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Quantification, caractérisation, et propriétés antioxydantes des polyphénols dans la pomme de terre de la région d'El Oued

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Acknowledgments

Abstract

Our work focuses on the study of the phytochemistry and antioxidant activities of four different varieties of Solanum tuberosum L (red and yellow), these varieties are grown in different area of El-Oued region. To extract the bioactive compound, we study the effect of two different commonly applied extraction techniques; Soxhlet extraction (SE) and ultrasonic-assisted extraction combine with maceration (UAE-M), with two ratios of ethanol as solvent (100% ethanol - 70/30 ethanol/water). The use of UAE-M proved to be faster and more efficient process when compared to Soxhlet extraction with 100 % ethanol. The phytochemical screening has highlighted the presence of alkaloids, coumarins, flavonoids, phytosterols. The qualitative analysis of Solanum tuberosum L for the whole root by high-performance liquid chromatography has revealed the presence of gallic acid, ascorbic acid, vanillin, and chlorogenic acid in the majority of the tested extracts. The methods used to quantify the phytochemical compounds are; Folin-Ciocalteu assay for polyphenols and tricholorur aluminum assay for the flavonoids. The measurement of antioxidant capacities is determined by two techniques: spectrophotometry using DPPH, FRAP, and voltammetric using superoxide anion radical ($O_2^{\bullet-}$). The correlation between the antioxidant activity and the content of phenolic and flavonoids compounds has also been investigated, the results of the analysis show a positive correlation between them. All results of this work have statistical significance and enabled us to assert that the extracts of Kondor variety are studied have a very high antioxidant property and reveal that their wealth in phytochemical content. Generally; extracts of the whole root of Kondor variety was more active and show that the antioxidant activity in is higher than interest than the flesh of sample.

Keywords: antioxidant capacity; El-Oued; potatoes; flavonoids; polyphenols; Cyclic voltammetry.

الملخص

تعتبر البطاطس مخزون غذائي شائع على سطح الارض، و نظرا لهذه الاهمية أوجبت دراستها تحليليا للتعرف على محتوياتها الغذائية. و لهذا تعرضنا في هذا العمل الى استخلاص عصارة اربعة انواع منها الشائعة الاستعمال في منطقة الوادي جنوب شرق الجزائر.

للاستخلاص المواد ذات الفعالية الحيوية استعملنا طريقتين: الأولى حوض المواج الفوق صوتية مرفقة بالنقع و الثانية طريقة سوكسلي، و ذلك باستعمال مذيبين الاول كحول ايثيلي 100% و الثاني مزيج من الكحول الايثيلي مع الماء بنسبة (70-30). ان استعمال الطريقة الولى اثبت أنها أسرع و أكثر فعالية من حيث استخلاص المواد الفعالة من الطريقة الثانية و ذلك باستخدام الكحول ايثيلي بنسبة 100% كمذيب.

و قد افضى التحليل الى اظهار المواد التالية: الفلافانويدات، الكومارين والفيتوستيرول، و عند استعمال تقنية الكروماتوعرافيا السائلة ذات الجودة العالية تم التعرف على العناصر التالية: حمض الغاليك، حمض الاسكوربيك، الفانيلين و حمض الكرونولوجيك في اغلب الانواع.

أثبتت دراسة الفعالية المضادة للاكسدة المقاسة بتقنيتين: الطيفية (FRAP، DPPH) و التقنية الكهروكيميائية (O_2^-) للمستخلصات الاربعة. كما أكدت لنا نتائج هذا العمل بأن مستخلصات البطاطس المدروسة تمتلك فعالية مضادة للأكسدة جيدة اذ تكون عالية في درنة كوندور أكثر من باقي الدرنات تليها بارتينا ثم سبونتا و أخيرا كيرودا . من جهة أخرى توصلنا الى أن درنة البطاطس كاملة أغنى بمضادات الأكسدة من لبها. كما قمنا أيضا بتقييم العلاقة بين الفعالية المضادة للأكسدة،الفعالية و كمية البوليفينولات و الفلافانويدات.

الكلمات الدالة: البطاطس، الفلافانويدات، حوض المواج الفوق صوتية، التقنية الكهروكيميائية، الفعالية المضادة للأكسدة.

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List of Abbreviations

Abs	Absorbance
ABTS	2,21azinobis(3-ethylbenzothiazoline)-6-sulfonate
ANOVA	Analysis of variance
BHA	Butylhydroxyanisole
BHT	Butylhydroxytoluene
CV	Cyclic voltammetry
D	Diffusion coefficient
DMF	N, N-dimethylformamide
DNA	Deoxyribonucleic acid
DppH	1,1-diphenyl 1-2-picrylhydrazyl
Ep	Potential peak
Ep_a	Anodic oxidation potential
Ep_c	Cathodic oxidation potential
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FRAP	Ferric-reducing antioxidant power
GAE	Gallic acid equivalent
GLM	General linear model
HPLC	High-performance liquid chromatography
Ip_a	Anodic potential peak
Ip_c	Cathodic potential peak
LDL	Low-density lipoproteins
NADH	Nicotinamide adénine dinucléotide réduit
TAC	Total antioxidant capacity
TBFB	Tetrabutylammonium tetrafluoroborate (Bu4NBF4)
TFC	Total flavonoids content
TPC	Total phenolics content
TPTZ	2,4,6-tris(2-pyridyl)-1,3,5-s-triazine
UAE-M	Ultrasound-assisted extraction combined with maceration
UV	Ultraviolet

General introduction

Because of today's chase for healthy products, the production and purification of vegetable extracts is an area of interests to the industry and academia. Recently, researchers have shown a proof for the role of antioxidants of plant origin on scavenging of free-radical, this property could have great importance as therapeutic agents in several diseases caused due to oxidative stress such as heart diseases and cancers[1]. However, plant constituents and phytochemical compounds were to be found effective as radical scavengers and inhibitors of lipid peroxidation.

The cellular functions and normal respiration produce a free radical. Under normal physiological conditions, approximately 2% of oxygen used up by the human body during respiration is transformed into superoxide anion free radical, with a negative charge $(O_2^{\bullet-})$ [2] which considered as one of a large group of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This oxygen environment is unfriendly to the living organisms. To survive, this organism produces a water- and lipid-soluble antioxidants that can neutralize these highly reactive free radicals [3]. If the body's antioxidant mechanism does not operate optimally, excess free radicals can damage various bimolecular, including lipids, proteins, carbohydrates, and nucleic acids.

A variety of antioxidants is found in dietary sources like fruits and vegetables which were known as a phytochemicals compounds. These plant constituents are classified as primary or secondary components, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [4].

They are present in all parts of higher plants (roots, stems, leaves, flowers, pollen, fruit, seeds and wood) and are involved in many physiological processes such as cell growth, rhizogenesis, germination of the seeds or fruit maturation. The most represented are the anthocyanins, flavonoids, and tannins [5].

That is why we are interested to make a phytochemical and quantification study of the plant **Solanum tuberosum L** (potato), which is considered as an important source of polyphenolic and flavonoid compounds, most recent studies focused on the evaluation of its antioxidant activities and its phenolic and flavonoid contents [6]. Until now no studies related

to the antioxidant activity of the potato in the region of El Oued (south-east of Algeria) have been conducted. According to the national statistics, the production of potato is growing very fast in this area which may lead her to become the first producer in Algeria and that since 2011.

Our study has been divided into two parts, the first one contains Bibliographic research presented in three chapters: the first one is a generality and description of the potato plant, the second is devoted to a free radical, the Polyphenols and the antioxidant activity, and the last one describes the equipment and methods used in this work.

The second one is the experimental part which contains two chapters: the fouth chapter discuss the results of different methods used by focuses on:

✓   the solid-liquid extraction by Ultrasound-assisted, maceration, and soxhlet techniques.
✓   determination of the total polyphenols, flavonoids, by colorimetric methods and liquid chromatography of high performance (HPLC).

The fifth chapter presents the results obtained and their discussions in two experiments; the first one is Study of the antioxidant activity of extracts from four different varieties, by spectrophotometry Methods: ferric reducing antioxidant power (FRAP) assay, and the inhibition of the free radical (DPPH$^\bullet$). Electrochemical methods (cyclic voltammetry (CV)) to evaluate the antioxidant capacity and the inhibition of superoxide anion ($O_2^{\bullet-}$). Finally, the experiment two present more phytochemical analysis for the richness variety

We will complete this piece of research with a general conclusion giving a summary of the main results obtained during this work as well as the perspectives which will be the objectives of the future work.

2

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Chapter I

Free radical

I) Free radical

I.1) Generality

A free radical is an atom or molecular species capable of liberated in existence that hold at least one or more unpaired electrons in the outermost shell configuration [1]. Free radicals are highly reactive, short-lived species generated by a variety of biological mechanisms, including inflammation [2], or as a side effect of the reactions occurring during normal oxidative metabolism [3]. During their short lifespan, they readily interact with macromolecules, including lipids, proteins, and nucleic acids, damaging their structures and often modifying their functionality, the most common free radicals are Superoxide anion radical, Hydroxyl radical, Hydroperoxyl radical, Nitric oxide and Lipid radical [4].

I.2) Sources of Free Radicals

In general, free radicals can be produced from either endogenous sources or exogenous sources. They are continuously forming in cells and environment [5].

I.2.1) Endogenous source

Under normal conditions, the Mitochondria consume oxygen associated with the process of oxidative phosphorylation, approximately 95-97% of the oxygen is reduced to water; a small fraction of the oxygen consumed (3-5%) is reduced equivalently to superoxide anion ($O_2^{\bullet-}$), as shown in figure II.1, Coenzyme Q or ubiquinone is an electron-carrying in the respiratory chain and it collects electrons from complex NADH and complex $FADH_2$, and donates electrons to complex III cytochrome bc1 segment. This process results in the formation of QH_2, the reduced form of Q, which generates coenzyme Q via an intermediate semiquinone anion. The Q-cycle leads to the formation of superoxide radical by transferring electrons to oxygen molecular [6].
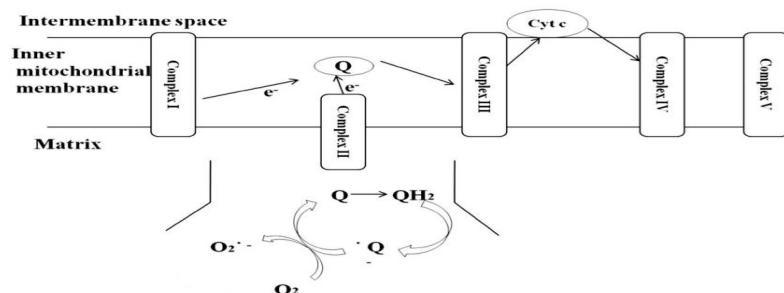
Figure I.1: Mitochondrial ROS production

The most common endogenous sources of free radicals are summarized in table I.1[4].

Table I.1: Endogenous Sources of Free Radicals

Physiological Process	Comment
Infection	May produce free radicals because the immune system may try to neutralize invading microorganisms with a burst of free radicals.
Enzymatic reaction	Many enzymatic reactions involving xanthine oxidase, lipoxygenase, aldehyde oxidase, etc. can generate free radicals.
Metal Ions	Metal ions such as copper ion and ferrous ion, which are essential for the body, can react with hydrogen peroxide to produce free radicals.

I.2.2) Exogenous source

Exposure to Solar radiations UV radiations, X-rays, gamma rays and microwave radiations, has a capacity to generate reactive chemical species, including free radicals, in cells [7,8,4]. Table I.2 summarizes most exogenous sources [9,10].

Table I.2: Exogenous Sources of Free Radicals

External sources	Comments
Air pollution	Exposure to particulate matters in polluted air can produce significant oxidative stress, increasing the risk of asthma, cardiovascular diseases, chronic pulmonary obstructive disease (COPD), and lung cancer.
Tobacco smoking	Oxidants present in tobacco smoked can damage lungs, causing COPD and even raising the risk of lung cancer.
Some medications	Medications such as bleomycin, adriamycin, and sulfasalazine may produce oxidative stress.

I.3) Major types of free radicals

I.3.1) Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) is a term used for different reactive molecules derived from molecular oxygen. They are devised in radical derivatives such as superoxide, hydroxyl radical, nitric oxide, and non-radical derivatives of high reactivity as singlet oxygen, hydrogen peroxide, peroxynitrite, hypochlorite [11].

I.3.1.1) Superoxide oxygen ($O_2^{\bullet-}$ one electron):

$O_2^{\bullet-}$ is produced in vivo in a variety of ways, the major source being the electron chain in mitochondria [12,13]. $O_2^{\bullet-}$ is not highly reactive with biological substrates in an aqueous environment. Once formed, $O_2^{\bullet-}$ quickly undergoes dismutation to generate H_2O_2. This reaction is accelerated by the antioxidative enzyme, the SOD [14,15], In addition to SOD, several other enzymes that generate H_2O_2 also exist in human tissues.

I.3.1.2) Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) itself is not especially toxic unless it is present in high concentrations within cells. H_2O_2 readily diffuses through cellular membranes and can thereby reach sites distant from where it was generated. Also, in the presence of transition metals, mainly Fe^{2+} but also Cu^{1+}, H_2O_2 is reduced to the hydroxyl radical (OH°) via either the Haber–Weiss or Fenton reactions [16-18]. In most cells, H_2O_2 is converted to innocuous products by the actions of two important antioxidant enzymes, that is, catalase and selenium-dependent glutathione peroxidase (GPx). GPx utilizes H_2O_2 hydroperoxides as substrates during the conversion of reduced glutathione (GSH) to its sulfide (GSSG) [19].

I.3.1.3) Hydroxyl Radical ($^\bullet$OH)

The hydroxyl radical ($^\bullet$OH) is a high reactive radical. under normal physiological conditions, the generating of hydroxyl radicals can be produced with the combination of both H_2O_2 and $O_2^{\bullet-}$ in presence of certain metals [20]. According to the Haber-Weiss reaction of $^\bullet$OH, it consists of the following two reactions:

In the first reaction

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2 \qquad (1)$$
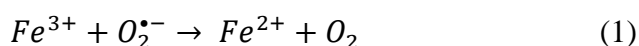
In the second reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^\bullet \qquad (2)$$

I.3.2) Reactive Nitrogen Spices (RNS)

Reactive nitrogen species (RNS) is a term used for different reactive molecules nitric and in particular nitric oxide radical considered as major components of oxidative burst and redox state regulation [21]. This spices is also devised into radical spices such as Nitric Oxide (NO$^\bullet$), Nitrogen dioxide (NO$_2^\bullet$), and non-radical spices Peroxynitrite (ONOO$^-$) and Alkyl peroxynitrites (ROONO)[22].

I.3.2.1) Nitric oxide (NO$^\bullet$)

Nitric oxide (NO$^\bullet$) contains an unpaired electron, thus it is a paramagnetic molecule and a free radical. The convert of L-arginine to L-citrulline with nitric oxide synthases (NOSs). produce a NO$^\bullet$ radical via a five-electron oxidative reaction [23] (equation 3).

$$L\text{-Arginine} + O_2 + NADPH \xrightarrow{\ NOS\ } L\text{-Citrulline} + NO^\bullet + NADP^+ \qquad (3)$$
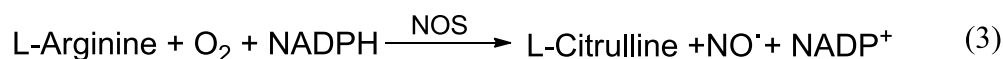
It is moderately soluble in water (7.4 ml/dl at 0°C) and is more soluble in organic solvents, therefore it can readily diffuse through the cytoplasm and plasma membranes [24]. It has also a greater stability in an environment with a lower oxygen concentration.

I.3.2.2) Peroxynitrite Anion and Nitrogen dioxide

The combination of NO$^\bullet$ with $O_2^{\bullet-}$ produce the peroxynitrite anion (ONOO$^-$) [25] (Eq 4). It is this latter molecule that accounts for much of the toxicity of NO$^\bullet$. ONOO$^-$ it's high reactive approximately the same as that of $^\bullet$OH. The toxicity of ONOO derives from its ability to directly nitrate and hydroxylate the aromatic rings of amino acid residues [26].

$$NO^\bullet + O_2^{\bullet-} \rightarrow ONOO^- \qquad (4)$$

$$OONO^- + H^+ \leftrightarrow HOONO \rightarrow HO^\bullet + NO_2^\bullet \qquad (5)$$
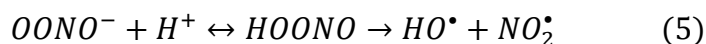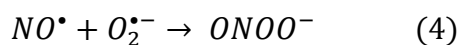
In an aqueous environment, the protonated peroxynitrous acid forms and can rapidly separate into nitrogen dioxide (NO$^\bullet_2$) and a hydroxyl radical ($^\bullet$OH)[27,28] (Eq 5).

I.4) Free radicals reaction:

With a wide range of free radicals that can be generated in the cell and in the microcirculation, it is clear that the free radicals attack all cellular (intracellular or extracellular) molecules. It can be considered that hydroxyl radical (HO$^\bullet$) and singlet oxygen are more reactive than superoxide anion (O$_2^{\bullet-}$) and hydrogen peroxide (H$_2$O$_2$). However, in a

suitable biological setting, the two last species may display considerable chemical reactivity leading to damage of various biomolecules [29].

Generally, this damage results from the involvement of free radical in chain reactions, a series of reactions lead to regenerate a radical that can begin a new cycle of reactions [30].

Free radical reactions take three distinct identifiable steps:
- ✓ Initiation step: formation of radicals. (eq 6)
- ✓ Propagation step: in this step required free radical is regenerated repeatedly as a result of chain reaction, which would take the reaction to completion. (eq7.8)
- ✓ Termination step: the destruction of radicals (eq 9)

$$RH \rightarrow R^\bullet + H^\bullet \qquad (6)$$
$$R^\bullet + O_2 \rightarrow ROO^\bullet \qquad (7)$$
$$ROO^\bullet + RH \rightarrow R^\bullet + ROOH \quad (8)$$
$$\left.\begin{array}{r} R^\bullet + R^\bullet \\ R8 + ROO^\bullet \\ ROO^\bullet + ROO^\bullet \end{array}\right\} \rightarrow \text{non} - \text{radical products (9)}$$
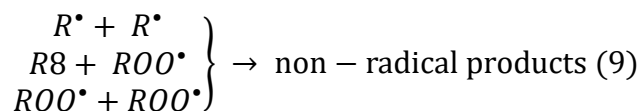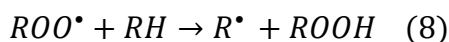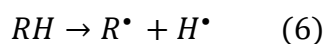
I.5) Oxidative Stress

Free radicals are highly reactive spices, a low concentration of ROS is essential for normal physiological functions like gene expression, cellular growth, and defense against infection. They act as the second messenger in intracellular signaling cascades that mediate several responses in plant cells [31].

However, with more formation of free radicals in cells leads to damaging almost all types of biomolecules (Proteins, lipids, carbohydrates & nucleic acid). The excess of free radicals leads to an imbalanced phenomenon between formation and neutralization of ROS/RNS called oxidative stress. If the body can't regulate properly the oxidative stress can lead to several diseases including cancer atherosclerosis, malaria... etc[32].

I.6) The Body's Antioxidant Defense

The body has two mechanisms either enzymatic or non-enzymatic [33]. To balance the damage effects of ROS. those mechanisms generate antioxidants from a natural source (endogenous) or through foods (exogenous) source.

The excess of free radicals is neutralized by antioxidants to protect the cells against their toxic effects and to contribute to disease prevention [34].

An antioxidant is "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate" [35]. In another term, the antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule" [36].

I.7) Classification of antioxidants

I.7.1) Enzymatic (Endogenous):

The body depends on diverse endogenous defense mechanisms to protect the cells against free radical damage. The antioxidant enzymes are:

a) Superoxide dismutases SOD is an antioxidant enzyme which scavenges superoxide anion into oxygen and hydrogen peroxide [37].
b) catalase (CAT) cytoplasmic enzyme, which converts the hydrogen peroxide (H_2O_2) into H_2O and O_2, by disproportionation[38].
c) Glutathione Peroxidase enzyme occurs in the mitochondria during the reaction, hydrogen peroxide (H_2O_2) is reduced to water and glutathione (GSH) is oxidized to glutathione disulfide (GSSG)[39].

I.7.2) Non-Enzymatic (Exogenous)
I.7.2.1) Ascorbic acid

Ascorbic acid is also known as Vitamin C (Figure I.2), considered as plasma antioxidant [40]. is found in our body in the form of ascorbate. It is located in the aqueous compartments of tissues due to its high solubility.
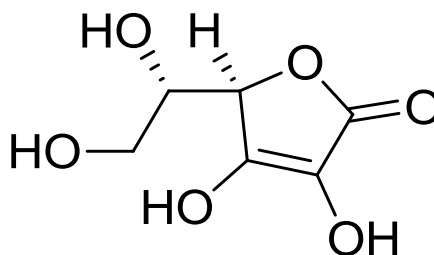
Figure I.2: Ascorbic acid

Ascorbate is a primary antioxidant in that it directly neutralizes radical species, acts as a reducing agent and reducing transition metals (Fe^{+3} and Cu^{+2}) present in active sites of enzymes or in free form in the body. Can be oxidized by free radical, also can be a good reducing agent, which arrives or is in tissue in an aqueous medium and in the reaction with free radicals, form a radical (semi- dehydroascorbate), which is little reactive. The ascorbate can act as an antioxidant against lipid peroxidation plays a major role in preventing cardiovascular disease and in conjunction with the alpha-tocopherol [38].

