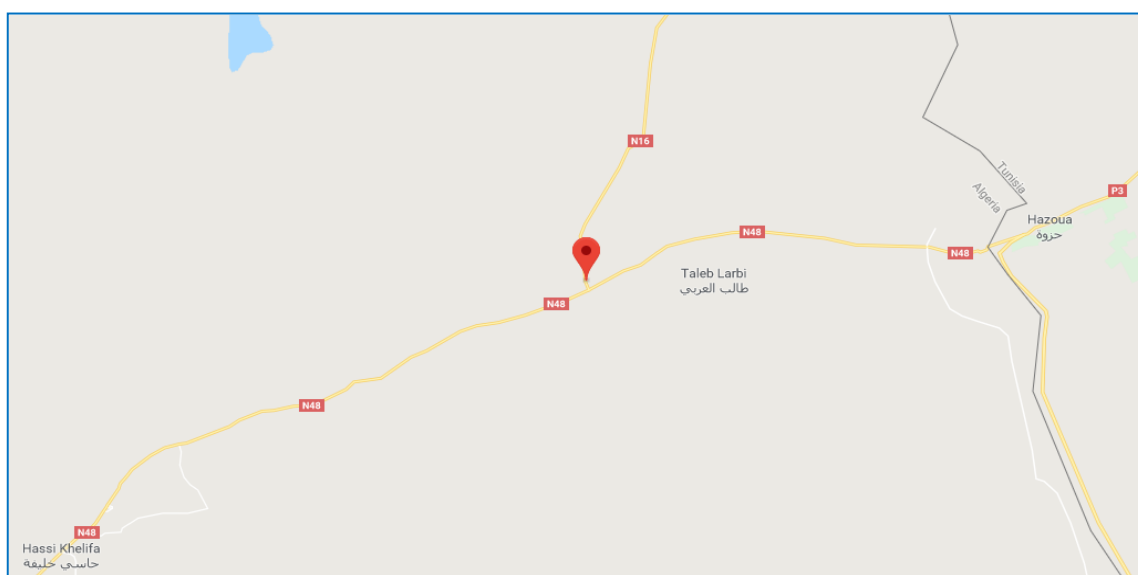


Figure (16): the place of collecting the flowers of *C. tinctoria*

1-2- Materials and methods used in preparing the plant materials:

In order to prepare the plant sample, we followed the steps in the table below:

Table (03): Materials and methods used for preparing the plant materials

Methods used		Materials
Collecting	The flowers of the plant have been collected during the flowering period.	- Scissors. - Plastic bags.
Drying	The collected plant material was air-dried at room temperature for 3 to 4 weeks.	- Clean cloth.

Chapter I: Materials & Methods

Grinding	After drying, the plant material has been ground by mechanical grinder to get a fine powder, this powder was stored at room temperature in airtight containers protected from bright light until the beginning of the experiment.	<ul style="list-style-type: none">- Mechanical grinder.- Airtight containers.
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2- Methods:

2-1- Preparation of the methanolic extract of *C.tinctoria*:

Ten grams (10g) of dry plant were macerated with 150 ml of methanol at room temperature in dark for 24 hours. After filtration, using the filter paper, the solvent was evaporated to dryness under reduced pressure in a rotary evaporator at 50°C, to get the crude extract, which was stored in a place protected from bright light and humidity (Bouchouka, 2016).

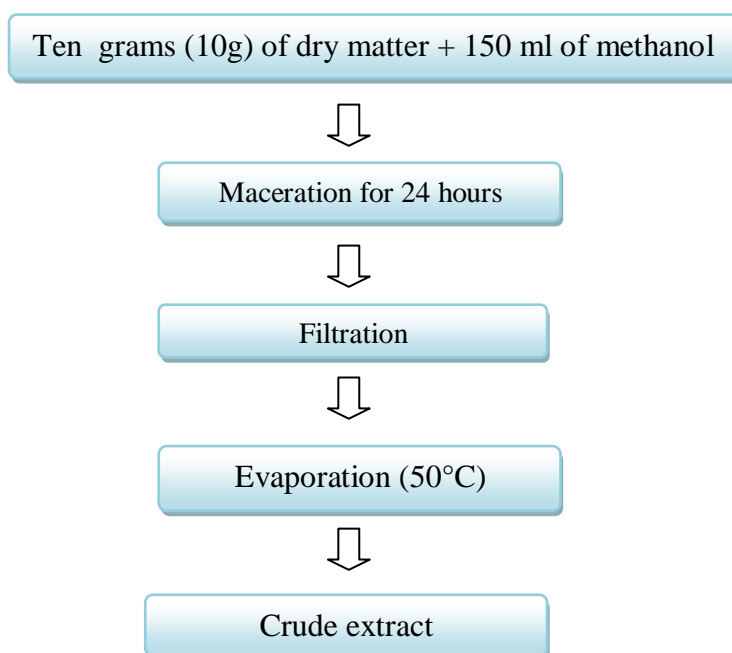


Figure (17): Protocol of preparation of crude extract.

Chapter I: Materials & Methods

2-2- Extraction of Flavonoids:

Ten grams (10g) of dry matter were macerated with 150 ml of methanol at room temperature in dark for 24 hours. After filtration, using the filter paper, the solvent was evaporated under reduced pressure in a rotary evaporator at 50°C until we get the methanolic extract. Then, we added to this extract a volume of 150 ml of warm distilled water and 150 ml of Ethyl acetate and we put the new mixture in a separator funnel. After the separation of the mixture, we got two phases: Ethyl acetate phase and Aqueous phase, then we take the first one and evaporated it in a rotary evaporator at 50°C to get the Flavonoids extract (phase Ethyl acetate). (Bekkara et al., 1998).

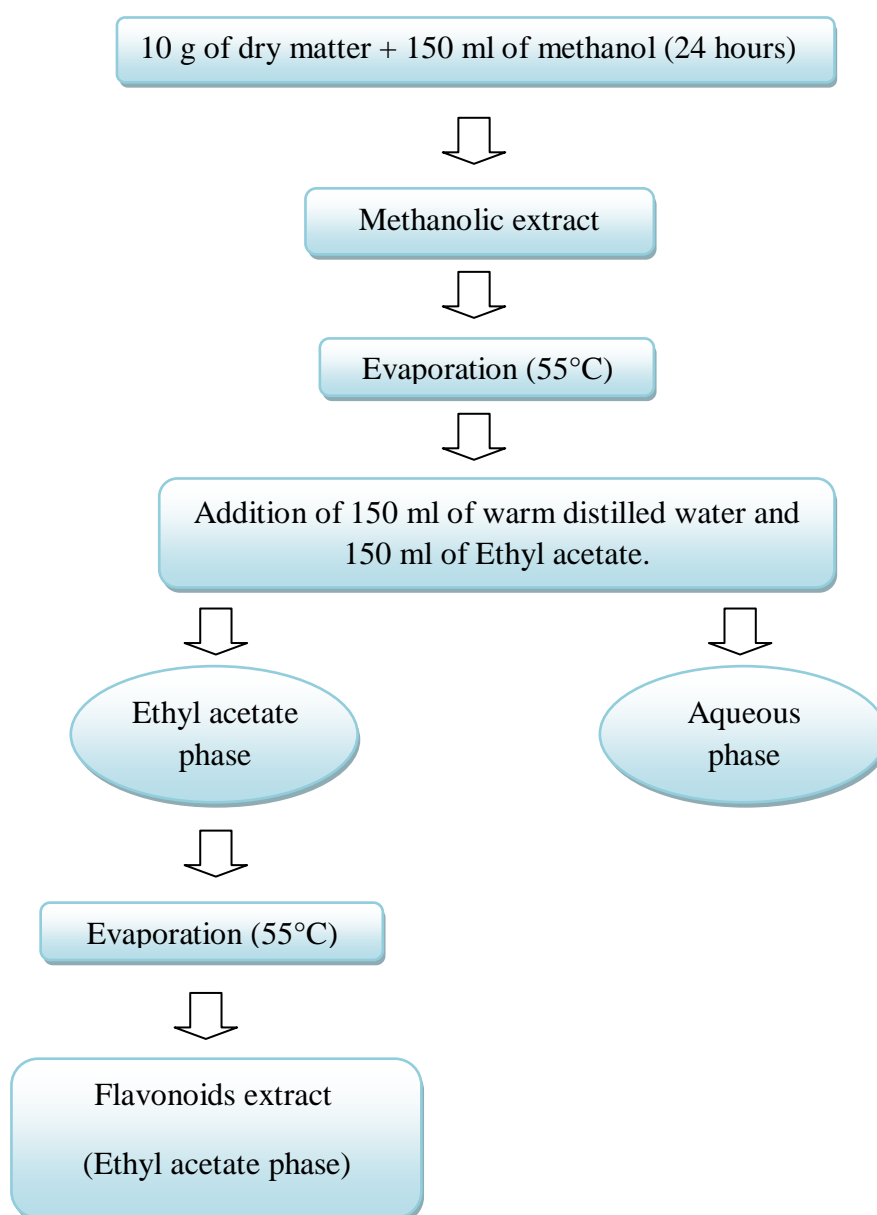


Figure (18): Protocol of extraction of flavonoids.

Chapter I: Materials & Methods

2-3- Extraction of Tannins:

Thirty grams (30g) of dry matter were macerated with 60 ml of distilled water and 140 ml of Acetone at room temperature in dark for 72 hours (3 days). After filtration, using the filter paper, the solvent was evaporated under reduced pressure in a rotary evaporator at 50°C to remove acetone. Then, we added 150 ml of Dichloromethane to the remaining solution to remove lipid-soluble substances, then, we put the whole mixture in a separatory funnel for about 2 hours. After the separation of organic phase (upper phase) and aqueous phase (lower phase) by separatory funnel, the first one (upper phase) was further extracted with Ethyl acetate (150 ml) and evaporated to dryness at 50°C. This extraction has been done according to the modified method described by Zhang *et al* (2008).

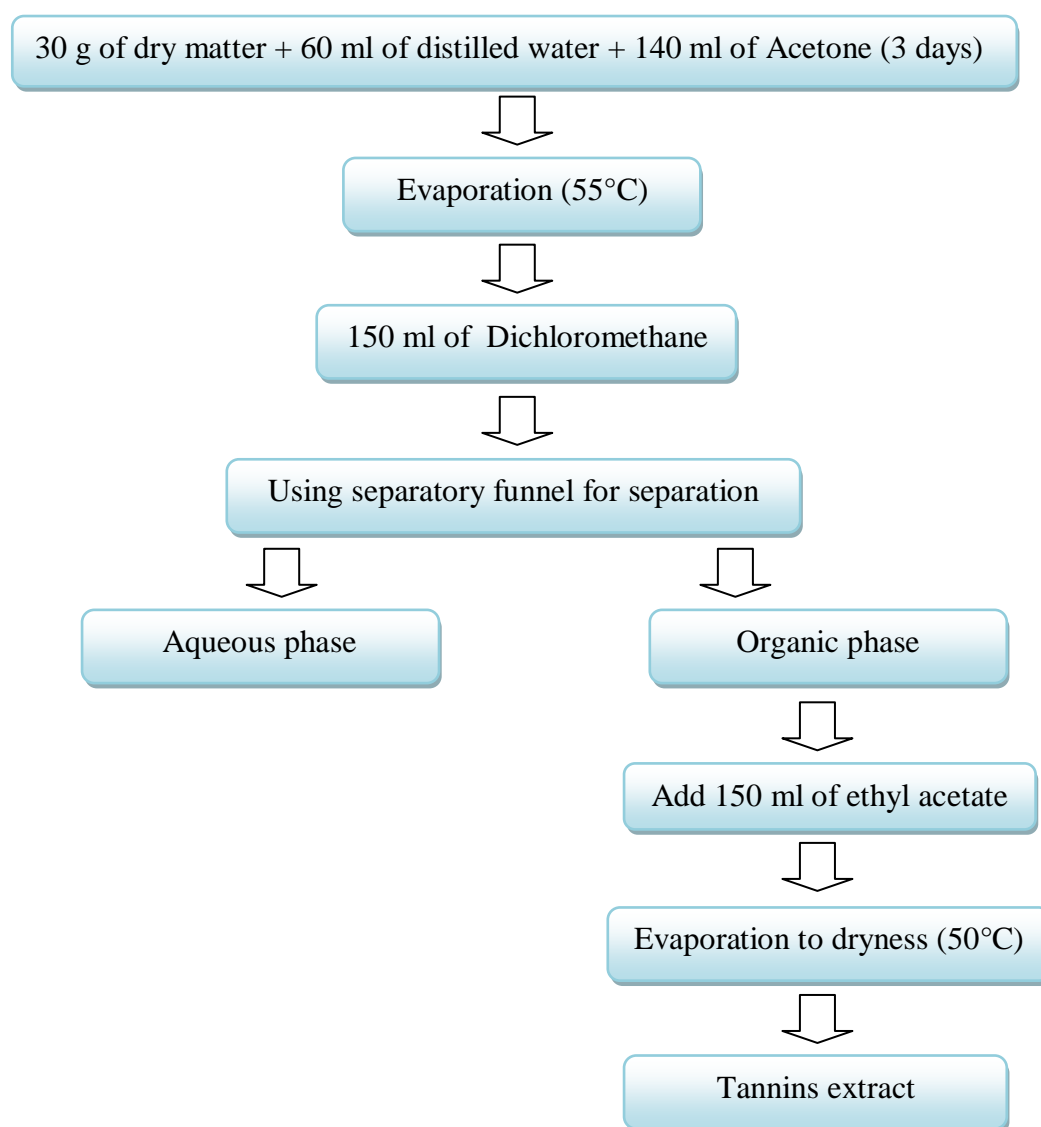


Figure (19): Protocol of extraction of Tannins.

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2-4- Determination of extraction yield:

According to Truong *et al* (2019), The extraction yield is calculated by the following formula:

$$\text{Extraction yield \%} = (\text{weight of the extract} / \text{weight of the initial matter}) \times 100$$

2-5- Determination of total phenolic contents (TPC):

The total phenolic contents of the crude extract were determined according to the Folin-Ciocalteu's phenol reagent method of Singleton-Rossi with some modification, this method is based on the redox reaction of the reagent forming a blue color pigment (Silberstein *et al.*, 2019).

As a first step and in a test tube, we mixed 0.2 ml of the extract with 1 ml of Folin-Ciocalteu reagent diluted 10 times with water, then we added 0.8 of sodium carbonate solution (7.5%). After stirring the test tubes we let them rest for 30 min, the absorbance was measured at 765 nm using the spectrophotometer (Rahmani *et al.*, 2018).

The same procedure was repeated for all standard Gallic acid solutions at different concentrations.

We used Gallic acid as a standard for the calibration curve. The total phenolic content was expressed as Gallic acid equivalents (mg of Gallic acid equivalents in 1 g of extract).

2-6- Determination of total flavonoid contents:

According to Boudjouref *et al* (2014), in a test tube, we blended 1 ml of the sample solution with 1 ml of aluminum trichloride methanolic solution (2%). After stirring the test tubes, we let them rest for 10 to 15 min at room temperature.

The same procedure was repeated for the standard Quercetin solutions at different concentrations.

Based on the measured absorbance 430 nm, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in the extract was expressed in terms of Quercetin equivalent (mg of QE/g of extract).

2-7- Evaluation of Antioxidant activity:

Antioxidant activity is a complex procedure usually happening through several mechanisms and is influenced by many factors, which cannot be fully described with one single method. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action. In this study, three complementary tests were used to assess the antioxidant activity of *Cistanche tinctoria*: the first one is DPPH assay, which is one of the most widely used method for screening the antioxidant activity of plant extract *In vitro*, while the second test was Hemolysis test as *In vivo* test, and the last one was Reducing power assay.

2-7-1- DPPH scavenging assay:

DPPH (2,2-diphenyl-1-picrylhydrazyl) is stable chromogenic radical with a deep purple color, and because of its simplicity and sensitivity, The DPPH assay has become one of the most frequently used methods and offers the first approach for evaluating the antioxidant activity.

The DPPH scavenging assay is based on electron donation of antioxidants to neutralize DPPH radical. The reaction is accompanied with color change of the DPPH measured at 517 nm, and the discoloration acts as an indicator of the antioxidant efficacy (Chavan, 2018).

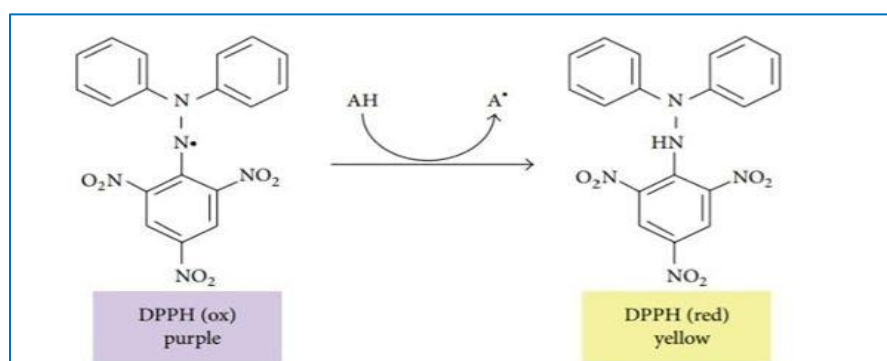


Figure (20): Principle of DPPH radical scavenging capacity assay (Teixeira et al., 2013)

❖ DPPH protocol:

The DPPH scavenging activity of the extracts was measured by using the modified method of Brand-Williams et al (1995). A volume of 1 ml of each extract at different concentrations was added to 1 ml of DPPH solution (0.1mM: 4mg/100ml) in methanol.

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After incubation for 10-15 min at room temperature, the absorbance of the reaction mixture was measured at 517 nm. In this test, Ascorbic acid (AA) was used as a reference standard.

According to Monowar et al (2019), the percentage of inhibition was calculated by using the following formula:

$$\text{DPPH radical scavenging inhibition \%} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

Where:

- A_{control} : Absorbance of the control reaction.
- A_{sample} : Absorbance of the reaction mixture containing DPPH and sample.

❖ Determination of IC₅₀ values:

IC₅₀ values were calculated from the plotted of scavenging activity against the concentrations of the samples. IC₅₀ is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50 %. IC₅₀ was calculated for all the extracts based on the percentage of DPPH radicals scavenged (Gaikwad et al., 2011).

2-7-2- Hemolysis assay:

This test is used to determine the ability of the plant extracts to protect the erythrocyte blood cells from damage or disruption of the cell membrane after exposing them to oxidative stress and free radicals by measuring the percentage of dissolved erythrocytes.

❖ Hemolysis assay protocol:

According to Abirami et al (2014), a volume of 40 µl of erythrocytes of blood (the blood sample was obtained from healthy human) was mixed with 2 ml of plant extract, this solution was conserved for 5 min at 37 °C. Then, we added 40 µl of H₂O₂ (30×10⁻³ Mol), 40 µl of FeCl₃ (80 ×10⁻³ Mol) and 40 µl of Ascorbic acid solution (50 ×10⁻³ Mol) respectively to the jumble.

After 1 hour of incubation at 37°C, the mixture was centrifuged with 700 Tour/min for 10 min. The absorbance of the upper layer (supernatant) was read at λ=540 nm.

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The percentage of hemolysis was determined using the following formula:

$$\text{Hemolysis\%} = (A_{\text{control}} / A_{\text{sample}}) \times 100$$

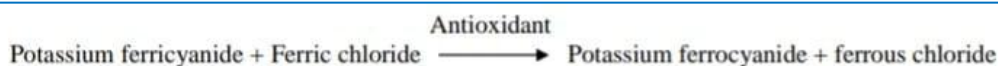
Where:

- A_{control} : is the absorbance in the absence of the extract.
- A_{sample} : is the absorbance in the existence of the extract.

2-7-3- Reducing power assay:

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. The presence of antioxidants in the extracts resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}) (Irshad et al., 2012).

(Singhal et al., 2011).