I.7.2.2) α-tocopherol

α-tocopherol (Figure I.3) is a form of the antioxidant vitamin E that is found in vegetable oil, nuts, and seeds.
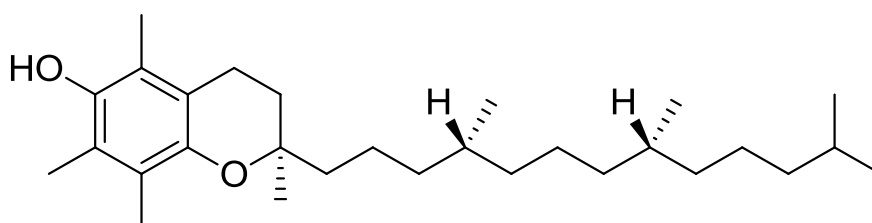
Figure I.3: α-tocopherol

alpha-tocopherol is the most active spices in tocopherols family. It acts as an important antioxidant in the inhibition of lipid peroxidation, and its efficiency, in the presence of ascorbate (cell membranes) and ubiquinone (mitochondrial membrane) [40]. Alpha-tocopherol is active in cell membranes, where reduces ROS and lipid peroxidation from the radical attack [41].

According to [42], deficiency of this vitamin alone or deficiency associated with exercise causes a significant increase in the production of free radicals and thus an increase in cellular disorders such as lipid peroxidation, loss of sarcoplasmic reticulum and mitochondrial uncoupling [41,42].

I.7.2.3) Carotenoids

Vitamin A or carotenoids their chemical structure is composed of conjugated double bonds, which are responsible for its color and for its biological functions. They are responsible for sequester singlet oxygen (free radical) and remove the radical peroxides (Free Radical) [43].
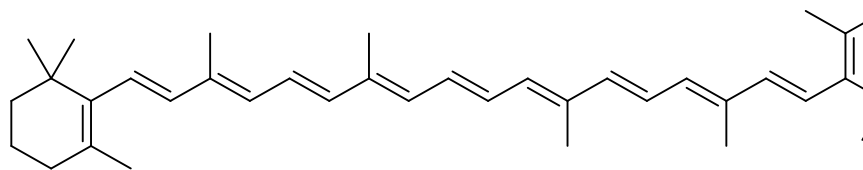
Figure I.4: β-carotene

The function to disable the singlet oxygen, carotenoids can sequester the peroxyl radicals [40,43] stated that carotenoids like beta-carotene (Figure II.4), lycopene, zeaxanthin, and lutein, exercise the functions of antioxidants in the lipid phase, blocking the free radicals that cause lesions on the lipoprotein membranes. According to [43], lycopene currently appears as one of the most potent antioxidants, mainly in the prevention of carcinogenesis and atherogenesis for protecting molecules such as lipids, low-density lipoproteins (LDL), proteins and DNA. The main sources of carotenoids are found in carrots and pumpkins (alpha and beta-carotene), tomatoes and derivatives (lycopene) and spinach (lutein) [43].

I.7.2.4) Phenolic Compounds

Phenolic compounds are a large family of phytonutrients. It has more than 2,000 family members [44]. distributed in the plant kingdom and those are the secondary metabolites. It can be classified in a number of ways: Harborne and Simmonds [45] classified these compounds into groups based on the number of carbons in the molecule. Apart from antioxidant activity, they also possess antifungal, anti-infective and antiseptic properties [46].

Phenolic structures are known, ranging from simple molecules such as the phenolic acids to substances highly polymerized as the tannins. As indicated in their name, they are compounds having one or more aromatic nuclei with one or several groups of hydroxyl [47].

I.7.2.4.1) Phenolic acid

One of the substances that compose the group of phenolic compounds is the phenol-acid, which is characterized by conferring antioxidant properties both in food and in organisms being used in the treatment and prevention of diseases. This substance is found in plants in free form or bound to sugars and protein [44].

Phenolic acids (C6-C1or C6-C3) with the sample structure are formed with an aromatic ring substituted by an alcohol in one or more positions, they are hydroxylated derivatives of benzoic and cinnamic acids (Figure II.5) [48].

(a)

(b)

Salicylic acid	$R_1, R_2, R_3 = H, R_4 = OH$	Ceramic acid	$R_1, R_2, R_3, R_4 = H$
p-Hydroxybenzoic acid	$R_1, R_3, R_4 = H, R_2 = OH$	o-Coumaric acid	$R_1 = OH, R_2, R_3, R_4 = H$
Gentisic acid	$R_2, R_4 = H, R_1, R_3 = OH$	m-Coumaric acid	$R_2 = OH, R_1, R_3, R_4 = H$
Protocatechuic acid	$R_3, R_4 = H, R_1, R_2 = OH$	p-Coumaric acid	$R_3 = OH, R_1, R_2, R_4 = H$
Gallic acid	$R_1, R_2, R_3 = OH, R_4 = H$	Caffeic acid	$R_2, R_3 = OH, R_1, R_4 = H$
Vanillic acid	$R_1 = OCH_3, R_2 = OH, R_3, R_4 = H$	Ferulic acid	$R_2 = OCH_3, R_3 = OH, R_1, R_4 = H$
Syringic acid	$R_1, R_3 = OCH_3, R_2 = OH, R_4 = H$	Sinapic acid	$R_2, R_4 = OCH_3, R_3 = OH, R_1 = H$

Figure I.5: phenolic acid derivatives of (a) benzoic and (b) cinnamic acids

Barreiros, et al [40] reported that phenolic antioxidants act as radical captors, and occasionally as metal chelators, acting both in the initiation stage and propagation of the oxidative process [40,44]. They are found in soybeans, defatted soybean flour and isolated soy protein concentrate [44].

I.7.2.4.2) Flavonoids

Flavonoids constitute one of the most wide-ranging groups of all plant phenolics. They are formed by a basic skeleton to 15 carbon atoms. The flavonoids are derivatives of the flavone or 2-Phenyl chromen, bearing of the functions free phenols, ethers or glycosides. all of which have the structure C6-C3-C6, two six-membered rings linked with a three-carbon unit, which may or may not be parts of a third ring (Figure I.5). The rings are labeled A, B, and C.
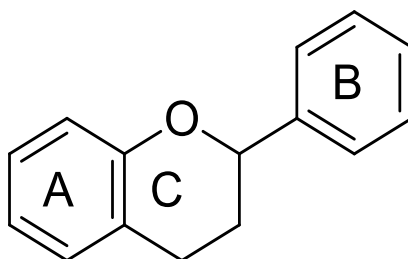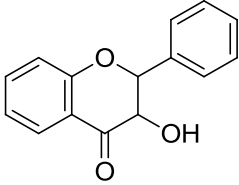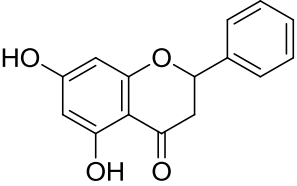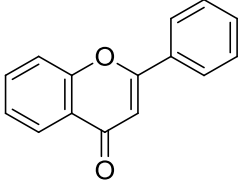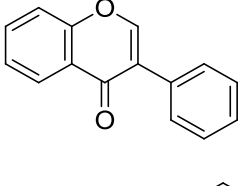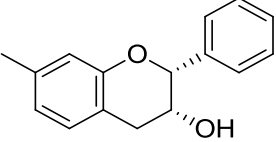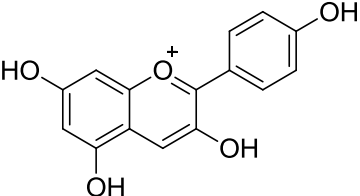
Figure I.5: flavonoids structure

These compounds represent a group of phenolic compounds the most diversified which are classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, proanthocyanidins. (Table I.3) [49].

Table I.3: Chemical structures of the main classes of flavonoids

Flavonoids	Basic structure
Flavonols	
Flavanones	
Flavones	
Isoflavones	
Flavanols	
Anthocyanidins	

In plants, flavonoids are usually glycosylated mainly with glucose or rhamnose, but they can also be linked with galactose, arabinose, xylose, glucuronic acid, or other sugars [50].

The flavonoids are solid, crystallized substances. Their colors are ranging from white to yellowish. They are soluble in hot water, alcohol, and polar organic dissolvents [51]. The flavonoids are rapidly oxidizable substances more than other types of substances [52].

However, the capacity of the flavonoids decreases with the substitution of hydroxyl groups for sugars, being the glycosides less antioxidants than their corresponding aglycons [53].

I.7.2.4.3) Tannins

Tannins are polyphenolic secondary metabolites of higher molecular weight. They are ranging from (500-3000 D) which may classify into two groups hydrolyzable tannins and non-hydrolysable or condensed tannins [54]. Also, there is another group of tannin, phlorotannins, rarely consumed by humans. It is only found in brown seaweeds [55].

Figure I.6 shows the chemical structures of casuarictin (hydrolyzable tannins) and proanthocyanidins (nonhydrolyzable or condensed tannins).
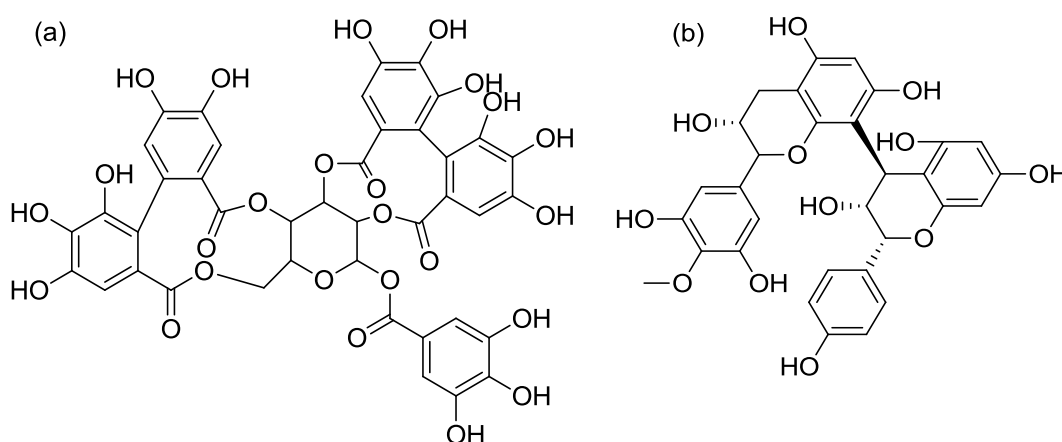
Figure I.6: Chemical structures of (a)casuarictin and (b) proanthocyanidins

a) Hydrolyzable tannins:

The hydrolyzable tannins have a center of glucose or a polyhydric alcohol partially or completely esterified with gallic acid or hexahydroxydiphenic acid, forming gallotannin and ellagitannins, respectively [56]. These metabolites are readily hydrolyzed with acids, bases or enzymes. However, they may also be oxidatively condensed to other galoil and hexahydroxydiphenic molecules and form polymers of high molecular weight. The best known hydrolysable tannin is the tannic acid, which is a gallotannin consisting of a pentagalloyl glucose molecule that can additionally be esterified with another five units of gallic acid [57].

b) Non-hydrolyzable tannins:

The condensed tannins are polymers of catechin and/or leucoanthocyanidin, not readily hydrolyzed by acid treatment, and constitute the main phenolic fraction responsible for the

characteristics of the astringency of the vegetables. Although the term condensed tannins are still widely used, the chemically more descriptive term "proanthocyanidins" has gained more acceptance. These substances are polymeric flavonoids that form the anthocyanidins pigments. The proanthocyanidins most widely studied are based on flavan-3-ols (-)-epicatechin and (+)-catechin [58].

I.7.2.5) Minerals

Other potent antioxidants include minerals such as copper, manganese, selenium, zinc, and iron. These minerals exercise their antioxidant function in diverse processes and metabolic steps in the organism

I.7.2.5.1) Copper

Copper has a role as an antioxidant, protecting the cells from the toxic effects of free radicals, and it helps to fix calcium and phosphorous, also participates in functions with antioxidant features of the enzyme family denominated Superoxide dismutase (SOD), which is responsible for eliminating the superoxide anion [59].

I.7.2.5.2) Iron

Iron forms part of the organism's antioxidant system because it contributes to eliminating the peroxide groups. However, its capacity to change valence with ease (2+/3+) renders that it can also intervene, depending on the environment, in the formation of Free radicals [60].

I.8) Synthetic antioxidants

In the food industry, naturally occurring antioxidants impart a certain level of protection against oxidation. However, during the food processing or storage, the natural antioxidants are often lost which necessitate the addition of exogenous antioxidants. Most of the synthetic antioxidants whose skeletons are often derived from natural antioxidants are of phenolic type[61].

The differences in their chemical structures reflect their antioxidant activities which also influence their physical properties such as volatility, solubility, and thermal stability. The commercially available synthetic antioxidants are butylated hydroxyl toluene (BHT), 2-ferf-butylhydroquinone (M3HQ), 2-fer£-butyl-4-hydroxyanisole (BHA), and esters of gallic acid, e.g., propyl gallate. Most of the synthetic phenolic antioxidants used in processed foods are

regulated by the FDA. Antioxidants must fulfill the following conditions: they must not be toxic, and highly active at low concentrations at the level of 0.02% of fat or oil content of the food [62].
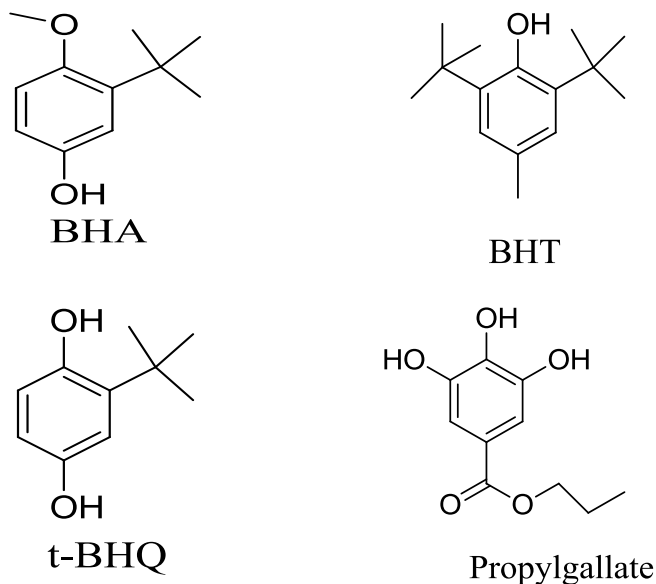
Figure I.7: Chemical structure of synthetic food antioxidants

Figure I.7 shows an example of these substances. These sterically hindered phenols are very effective antioxidants and react with free radicals. The phenoxy radicals are stabilized by delocalizing unpaired electrons in the aromatic ring.

I.9) Antioxidant mechanism

Antioxidants counteract oxidation in two different ways: by protecting target lipids from oxidation initiators or by stalling the propagation phase. In the first case, "the so-called" preventive antioxidants hinder ROS formation or scavenge species responsible for oxidation initiation (O_2, $^1O_2$, etc.). In the second case, "the so-called" chain-breaking antioxidants intercept radical oxidation propagators (LOO) or indirectly participate in stopping radical chain propagation. The mechanisms of action are sequentially reviewed here, but it should be kept in mind that antioxidants often act via mixed mechanisms that combine different types of antioxidation [63].

I.9.1) Preventive antioxidants

There are many different 'preventive' antioxidation pathways because of the diverse range of available oxidation initiators.

✓ Transient metal chelators

Chelators of transition metals such as copper and iron can prevent oxidation by forming complexes or coordination compounds with the metals. These are proteins such as transferrin, ferritin, and lactalbumin that sequester iron, or ceruloplasmin and albumin that sequester copper. However, this type of antioxidation depends directly on the oxidizable target being protected. The mechanism of action is minor for lipid peroxidation inhibition as compared to the anti-radical activity via ROS scavenging [64], but paramount in the inhibition of DNA strand breakage [65].

✓ Singlet oxygen quenchers

Carotenoids are the most efficient molecules for $^1O_2$ quenching. Hundreds of carotenoids compounds occur naturally in the environment, most of which have 40 carbon atoms. They can be pure hydrocarbons. carotenes (lycopene, b-carotene, etc.), or include an oxygenated functional group [36]. carotenoids, as many antioxidants, have antioxidant activity through several different but highly complementary mechanisms; chain breaking anti-oxidants and $^1O_2$ quenchers. This latter mechanism of action occurs through deactivation of $^1O_2$ into $^3O_2$ (eq 10) [66].

$$^1O_2 + \beta\text{-carotene} \rightarrow {}^3O_2 + \beta\text{-carotene}^* \quad (10)$$
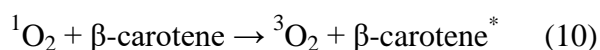
✓ ROS detoxification

ROS detoxification is a crucial oxidation prevention pathway, mainly mediated by endogenous enzymatic antioxidant systems. First, superoxide dismutase (SOD), a metalloenzyme that is omnipresent in eukaryotic organisms, catalyzes superoxide anion dismutation into hydrogen peroxide and oxygen (eq 11)

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (11)$$
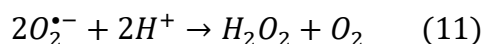
I.9.2) Chain-breaking antioxidants

In lipid peroxidation, chain-breaking antioxidants usually lose a hydrogen radical to LOO (eq12), thus halting radical oxidation propagation. This primarily involves mono-or poly-hydroxylated phenol compounds (tocopherols, flavonoids, phenolic acids....etc.) with different substituents on one or several aromatic rings.

$$A - H + LOO^{\bullet} \rightarrow A^{\bullet} + LOO - H \quad (12)$$
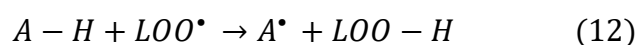
Chain-Breaking antioxidants induce a lag phase during which the substrate is not substantially oxidized. This phase continues until the antioxidant is completely consumed. Once the antioxidant has disappeared, the peroxidation rate rises sharply until it reaches the same rate as during uninhibited oxidation. Conversely, retarder antioxidants reduce the peroxidation rate without inducing a distinct lag phase [63].

I.10) Antioxidants benefits

The human body naturally produces reactive oxygen or nitrogen species which are known as free radicals. The overproduction of these compounds leads to oxidative damage to large biomolecules such as lipids, DNA, and proteins. This damage is responsible for Chronic diseases like cardiovascular diseases, diabetes, and cancers. However, it has been demonstrated that phytochemical compounds play a significant role in the protective effect against the development of these chronic diseases. In order to benefit the body, the phytochemical compounds neutralize and remove the free radicals [67].

The phytochemical compounds as Antioxidant exist widely in fruits, vegetables, cereal grains and medicinal plants [68]. Besides, wild fruits and fruits wastes (peel and seed) also contain high contents of antioxidant phytochemicals. This antioxidant distributed in a wide range in nature and classified in groups, such as phenolics, flavonoids, and carotenoids...etc.

The polyphenols have a beneficial effect on the plasma. Some studies found that consuming 10 servings of fruit and vegetables each day for 15 days increase their antioxidant capacity [69]. In addition, the binding of polyphenols with red blood cells also leads to improving the total oxidant-scavenging capacities of human blood [70], this role of polyphenols has an effect on blood pressure which could also protect the cardiovascular system.

Furthermore, anthocyanins and flavonoids were also proved to have protective actions against several cardiovascular risk factors [71]. Several other phytochemicals compounds such as caffeic acid and chlorogenic acid possess potent anti-obesity activity.

Antioxidant phytochemicals could possess protective actions against many other chronic diseases besides the diseases mentioned above. Recent studies have suggested that the appropriate lifestyle modifications could prevent more than two-thirds of human cancers and the diet contributes to about 35% of human cancer mortality [72]. Therefore, phytochemicals compounds as an antioxidant can be good candidates for the prevention and treatment of human body diseases.

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Chapter II

Potato

Generality

II) Potato (*Solanum tuberosum* L.)

II.1) Generality

Potatoes (*Solanum tuberosum* L.) Figure I.1 are grown and eaten in more countries than any other crops; they are grown in all the continents except Antarctica [1]. The potato is without doubt of ancient origin, although our knowledge of its early stages of domestication is not so precise as that of some other crops, such as wheat and barley. We know, however, that it was domesticated in South America and that it had been dispersed by man over a considerable area by the time the Spaniards arrived in the sixteenth century.[2] In the sixteenth century, Spanish travelers brought a potato to Europe and from there, its cultivation spread to other parts of the world [3].

In the global economy, they are presently the fourth most important food crop in the world after maize, wheat, and rice, with a production of 365 million tons. Algeria is the 14th producer among the top 25 potato producing countries in the world with a total production of 5 million tons [4].

Figure II.1: A selection of potato cultivars

II.2) Origin and history

The white potato is a native of South America and was first cultivated by South American Indians some 8,000 years ago [5-7]. It has been the staple commodity of the inhabitants of Chili, Columbia, Peru, Ecuador, and Bolivia. In 1537 the first recorded contact of the white man with the potato was made by Gonzal Jiminez De Quesada, stated that "the potato has floury roots of good flavor, a gift very acceptable to Indians and a dainty dish for Spaniards" [8]. The data in Table I.1 show significant happenings in the history of the potato.