In reducing power assay, antioxidants cause the reduction of the Fe^{3+} into Fe^{2+} , thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds. Strong reducing agents, however, formed a blue color and absorbed at 700 nm.

❖ Reducing power protocol:

In test tubes, a volume of 0.5 ml of the extracts was added to 1.25 ml of a phosphate buffer (0.2 M, pH 6.6) mixed with 1.25 ml of potassium ferrocyanide (1%). The mixture was incubated in a hot bath at 50°C for 20 min. During this time of incubation, the reaction of reduction of Fe^{3+} to Fe^{2+} of each sample proceeds perfectly.

After 20 min of the incubation, the reaction is stopped by adding 1.25 ml of trichloroacetic acid (10%), the tubes were then centrifuged at 3000 rpm for 10 min. We collected a volume of 1.25 ml of the upper layer (supernatant), mixing it with 0.25 ml of FeCl_3 (0.1%) and diluting it with 1.25 ml of distilled water, the optical density was read at 700 nm (Kemewele Saague et al., 2019). Ascorbic acid (AA) was used as a positive control.

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2-8- High Performance Liquid Chromatography (HPLC):

High Performance Liquid Chromatography, which is also known as High Pressure Liquid Chromatography. It is a popular technique used the separation, identification and quantification of each constituent of a mixture (Thammana, 2016). HPLC is based on the interaction between the analytes, a solid stationary phase and liquid mobile phase. By choosing the proper mobile phase and stationary phase chemistries, analytes can be separated based on hydrophobicity, size, charge and many other properties (Belkacem, 2009).

❖ Principle of work:

According to Chouikh *et al* (2018), the principal work of HPLC was:

- 20 µl of plant extract solution (crude extract) was injected into the flow of mobile phase.
- Adjusts the high pressure that drives the mobile phase solvent in the column that separates the compounds of the mixture on the basis of their polarity by using a pump.
- The separated compounds shall be determined using the detector which connected to the column and to the computer which records the results as curves characterized by the numbers of the sample's compounds.

Chapter II

Results

&

Discussion

Chapter II: Results & Discussion

1- Results:

1-1- Determination of extraction yield:

After the extraction processes, the extraction yield had been determined using the formula described by Truong *et al* (2019) and the results are shown in the table below:

The extract	Yield %
Crude extract	11.26
Flavonoids extract	1.34
Tannin extract	0.505

Table (04): Percentage yield of each extract.

According to these results, the yield of the three extracts (Crude, Flavonoid phase Ethyl acetate and Tannin) was estimated with a variance in its values, the highest value recorded in the Crude extract (11.29 %), followed by Flavonoids extract (Ethyl acetate phase) (1.34%), however the Tannins extract got the lowest value (0.505%).

1-2- Total phenolic and flavonoid contents:

TPC contents is the process to figure out the amount of phenolic content in the samples. The phenolic level was determined by the Folin-Ciocalteu assay, as described by Rahmani *et al* (2018).

A calibration curve of standard reference was established using Gallic acid as a standard reference plotted.

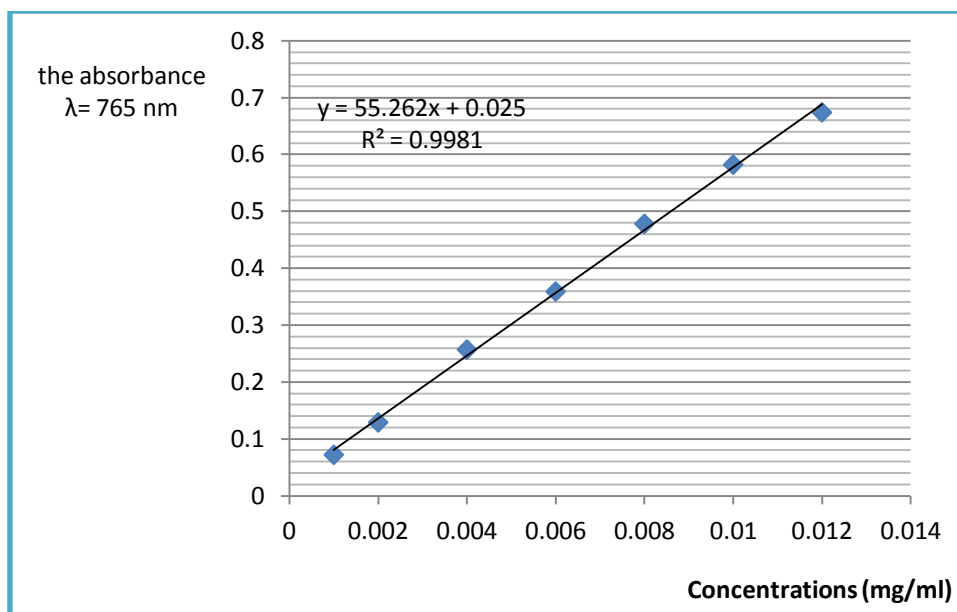


Figure (21): A linear calibration curve of Gallic acid (standard) in the total phenolic assay.

TPC was revealed as Gallic acid equivalents (the mg of Gallic acid corresponding to the polyphenols present in 1 g of extract).

Total flavonoids were determined using the modified aluminum chloride colorimetric method of Boudjouref et al (2014), where Quercetin was used as a reference standard to estimate flavonoid content.

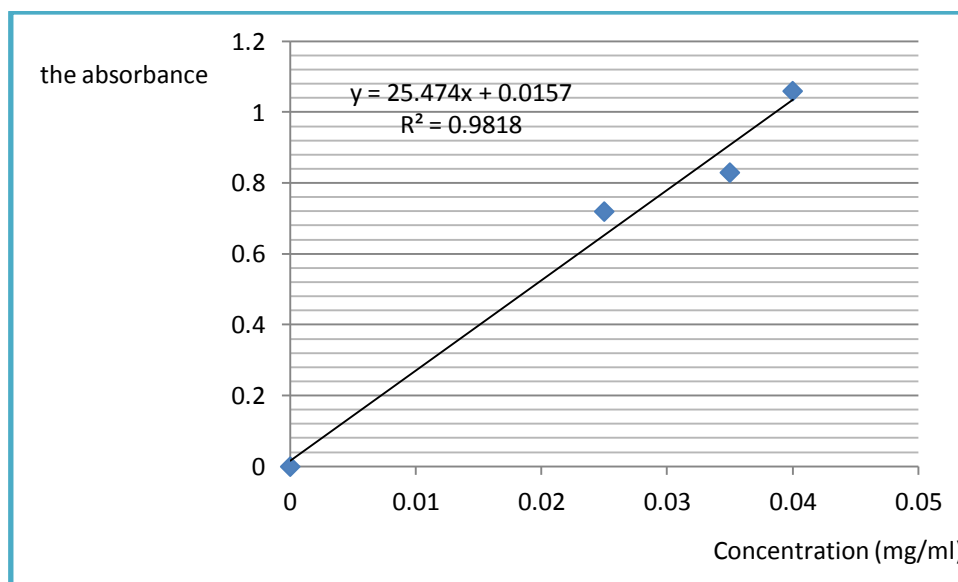


Figure (22): A linear calibration curve of Quercetin (standard) in the total flavonoid assay.

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According to the results shown in the table (05), the total phenolic contents in the Crude extract were 167.74 ± 24.36 (mg GAE/g Ex) and the content of flavonoids in was 26.76 ± 2.69 (mg QE/g Ex).

Table (05): Total phenolic and flavonoid contents in crude extract.

Sample extract	Total phenolic contents (mg GAE/g Ex)	Total flavonoid contents (mg GAE/g Ex)
Crude extract	167.74 ± 24.36	26.76 ± 2.69

1-3- Evaluation of Antioxidant activity:

There are a number of *in vitro* and *in vivo* assay methods for evaluating antioxidant properties of compounds. In the present study, DPPH radical scavenging assay, Hemolysis assay and reducing power assay were carried out to assess the antioxidant potentials of the extracts of *Cistanche tinctoria*.

1-4-1- DPPH scavenging assay:

In order to evaluate the antioxidant activity of *C. tinctoria*, we used the DPPH test because it is one of the best-known, accurate and frequently employed methods for evaluating antioxidant activity. In this study, Ascorbic acid (AA) was used as a standard compound. (Figure 23).

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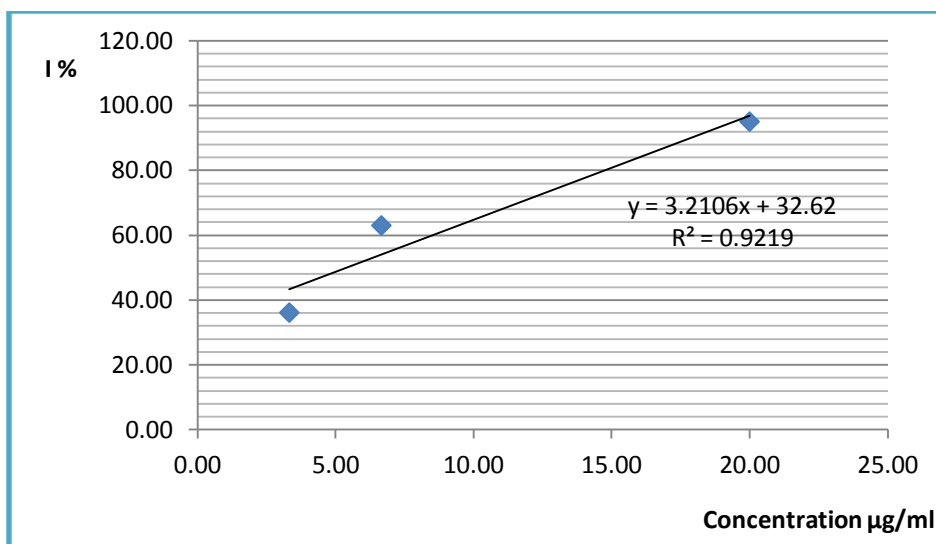


Figure (23): A linear calibration curve of Ascorbic acid (standard) in the DPPH radical scavenging assay.

The following chart presents the DPPH radical scavenging capacity of all the extract (Crude, Flavonoid and Tannin) and the positive control AA at concentration 10 $\mu\text{g/ml}$.

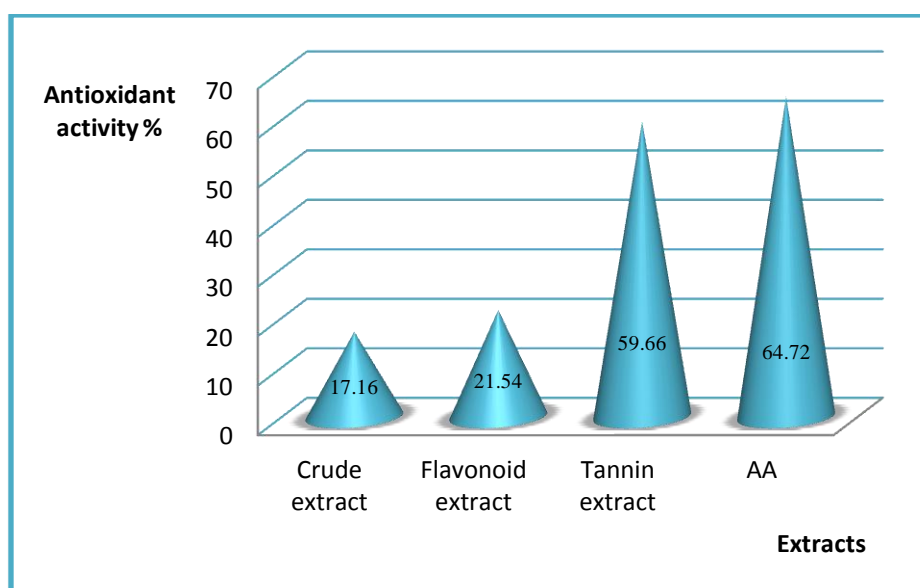


Figure (24): Values of antioxidant activity of extracts of *Cistanche tinctoria* and standard compound (AA) with C =10 $\mu\text{g/ml}$

At concentration 10 $\mu\text{g/ml}$, The results showed that the extracts and the standard compound had a different DPPH radical scavenging activity, the Tannins extract marked a high value (59.66%) and it was so close to the value marked by of Ascorbic acid (64.72%). On the other hand, the other two extracts (crude extract and flavonoid extract) had recorded low and convergent values (17.16% and 21.54%), respectively.

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The IC_{50} value was determined from the plotted graph of scavenging activity against various concentrations of extracts, which is defined as the efficient concentration of antioxidant necessary to decrease the initial DPPH radical concentration by 50 % (Figure 25). The lowest IC_{50} indicates the strongest ability of the extracts to act as DPPH radical scavengers

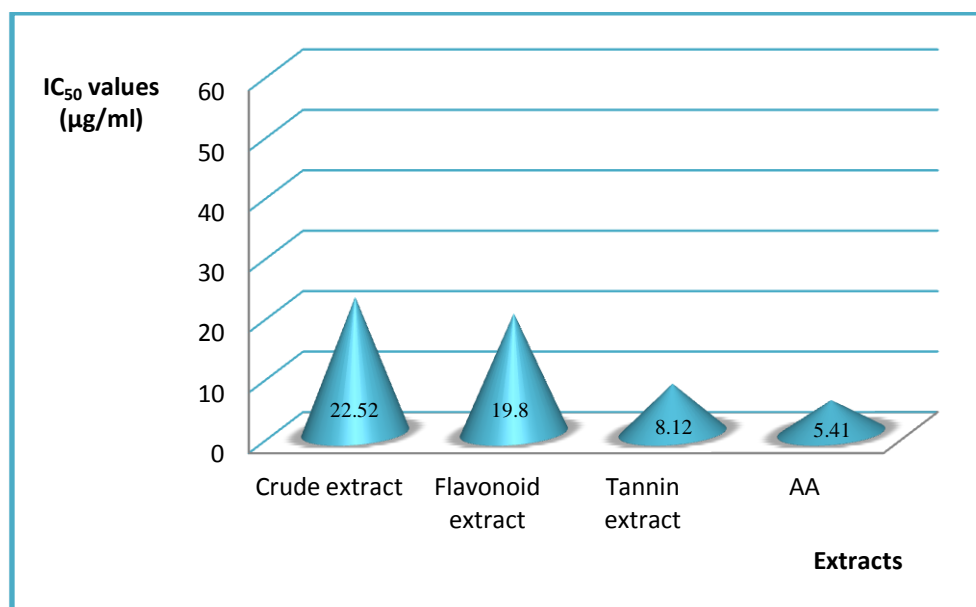


Figure (25): The IC_{50} values (DPPH- test) ($\mu\text{g/ml}$) of the extracts of *C.tinctoria* and the standard compound (AA).

According to figure (25), out of all the extracts, Tannin extract showed the lowest IC_{50} (8.12 $\mu\text{g/ml}$), whereas Crude extract and Flavonoid extract showed low and convergent values (22.52 $\mu\text{g/ml}$ and 19.8 $\mu\text{g/ml}$), respectively.

Ascorbic acid showed the highest DPPH radical scavenging with IC_{50} of 5.41 $\mu\text{g/ml}$.

1-4-2- Hemolysis assay:

In order to evaluate the antioxidant activity of extracts of *Cistanche tinctoria*, we used the Hemolysis assay because it is one of the most easiest assays for this purpose, the percentage of Hemolysis has been identified in the whole extracts (figure 26).

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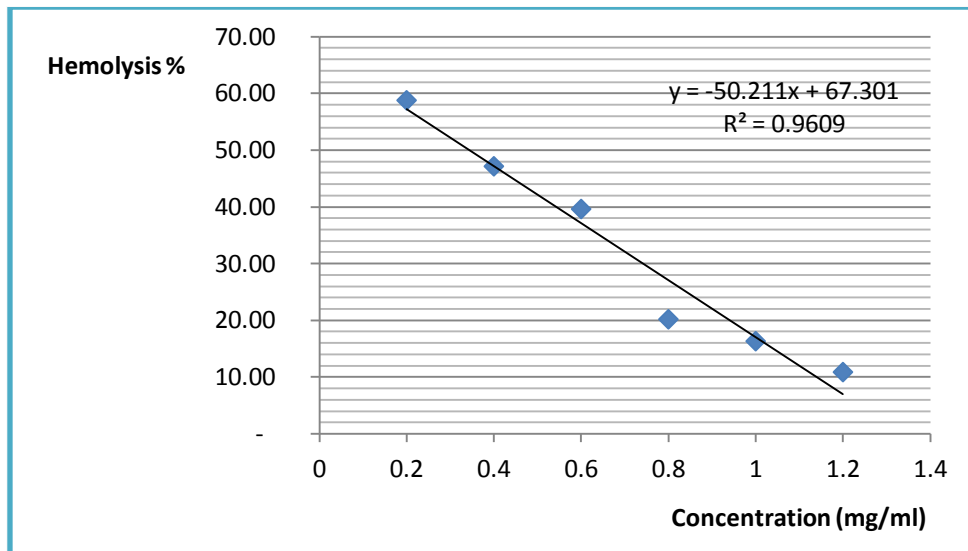


Figure (26): A linear calibration curve of Ascorbic acid (standard) in Hemolysis assay.

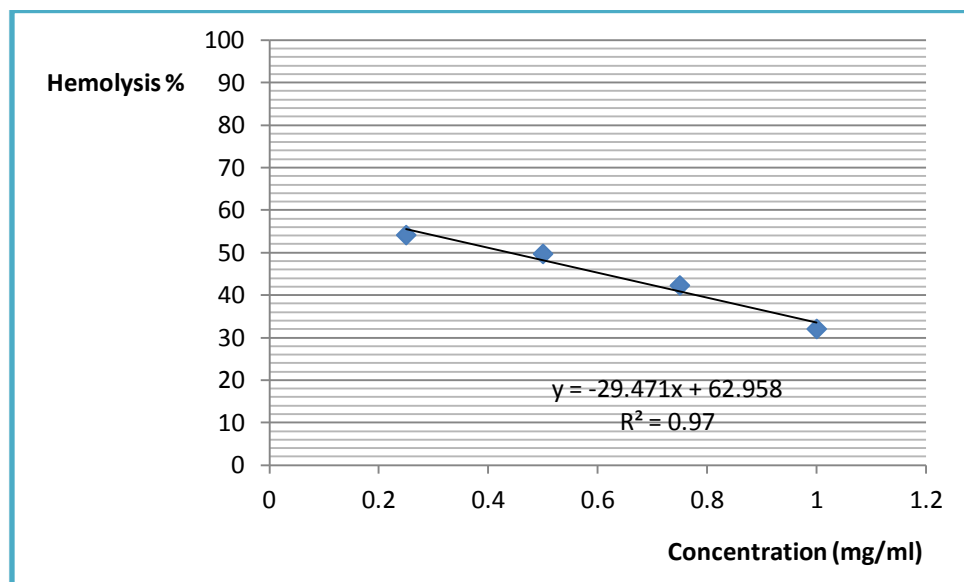


Figure (27): A linear calibration curve of Crude extract in Hemolysis assay

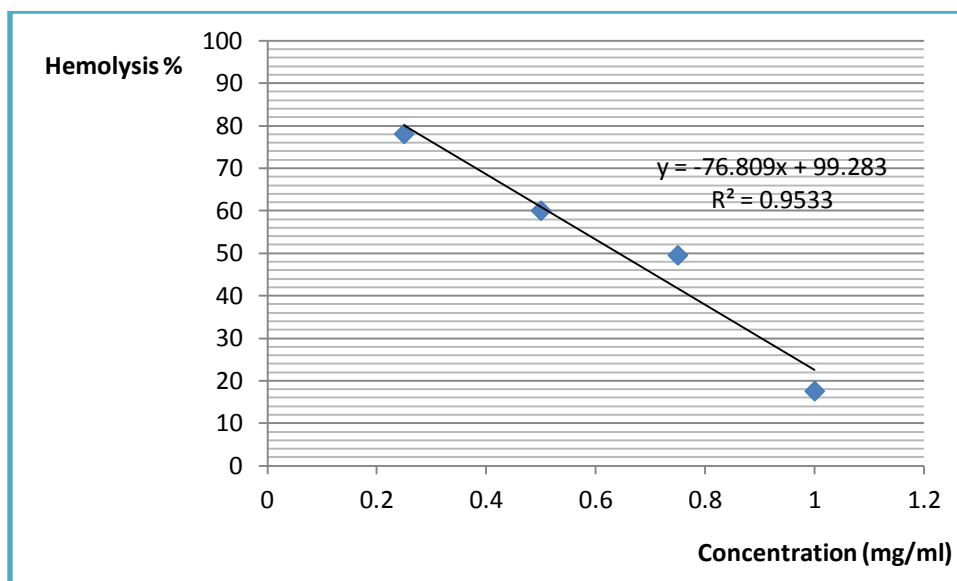


Figure (28): A linear calibration curve of Flavonoid extract in Hemolysis assay.