The original name of potato tuber came from the latina word batata (name of sweet potato) because the European couldn't adopt the word papa (name from the Inca empire) whose name came with the Spaniards one of many Latin names such as amka (in Bolivia) and poni (in Chile). [2]

Table II.1: Significant happenings in the history of the potato

Dates(Year)	Activities
1536	Potatoes were first encountered by Spanish Conquistadors in The Andes
1586	Sir Walter Raleigh brought potatoes to England
1620	Bauhia named potato Solanium Tuberosum
1662	Royal Society recommended the plantation of potatoes to prevent famine
1731	Miller mentioned two varieties of sorts, the red and the white
1814	The National Society of Agriculture of France gathered and displayed a collection of 115 to 120 varieties of potatoes

After *Solanum tuberosum*'s introduction to Algeria, in the mid-1800s, potatoes were grown mainly for export to French markets. By national independence from France, in 1962, farmers were harvesting on average 250 000 tonnes a year, with about one third marked for export.[9]

II.2) Scientific classification

Plant classification is the placing of known plants into groups or categories then grouping successive categories into a hierarchy. The act of classification can be defined as 'the grouping of individuals so that all the individuals in one group have certain features or properties in common' According to the rules of taxonomy potatoes are classified as:

Order: Solanales

Family: Solanaceae

Subfamily Solanoideae

Genus   Solanum L.

Species Solanum tuberosum L.

Potato is part of a group of plant family Solanaceae. The Solanaceae includes 95 genera, with Solanum, from which the family gets its name, being the largest and most economically significant. In addition to potato, the Solanaceae includes other significant crop plants including tomato (Solanum lycopersicum), eggplant (Solanum melongena)[10]. The genus Solanum ranks among the five largest of plant genera, estimated at 1,000 to1,700 species [11,12].

II.3) Botanic description

The potato is a plant whose growth dies down annually but whose roots or other underground parts survive. The figure I.2 shows potato plant is devised into an above-ground portion which contains flowers, leaves, fruits, and stems, and a below-ground portion contains tubers and roots.
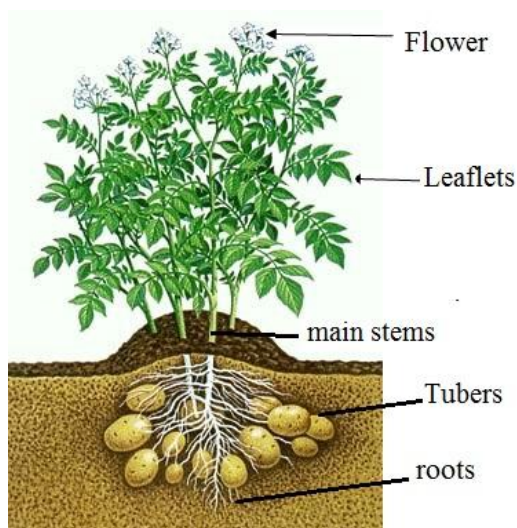
Figure II.2: The potato plant

II.3.1)The above ground portion

II.3.1.1) Flowers

Potato flowers obtain all four essential parts of flower calyx, corolla, male and female elements (flowers are bisexual). The corolla consists of five petals. These are also joined at their base and form a short tube and flat five-lobe surface. Each lobe ends a triangular point. The form of the corolla is generally round. Some primitive cultivars have pentagonal or star-like corollas. The corolla color may be either white, light blue, blue, red and purple with different tones and intensities[13].

II.3.1.2) Leaflets

The potato leaflets are attached along a common axis; there is a terminal leaflet and therefore an odd number of leaflets. Three to four pairs of leaflets are generally present. The leaflets are borne on petiolules; they are ovate (i.e. they have a tapering point). Secondary leaflets fill the spaces between the primary leaflets and serve to increase the amount of light intercepted [14].

The most important role of the leaves is photosynthesis by absorbing sunlight, which may make the leaves the most active organs of the potato plant. The plant has a rosette or semi-rosette habit because of the arrangement of the most of the leaves at or near the base of short stems and is near the soil surface.

According to one variety, the leaf may be long or short, also its colors may be light, dark or grey-green [15].

II.3.1.3) Stem

Stem color is generally green or mottled green (Figure I.2); occasionally it may be red-brown, or purple. Main stems show partial apical dominance, plays a major role in determining tuber yield. This is achieved through interstem competition, whereby stems compete for water, nutrients, and light. The intensity of competition controls the number of tubers formed per stem and then goes on to influence the size distribution of the tuber crop [14].

II.3.1.4) Fruits

Some potato cultivars will produce small green tomato-like fruits in addition to tubers. These fruits typically contain 25 to 200 seeds each. When mature, such fruits can be harvested, and their seeds extracted. When stored in a dry cool place these seeds can maintain viability for ten years or more [13].

II.3.2) The below-ground portion

II.3.2.1) Tubers

A tuber is a botanical name for the swollen end of a fleshy underground stem (a stolon), which arises from a below ground axil at the base of the stem. Not all stolons will form tubers. Stolon tips not covered by soil develop into vertical stems with normal foliage. Tuber formation is basically the result of translocation and storage of excess food and is dependent upon several genetic and environmental factors [13].

The shape of tuber can range from very long fingerling types to round. All tubers, even round ones, have two ends: the heel, or stem end, where the tuber is attached to the stolon, and the apical, rose, or seed end. The eyes are located on the axils of scalelike leaves or "eyebrows." Morphologically the eyes correspond to the nodes of stems. Each eye contains several buds [14].

II.3.2.2) Roots

A much branched fibrous root system is formed either by the seedling tap root or by adventitious roots in tuber-grown plants. In early stages of growth, the root system is restricted to surface soil, the roots turning downward after extending for some distance horizontally; this leaves the area of subsoil directly below a plant almost free of its own roots [16].

II.4) Varieties

The cultivation of potato around the world for years under different climate conditions [17] has effects on shape, size, color, flavor, and nutritional content of potato. Actually, there are more than 4000 distinct varieties worldwide, but only about 100 are suitable for commercial use and are consistently available in consumer markets [18]

Potato varieties can be classified and grouped according to size, shape, color, texture, taste, and cooking characteristics. Potatoes are also classified on the basis of their skin color (Red, yellow, and white); texture (smooth, slightly netted); tuber shape (round and oval to round); and flesh color (cream and light yellow).

According to [18] the potato varieties classified according to one of seven subcategories (Figure II.3): Russet, yellow, white, red, fingerling, purple and blue, and petites/other.

(c)

(a)   (b)

(d)   (e)   (f)

(g)
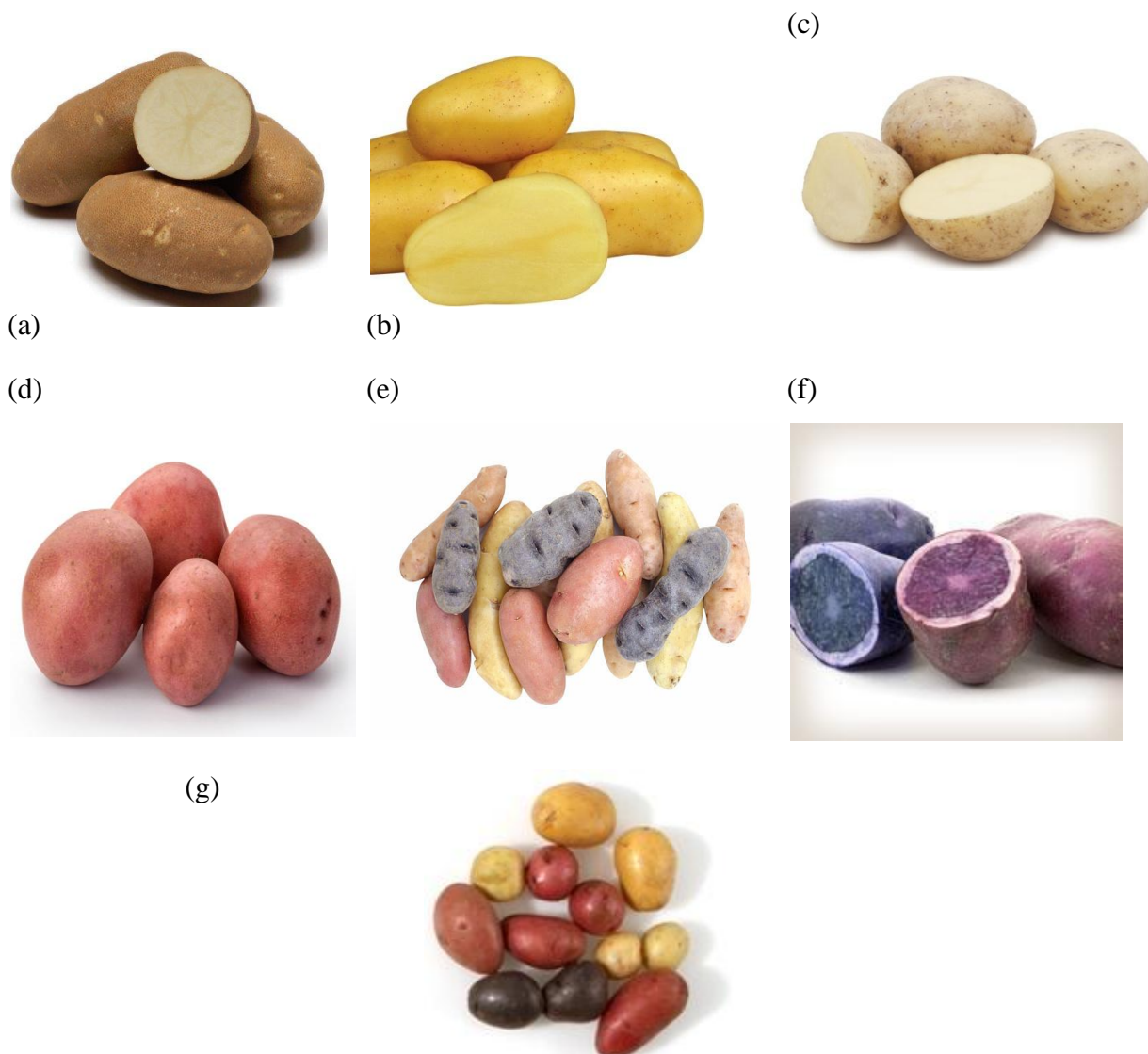
Figure II.3: Different potato variety

II.4.1) Russet (a)

These bulky, oval potatoes are characterized by netted, brown skin and white flesh. In general, Russet-type varieties exhibit high starch content, containing between 60 and 70% of starch. Russets also contain low moisture levels [19].

Common Russet varieties are Russet Burbank, Kennebec, Russet Norkotah, Canela Russet, Ranger Russet [20].

II.4.2) Yellow (b)

The yellow potato category is gaining popularity for its diversity. Yellow potatoes vary in size from 1 oz to larger sizes comparable to a Russet. The category gets its name from the yellow interior flesh, though the exterior skin is typically light brown to a yellow-golden

color. The interior of a common yellow variety will be smooth and creamy, Moderate levels of starch and sugar make many yellows a popular choice for a variety of uses.

Common yellow varieties are Yukon Gold, Satina, and Dutch Baby [20].

II.4.3) White (c)

Whites are typically small-medium size and many varieties are oval or oblong in shape. The interior flesh is white and the delicate exterior skin can range from nearly colorless to shades of tan. Common white varieties frequently have a slightly creamy, dense texture and average starch levels and low-sugar content [18].

Common white varieties are Atlantic, Snowden, Dakota Pearl, Superior, Kennebec, Shepody, Cal White, Reba, and Pike [20].

II.4.4) Red (d)

The skin of red potatoes tends to be thin, and the tubers are often smaller and rounder than other varieties. The interior flesh is commonly cream or white in color and is firm. Also in many red varieties known with her higher levels of sugar and lower starch levels.

Common red varieties are Sangre, Norland, Dakota Rose, Chieftain, Red La Soda, Red Pontiac, Cherry Red, and Modoc [20].

II.4.5) Fingerling (e)

Fingerling varieties are named for their comparatively slender and long appearance. Average fingerlings are approximately 10 cm in length, although sizes vary greatly by the variety and desired end use. The term "fingerling" characterizes potatoes of a particular shape and size, rather than a specific subset of varieties. Thus, there is more diversity in color, texture, flavor, nutrition, starch and sugar content within this category of potatoes compared to others (e.g. Russet, white, etc.). External fingerling colors range from brown/tan, red, orange, yellow, white, and even purple. Interior colors are often cream or white [19].

Common fingerling varieties are Russian Banana, French, Rose Finn Apple, and Purple Peruvian [21].

II.4.6) Purple and Blue (f)

These colorful potatoes are eye-catching and come in a range of hues of blue, purple, and even red. The skin and the flesh of these varieties tend to be highly pigmented and contain relatively high levels of antioxidants. Category members tend to be small to medium size and may range in shape from round to oblong, and even fingerling size.

Common purple and blue varieties are: Purple Majesty, Purple Peruvian, All Blue, Mountain Rose, Adirondack Blue, Congo Blue, and True Blue [20,21].

II.4.7) Petites/Other (g)

The category of "Petites" refers to smaller versions of full-sized potatoes. The unifying characteristic is the C-size class distinction, which is no larger than 3.8 cm in diameter, roughly marble or pearl-sized. Category members range significantly in terms of starch and sugar content, flavor, color, and texture. Attractive medleys of yellow, red, and purple or blue flesh are increasingly available in US grocery stores [18].

Common petite varieties are: smaller sizes of most potato varieties, Dutch Babies, and Rosara/Klondike Rose.

II.5) Tuber Composition

The composition of a potato tuber is dependent on a number of factors, although the innate genetic characteristics associated with each cultivar are thought to be among the most significant [22]. Other factors are known to influence potato chemical composition include the geographic growing location, soil type and climate [23], agronomic factors such as fertilizer or pesticide use [24], the impact of potato pests or diseases [25] and age or maturity at harvest [24]. In addition, processing or preparation techniques [26], can affect the actual quantity of the nutrient that is present at the time the potato is eaten. Finally, at the time of consumption, factors such as the bioavailability of the nutrient, or other foods eaten in conjunction with the potato, can influence how well the nutrient is able to be utilized for nutritional or health benefits.

The nutritional content of potato tuber is important for human health Table II.2 shows the nutritional content of a whole potato tuber 100 g mass provides:

Table II.2: Proximate composition of edible portion of potatoes

Component	Value/100g
Water	78.58
Total carbohydrate (by difference)	18.07
Starch	15.86
Dietary fibre	1.30
Sugars	0.61
Protein	2.14
Total lipids	0.08
Ash	1.13

About 80% water (Table II.2). A major portion of potato solids is starch, followed by protein, and fiber. Table I.3 gives the proximate composition of an edible portion of a typical potato tuber. Approximately 6% of a potato tuber consists of peel having slightly higher amounts of ash, crude fiber, protein, riboflavin, and folic acid than the flesh.

Table II.3: Selected nutrients and vitamins in flesh and peel of raw potatoes (based on 100 g wet basis)

Component	Potato flesh	Potato peel
Dry matter (%)	21.6	17.0
Ash (%)	0.86	1.67
Crude fiber (%)	0.37	1.83
Protein (%)	1.81	2.67
Ascorbicacid(mg)	14.4	11.8
Thiamine (mg)	0.10	0.03
Riboflavin (mg)	0.036	0.058
Niacin (mg)	1.57	1.05
Folic acid (mg)	12.8	17.3
Vitamin B6 (mg)	0.22	0.21

Although potato contains most of the minerals (Table II.4) iron, zinc, calcium, magnesium, selenium, manganese of nutritional significance in relatively small amounts, it is considered a valuable source of potassium (563.5 mg/100 g of edible portion) in our diet. However, Potato varieties differ in their mineral compositions [27].

Table II.4: Nutrition information of potato (dry weight)

Ingredient name	Content [a] (mg/100 g)
Energy (kJ/100 g)	1492 ± 0.00
Vitamin B1 (thiamine)	0.76 ± 0.00
Vitamin B2 (riboflavin)	0.40 ± 0.01
Vitamin B3 (niacin)	9.04 ± 0.02
Vitamin C	28.7 ± 0.20
Na	2.74 ± 0.15
Mg	76.2 ± 0.03
K	563.5 ± 0.00
P	301.9 ± 0.00
Ca	14.0 ± 0.00
Fe	4.32 ± 0.00
Cu	3.76 ± 0.00
Zn	10.3 ± 0.07
Mn	4.84 ± 0.00
Se (µg/100 g)	3.87 ± 1.30

[a] Potato cultivar: Shepody

Also contain phytochemicals compounds, besides a combination of vitamins and minerals [28]. Potato is considered as an important source of phenolic compounds diverse in groups of tens of thousands of different compounds, some of which are effective against malady or have other health-promoting qualities containing effects on longevity, mental acuity, cardiovascular disease, and eye health [29-31].

The phytochemical compounds are present in the whole potato tuber peel and flesh; however, the peel is indicated to have the highest amounts [32]. These phytochemical compounds presented in potatoes as phenolic acids and flavonoids including flavonols, and Anthocyanidins (Table II.5)

Phenolic acids such as chronologic acid, gallic acid, p-hydroxybenzoic acid and vanillic acid with a content range from 0 to 34 mg [33-35], for flavonoids compounds including flavanols such as rutin and quercetin-3-o-glu-rut, with a content range from 0 to 12.2 mg [36,37], also anthocyanins [38].

Table II.5: Concentration levels of the main phenolic compounds in potatoes

Phenolic Classes	Phenolic Compounds	Range (mg/100 g Dry Extract)
Phenolic acids	gallic acid	0–1.0
	p-hydroxybenzoic acid	0–7.8
	vanillic acid	0.6
	chlorogenic acid	0.4–34.0
Flavonols	Rutin	0–12.2
	quercetin-3-o-glu-rut	2.5
Anthocyanidins	Anthocyanins	1.4–163.3

For carotenoids, such as lutein, zeaxanthin, violaxanthin, and neoxanthin (Figure II.4) are major carotenoids found in potatoes and b-carotene is found in trace amounts according to [32].
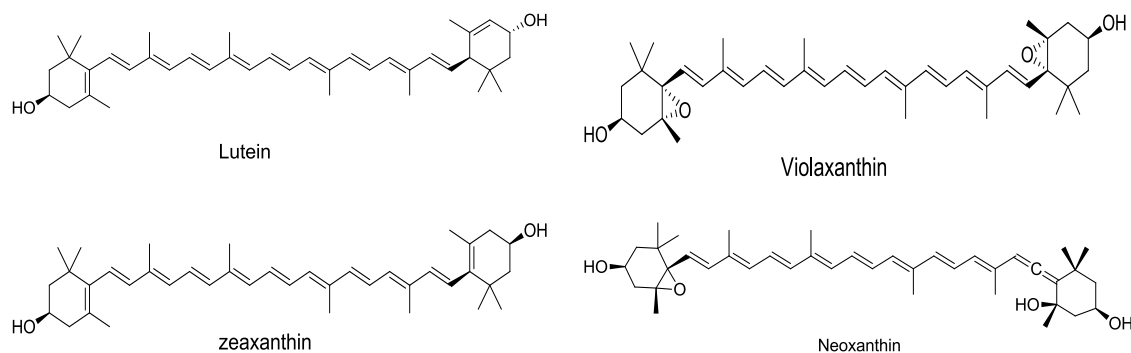
Figure II.4: Major carotenoids in potatoes.

However, another study of potato tubers along with other foods reports of total phenolic amounts found in other plants, these potatoes have more phenolics content than tomatoes, onions, carrots, Snapbeans [39]. These amount of phenolic acids can be higher or less from the same variety depending on the cultivar condition [40-42].and different variety of the potato cultivars [43].

Potato contains glycoalkaloids, α-chaconine, and α-solanine. Because of food poisoning concerns, new potato varieties are tested for glycoalkaloid levels, and if the amount (total of α-chaconine and α-solanine) exceeds 20 mg glycoalkaloid /100 g, or 200 mg/kg tuber on a wet weight basis, the variety will most likely not be released [44].The levels of these glycoalkaloids can vary in potato varieties. The skin contains significantly more glyco alkaloids (12.4–543 mg/kg of wet peel) than the flesh (1.3–148 mg/kg).The glycoalkaloid content of potatoes is not affected by baking, cooking, and frying. Thus, potato products having high skin content should be watched [45]. Postharvest factors such as low storage temperature, the light greening of potato (which is a sign of glycoalkaloid), and mechanical injury can increase glycoalkaloid content in potatoes [46-48].

II.6) Cultivation

The potato plant can be grown from a potato or piece of potato. which makes the potato exceptional in agriculture. The new plant can produce 5-20 new tubers. They have genetic clones of the original plant, also the plant produces flowers and berries that contain 100-400 botanical seeds. These can be planted to produce new tubers, which will be genetically different from the original plant [49].

The development of potatoes can be broken down into three distinct growth stages: The first stage begins with sprouts developing from the eyes and ends at emergence from the soil. The seed piece is the sole energy source for growth during this stage.

The second stage, at this point the vegetative parts, leaves, branches, roots, and stolons start of formation and last until tubers start to develop. The first and the second stages last from 30 to 70 days depending on planting date, soil temperature and other environmental factors,

The last stage is Growth Stage, tubers are forming at salon tips, but are not yet enlarging. This stage will last approximately 2 weeks. Tuber cells expand with the accumulation of water, nutrients, and carbohydrates. Tuber bulking is the growth stage of longest duration. Depending on the date of planting and cultivar, bulking can last up to three

months. Vines turn yellow and lose leaves, photosynthesis gradually decreases, tuber growth rate slows and the vines die [50].

II.7) Production

Since 1960's, the production of potato has rapid growth, in 2005 the combination of potato production in Africa, Asia, and South America exceeds that of Europe and the USA [51,52], with 200 million cropping area globally about the half is concentrated in Asia which include China, India, and Russia the first three producing countries (Figure I.5) with 96.1, 46, and 32 million tonnes respectively [53].