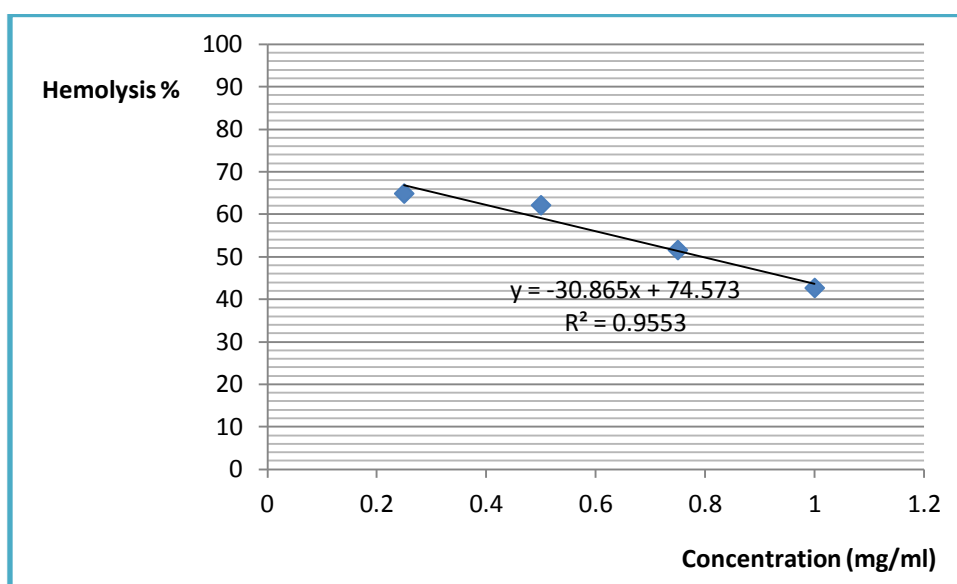


Figure (29): A linear calibration curve of Tannin extract in Hemolysis assay.

According to the results shown in figures 26, 27, 28 and 29, we noticed that there is an inverse relation between the percentage of Hemolysis and the concentration of extracts, which means, whenever the concentration of the extract get increased, the percentage of hemolysis decreases.

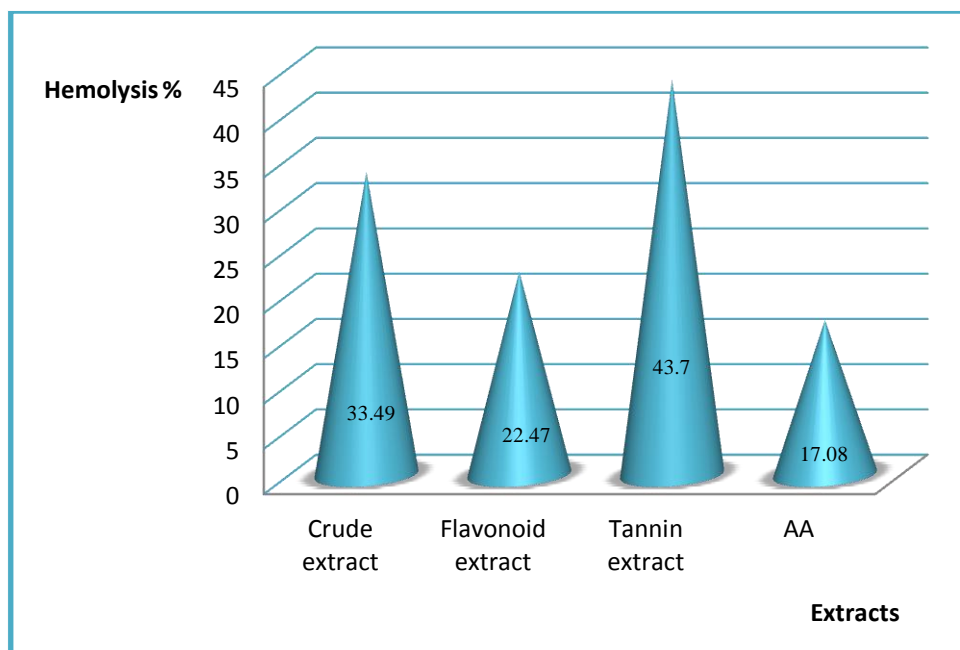


Figure (30): Rate of Hemolysis of the extracts of *Cistanche tinctoria* and Ascorbic acid with C=1mg/ml

The figure (30) shows the percentage of hemolysis at concentration 1 mg/ml, according to the results described in it, the Ascorbic acid got the lowest percentage of hemolysis (17.08%), while the three extracts: crude, flavonoid and tannin extract had a convergent disintegration; their percentage of hemolysis were: 33.49%, 22.47 % and 43.71%, respectively.

1-4-3- Reducing power assay:

In the reducing power assay, the presence of reductants (antioxidants) in the fractions would bring about reduction of ferricyanide complex (Fe^{3+}) to the ferrous form (Fe^{2+}) by giving away an electron. Increasing the absorbance at 700 nm implies an increase in its ability to reduce, so the purpose of reducing power assay is to evaluate the ability of *Cistanche tinctoria* extracts to reduce Fe^{3+} to Fe^{2+} . In this assay we used the Ascorbic acid as a reference standard.

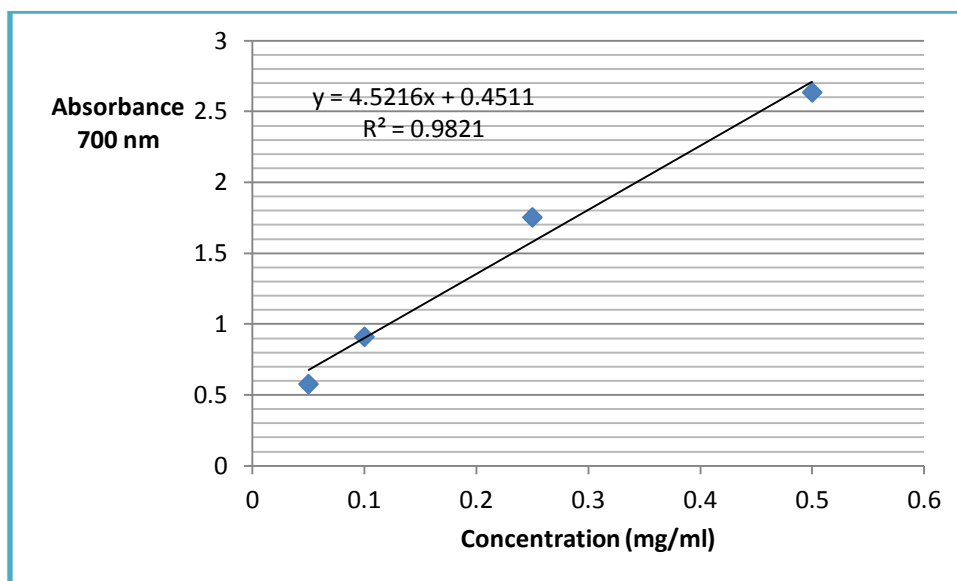


Figure (31): A linear calibration curve of Ascorbic acid in Reducing power assay.

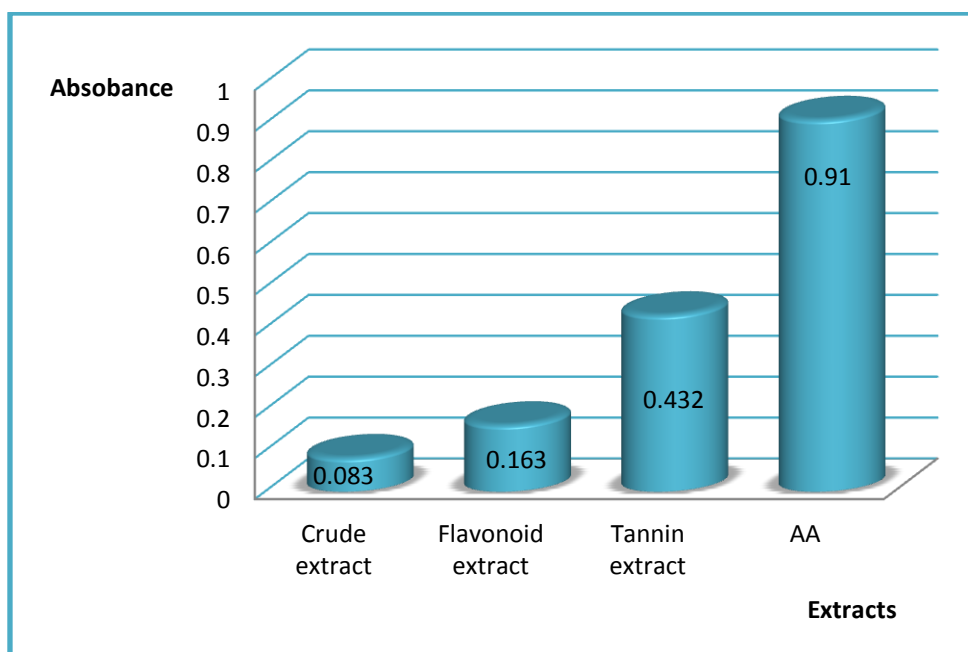


Figure (32): Column graph of reducing power assay of extracts and AA with

C = 0.1mg/ml

Figure (33) shows that the tannin extract had the highest reducing power (for iron ions) between the extracts of *Cistanche tinctoria* (Desf). Beck, with C = 0.1 mg/ml, and it showed that the reducing power of all extracts increases with increase in concentration. These results were calculated using the annex number 3.