Algeria is the 14th producer among the top 25 potato producing countries in the world and the first in Africa with a total production of 5 million tons [4]. The potato is grown over an area of over 160 000 ha and can be planted and harvested somewhere in Algeria in virtually any month of the year. Recently the county of El Oued southeast Algeria become the first producer in the country with 1.1 million tons which represent 35% of the national production [54].
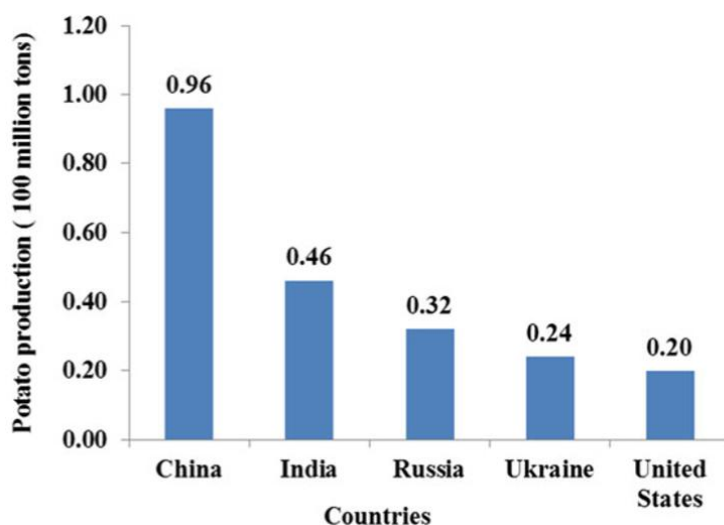
Figure II.5: Top five potato-producing countries in 2014 (FAO 2016)

II.8) Non-Food Uses of Potato

From the economic side, the potato is considered as the most widely grown tuber crop in the world, which used for human consumption as the main purpose. In addition to its use in the industrial field, like potato starch, alcohol, etc.

In the nutritional side, the potatoes are known for their carbohydrate content the predominant form of this carbohydrate content is starch. A small but significant portion of the starch in potatoes called 'resistant starch', which resist the digestion by enzymes in the

stomach and small intestine, and so reaches the large intestine essentially intact, also considered to have similar physiological effects and health benefits of fiber by offer protection against colon cancer, improve glucose tolerance and insulin sensitivity [55].

Potato starch is a promising biopolymer for different food, pharmaceutical, and biomedical applications because of its higher water solubility that raises its degradability and speed of degradation; non-toxicity, easy availability, and abundance

Presently, in nanoscience, the potato starch is converted to glucose through enzymatic hydrolysis, which is a raw material for the production of lactic acid. Then the lactic acid is polymerized to produce polylactic acid (PLA). PLA is becoming increasingly popular in the production of a wide range of biodegradable materials (board, sheet, films, fiber, paint, etc.) because of low energy requirements during its production compared to other plastics of petroleum origin. Fiber-reinforced PLA composite materials have been used to interior components for automobiles [56]. However, the cost of production of these plastics is significantly high as compared to those of petroleum origin and it is essential to develop a technology that uses energy more efficiently and is cost-effective.

II.9) Potatoes information

As a matter of fact, the potato has always been a staple food around the world and the second one in developed countries like European countries and the USA. Therefore, conducting the researchers on the potato as a staple food and industrial development became an inevitable tendency for complying with the continuously growing nutritional and healthy demand [57].

The potato samples were analyzed for nutrient content by a number of studies, Provision of information has been shown that this crop contains essential amino acids, vitamins, and minerals, and is thus reported to play a significant role in human nutrition [58]. Many varieties of potatoes offer nutritional quantities of ascorbic acid (up to 42 mg/100 g), potassium (up to 693.8 mg/100 g), dietary fiber (up to 3.3%),

other bioactive components, such as protein with lower amounts (0.85%–4.2%) [59]. However, almost 50 % of potatoes' phenolic compounds are located in the peels and adjoining tissues [60,61], since peels have more phenolic compounds than tubers, these compounds could be used in food and non-food applications [62].

In the last decade, there has been increasing attention given to new sources of natural antioxidant phytochemicals as a result of their potential health benefits, in addition to their

functional properties in traditionally commercialized products such as preserving color and flavor and hence improving shelf life [63].

Antioxidant activity in potato tubers has been extensively reported [64], pigmented potato genotypes (mainly cultivars with purple and red flesh), as compared to those with white and yellow flesh, have been shown to contain significantly higher levels of antioxidants [65]. At present, the potato grown in the El-Oued county is mainly consumed in the form of fresh tuber in Algeria.

II.10) Materials references and goals

Little is known about the chemical composition or antioxidant capacity of potatoes' El-Oued county, but a great variation in cultivar condition and difference of genetic characteristics between varieties may be lead to diverse in their chemical composition.

Although there may be as many potato varieties cultivars in this region. The most four popular cultivars of (Solanum tuberosum L), were chosen to provide a reference basis for chemical composition values.

Four potatoes cultivars 'Bartina, Kondor, Kuroda, and Spunta' were collected in 2015 from different areas located in the region of El-Oued south-east of Algeria (Table II.6), were planted for three months before being used. Table II.7 describes the tuber physical characteristics of the four cultivars analyzed and Figure II.6 gives a visual representation of the five chosen cultivars.

Table II.6: location of four varieties of potatoes

Varieties	Area's name	Area's located
Bartina	Ourmas	33° 24' 32.7" N, 6° 46' 44.06" E
Kondor	Guemar	33° 30' 17.6 " N, 6° 47' 50.7" E
Kuroda	Trifaoui	33° 25' 1.6" N, 6° 56' 0.5" E
Spunta	Aarair	33° 17' 20.3" N, 6° 46' 19.5" E

Table II.7: Tuber physical Characteristics of the four potato cultivars investigated.

Potato Cultivar	Skin color	Flesh Color	Tuber skin texture	Tuber Size & Shape	Eye Depth
Bartina	Red	Light yellow	Smooth	Large to very large, Oval to round	Medium
Kondor	Red	Cream	Smooth	Large to very large, Oval to long	Medium
Kuroda	Red	Light yellow	Intermediate	Large to very large, Oval	Medium
Spunta	Yellow	Light yellow	Smooth	Large to very large, Long	Shallow

Note: Information for this table was gathered from THE EUROPEAN CULTIVATED POTATO DATABASE.
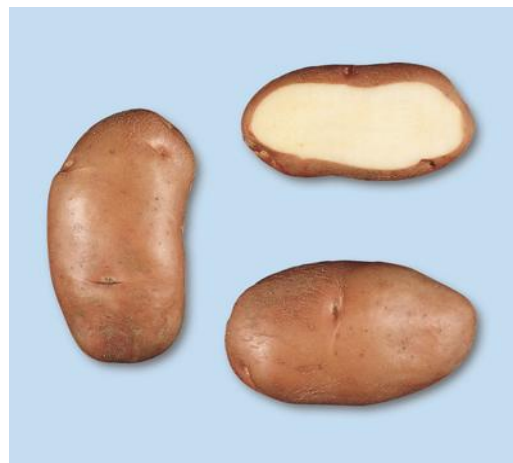
Bartina   Kondor

Kuroda   Spunta

Figure II.6: The visual appearance of the four potato tuber varieties analyzed in this thesis.

The overall goals of this thesis are threefold

1) To choose an extraction technique and a ratio of solvent which gives high yield extraction of bioactive compounds from one cultivated potato variety.

The literature research shows that the use of proper methods has an influence on the level of phytochemical compounds extracted from plant sample. Also, the extraction process is affected by several factors as solvent type, contact time, and others. These will be discussed in chapter IV, by focusses on the level of total phenolic and flavonoids content extracted which leads to conclude the best technique.

2) To investigate and quantification the chemical composition and antioxidant activity of the whole roots of four different variety of potatoes which are present the most popular cultivated in the region of El-Oued (Bartina, Kondor, Kuroda, Spunta). However, there are large differences in quantity of phytochemical compounds between potatoes cultivars. Thus, chapter V will aim to quantify the total phenolics and total flavonoids content, this investigation will complete with HPLC analysis.

The antioxidant activity of four samples measured by two techniques the first one is spectrophotometer assay and the second one is electrochemical (cyclic voltammetry) assay. Also, we will emphasize on comparing the results obtained by linear regression analysis.

3) To incorporate the characteristics of the bioactive compounds and antioxidant activities found in one variety which shows the highest level in phytochemical compounds, thus we will measure the potential antioxidant activity with other spectrophotometers assays.

As discussed in the first experiment (chapter V). The Kondor variety shows the highest phytochemical compounds we will undertake in order to assess the potential acceptability of peeled and unpeeled Kondor variety and compare the results in the second experiment in chapter V.

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Chapter III

Methods

III.1) Introduction :

For most analytical protocols, the procedure for the effective extraction of the specific compounds from the bulk matrix is one of the most important processes in the analytical mechanism [1]. The composition and nature (simple and/or complex) of the phytochemical compounds to be extracted determine the condition of the extraction process.

The extraction process is affected by the chemical structure of the phytochemical compounds (simple or complex compounds), the extraction technique employed by solvents, solid-phase extraction, and supercritical extraction, the conditions of storage time and temperature and the presence of the substance [2]. the polymerization of the phytochemical content is ranged from simple to high polymerized substance. Some plants contain different phenolic acids, phenylpropanoids, anthocyanins, and tannins, which can interact with other plant components such as primary metabolism substances (protein and carbohydrates) these compounds might be insoluble. That is why it is difficult to develop a process capable of recovering all the phytochemical substances contain in a plant matrix [3], which makes the choice of the condition extraction solvent and technique an important factor.

III.1) Methods:
III.1.1) Soxhlet:

Soxhlet extraction is a common example of an exhaustive solid-liquid extraction technique and its principle of operations depend on the transfer of the target compound from the plant (solid) to appropriate organic solvent(s) by ensuring that the extraction solvent (liquid) maintains continuous contact with the sample at the time of the heat-aided extraction process[4].

Figure III.1, shows the traditional Soxhlet extraction apparatus generally contain a distillation flask, the sample holder (thimble), siphon, and condenser. In practice, a preweighed and sufficiently dried solid sample is loaded on filter paper and placed inside the thimble. Solvent vapors generated from the heated flask containing the extraction solvent(s) pass through the thimble and become liquefied in the condenser.

The extraction process begins the moment the liquefied solvent maintains contact with the sample in the thimble. The level of the condensed solvent, therefore, continues to upgrade until it reaches the overflow level. At this point, an instant siphon effect rapidly makes the extract (solute + solvent) to fall back into the distillation flask.

Within the flask, only the fresh solvent evaporates resulting in a continuous cyclical

movement of the extraction solvent. A complete cyclical movement of fresh solvent from the distillation flask until it returns back to the flask as an extract is indicated to as a "cycle." The operation is repeated until complete extraction is achieved [5].

The cyclical movement of evaporated solvent from the flask that is subsequently condensed facilitates the mass transfer of the target analyte in every cycle completed during the extraction. Generally, the more the number of cycles completed, the faster the rate of extraction of the target analyte from the sample matrix [4].
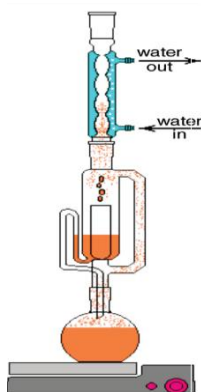
Figure III.1: Typical conventional soxhlet extractor

III.1.2) Maceration:

Maceration is a method used a long time ago in winemaking and widely used in medicinal plants research to extract essential oils and bioactive substance. this technique generally has several stages [6]. Firstly, crushed or cut the plant materials into small pieces to increase the surface area for proper mixing with solvent. Secondly, adding the menstruum (the appropriate solvent) in a closed container. Thirdly, 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. to facilitate the extraction and increase the yield, we shake the container this procedure increase the diffusion and bringing new solvent to the menstruum by removing the concentrated solution from the sample surface. the procedure of maceration is a slow process, taking a few hours up to several weeks, also can consume large volumes of solvent.

III.1.3) Ultrasound-assisted extraction

Most applications of ultrasound-assisted leaching contain systems using the bath or ultrasonic probe. This kind of equipment has been used for leaching organic and inorganic compounds with frequencies more than 20 kHz [7]. The plant materials placed in a flask which fixed an ultrasonic bath, the mechanical stress produced by ultrasonic on the cells through the production of cavitations in the sample made the cellular breakdown. this damage increases the solubilization of metabolites in the solvent and develops extraction yields [8]. The condition of sonication; frequency, time and temperature effect on the level of the extraction.

III.1.4) Several variables effect on extraction system

In general terms, the extraction productivity of a target compound is usually a function of several process variables. The most important factors concerning the extraction of phenolic compounds natural products are the solvent type, temperature, contact time, solvent-to-solid ratio, particle size, and among others.

The positive or negative effect of each variable on the mass transfer phenomenon, which governs the extraction process, is specific to different varieties of the vegetable matrix and is not always obvious. the extraction of soluble phenolic compounds can be performed by promoting their dispersion from plant tissue using a solvent.

Each vegetable material possesses unique properties that might interfere with the phenolic compounds' extraction. so it is important to develop optimal extraction technique for their quantification and identification [9].

III.1.4.1) Solvent

Solvent composition is always an important variable to be considered when dealing with the extraction process.

This variable should always be optimized in order to produce good extraction yields in an economically advantageous process. Many solvents can be used to extract phenolic compounds from plant matrices, ethanol, isopropanol, water, and their combination; these substances are classified with the GRAS status and, for that reason, water, isopropanol, and ethanol are suitable for the recovery of nutraceuticals [1].

Ethanol is reported to be appropriate solvent to recovery the phenolic compounds and, for that reason, it is usually used for the obtaining of this group of compounds, especially

when it comes to the production of nutraceuticals, which is related to its GRAS classification [1]. Ethanol, water, and their mixtures are the preferable solvent systems currently used for natural product production.

In that context, infusions (immersion in hot water) continue to be an interesting way to produce extracts with high contents of antioxidant compounds. further, the use of ethanol should also be considered [10].

III.1.4.2) Solvent-to-feed-ratio

According to mass transfer principles, the driving force during mass transfer is the concentration gradient between the solid and the bulk of the liquid, which is increased when the ration solvent to solid creased.

Therefore, according to mass transfer principles, independent of the extraction solvent used, the higher the solvent-to-solid ratio, the higher the total amount of solids obtained [11].

However, from economic side, the effect of consumption of the solvent on the extraction process cost, this variable should be carefully analyzed and optimized.

There for several studies have reported that the effect of the solvent-to-feed (S/F) ratio variation from 6:1 to 18:1 was evaluated in terms of recovery of the antioxidant agents from several plants [12]. The effect of the solvent-to-solid ratio on the recovery of phenolic compounds from different plant matrices was well studied by several authors [13-15].

III.1.4.3) Temperature

Many phytochemicals are heat stable and extraction at high temperature has no adverse effects; however there are some phytochemicals which are heat labile, In general, a higher extraction temperature causes an increase in the rate of diffusion of the soluble plant phytochemicals into the extraction solvent An increase in temperature can cause an augment in the content of some phytochemicals, which is possibly due to an increase in the solubility of many of these bioactive compounds [16-17].

Overall some phytochemicals such as anthocyanins the heat had an effect on anthocyanins degradation [18-19], therefore high temperatures for long extraction times would not be suitable for extraction of plants containing these compounds. However, for phenolic compounds, attention should be paid to their stability during the process; phenolic

compounds, when kept above certain temperatures for certain periods of time, can suffer thermal degradation (oxidation) and activity loss [20].

In this study, the temperature was ranged from 33°C in UAE extraction which reached by the extraction performed by UAE [21], to 70 °C (Ethanol boiling point) for Soxhlet extraction.

III.1.4.4) Time

The time necessary for the extraction of phytochemicals compounds from plant tissue will vary depending on the plant species to be extracted [10], According to Durling et all The concentration of the target compounds in the extract obtained after a 6-h extraction time was 20% higher than that observed for the sample related to the extraction period of 1 h [12]. Increased contact time between solvents like ethanol and solid matrices might lead to a progressive release of solute from the solid matrix to solvent [22].

Generally, the extraction time using for UAE process ranged from 10–60 min, and for Soxhlet extraction ranged from 6–24 h [23-25].

III.1.4.5) Particle size

The variation of in particle size of the sample has a strong influence on The quantity of polyphenol recovery from plant materials.

Mass transfer can be improved by the use of smaller particles to improve the penetration of solvent in the solid matrix [10]. The bigger the particle size of the material the longer the path that the solvent has to travel, which increase the time for maximum phytochemical content to be extracted [26].

Also, the damage of the plant cells caused by the crushing or milling the plant material lead to increased extraction of phytochemical compounds.

III.2) Estimation of the water weight percentage in potato roots

No agricultural product in its natural state is completely dry. Some water is always present. The percent moisture is an expression used for the description of the quantity of water in plant material, and for this purpose, we usually use two methods.

These methods are the wet basis and dry basis. In addition, the content may be expressed as a percent or as a decimal ratio [27]. In our work, we will use one form (percent) in analyzing moisture in potatoes roots calculating by the following equation.

$$W_a = \left(\frac{m_0 - m_1}{m_0}\right) \times 100 \qquad (1)$$

where:

W_a is the moisture content of the sample, (m/m)%

m_0 is the sample weight before drying, g

m_1 is the sample weight after drying, g

Two or three parallel measurement has to prepare. The final sample moisture content will be the mean of the two or three parallel measurements.

III.3) Phytochemical investigation

Analyses of plant materials based on two methods non-chromatographic spectrophotometric methods or chromatographic methods. Spectrophotometric methods are based on the ability of the phytochemicals that absorb light in the ultraviolet (UV) or visible range of the spectrum (e.g. total flavonoids content), or the ability to form such chromophores after reacting with certain reagents such as total phenolic content, and this quantification depending on Beer-Lambert law. This approach does not require separation of individual compounds in the extract, and often quantification is done as the total amount of similar compounds in the extract.

The advantages of the non-chromatographic spectrophotometric methods are simple, fast, and of low cost, but these methods lack the specificity for individual compounds and the results are less precise [28].

III.3.1) Screening chemistry

Phytochemical analysis was determined for all the extracts using the standard methods.

III.3.1.1) Detection of flavonoids:

The detection of flavonoids done by Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.[29,30]

III.3.1.2) Detection of phytosterols :

a) Liberman and Burchurd's: The test was done after the extraction and reflux of the plant material. 2 ml extract taken in a test tube. 2 ml Chloroform, 2 ml Acetic Anhydride and 2 ml concentrate Sulphuric acid was added to it; translucent green color shows the presence of steroids and formation of deep red color indicates the presence of triterpenoids [31,32].

b) Salkowski's test: Dissolve the extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow to stand for some time, red color appears in the lower layer indicates the presence of sterols and formation of yellow colored lower layer indicating the presence of triterpenoids [32].

III.3.1.3) Detection of tannins:

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration [33]

III.3.1.4) Detection of carbohydrates:

Fehling's Test: Filtrates were hydrolyzed with diluted. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.[30,34]

III.3.1.5) Detection of alkaloids:

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.[32]

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.[32]

III.3.1.5) Detection of coumarins:

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow color indicates the presence of coumarins [31,35]

III.3.2) Total flavonoids content (TFC)

a) Principle

The estimation of the total flavonoids content generally used by aluminum chloride method. This method is based on flavonoids' capability of forming a stable complex with Al ions in a solution.

The color of the complex depends on the ratio of the Al ions to the flavonoid molecules and the hydroxylation pattern of the latter. For this reason, the spectrophotometric readings used in this method can vary from 367 to 510 nm in different experimental procedures.[36]

b)Protocol

Total flavonoids content was determined as described previously by ZOU et AL (2004) [37]. A mixture of 1 ml of extract, 2 ml of nanopure water and 0.15 ml of 5% $NaNO_2$ was prepared and allowed to react for 6 min. Then 0.15 ml of 10% $AlCl_3$ solution was added and mixed thoroughly. After 6 min, 2 ml of 4% NaOH solution was added and allowed to stand for another 15 min. The absorbance of the mixture was measured at 510 nm versus prepared blank. Rutin was used as a standard compound for the quantification of flavonoids content. Results were expressed in mg of rutin equivalents/ g of extract

III.3.3) Total phenolics content (TPC):

a)Principle

The Folin-Ciocalteu (FC) assay is the most widely used method for the estimation of total phenolic content (TPC) in extracts fruits, vegetables, grains, and other foods. The FC reagent consists of an oxidizing mixture of phosphotungstic acid and phosphomolybdic acid which, when reduced, produce a mixture of blue molybdenum and tungsten oxides (λ max 765 nm). Technically all compounds that can be oxidized by the FC reagent will be measured [38].

b)Protocol

Total phenolics content was determined with the Folin-Ciocalteu reagent according to a method described by Singelton-Ross (1956)[39]. Briefly, 100 µl of the appropriately diluted extract was added to 0.5 ml of freshly diluted 10-fold Folin-Ciocalteu reagent. Then 2 ml of

20% Na_2CO_3 solution. The reaction mixture was kept in the dark for 30 min, and its absorbance was measured at 760 nm against a blank. Gallic acid was used as a reference standard and the results were expressed as gallic acid equivalents/ g of extract.

III.3.4) HPLC analysis:

liquid chromatography is a powerful separation method used in phytochemical studies. Practically, this technique can be able to resolve mixtures with a large number of similar analytes, HPLC is very efficient, it yields excellent separations in a short time [40].

III.3.4.1) Principe

The procedure of HPLC is depended on the flow of solutes through two non-miscible phases inside column (mobile phase and stationary phase) to be separated [41]. For this, the sample is dissolved in the mobile phase, However, this phase is in constant movement relative to the stationary phase. this movement due to the interactions of the constituents with the stationary phase, they separate after sufficient running time and they appear at the end of the column at different times [40], this detection expressed as chromatograms.

III.3.4.2) HPLC system

According to the basic diagram shown in Figure 2 an HPLC system contains the following elements:
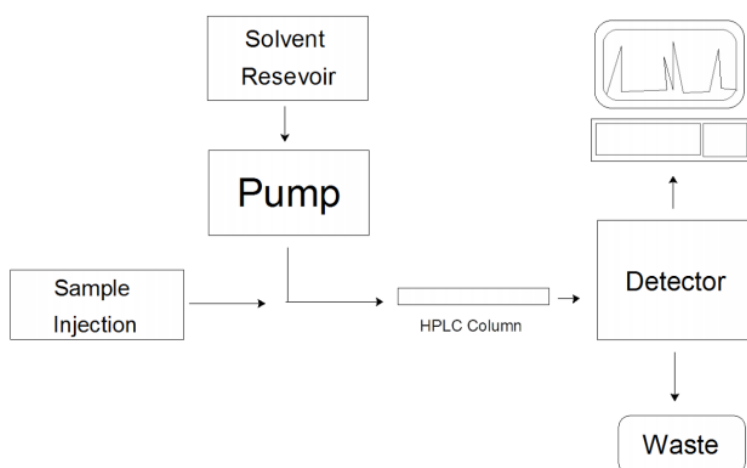
Figure III.2: Block Diagram of an HPLC

a) Solvents

Requirements for the solvents used in HPLC are filtering and degassing. Filtering the solvents will get rid of particulates that might contaminate the sample and can block the flow through the HPLC column. Particulates that are large enough can cause permanent damage to the column. Degassing helps to remove dissolved gases that are in the solvent. This is accomplished by pulling a vacuum on the solvent container while vibrating or sonicating. Both the particulates and the dissolved gas bubbles can also cause the efficiency of the separation to go down. Dissolved gas bubbles reduce separation efficiency by causing band broadening and interfering with some detection systems [42].

b) Pumps.

Pumps. These are devices that deliver the mobile solvent at a controlled flow rate to the separation system. The pumping system needs to be of a high enough quality that it can consistently produce a high-pressure [43]. High-pressure gradients that are reached by these pumps provide more accurately different gradients maintained by microprocessor control. In the gradients, up to four solvents are presented in a constant relationship.

c) Injection systems

Injector this device allows the addition of the liquid sample to the mobile phase. Sample injection systems, which work automatically are preferred for prominent precision in sample introduction [44].

d) Column

In the analytical model, stainless steel columns generally have an internal diameter of 4.6mm. The length is 5, 10, 15, or 25cm. The filling (silica, grafted silica or polymeric particles) has a particle size of 3, 5, or 10 u.m. The internal diameter of a column is usually 4 or 4.6 mm. If pure substances must be collected at the end of the chromatogram large diameter columns will be required [45].

e) Detectors

An ideal detector of any type is sensitive to low concentrations of every analyte, provides a linear response, and does not expand the eluted peaks. according to the characteristic of the molecules investigated, various detectors are used, such as its absorption in UV range or its fluorescence. This is the most frequent method used, and its application allows for over 70 % of detections [45].

III.3.4.3) Protocol

The phenolic compounds were analyzed by HPLC. A LC-18 column (150 mm x 4.6 mm i.d. x 5 mm), maintained at 30 °C, was employed. Elution was performed at a flow rate of 1 mL•min-1, using a mobile phase composed of water/acetic acid (98:2, v/v) (solvent A) and acetonitrile (solvent B) in gradient elution mode. The elution gradient was: 0 - 5 min, 5 % B; 10 min, 10 % B; 11 min, 20 % B; 20 min 20 % B; 30 min 40 % B; 40 min 50 % B; 50 min 80 % B.

Chromatograms were acquired at 300 nm. Phenolic compound standards ascorbic acid, gallic acid, chlorogenic acid, vanillin, rutin were dissolved in extraction solvents and used for identification of phenolic acids presents in different sample extracts. Peak identification in HPLC analysis was achieved by comparison of retention time and UV spectra of reference standards.

Quantification of individual phenolic compounds in the extracts was done using the peak area of reference compounds and reported as µg/mg of extract

III.4) Antioxidant activity

According to its definition, an antioxidant should have a significantly lower concentration than the substrate in the antioxidant activity test. Depending on the type of reactive oxygen species (ROS) and target substrate, a certain antioxidant may play a completely different action or have a completely different role/performance. In line with this, some studies support the use of a selection of methods to measure the antioxidant activity [46-47]. The choice of substrate is very important in an antioxidant activity test. Depending on the type of substrate and its amount/concentration, different results will be achieved.

In our study, the methods used to determine total antioxidant activity devised in two techniques, First one is spectrophotometers methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and ferric ion reducing antioxidant parameter (FRAP) assay. Secondly, an electrochemistry technique, in this process we used cyclic voltammetry methods to determine the antioxidant activity.

III.4.1) Spectrophotometers assay

The Spectrophotometry is a quantitative analytical method that involves measuring the absorbance or optical density of a given chemical substance in solution. More this species is concentrated plus it absorbs light within the limits of proportionality laid down by the law of Beer-Lambert. The optical density of the solutions is determined by a spectrophotometer previously calibrated to the absorption wavelength of the chemical species to be studied. when intensity light I_0 passes through a solution, part of it is absorbed by the solute (s). Therefore, the intensity I of the transmitted light is less than I_0 [48].

The absorbance of the solution is a positive value and defined as follows:

$$A = Log_{10}\left(\frac{I_0}{I}\right)$$

Or $\quad\quad A = -logT \quad\quad\quad\quad$ When $\quad\quad\quad T = \frac{I}{I_0}$

III.4.1.1) DPPH assay

a) Principle

The 1,1-diphenyl 1-2-picrylhydrazyl (DPPH$^{\bullet}$) - which is also known as α,α-diphenyl-β- picrylhydrazyl, 2,2-diphenyl -1- picrylhydrazyl or 2, 2-Diphenyl -1-(2,4,6-Trinitrophenyl hydroxyl) assay originally described by Boils (1958) was designed to take advantage of a common electron spin resonance reagent, a stable free radical with an odd, unpaired valence electron to study antioxidant activity . With its odd electron, DPPH$^{\bullet}$ can be stabilized by accepting an electron or hydrogen radical from an antioxidant molecule such as a gallic acid as a reducing agent. DPPH$^{\bullet}$ is known for its deep violet color and the strong absorbance at 517nm when dissolved in ethanol at concentrations between 1mM and 22.5µM [49-51]; this absorbance is decreased with the decolorization of DPPH$^{\bullet}$ which accompanies the pairing of the lone electron.

b) Protocol

The scavenging activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method according to Ohinishi and others [52]. with some modifications, a 1 ml of diluted extract and 1 ml of DPPH (0.25 mM) were mixed, and after 30 min the absorbance was measured at 517 nm. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. Results were expressed as radical scavenging activity percentage (%) of the DPPH according to the formula:

$$I\% = [(A_0 - A_s)/A_0] \times 100$$

Where:

A_0: absorbance of the control.

A_s: absorbance of the sample.

III.4.1.2) Evaluation of total antioxidant capacity (TAC) by Phosphomolybdenum method

a) Principle

The total antioxidant capacity of the ethanol extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al. [53].

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH.

c) Protocol

The antioxidant activity of different samples was evaluated by the phosphomolybdenum method of Prieto et al [53]. An aliquot of 100 μl of the sample, was combined with 1ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. After the samples were cooled to room temperature, the absorbance was measured at 695 nm. The antioxidant capacity was expressed as equivalent to ascorbic acid (mg ascorbic acid/ g of extract).

III.4.1.3) Ferric-reducing antioxidant power (FRAP) assay:

a) Principle

The FRAP assay is based on the single electron transfer by an antioxidant to reduce the ferric to ferrous ion; when the ferric- tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous counterpart, the complex absorbs at 593 nm with an intense blue color [55].

b) Protocol

The FRAP assay was performed based on the procedure described by Benzie and Strain [54], 100 μl of the diluted sample was added to 300 μl water, then 3 ml of the FRAP reagent and the reaction was monitored after 30 min in the dark at 593 nm. The results were expressed as mg Fe(II)/ g of extract

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III.4.1.4) Beta-carotene bleaching

a) Principe

This method first developed by Marco [55] and modified by Miller [56].

This technique is sensitive and mainly based on the principle oxidation of linoleic acid, which is an unsaturated fatty acid, by "Reactive Oxygen Species" (ROS) produced by oxygenated water. This complex formed will oxidate the b-carotene, this oxidation will lead to discoloration. The retarding of this oxidation correlate with the addition of an antioxidation compounds [57].

The degradation of b-carotene by linolic acid catalyzed with heat 50 °C in the aqueous phase, the activity is measured at 470 nm [58].

b) Protocol

Two milliliters of a solution of β-carotene in chloroform (2mg/10ml) was pipetted into a flask containing 40mg of linoleic acid and 400 mg of Tween 80.The chloroform was removed by rotary vacuum evaporator at 45℃ for 4min, and 100 ml of distilled water was added slowly to the semisolid residue with vigorous agitation to form an emulsion. A 4.8ml aliquot of the emulsion was added to a tube containing 0.2ml of the antioxidant solution, the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene [59]. The tubes were placed in a water bath at 50℃, and the absorbance measurements were conducted again at 15 min intervals up to 120min.All determinations were carried out in triplicate [60]. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β-carotene using the following formula:

$$AA = [1 - (A_0 - A_t)/(A_0' - A_t')] \times 100$$

where:

A_0 is the absorbance of values measured at zero time of the incubation for the test   sample

A_0' is the absorbance of values measured at zero time of the incubation for test control

A_t is the absorbance's measured in the test sample.

A_t' is the absorbance's measured in the test control.

After incubation for 120 min, the total antioxidant activity of acid ascorbic and Gallic acid was also measured by inhibition of β-carotene bleaching method for comparison

III.4.2) Electrochemical techniques

III.4.2.1) Principle

Recently, the Electrochemical technique was applied to determine the antioxidant content and antioxidant capacity in the phytochemical analysis, these interest based on their advantages of sensitivity and fastness process [61]. The broadest technique in electrochemistry used for redox system is Cyclic voltammetry. The CV record the anodic current produced by the antioxidant compounds which are oxidized on the surface of the working electrode, while the potential of a working electrode is linearly scanned from an initial value to a final value and back [62]. The current produced is proportional to the combined concentration of the antioxidants [63,64]. This process can happen multiple times during a single experiment. The current produced is plotted versus the applied potential to give the cyclic voltammogram as shown in Figure III.3.
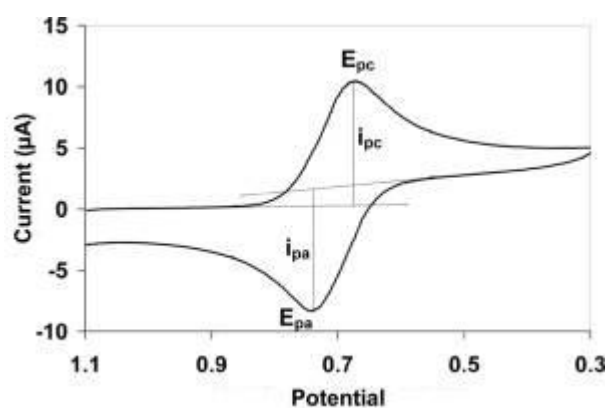
Figure III.3: cyclic voltammogram for redox system

The cyclic voltammogram contains the cathodic peak Ic and anodic peak Ic, the anodic oxidation potential (Ea), and the cathodic oxidation potential (Ec). If the only cathodic peak is present and anodic peak is absent then the process is irreversible and for the reversible system, the values of Epc and Epa are equal [65].

III.4.2.2) Protocol of antioxidant activity

Electrochemical measurements were performed on a Potentiostat. voltammetric signals of Cyclic Voltammetry and square-wave voltammetry were recorded at room temperature in an electrochemical cell with a volumetric capacity of 25 mL containing a working electrode was glassy carbon, a saturated calomel $Hg/Hg_2Cl_2/KCL$ electrode was used as a reference and a platinum wire was used as counter electrode. The glassy carbon was polished before every

experiment. The antioxidant capacity measured of sample varieties was in a buffer solution of acetate (300 mM pH=3.6), Cyclic voltammograms were obtained by a single cycle performed at a scan rate of 100 mVs^{-1} and potentials range +200 to +1400 mV. Gallic acid was used as a standard in the calculation of antioxidant capacity of the studied sample of potatoes because of its wide-spreading in nature and also because of its anodic area display excellent linearity toward gallic acid concentration [66].

III.4.2.2) Protocol for scavenging activity of superoxide anion $O_2^{\bullet-}$ radicals

Superoxide anion radical was generated electrochemically by one electron reduction in the commercial molecular oxygen in ACN containing 0.1 M TBFB at room temperature (28 ± 1 °C). The samples of each extract were added to this reaction medium, and the cyclic voltammograms were recorded following a modified method of Le Bourvellec et al [67]. Experimentations were carried out using voltalab40 PGZ301 potentiostat/galvanostat (radiometer analytical SAS) in a double-walled electrochemical cell of 25 mL, and the conventional three-electrode system was employed. A glassy carbon (GC) working electrode (radiometer analytical SAS), having area 0.013 cm2, a platinum wire counter electrode and a Hg/Hg2Cl reference electrode (3.0 M KCl) were used. Data acquisitions were accomplished with a Pentium IV (CPU 3.0 GHz and RAM 1 Gb) microcomputer using VoltaMaster4 software version 7.08 (radiometer analytical SAS). The scan rate was kept at 100 mV/s, and potential window was from −1.4 to 0.0 V. The studied extract samples were added to the in situ generated superoxide anion radical $O_2^{\bullet-}$, and the cyclic voltammograms were recorded

III.5) Calibration curve

Evaluation of the linearity of the calibration curve is favored to prove the acceptability of any analytical method [68]. Generally, to verify the linearity, five concentration levels of standard solutions are required to construct the regression line of the calibration curve. In this study, a linearity test was performed by plotting the calibration curve between the standard concentration and the absorbance wave for spectrophotometers analysis and current peaks for electrochemical analysis. The linearity can be investigated through the correlation coefficient (R), usually used to determine the linearity of the calibration curve. In chemical correlation analysis, the coefficient of determination (R^2) is the more exact term used [69]. In this study, the linearity criterion was chosen using R^2 of the regression line, which is suggested to be 0.90 or more.

III.5.1) Totale phenolic content

The linearity of the calibration curve was tested at ranges of concentrations (0.03 – 0.3 mg/mL) prepared of Gallic acid as standard solutions of total Phenolic content the equation obtained from the linear calibration graph in the studied concentration   Y = 3.14x+0.046 (R² = 0.990) where y represents the value of the Absorbance and x, the value of standards concentration.
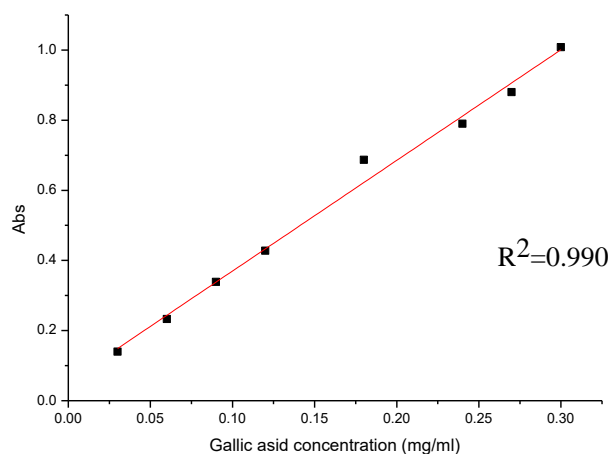
Figure III.4: The calibration curve obtained for Gallic acid

III.5.2) Total Flavonoid content

The linearity of the regression line of the calibration curve was evaluated by preparing standard solutions of Rutine in ethanol at concentration ranges of 0.05 – 0.25 mg/mL for total Flavonoids content. The equation obtained from the linear calibration graph in the studied concentration Y=1.92X-0.00567 (R² = 0.997) where y represents the value of the Absorbance and x, the value of standards concentration.
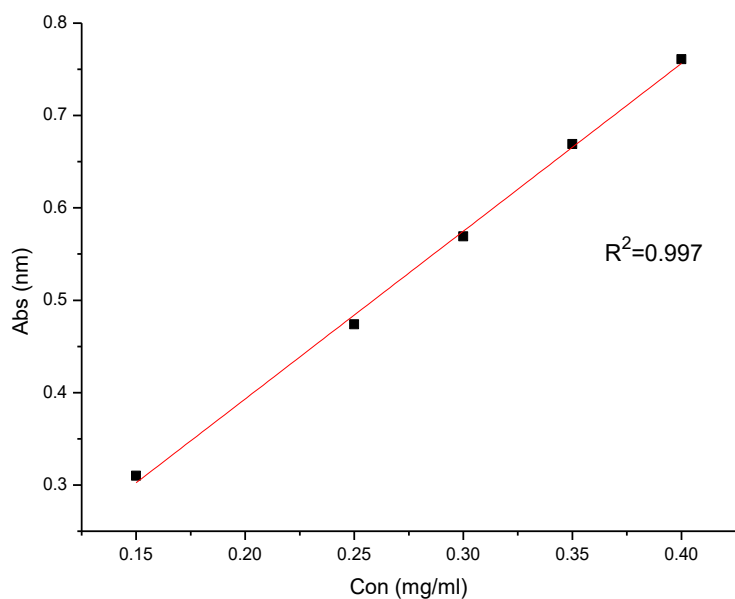
Figure III.5: The calibration curve obtained for Rutine

Figure I. 21Figure III.5: The calibration curve obtained for

III.5.3) FRAP assay

Ferrous sulfate ($FeSO_4$) at concentration ranges 0.05 – 0.4 mg/mL were prepared as standards solution to assess the linearity of the calibration curve for FRAP antioxidant assay. The equation obtained from the linear calibration graph in the studied concentration Y= 2.34746X-0.03929 (R^2 = 0.993) where y represents the value of the Absorbance and x, the value of standards concentration.
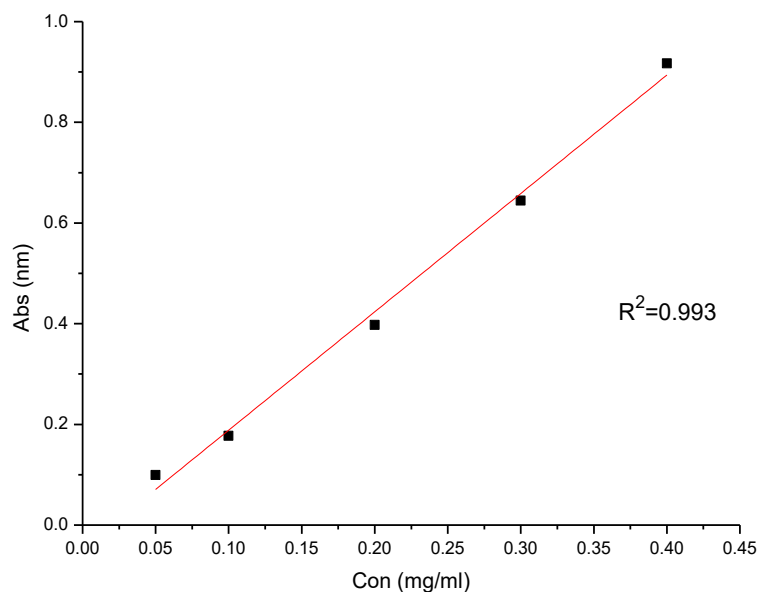
Figure III.6: The calibration curve obtained for Ferrous sulfate

III.5.4) Total antioxidant capacity

Linearity was evaluated through the regression line of the calibration curve. The calibration line of Ascorbic acid at a different concentration from 0.07 to 0.3 mg/mL for Total antioxidant capacity. The equation obtained from the linear calibration graph in the studied concentration Y= 3.20228X+0.03727 (R^2 = 0.990) where y represents the value of the Absorbance and x, the value of standards concentration.
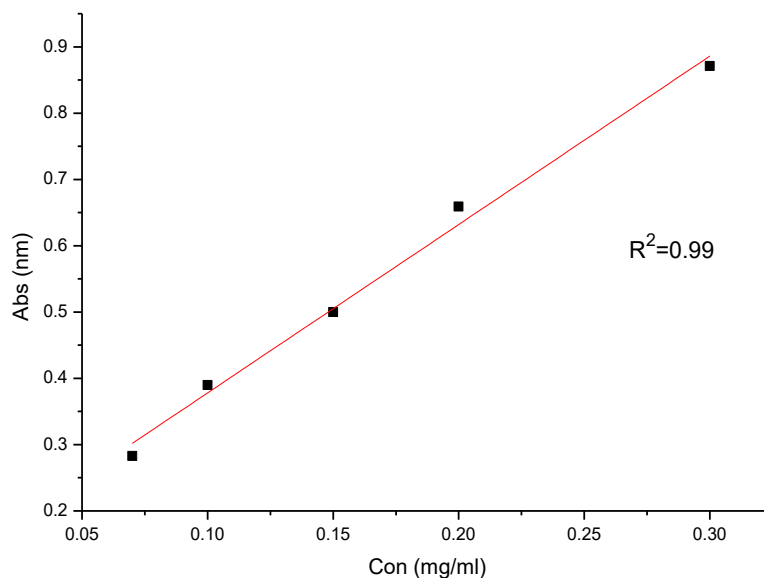
Figure III.7: The calibration curve obtained for Ascorbic acid TAC