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1-5- High Performance Liquid Chromatography (HPLC):

HPLC is one of the most important technique used for analysis and separation of the various phenolic compounds. The chromatogram of the methanolic extract (crude extract) of *C. tinctoria* shows that it contains some phenolic compounds. (Figure 33 , tables 06 and 07).

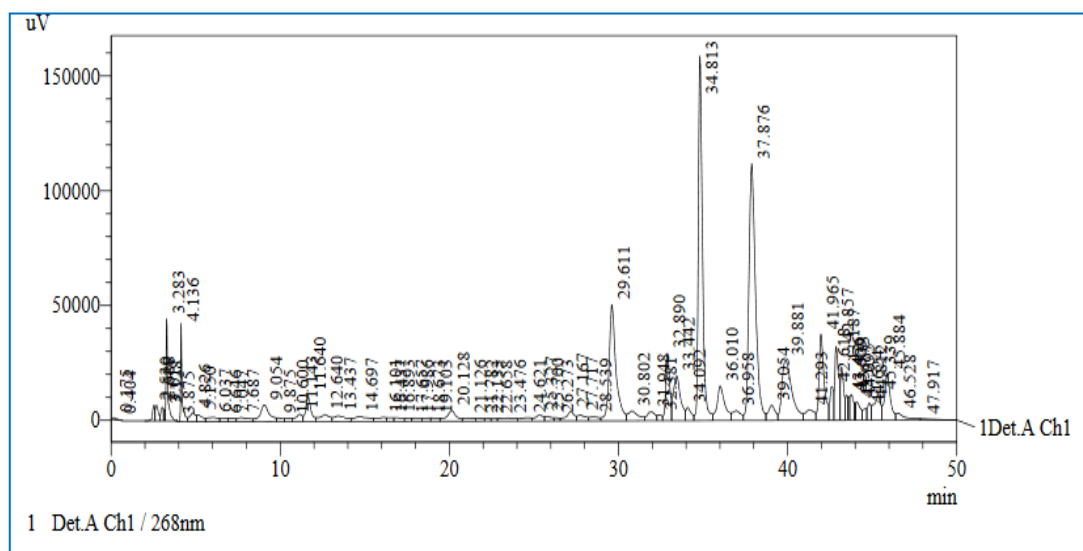


Figure (33): Chromatogram of methanolic extract of *C.tinctoria*

Table (06): Some phenolic compounds of methanolic extract *C.tinctoria*

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.175	11788	878	0.067	0.104
2	0.404	4366	400	0.025	0.047
3	2.530	64464	6902	0.365	0.818
4	2.688	80195	6846	0.454	0.811
5	3.017	61384	5974	0.347	0.708
6	3.128	27201	5135	0.154	0.608
7	3.283	299949	44455	1.697	5.266
8	3.875	2449	628	0.014	0.074
9	4.136	252514	42811	1.429	5.071
10	4.826	92381	3907	0.523	0.463
11	5.150	71207	2929	0.403	0.347
12	6.037	84382	1894	0.478	0.224
13	6.646	44219	1530	0.250	0.181
14	7.047	39648	1442	0.224	0.171
15	7.687	85387	1743	0.483	0.206
16	9.054	277223	7288	1.569	0.863
17	9.875	39889	1612	0.226	0.191
18	10.600	37778	1427	0.214	0.169
19	11.143	87140	3239	0.493	0.384
20	11.640	237241	11274	1.342	1.336
21	12.640	125909	3036	0.712	0.360
22	13.437	124803	2429	0.706	0.288
23	14.697	126121	2149	0.714	0.255
24	16.101	68710	1719	0.389	0.204
25	16.437	40214	1660	0.228	0.197
26	16.893	51947	1553	0.294	0.184

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27	17.657	33857	1406	0.192	0.167
28	17.986	48201	1451	0.273	0.172
29	18.673	44054	1441	0.249	0.171
30	19.103	44409	1435	0.251	0.170
31	20.128	181040	4663	1.024	0.552
32	21.126	59806	1373	0.338	0.163
33	21.782	41897	1378	0.237	0.163
34	22.133	25823	1251	0.146	0.148
35	22.658	24406	1070	0.138	0.127
36	23.476	83614	1339	0.473	0.159
37	24.621	65824	1446	0.372	0.171
38	25.327	85293	2551	0.483	0.302
39	25.700	56705	1970	0.321	0.233
40	26.273	29900	1394	0.169	0.165
41	27.167	137978	4037	0.781	0.478
42	27.717	87071	2473	0.493	0.293
43	28.539	66230	1649	0.375	0.195
44	29.611	1417592	50718	8.022	6.008
45	30.802	185524	4246	1.050	0.503
46	31.948	126522	3765	0.716	0.446
47	32.381	46246	2475	0.262	0.293
48	32.890	473758	29349	2.681	3.477
49	33.442	579151	19478	3.277	2.307
50	34.092	123829	5789	0.701	0.686
51	34.813	2660302	158839	15.054	18.816
52	36.010	481284	15059	2.723	1.784
53	36.958	150324	4289	0.851	0.508
54	37.876	2990284	111857	16.921	13.250
55	39.054	191115	6575	1.081	0.779
56	39.881	1081860	26106	6.122	3.093
57	41.293	172289	4640	0.975	0.550
58	41.965	773613	37546	4.378	4.448
59	42.618	218998	14749	1.239	1.747
60	42.857	506263	32158	2.865	3.809
61	43.187	298579	23233	1.690	2.752
62	43.476	103346	10857	0.585	1.286
63	43.609	61532	10419	0.348	1.234
64	43.773	158881	11173	0.899	1.324
65	43.977	42061	7688	0.238	0.911
66	44.095	145609	7971	0.824	0.944
67	44.624	79845	5118	0.452	0.606
68	44.812	104728	7488	0.593	0.887
69	45.339	304215	11376	1.721	1.348
70	45.884	484781	20385	2.743	2.415
71	46.528	123151	3059	0.697	0.362
72	47.917	31337	583	0.177	0.069
Total		17671638	844175	100.000	100.000

Based on the results of qualitative analysis of methanolic extract of *C. tinctoria*, which obtained by HPLC method (High Performance Liquid Chromatography), we noticed a few known phenolic compounds (2 compounds)

Table (07): Concentration of some known phenolic compounds of methanolic extract of *C.tinctoria*

Compounds	Concentration ($\mu\text{g} / \text{mg Ext}$)
Chlorogenic acid	1.152
Naringin	27.456

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According to table (07), there is a contrast between the concentration of the two compounds: chlorogenic acid and naringin (1.152 $\mu\text{g}/\text{mg}$ Ext and 27.456 $\mu\text{g}/\text{mg}$ Ext), respectively.

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2- Discussion:

2-1- Extraction yield:

In the extraction process, the most important criterion to be evaluated is the extraction yield (quantity in percentage). Based on the results shown in this study, there are quantitative differences between *Cistanche tinctoria* extracts, this fluctuation in the extraction yield could be explained by the reasons below:

- ✓ Solvent characteristics: the characteristics that should be considered include the type of solvent used and its polarity it is known that Ethyl acetate and characterized by a weak polarization compared to Methanol, which explains the low yield at the Ethyl acetate extract. (Lee et al., 2003),
- ✓ The conditions of plant collection are among the factors affecting the extraction yield, as well as the drying process and storage conditions of the plant. Also, the type of extraction process (Maceration) and quantity of the solvent for the plant material as well as the duration of the extraction process will determine the value of the yield (Madi, 2010; 2015، جيدل).
- ✓ It may be due to the exposure of the plant to the different stresses that play a role in the change of its physiology, thereby changing the nature and quality of plant compounds (Ibrahim et al., 2008).
- ✓ It may be due to the age of the plant at the time of study, because, the yield of chemical compounds of perennial plants retracts with the increasing in plant life.
- ✓ It can also traced back to the geographical location and nature of the climate prevailing in the plant growth and presence environment, which can determine the quality and quantity of its compounds (Sideney et al., 2016).

2-2- Total phenolic and flavonoid contents:

Phenolics and flavonoids are secondary plant metabolites that are ubiquitously observed among the plant *Cistanche tinctoria*. The presence of such metabolites enhances the level of antioxidant.

In the present study, the total phenolic content of crude extract of *Cistanche tinctoria* was determined using the Folin-Ciocalteu method and the results obtained were 167.74 ± 24.36 (mg GAE/g DM). Beside phenolic compounds, the biological effects of many plant raw materials depend on flavonoids; therefore, studies of the variation in their content are

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important and relevant. This study determined the total flavonoid contents in the crude extract of *Cistanche tinctoria* (flowers), which was 26.76 ± 2.69 (mg QE/g DM). These high results may be due of using methanol as a solvent, because methanol is the most suitable solvent for the extraction of phenolic compounds due to its ability to inhibit the reaction of polyphenols oxidase that cause the oxidation of phenolics and its ease of evaporation as compared as water (Yao et al., 2004).

Actually, the total content of phenolics or flavonoids is controlled by many parameters and conditions, one of them is the environmental factors in which plant grows such as: the season and date of collecting, soil composition, climate, temperature, light, humidity, salt and water stress (Hermans et al., 2006). Also, the quantity and quality of the compound are greatly affected by the plant age and its stage of growth. Drying and extraction methods have an effective role in the total contents of phenolic and flavonoid in the extracts (Toledo et al., 2011).

The data on the patterns of variation in the total phenolic and flavonoid contents of flowers of *Cistanche tinctoria* are scarce. Therefore, this study provides new knowledge about total phenolic and flavonoid contents in *Cistanche tinctoria*.

2-3- Antioxidant activity:

In this study, three different assays were used to evaluate antioxidant activities of *Cistanche tinctoria* (flowers). Each of them employs a different mechanism and reaction to detect antioxidant compounds. We applied these three assays because there is no single assay, which will accurately reflect all antioxidants and radical sources in such an intricate system due to their complex composition (Nuutila et al., 2003), Prior et al (2005) also assert that there is no simple universal antioxidant assay by which antioxidant capacity can be measured accurately and quantitatively.

2-3-1- DPPH assay:

DPPH is stable nitrogen-centered free radical, it is largely used for evaluating scavenging activity of antioxidant standards and plant extracts with a characteristic absorbance at 517 nm, which decreases in the presence of free radical scavengers. By accepting hydrogen from a corresponding donor, the DPPH turns from blue violet to light yellow in color. Lighter color indicates a better antioxidant ability. This scavenging activity has been widely used as a quick and reliable parameter to evaluate the general in vitro antioxidant activity of plant extracts (Chen et al., 2018).

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Based on the results obtained, there is a fluctuation in the scavenging activity between the extracts, Tannin extract exhibited the highest inhibition of DPPH radical scavenging activity as compared to other extracts. However, all the extracts were considered as having low scavenging activity if compared to the standard "ascorbic acid".

We know that "The low IC_{50} value indicates the greater radical scavenging activity under the same testing conditions" (Johari and Khong, 2019); so, according to the results we had, we could say that the percentage of DPPH scavenging activity of the extracts is lower than the one of the positive control (Ascorbic acid), these results don't support the ones obtained by Ould Saadi (2016), who found that the Ethyl acetate extract was the most potent in terms of IC_{50} values of DPPH scavenging activity when it compared to the two standard compounds: BHT and Ascorbic acid.

The fluctuation in the scavenging activity between the extracts could be because the structure and kind of phenolic compounds and their concentration in the tested samples. The content of polyphenols is one the main reasons of the antioxidant activity of the plant, because the recent studies showed that these compounds possess powerful antioxidant activity due to the hydroxyl groups in the aromatic ring (Debouba *et al.*, 2012; Diplock, 1997), also the content of flavonoids had an effect on it, according to the research conducted by Zheng *et al* (2010) about the inhibition effect of 13 flavonoids on DPPH radical, they found that the hydroxyl groups and their situation have a big role in the inhibition effect, due to their ability of giving the hydrogen, and contain of double bond between the two carbon atoms (C_2 and C_3) (Javanmardi *et al.*, 2003; Cai *et al.*, 2004)

Ascorbic acid was used as a positive standard in this assay due to doubts concerning the direct determination of DPPH obtained from calibration curve (Molyneux., 2004). Moreover, Ascorbic acid has already proven as a good antioxidant and radical scavenger. Previous research by Brand-Williams *et al* (1995), Kim *et al* (2002) and Truong *et al* (2019), also used ascorbic acid as standard for DPPH radical scavenging assay.

2-3-2- Hemolysis assay:

Hemolysis assay is one of the easiest methods used for evaluating antioxidant activity, in this assay, the red blood cells were selected as the study model of interactions between oxidants and antioxidants because they are characterized by a membrane rich in unsaturated fatty acids, which are so sensitive to free radicals, thus oxidizing them and they are also responsible for oxygen transport to the hemoglobin molecules (Cooper, 1991; Aibirami *et al.*, 2014).

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In general, oxidative stress causes lipid oxidation of red blood cell membranes, so causing dysfunction for them by influencing their fluidity and the functions of receptors in membranes, these imbalances are the cause of RBCs decomposition (Dolci and Panteghini, 2014).

In this study, the ability of red blood cells to resist free radicals in the extracts had been tracked by spectrophotometry. The results obtained showed that there is a fluctuation between them, but it was a big one compared to the standard compound which is Ascorbic acid. Actually, this result supports the one obtained when we applied the DPPH assay that the plant has a weak antioxidant activity in comparison with the one of Ascorbic acid, this activity of plant might be due to its phenolic compounds, because they raise the possibility of protecting biofilms by preventing them from oxidation by free radicals, which means they are antioxidant agents which can act as free radicals terminators (Judith, 2005; Kalaivani et al., 2011).

2-3-3- Reducing power assay:

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, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of extract (Makni et al., 2018).

The presence of reducers in sample causes the reduction of the Fe^{3+} to Fe^{2+} . Thus, the higher the reducer concentration, the higher amount of Fe^{2+} which becomes evident with high absorbance of the sample. These reducers show their antioxidant action by breaking the free radical chain by donating a hydrogen atom and also react with certain precursors of peroxide, which in turn prevents peroxide formation (Makni et al., 2018; Benzie and Strain, 1999; Lahmar et al., 2017).

Some earlier workers have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts (Guo et al., 2011). In the present study also, reducing power of all extracts increases with increase in concentration which is in close correlation with its observed antioxidant activity. Thus, these reducers must be responsible for the antioxidant property of extracts (Kemewele Saague et al., 2019).

This assay further confirmed the antioxidant properties of the extracts observed from the DPPH assay. The correlation between reducing power and DPPH values could be due to the same mechanism on which these methods rely (Jothy et al., 2012).

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1-4- High Performance Liquid Chromatography (HPLC):

In this study, the chemical composition of *Cistanche tinctoria* was analyzed using an HPLC system with detection at 268 nm. Based on the results obtained by this test which was applied on the crude extract of *Cistanche tinctoria*, we figure out two kinds of phenolic compounds: Chlorogenic acid and Naringin.

According to Gil and Wianowska (2017), chlorogenic acid is an ester formed from cinnamic acids and quinic acid and is also known as 5-O-caffeoylquinic acid (5-CQA), it exhibits many biological properties, including antibacterial, antioxidant and anticarcinogenic activities (Sanatana-Galves et al., 2017), while Naringin is a predominant flavanone glycoside (flavonoid) found in many plants, it has been reported to show various properties such as antioxidant and antimicrobial activities (Sivanesan et al., 2019).

Both of these compounds have health benefits, for example, in the case of Chlorogenic acid, these health benefits are a result of CGA donating hydrogen atoms to reduce free radicals and to inhibit oxidation reactions (Liang and D.kitts, 2015)..

Conclusion

Conclusion

In the recent years, there has been increasing interest in the valuation of wild plants, especially local ones, and their exploitation in several domains according to their compounds. So we conducted this phytochemical study which aims to valuing a one of the desert plants which is *Cistanche tinctoria* that grows in our local environment: Oued-souf region (located in the south-east of Algeria).

In this work we have prepared three extracts (Crude, flavonoid and tannin extract) using the Maceration method, accordingly we could estimate the extraction yield, where the highest value was recorded by the crude extract.

In order to determinate the total phenolic and flavonoid contents of the crude extract, we used the Folin-Ciocalteu and AlCl_3 as reagents, respectively. The results were by 167.74 ± 24.36 mg EAG/g Extract, 26.76 ± 2.69 mg EQu/g Extract.

For determinate the antioxidant activity we applied three assays, the first one was DPPH radical scavenging assay, which has become one of the most frequently used methods for evaluating the antioxidant capacity and it's based on determining the IC_{50} value, which is the total necessary to decrease the initial DPPH radical by 50%, the results of this assay showed that that tannin extract had the best inertial capacity than the other extracts with $\text{IC}_{50} = 8.12$ $\mu\text{g/ml}$, followed by the crude extract and flavonoid extract with $\text{IC}_{50} = 22.52$ $\mu\text{g/ml}$ and 19.8 $\mu\text{g/ml}$, respectively. The second was Hemolysis test, this test is applied to determine the ability of the plant extracts to protect the erythrocyte blood cells from damage or disruption of the cell membrane after exposing them to oxidative stress and free radicals by measuring the percentage of dissolved erythrocytes and its results showed that the Flavonoid extract got the lowest disintegration (22.47%) between the extracts, whereas crude and tannin extract were recorded: 33.49% and 43.71%, respectively. These tow assays were followed by a third one which id Reducing power assay, where the results obtained showed showed that the tannin extract had the highest reducing power between the extracts of *Cistanche tinctoria* (Desf). Beck.

Also, we knew some of phenolic compounds of methanolic extract of *Cistanche tinctoria* (Desf). Beck. (Flowers) using HPLC method that showed differences of concentrations and types of these phenolic compounds.

Conclusion

Finally, *Cistanche tinctoria* is one of the plants that has a few studies about it, so, we hope that the further studies will focus on detecting the compounds of other extracts of this plant and try to apply other experiences on other parts of it..

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Annexes

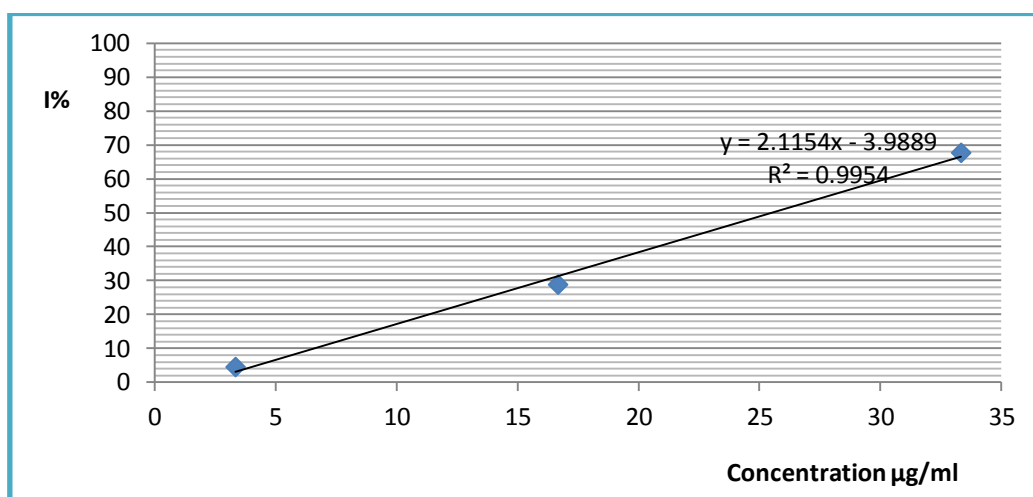
Annexes:

1- The materials used:

<p>BUCHI LAORTECHNIC AG CH-9230 FLAWIL 1/ SWITZERLAND</p> <p>Type: R-21 SN: 1000048012 Volt: 240-100VAC Frequ: 60 / 50Hz Power: 60W Built 2010 T 1.6 A L 250 V (2x)</p>	
<p>Rotary evaporator</p>	
<p>SHIMADZU CORPORATION MODEL UV mini-124 .No .CAT 24-206 ---- 38 SERIAL NO. A 10934603363 CD 220-240 V~ 60 / 50Hz 160VA MADE IN JAPAN</p>	
<p>Spectrophotometry</p>	

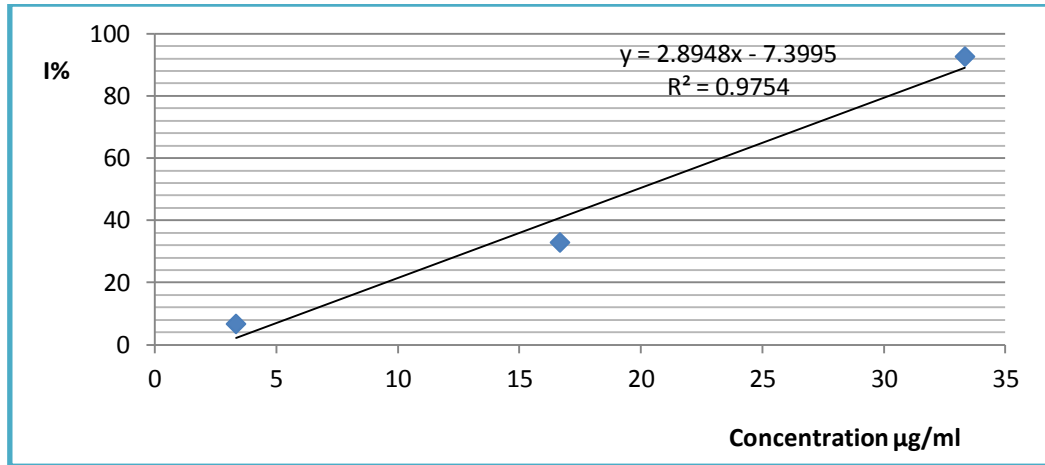
<p>CHIMADZU CORPORATION :Model A20-CTO .NO .CAT - 1 45-228 38 .NO SERIAL 20214806938L ~V 240-220 Hz 60 / 50 600 JAPAN MADE IN VA</p>	
<p>CHIMADZU CORPPORATION Model A20-SPD .NO .CAT -3 45-228 38 .NO SERIAL 20134813938L 24- 23 Hz 60 / 50 VA 16 JAPAN MADE IN</p>	
<p>HPLC</p>	

2- The linear calibration curves of DPPH assay:

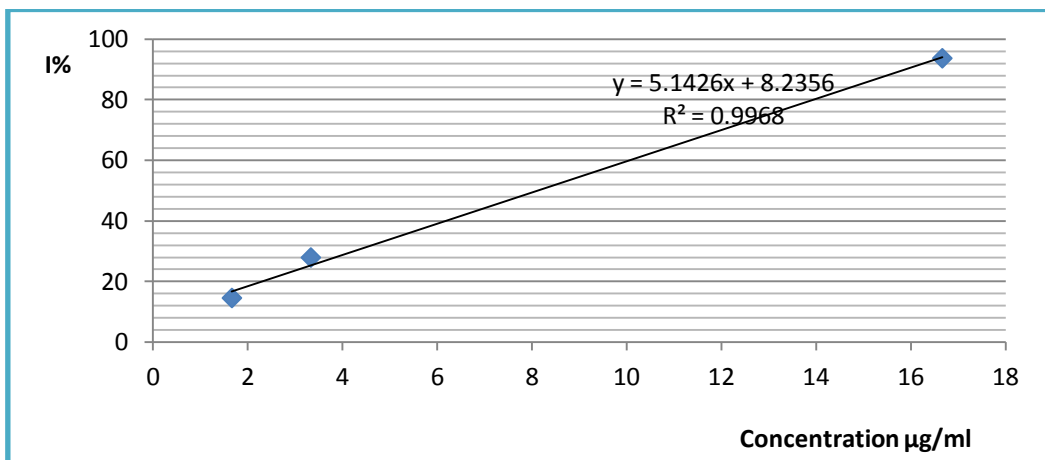


A linear calibration curve of the crude extract (DPPH assay)

Annexes

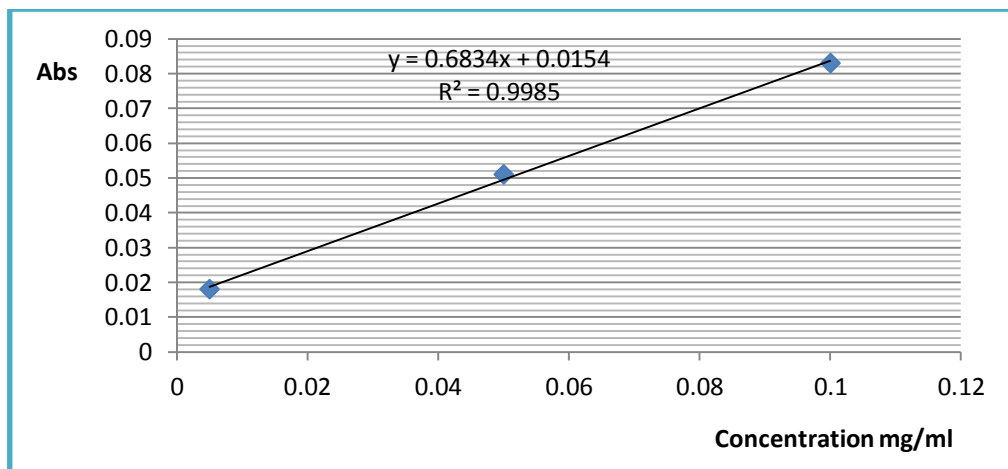


A linear calibration curve of Flavonoid extract (DPPH assay)



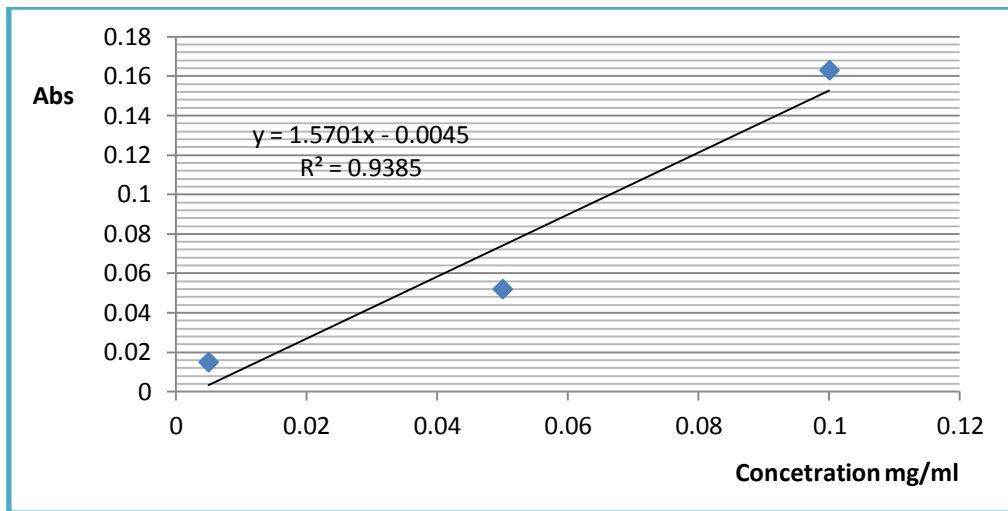
A linear calibration curve of Tannin extract (DDPH assay).

3- The linear calibration curves of Reducing power assay:

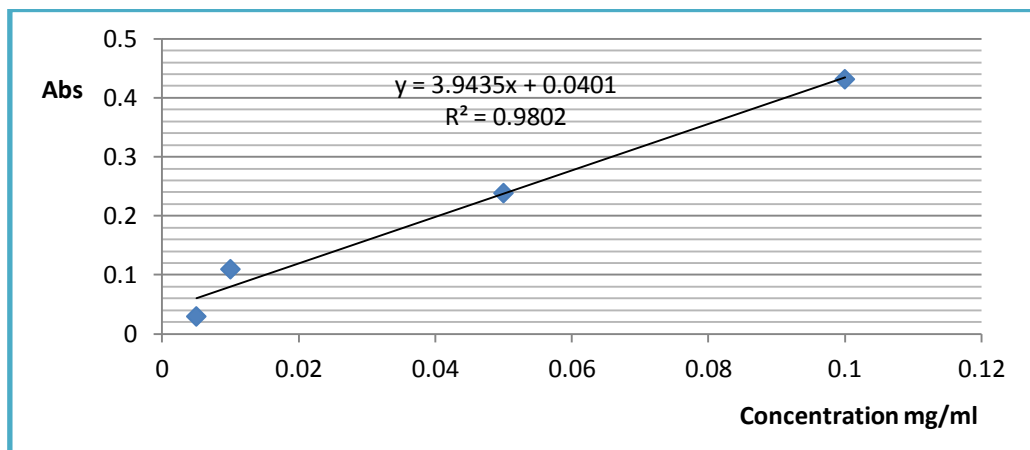


A linear calibration curve of the Crude extract (Reducing power assay).

Annexes



A linear calibration curve of Flavonoid extract (Reducing power assay).



A linear calibration curve of Tannin extract (Reducing power assay).



Université Echahid Hamma Lakhdar El Oued
Faculté des Sciences de la Nature et de la Vie
Département de Biologie



Le 06-07 Mars 2019

Attestation de Participation

Le directeur du 1^{er} Séminaire National sur la Biodiversité et Valorisation des Produits Biologiques
dans les Régions Arides et Semi-Arides atteste que:

CHENGUEL Aouatef

A présenté une communication affichée intitulée:

*Phytochemical study and biological activity of different extracts from flowers of parasitic plant
Cistanche tinctoria (Desf.) Beck*

Co auteurs: CHOUKHAlef

Directeur du Séminaire

Dr. HEMAM MWARA Djilani



Président du Comité d'Organisation

Dr. HANWAD Brahim