III.5.5) HPLC calibration curve

Figure 7 shows the chromatograms of seven mixed standards which analyzed in 300 nm waves length and concentration (1ug/ml), peaks from 1 to 7 were Ascorbic acid, Gallic acid, Chlorogenic acid, Cafiec acid, Quercetin, Vanillin and Rutine respectively. The linearity of standard preparations was tested. The calibration curves were constructed by plotting the peak area of each compound against the corresponding concentrations. The five points of the regression line offered good linear behavior in the ranges 0.02 – 0.1 and 0.2 – 1.0 µg/mL. The coefficient of determination (R^2) values as shown in the figures below were found to be acceptable and close to 0.990, which was the R^2 selected for the linearity criteria in this study. The amount of this standard in samples extracts was determined according to their calibration curves of retention time and area (Table III.1).
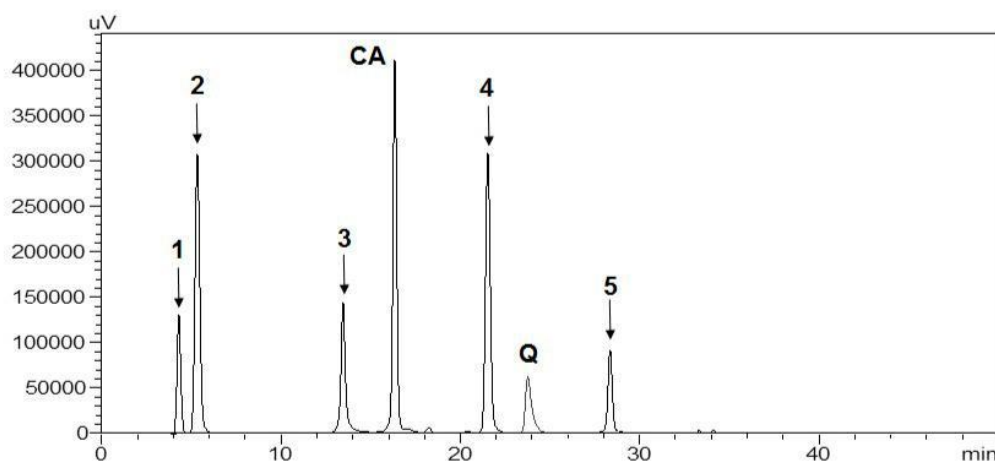
Figure III.8: Chromatograms of standard phenolic compounds:1ascorbic acid;
2.gallic acid; 3. chlorogenic acid; CA. caffeic acid; 4. vanillin; Q. quercetin; 5. rutin

Table III.1: Constituents Standard content analyzed by HPLC

Standard	R_t(min)	Equation	R^2
Ascorbic acid	4.21	Y=75728X	0.9614
Gallic acid	5.23	Y= 23616X-7232	0.9986
Chlorogenic acid	13.62	Y=39775X-1881	0.9983
Caffeic acid	16.3	Y=72328X	0.9986
Quercetin	20.37	Y=548X-2823	0.996
Vanillin	21.46	Y=82773X-1423	0.9984
Rutine	28.22	Y= 2411298X-1060	0.995

The equation obtained from the linear calibration graph in the studied concentration range for gallic acid in Cyclic Voltammetry (Figure 2b), $Y = 215.3X - 0.2432$ ($R^2 = 0.999$) where y represents the value of the area of the anodic wave and x, the value of standards concentration, expressed as mg/ml.
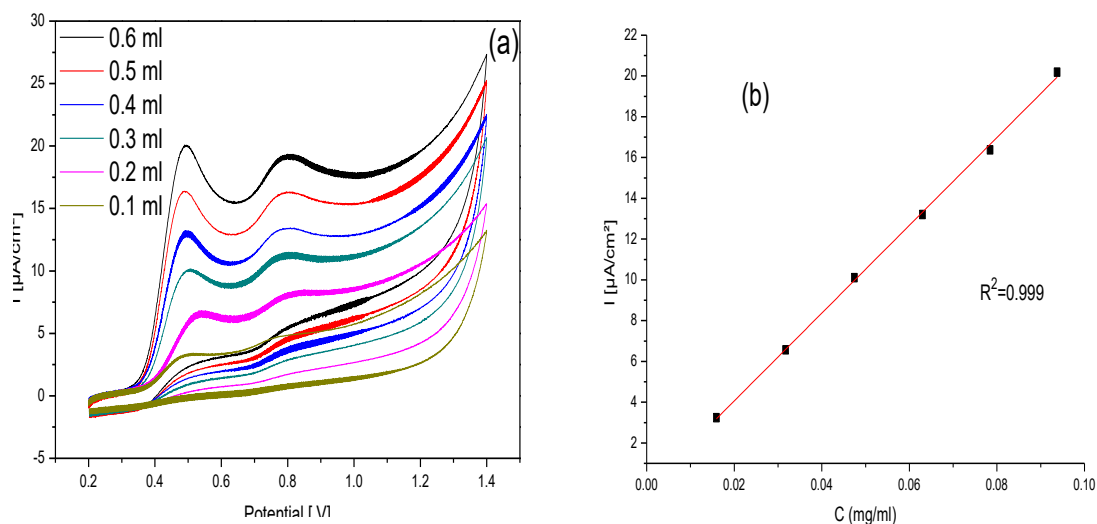
Figure III.9: Voltammograms obtained for gallic acid (a) and (b) The calibration curve obtained for gallic acid by cyclic voltammetry in pH=3.6 acetate buffer

III.6) Determination of IC$_{50}$ value

On the basis of screening results of triplicate measurement of the extract, the inhibition concentration (IC$_{50}$) value was determined from extrapolating the graph of scavenging activity versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50% value higher the antioxidative effects [70]. The effective concentration having 50% radical inhibition activity expressed as mg extract/ml.

III.7) Statistic analysis

The statistical analysis is descriptive methods aim to summarise empirical data expressed in the quantitative form. The interpretation of collecting data for any kinds of topics in technology information used statistical tools such as mean, median, mode and standard deviation [71].

III.7.1) Linear regression analysis

Linear regression analysis is the most widely used of all statistical techniques: it is the study of linear, additive relationships between variables. Let Y denote the "dependent" variable whose values you wish to predict, and let X_1, …,X_k denote the "independent" variables from which you wish to predict it, with the value of variable X_i in period t (or in

69

row t of the data set) denoted by X_{it}. Then the equation for computing the predicted value of Y_t is:

$$Y = b_0 + b_1 X_{1t} + b_2 X_{2t} + \cdots + b_k X_{kt}$$

This formula has the property that the prediction for Y is a straight-line function of each of the X variables, holding the others fixed, and the contributions of different X variables to the predictions are additive. [72]

III.7.2) Main effect plot and interaction plot

For two-way data, an interaction plot shows the mean value of the response variable for each combination of the independent variables. This type of plot gives us some understanding of the effect of the main factors and their interaction. When main effects or interaction effects are statistically significant, post-hoc testing can be conducted to determine which groups differ significantly from other groups.[73]

III.7.3) p-values and R-squared values.

p-values and R-squared values measure different things. The p-value indicates if there is a significant relationship described by the model, and the R-squared measures the degree to which the data is explained by the model. It is, therefore, possible to get a significant p-value with a low R-squared value. This often happens when there is a lot of variability in the dependent variable, but there are enough data points for a significant relationship to be indicated [74].

In this study, all the experiments data were performed in triplicate, and the results were expressed as mean ± SD (standard deviation) calculated by Excel 2007. These means were compared using analysis of variance, (ANOVA) using the Minitab version 17 (SAS) and Linear regression, the data obtained were considered statistically significant at $p < 0.05$ and very statistically significant at $p < 0.005$.

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Chapter IV Comparative study of extraction methods

IV.1) Introduction

Sample preparation is a critical step of a successful analytical method. Again, due to the large variations in sample matrices, for example, plant materials, food formulations, biological fluid and tissue samples, the diverse chemical structures, and physicochemical properties of the phytochemicals, it is unrealistic to develop any definitive procedure or protocol for all types of sample matrices. However, there are important common precautions that must be taken for better preparing samples for the subsequent analyses. The overall purposes of sample preparation are to concentrate or dilute the samples so the analytes can be detected and quantified within the detection limit and linear range; to rid any interference that might affect the detection of the compounds of interest. Therefore, techniques adopted to sample preparation must follow these two principles [1]. The aim of extraction is to maximize the yield of compounds of interest while minimizing the time and solvent.

IV.2) Aim

To decrease the yield of phytochemical compound extract from potato sample. We elected to compare traditional extraction techniques as Soxhlet extraction, maceration extraction and modern extraction techniques as Ultrasound-assisted extraction, this three techniques used to extract the bioactive compound from red potatoes cultivars, Kondor. In this chapter, we looked for technique with less time and solvent which gives a higher yield.

IV.3.2) Preparation of samples

Immediately after receiving, all samples of raw potatoes were peeled using a kitchen knife and prepared by simply cutting into 5 mm thick slices. 20 g of the slices of freshly peeled potatoes was extracted using 120 mL extraction solution (1:6, S-f-ratio)which is consisted of a mixture of ethanol and water at a ratio of (70:30 v/v). Subsequently, all the samples taken after the treatment were filtered before analysis for removal of potato particles, then the filtrate was concentrated under reduced pressure at 60 °C by rotary evaporator and stored in a refrigerator.

IV.3.3) Ultrasonic extraction

In this study the ultrasound-assisted extraction procedure was used for the extraction, thus samples were further submitted to ultrasonication in bath (40 kHz) at three levels of

power 30 %, 50 % and 70 %, the extraction being performed at T = 33 ± 4 °C. The extracts were kept to macerate for 5 min, 2 h and 24 h, in dark at room temperature.

IV.3.4) Soxhlet extraction

The sliced fresh potatoes (20 g) were continuously extracted with 120 mL using the appropriate solvent mixture for 3 h (5 cycles) at a maximum temperature of 70 °C in a Soxhlet apparatus.

IV.3.5) Results and discussion

IV.3.5.1) the total flavonoids content

Table IV.1 and 2 show the amount of TFC obtained from the extract using UAE with (70:30 ethanol: water) and 100 % ethanol as solvent.

Table IV.1: Total flavonoids content* obtained using ethanol: water (70:30)

Maceration time	ULTRASOUND			SOXHLET
	Amplitude			
	30 %	50 %	70 %	
5 min	9.3230 ± 0.043	13.073 ± 0.010	8.5319 ± 0.003	6.9446 ± 0.019
2 h	11.7648 ± 0.007	9.4793 ± 0.007	12.0140 ± 0.004	
24 h	14.4272 ± 0.002	13.0321 ± 0.043	11.0244 ± 0.007	

*Total flavonoids content was expressed as mg rutin / g extract

Table IV.2: Total flavonoids* content obtained using 100 % ethanol

Maceration time	ULTRASOUND			SOXHLET
	Amplitude			
	30 %	50 %	70 %	
5 min	63.3336 ± 0.01	41.1461 ± 0.027	17.1184 ± 0.013	11.3242 ± 0.024
2 h	61.1119 ± 0.006	51.9105 ± 0.006	23.7503 ± 0.005	
24 h	85.9383 ± 0.008	69.1670 ± 0.049	58.2295 ± 0.018	

*Total flavonoids content was expressed as mg rutin / g extract

The UAE showed an advantage over SE, but a ratio of 100 % ethanol favored the extraction process resulting in an increase of TFC for the two techniques. The amount of TFC

obtained after 5 min of ultrasound treatment was higher compared to SE at the two ratios 6.9446, 11.3242 mg rutin/g extract (Soxhlet, UAE at 70/30 solvent ratio) and 8.5319, 17.1184 mg rutin/g extract for (Soxhlet, UAE at 100 % solvent ratio), the three amplitude chosen by UAE increase the amount extracted from 17.1184 mg rutin / g extract at 70 % amplitude to 63.3336 mg rutin/g extract at 30 % of amplitude. Longer times of maceration following ultrasound extraction lead to the increase of the amount of TFC in the extract.

IV.3.5.2) The total phenolic content

The concentration of TPC in the extracts expressed as mg GAE/g sample depends on the solvent and the method of extraction, as shown in Tables IV 3 and 4.

Table IV.3: Total phenolic content* obtained using ethanol: water (70:30)

Maceration time	ULTRASOUND			SOXHLET
	Amplitude			
	30 %	50 %	70 %	
5 min	6.7781 ± 0.059	7.9936 ±0.067	5.1273 ± 0.032	6.7788 ± 0.017
2 h	9.3736 ±0.017	7.4522 ±0.094	6.9002 ±0.022	
24 h	10.3821 ± 0.007	8.1347 ± 0.018	7.2793 ± 0.007	

*Total phenolic content was expressed as mg gallic acid / g extract

Table IV.4: Total phenolic content* content obtained using 100 % ethanol

Maceration time	ULTRASOUND			SOXHLET
	Amplitude			
	30 %	50 %	70 %	
5 min	17.5053 ±0.053	14.4904 ± 0.003	6.9426 ± 0.016	13.9490 ± 0.023
2 h	15.2229 ± 0.065	14.4378 ± 0.016	9.2993 ± 0.029	
24 h	22.9299 ± 0.062	14.0127 ± 0.064	13.8694 ± 0.122	

*Total phenolic content was expressed as mg gallic acid / g extract

The content of phenolic compounds in the UAE extraction increases from amplitude 70 % (6.9426 mg gallic acid / g extract) to 30 % (17.5053 mg gallic acid / g extract). Also, the maceration time after sonication of the extract has an effect on the level of TPC in samples, e.g. 24 hours of maceration led to the highest TPC with 22.2999 mg gallic acid/g extract, however using 5 minutes maceration time gave 17.5053 mg gallic acid/g extract. The best

results were recorded using 100 % ethanol which showed an advantage on the ratio of 70:30 (ethanol: water). The results obtained from SE increased from 6.77 (using 70:30 ethanol: water) to 13.94 (using 100 % ethanol), but are remarkably lower than those obtained by UAE.

IV.3.5.3) The antioxidant capacity

The antioxidant capacity was evaluated using the phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm (Tables IV. 5 and 6). The results show an increase of TAC at a lower amplitude and higher maceration time CA = 10.6776 to 15.3348 mg ascorbic acid/g of extract for the solvent ratio (70:30 ethanol: water) and 36.3946 to 41.3346 mg ascorbic acid/g of extract for 100 % ethanol. It is clear that The TAC with UAE-M has an advantage over Soxhlet at two ratios of solvent.

Table IV.5: Total antioxidant capacity* obtained using ethanol: water (70:30)

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	10.6776 ± 0.006	10.6838 ± 0.013	9.9551 ± 0.017	13.8983 ± 0.233
2 h	15.0973 ± 0.031	11.0793 ± 0.008	13.2861 ± 0.039	
24 h	15.3348 ± 0.003	9.7450 ± 0.025	15.2847 ± 0.015	

*Capacity antioxidant was expressed as mg ascorbic acid / g of extract

Table IV.6: Total antioxidant capacity* obtained using 100 % ethanol

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	36.3946 ± 0.022	20.2811 ± 0.079	11.3493 ± 0.014	23.1124 ± 0.029
2 h	28.4836 ± 0.044	20.8804 ± 0.015	19.5108 ± 0.060	
24 h	41.3346 ± 0.057	39.0802 ± 0.103	25.3192 ± 0.064	

*Capacity antioxidant was expressed as mg ascorbic acid / g of extract

IV.3.5.4) Statistic analysis

Fig IV.1,2 and 3 shows the overall effect of maceration time (Fig 1a,2a, and 3a). amplitude (Fig. 1b,2b and 3b). method (Fig.1c, 2c, and 3c) and the solvent ratio (Fig.1d, 2d, and 3d) on the TFC, TPC extracted from the potatoes and their TAC. Table 7 shows relative between the amounts of TFC, TPC and TAC extracted using different conditions of extraction a further analysis of the variance of the main effects between the variables studied and their significance was performed using one-way ANOVAs. as it can be seen there is a statistically significant difference in the amounts of TFC, TPC and TAC using different maceration time ($p < 0.000$- see table 7) with the lowest amount obtained at 5 minutes maceration time. Furthermore, a decrease of amplitude lead to an increase in TFC, TPC extracted and TAC as can be seen in Fig 1b,2b, and 3b, respectively. The effect of this factor has a significant statistically with ($p < 0.000$ - table 7). However the use of UAE-M extraction had an effect on the amounts of TFC, TPC extracted from potato and TAC (Fig 1c,2c, and 3c) with the lowest amount obtained from soxhlet extraction Table IV.7 show a statistically significant with ($p<0.000$). It should be pointed out that the amount of TFC, TPC, and TAC increased with a higher ratio of ethanol with statistically significant ($p < 0.001$) (Table 7).

Table IV.7: Analysis of variance for the main effects of factors studied

	Factors	P value
TPC	Time of maceration	0.000
	Amplitude	0.000
	Method	0.000
	Solvent ratio	0.000
TFC	Time of maceration	0.000
	Amplitude	0.000
	Method	0.000
	Solvent ratio	0.001
TAC	Time of maceration	0.000
	Amplitude	0.000
	Method	0.000
	Solvent ratio	0.000
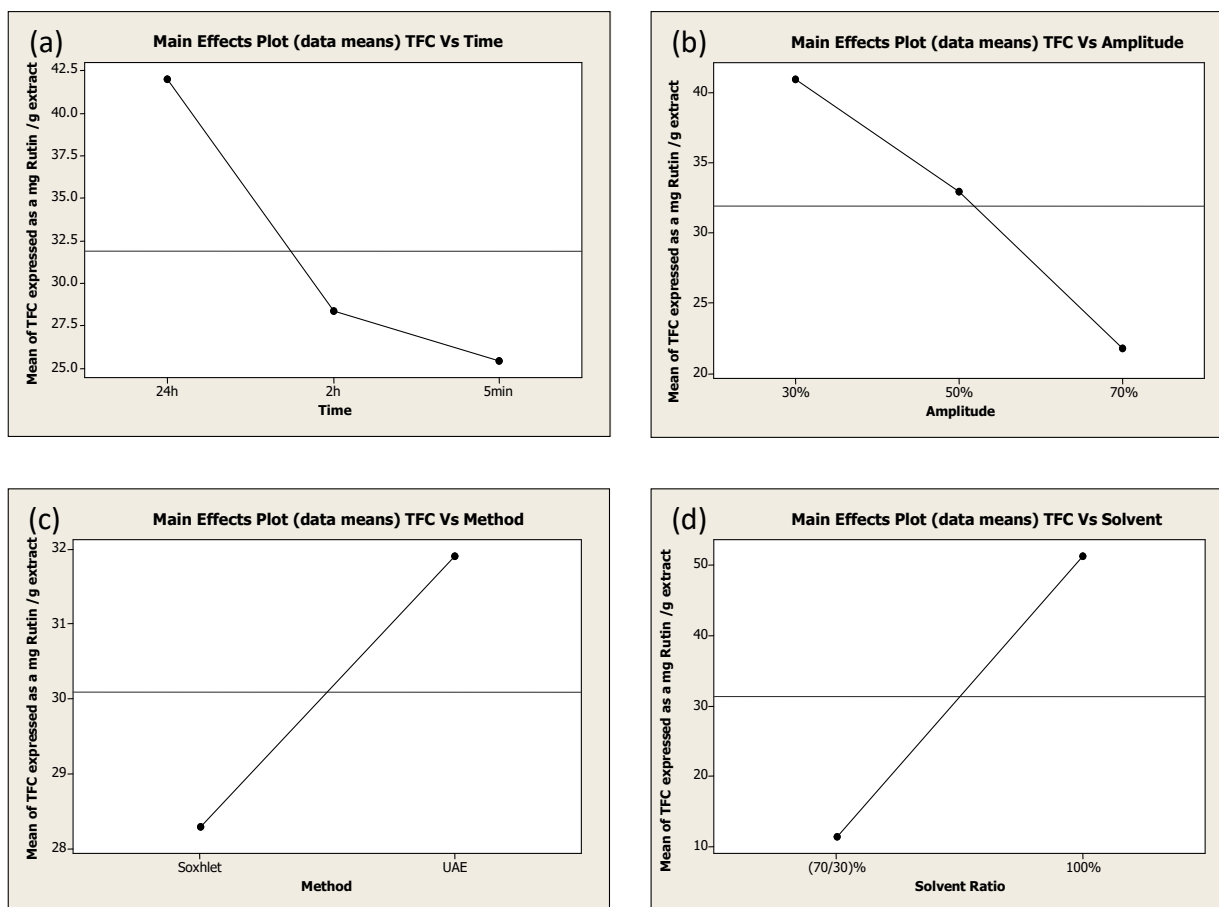
Figure.IV.1: Effect of maceration time (a), amplitude (b), method (c) and the solvent ratio (d) on the level of total flavonoids content. Y-axis shows Total Flavanoids content (expressed as a mg Rutin /g extract)
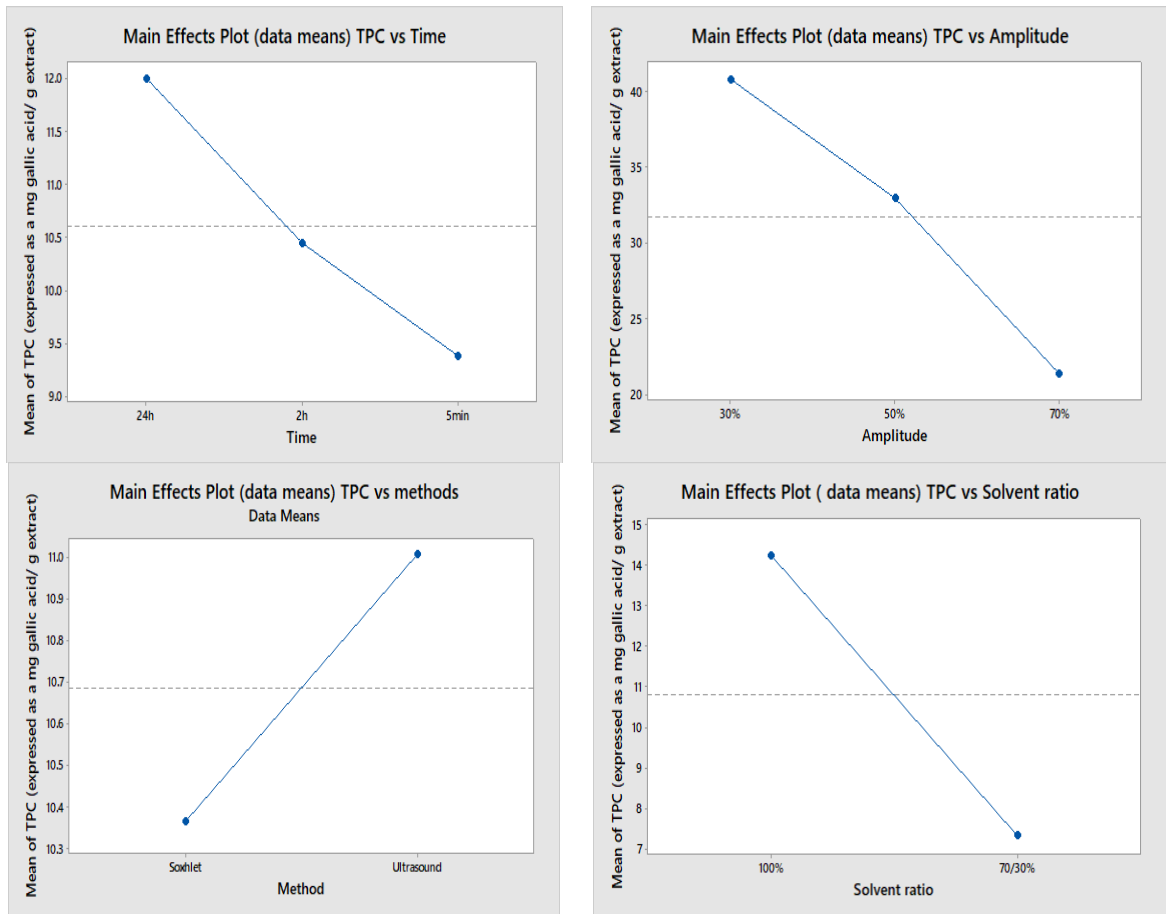
Figure IV.2: Effect of maceration time (a); amplitude (b); method (c) and the solvent ratio (d) on the level of total phenolic content. Y-axis shows Total phenolic content (expressed as a mg gallic acid/g extract)

).
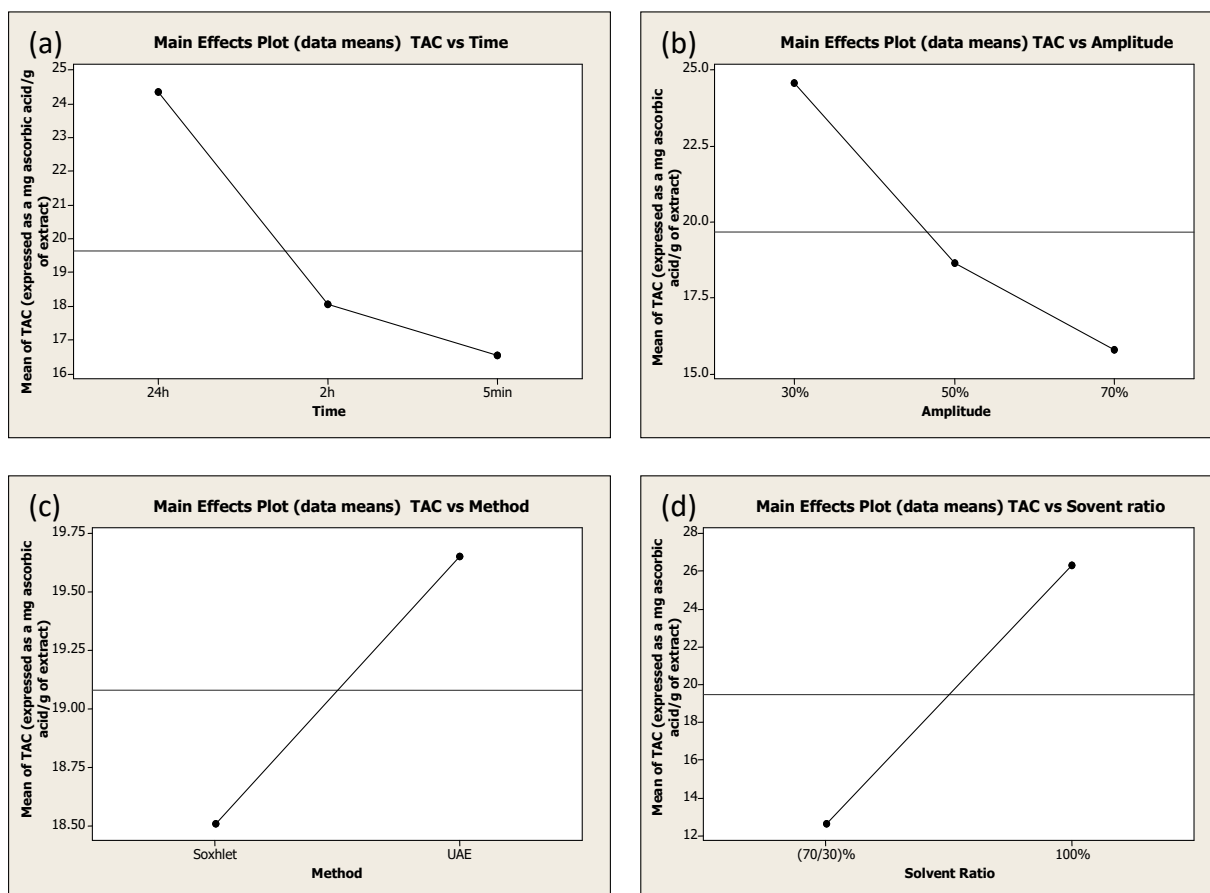
Figure IV.3: Effect of maceration time (a); amplitude (b); method (c) and the solvent ratio (d) on the level of total antioxidant capacity. Y-axis shows Total antioxidant capacity (expressed as a mg ascorbic acid/g of extract).

IV.3.5.4) HPLC analysis

The chromatographic profile of extract of the leaf of the variety Kondor represented in Level of the figure (4-7). The four samples A, C, B, and D were extracted by UAE-M extraction and Soxhlet extraction. The results of quantitative analyses of phenolic compounds Identified are represented in the Table IV.8. The analysis of these results shows that in The extract of this variety, the ascorbic acid range from 0.12 to 1.18 µg/mg, the Gallic acid range from 0.81 to 1.37 µg/mg, the Chlorogenic acid range from 0.27 to 1.53 µg/mg for all the sample extract. However, the Vanillin range from 0.004 to 0.18 µg/mg for extract samples A, C, D. We note that the Rutine (0.532 µg/mg) showed only in C sample. From the point of view of quantity, the A sample has the highest constituents in Ascorbic acid and Chlorogenic acid.

The Results shows that all the samples contain at least three bioactive compounds using either UAE-M or SE as an extraction technique and two ratios for the ethanol as solvent, all these variables have an effect on the level of the amount of bioactive compound in the Kondor sample.
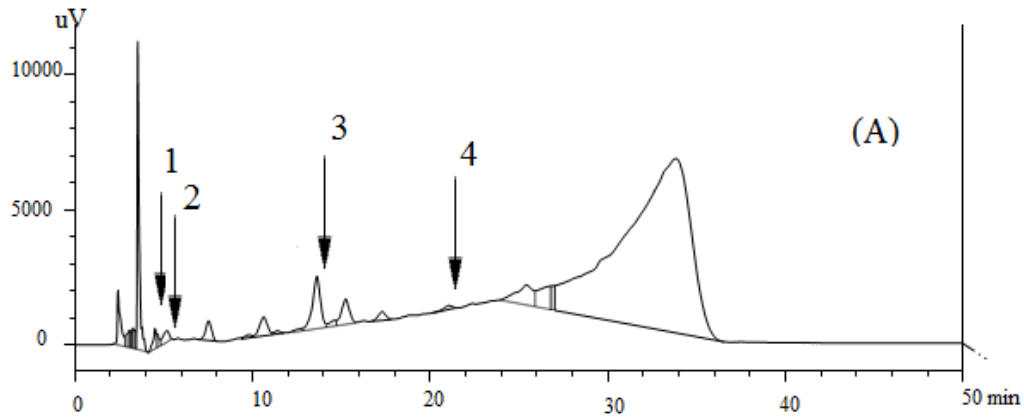
Figure IV.4: Chromatograms of sample extract A;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid;(4) Vanillin
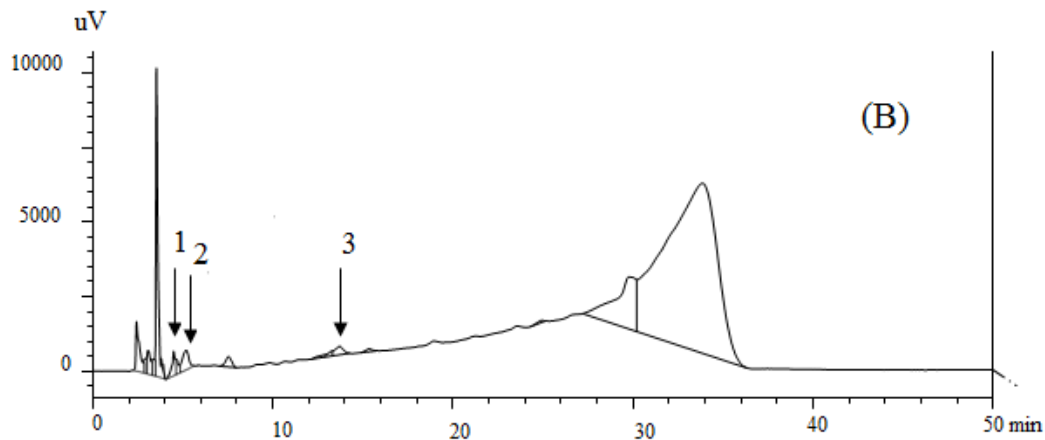
Figure IV.5: Chromatograms of sample extract B;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid
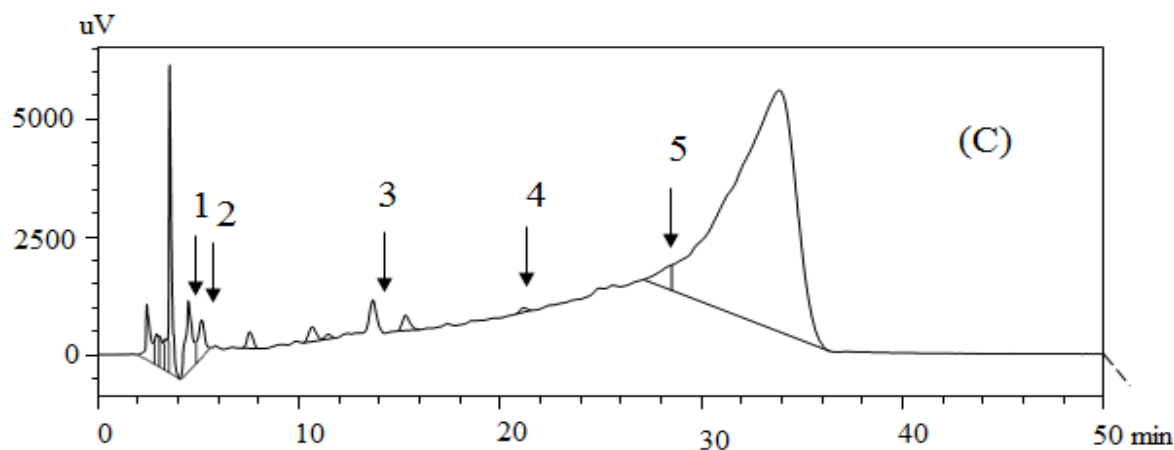
Figure IV.6: Chromatograms of sample extract C;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid;(4) Vanillin;(5)Rutine.
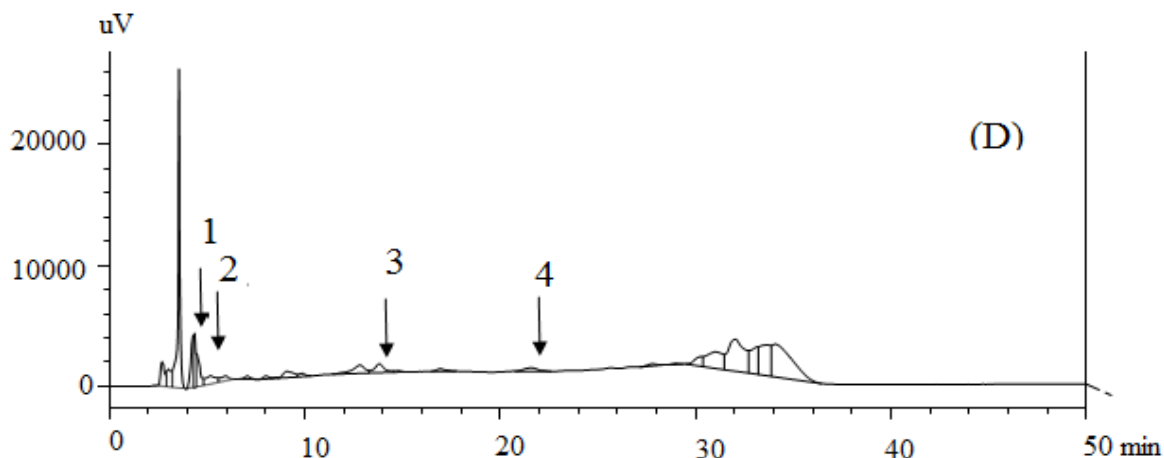
Figure IV.7: Chromatograms of sample extract D;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid;(4) Vanillin.

Table IV.9: Constituents content analyzed by HPLC

Method	Ascorbic acid [µg/mg]	Gallic acid [µg/mg]	Chlorogenic acid [µg/mg]	Vanillin [µg/mg]	Rutine [µg/mg]
A	1.1831	0.8108	1.5359	0.0702	-
C	0.4135	1.095	0.4768	0.0043	0.0095
D	0.3702	1.3752	0.6970	0.1869	
B	0.1288	1.0670	0.2768	-	-

A, C: sample extracted with UAE; 30 % amplitude, 24 h maceration and solvent ration [100 % ethanol, (70/30 ethanol/water)]

B, D: sample extracted with Soxhlet and solvent ration [100 % ethanol, (70/30 ethanol/water)]

IV.4) Discussion

According to the results previously mentioned, the extraction of bioactive compound from potatoes roots can be affected by several factors. One of them was the technique. These results indicated that the amount extracted from both total phenolic and flavonoids contents is remarkably improved when using UAE-M over Soxhlet extraction, also the yield of extraction improved when maceration is used after to ultrasonic-assisted extraction. This fact is in accordance with other data found in the literature which showed in an enhancement of the levels of phytochemicals extracted presented in different food matrixes after sonication [2-4].

However the UAE can induce a reduction in the size of vegetables and/or changes in the cell structure. Because of the ultrasonic cavitation, this structural disruption can provide a better contact between the solvents and cells, allowing an increase in the content of the extracted compounds [5]. Additionally the increase of total phenolic and flavonoids content depends on UAE conditions as an amplitude. The decrease in this factor leads to higher amount .

Secondly, the solvent ratio showed significantly affect the amount of TPC, TFC and TAC. A higher solvent ratio was used in the extraction had better content than (70/30; ethanol/water). Also this results observed by Zhou and Yu [6], all obtained results are confirmed by HPLC analysis. Finally, all these factors lead to the UAE combined with maceration to be a better technique than soxhlet.

The most efficient method for amount bioactive compound is to use UAE with 30% of amplitude, ethanol as solvent and kept the sample macerate for 24 hours. It should be noted that the amount of TFC, TPC and TAC was observed in 2 hours maceration of the samples sonicated with 30% of amplitude have higher results than a soxhlet extraction. This condition was used to extract bioactive compounds from four varieties of potatoes.

Chapter V
Phytochemical study of Solanum tubersum L.

V.1) Introduction

Potatoes can be a good source of phenolic and flavonoid compounds in the diet, despite its moderate levels compared to other plant sources. However, as mention above The composition of a potato tuber depends on a number of factors, although the innate genetic characteristics associated with each cultivar are thought to be among the most significant. Other factors known to influence potato chemical composition include the geographic growing location, soil type and climate [7]. Thus the nutrient screening of a range of different cultivars can help classify the potato variety by their phytochemicals compounds.

The proximate analysis estimates the moistures, phenolic, flavonoids, and other phytochemical compounds and antioxidant activity in vegetable or fruits is the method used to assist in determining potential end use and tuber quality of potato variety. Experiment one of this chapter will discuss the proximate analysis results of four potato tubers in order to qualify the major phytochemicals content of the tubers and to identify the most richness potato variety in phytochemicals compounds.

The phytochemicals value of the richness tuber will then be further quantified in Experiment two by assessing the quantity of phytochemical content and their antioxidant activity in the flesh and the whole tuber.

V.2) Experiment One

V.2.1) Aim

This experiment aimed to examine the presence and quantity of the certain phytochemical component in order to gauge health-promoting in four most popular variety of potatoes cultivated in El-Oued county 'Bartina, Kondor, Kuroda, and Spunta'. This was achieved by performing analyses to estimate and identify certain phenolic, flavonoid content by spectrophotometers assays and HPLC analysis. Also, we will take into our consideration the estimation of moisture/water, the presence of phytochemicals compounds by standard methods. The antioxidant activity measured by two techniques spectrophotometer assays and electrochemical assays. All these analysis results will discuss the proximate analysis results of four different variety of potato cultivars.

V.2.2) Estimation of the water weight percentage in potato roots

The water percentage was measured by weighing fresh roots into a crucible which was placed in an oven at 100 °C overnight. Later, the dried roots were weighed to calculate the amount of water lost. The (Table V.1) shows the moisture content (H_2O %) of four varieties of potato samples. The tuber from red cultivars Bartina showed higher moisture content 84.239% then the yellow tuber Spunta 83.10%. we regarding a high moisture content in potatoes roots which relates to materials such as fruits and vegetables, according to Wilhelm L et al [8]. Many of these products have moisture contents near 90% or more.

Table V.1: Determination of moisture content for four varieties of potatoes

Roots	Bartina	Kuroda	Kondor	Spunta
H_2O %	84.2394	77.2256	81.9591	83.1077

V.2.3) Screening chemistry (Chemical characterizations)

The present study revealed that the various alcoholic extracts of root parts of potatoes contained alkaloids, coumarins, flavonoids, phytosterols and carbohydrates (Table V.2). However, alkaloids, coumarins, and carbohydrates were detected in ethanolic extracts of all roots varieties and the detection of Phytosterols by Liberman and Burchurd's shows positive results with a formation of translucent green color in all varieties which indicate the presence of steroids for Salkowski's test shows negative results. for the detection of Flavonoids, all the roots extracts had a higher number of secondary metabolites with a high degree of precipitation (++) for Alkalin reagent.

Table V.2: Preliminary qualitative phytochemical analysis of various alcoholic extracts of root parts of potatoes

Test		Bartina	Kuroda	Kondor	Spunta
Alkaloids		+	+	+	+
Coumarins		+	+	+	+
Flavonoids	Alkalin reagent	++	++	++	++
	Hydroxyl	+	+	+	+
Phytosterols	Liberman	+	+	+	+
	Salkowski	-	-	-	-
Carbohydrates		+	+	+	+
Tanin		-	-	-	-

++: moderately present, +: Low, -: absent.

V.2.4) Phytochemical contents :

The Table V.3 shows the total phenolic contents (TPC) and the total flavonoid contents (TFC) of four kinds of potato samples unpeeled. The amount of TPC from the whole tuber ranged from 19.2144 to 08.8853 mg AG / g of extract, the results of different extracts showed that the amount of TFC varied considerably from 10.9129 to 25.0350 mg rutin / g of extract. Overall the samples showed the lower amount of TPC and TFC. The results indicate that all of the ethanolic extracts of four kinds has the amount of phenolics and flavonoids compound but their content in ethanolic extract increasing in order was :

Kondor > Bartina > Spunta > Kuroda

Table V.3: Determination of total Phenolics contents and total flavonoids of four kinds of potatoes.

Sample	Total Polyphenols (mg AG / g of extract)	Total flavonoids (mg rutin / g of extract)
Kondor	19.2144 ± 0.096	25.0350 ± 0.125
Bartina	18.9596 ± 0.094	23.5592 ± 0.235
Spunta	12.4628 ± 0.124	21.8057 ± 0.218
Kuroda	08.8853 ± 0.088	10.9129 ± 0.076

V.2.5) HPLC analysis

Identification and quantification of marker compounds were performed on the basis of the coinjections and retention time matching with standards. The HPLC fingerprint of ethanolic extracts of roots and leaf revealed peaks at the retention time 50 min at 300 nm. The quantitive analysis of the chromatograms showed in Table III.4

V.2.5.1) Chromatograms of Kondor sample

The chromatographic profile of unpeeled extract the variety Kondor represented in Level of the Figure V.1. The results of quantitative analyses of phenolic compounds Identified are represented in the Table V.4. The analysis of these results shows that in The extract of the variety Kondor, the ascorbic acid (0.189 µg/mg), the Gallic acid (5.294 µg/mg), the Chlorogenic acid (3.052 µg/mg), caffeic (0.532 µg/mg), Vanillin (0.059 µg/mg) and Quercetin (10.91 µg/mg), we note that the Quercetin is the more representative and found

only in the unpeeled Kondor sample. But from the point of view of quantity, the extract of this variety shows a richness in bioactive compounds more than the extract of other varieties.
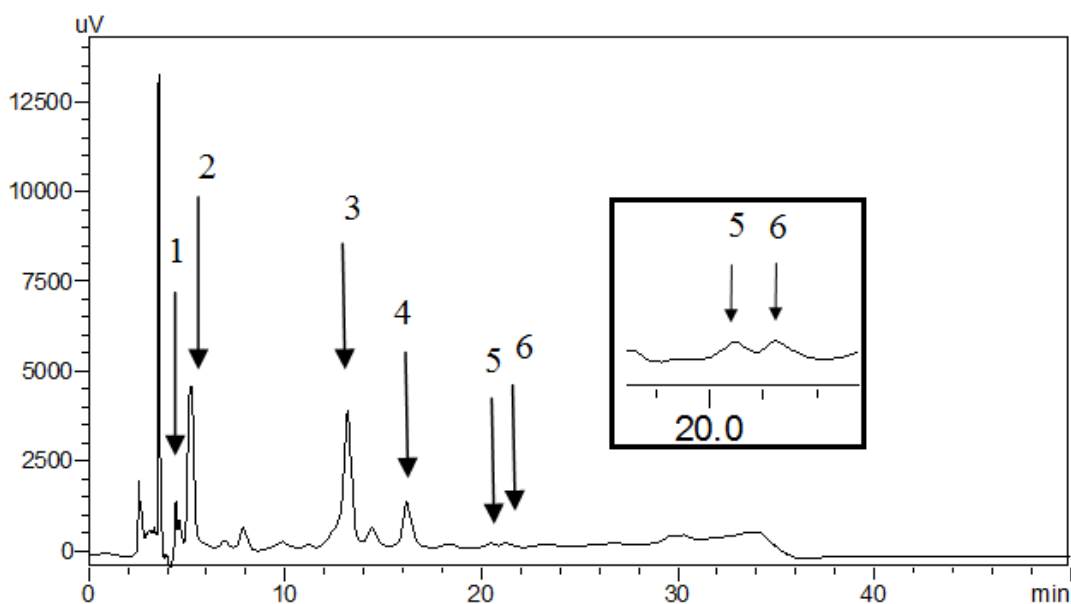
Figure V.1: Chromatograms of unpeeled Kondor extract;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid;(4) Caffeic acid; (5) Quercetin; (6)Vanillin.

V.2.5.2) Chromatograms of Bartina sample

Figure V.2 shows the chromatographic profile of extract of the unpeeled Bartina variety. The results of quantitative analysis of phenolic compounds Identified are represented in the Table V.4. The analysis of these results shows that in The extract of the variety Bartina, the ascorbic acid (0.317 µg/mg), the Gallic acid (7.493 µg/mg), the Chlorogenic acid (1.88 µg/mg), caffeic (0.021 µg/mg) and Vanillin (0.0310 µg/mg). We found that the Gallic acid is the more representative. But for Quercetin and rutin not exist in this variety.
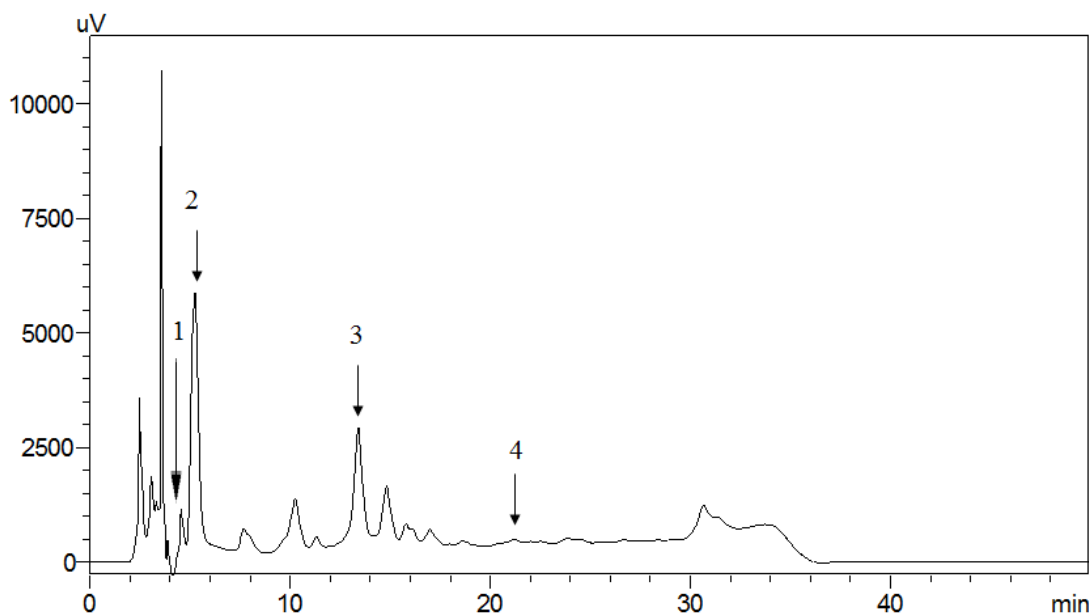
Figure V.2: Chromatograms of unpeeled Bartina sample extract;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid;(4) Vanillin.

V.2.5.3) Chromatograms of Spunta sample

The chromatograms qualitative and quantitative analyses of compounds Phenolic compounds of unpeeled extracts of Spunta are represented below (Figures V.3), and the quantitative assessment of individual compounds identified is a recovery in the Table V.4. The analysis of these results shows that in the extract of the variety, the ascorbic acid (0.025 µg/mg), the Gallic acid (7.804 µg/mg), the Chlorogenic acid (0.756 µg/mg). The Gallic acid is the more representative. For Quercetin, rutin and vaniline do not exist in this variety.
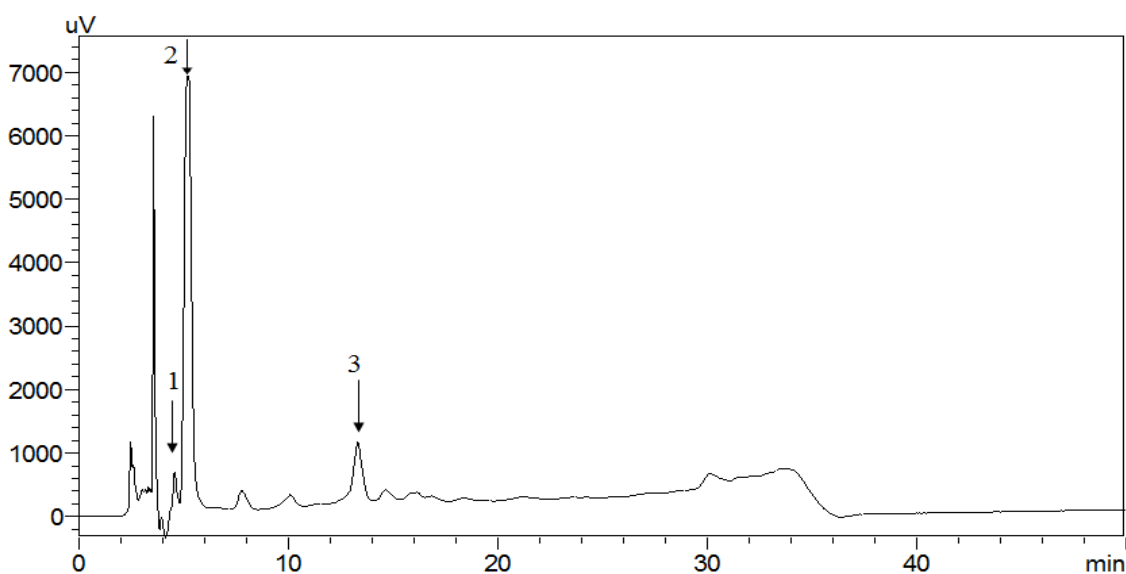
Figure V.3: Chromatograms of unpeeled Spunta sample extract;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid.

V.2.5.4) Chromatograms of Kuroda sample

The chromatograms of unpeeled extract of Kuroda variety shows in (Figure V.4). Qualitative and quantitative analyses of Phenolic compounds identified in the Table V.4. Through this table, it was note that the ascorbic acid (0.203 µg/mg), the Gallic acid (5. µg/mg), the Chlorogenic acid (1.49 µg/mg), and rutin (0.0016 µg/mg) for Kuroda only. from this analysis, the Gallic acid was the more representative compounds.
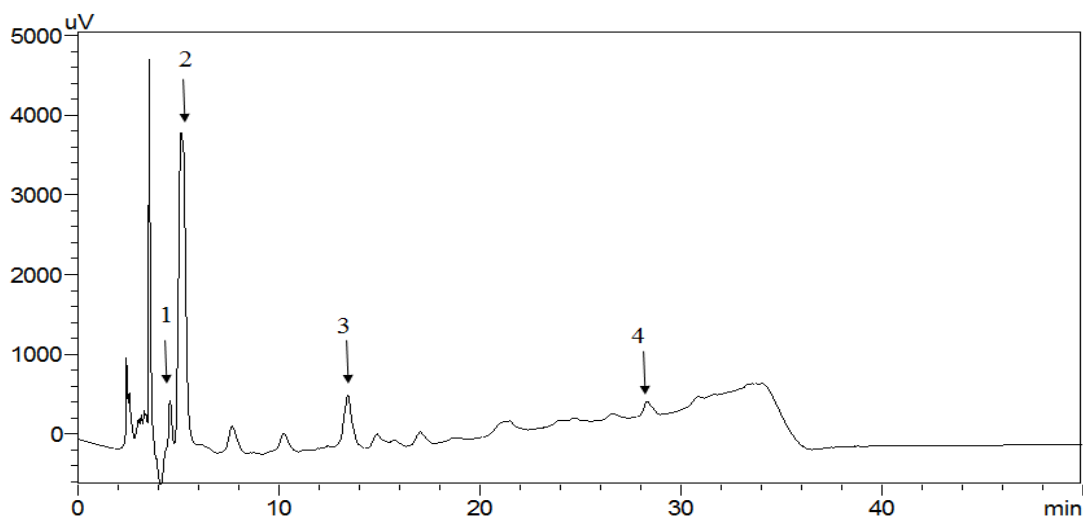
Figure V.4: Chromatograms of unpeeled Kuroda sample extract;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid;(4) Rutine.

Table V.4: Constituents content analyzed by HPLC

Sample extract	Bartina	Kondor	Kuroda	Spunta
Ascorbic acid [µg/mg]	0.3176	0.1896	0.2304	0.0256
Gallic acid [µg/mg]	7.4936	5.2946	5.5922	7.8047
Chlorogenic acid [µg/mg]	1.8864	3.0520	1.4900	0.7566
Caffeic acid [µg/mg]	-	0.5322	-	-
Quercetin [µg/mg]	-	10.9197	-	-
Vanillin [µg/mg]	0.0310	0.0596	-	-
Rutine [µg/mg]	-	-	0.0016	-

V.2.6) Antioxidant capacity

As mentioned above, the methods used to determine total antioxidant activity devised in two techniques, First one is spectrophotometers methods. Secondly, an electrochemistry

technique, in this process we used cyclic voltammetry methods to determine the antioxidant activity.

V.2.6.1) Spectrophotometers

V.2.6.1.1) FRAP assay

Ferric reducing antioxidant power (FRAP) is based on the ability of the antioxidant to reduce Fe3+ to Fe2+, according to Rodriquez & Hadley, the ethanolic extracts of potato tubers showed marked hydrogen-donating activity using reducing power in the Fe(III) Æ Fe (II) reaction in FRAP assay. [9]

Based on the FRAP assay (Table V.5), the results indicated that the whole root (unpeeled) samples had a reducing antioxidant power. However, the results indicated that Kondor extract had the highest level of reducing power with value of 38.8032 mg Fe(II)/ g of extract, this activity may due to the contents of flavonoids. The Kuroda extract indicates the lowest reducing antioxidant power with a value of 22.7442 mg Fe(II)/ g of extract. All the extract samples showed reducing antioxidant power but less than ascorbic acid.

Table V.5: Determination of reducing the antioxidant power of four kinds of potatoes.

Sample	FRAP (mg Fe(II)/ g of extract)
Kondor	38.8032 ± 0.043
Bartina	31.5543 ± 0.010
Spunta	29.6169 ± 0.007
Kuroda	22.7442 ± 0.005
Ascorbic acid	2512.985 ± 0.020
Gallic acid	7778.2511 ± 0.388

V.2.6.1.2) DPPH

DPPH scavenging activity (%) had increased with the increased concentration of the extract in all samples (Figure V 5, 6 and 7). The stable DPPH free radical accepts an electron or hydrogen radical from donors to form a stable molecule which could be seen as color reduction.

V.2.6.1.2.1) Calculation of radical inhibition

Kondor extract was the strongest electron or hydrogen donor due to the highest DPPH scavenging activity. The IC_{50} value of each extract is deducted from the equations of the curves of the variation in the percentage of inhibition I% as a function of the concentration of each extract as shown in Figure V.5 and annexe (A)

The IC_{50} of DPPH scavenging activity of the Kondor, Bartina, Spunta and Kuroda extracts were 1.21 mg/ml, 2.12 mg/ml, 2.54 mg/ml and 3.86 mg/ml respectively for unpeeled extracts. Gallic acid and ascorbic acid, however, showed the highest value 0.0069 mg/ml and 0.01 mg/ml. All the results have a very statistically significant with p<0.000 (Table V.6)
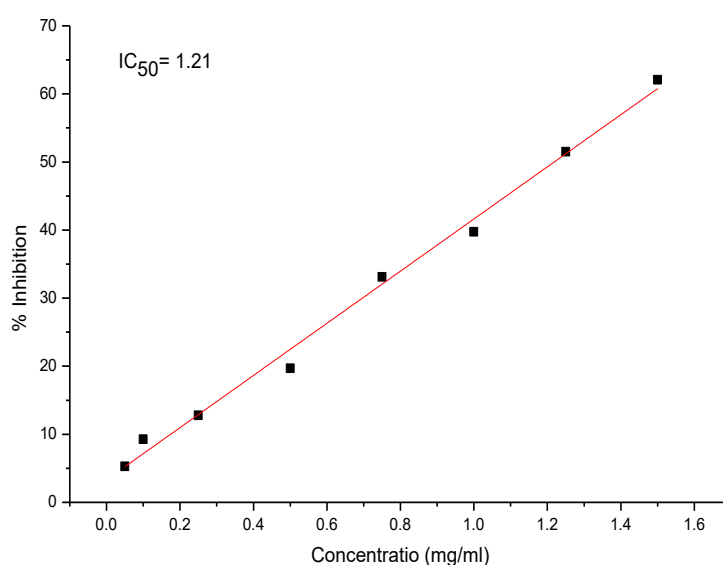
Figure V.5: The percent DPPH radical inhibition as a function of the concentration of of unpeeled Kondor

Table V.6: IC_{50} values of sample extracts using DPPH

Sample	DppH			
	Equation	R^2 value	IC_{50}*	P
Kondor	Y = 3.33 + 38.3 X	0.994	1.21	0.000
Bartina	Y = - 0.44 + 23.7 X	0.968	2.12	0.000
Spunta	Y = - 4.53 + 21.4 X	0.952	2.54	0.000
Kuroda	Y = - 5.30 + 14.3 X	0.973	3.86	0.000
Ascorbic acid	Y= 0.94 + 4720X	0.972	0.010	0.000
Gallic acid	Y=-6.78 + 8154X	0.982	0.0069	0.000

* inhibition expressed as mg/ml

V.2.6.1.2.2) Calculation of the binding constant

The electronic spectra of complexes extract–DppH were recorded in the range 200-900 nm, which showed intense absorption at range 515-518 nm   The determination of binding constant Kb of extract–DppH complex can be calculated from the inhibition of absorbance in UV-spectra after the addition of unpeeled sample extract (Figure V.6 and 7). Benesi-Hildebrand equation is used to evaluate binding constants spectrophotometrically [10-12]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \times \frac{1}{K_b[\text{extract}]}$$

Where

Ao and A are the absorbances of free and bound extract,

ε_G and ε_{H-G} are their molar extinction coefficients, respectively. (H and G correspond to host and guest).

A plot of Ao /(A-Ao) to 1/[extract] has shown linearity which is suggestive of 1:1 complex formation of both extracts with DppH. The intercept to slope ratio of this plot gives the value of binding constant "Kb " (Annexe). Kb values were calculated given in Table V.7

UV spectra of DppH showed a hypochromic[*] effect upon addition of sample extract the intensity decreased rapidly with increasing concentration of the extract were recorded in Figure III.5, 6, In the absence of sample extract the DppH displayed one absorbance peak at 517 nm, this peak intensity exhibited hypochromism up from 56% to 77% for 2 mg/ml concentration of sample extract.

The values of binding constant "Kb " were further used to calculate standard Gibbs free energy "ΔG" of extract–DppH complex, using the following equation;

$$\Delta G = -RT\ln K_b$$

Free energy changes of extracts values, Table V.7, and showed spontaneous of their binding with DppH. The p-value of the results was <0.05 which a significant statistically.

---

[*]   HYPOCHROMIC EFFECT: decrease in the intensity of a spectral band due to substituents or interactions with the molecular environment
Source: PAC, 1996, 68, 2223 (Glossary of terms used in photochemistry (IUPAC Recommendations 1996)) on page 2247.
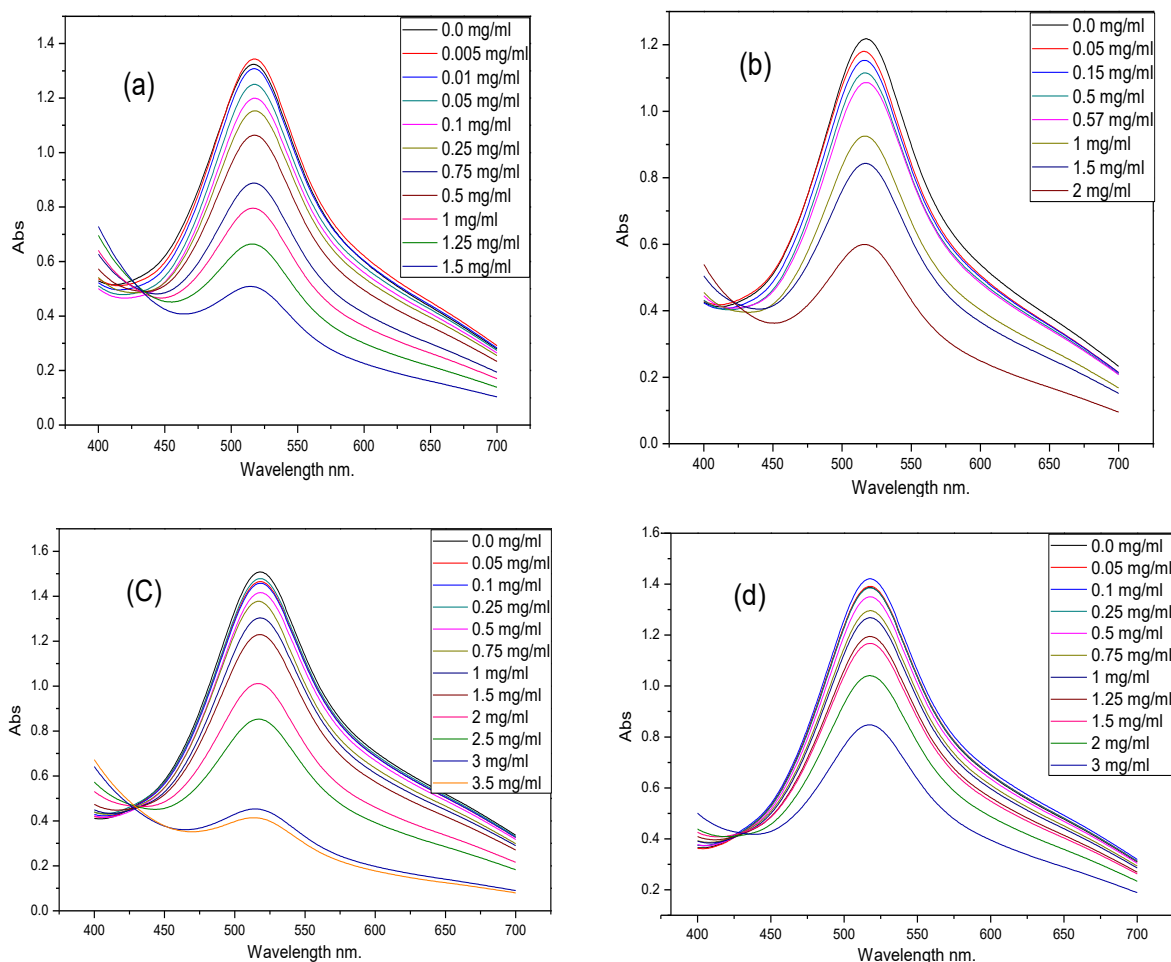
Figure V.6: Electronic absorption spectra of 0.25 mM of DPPH interaction with Roots of (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda

* a decrease in absorption intensity
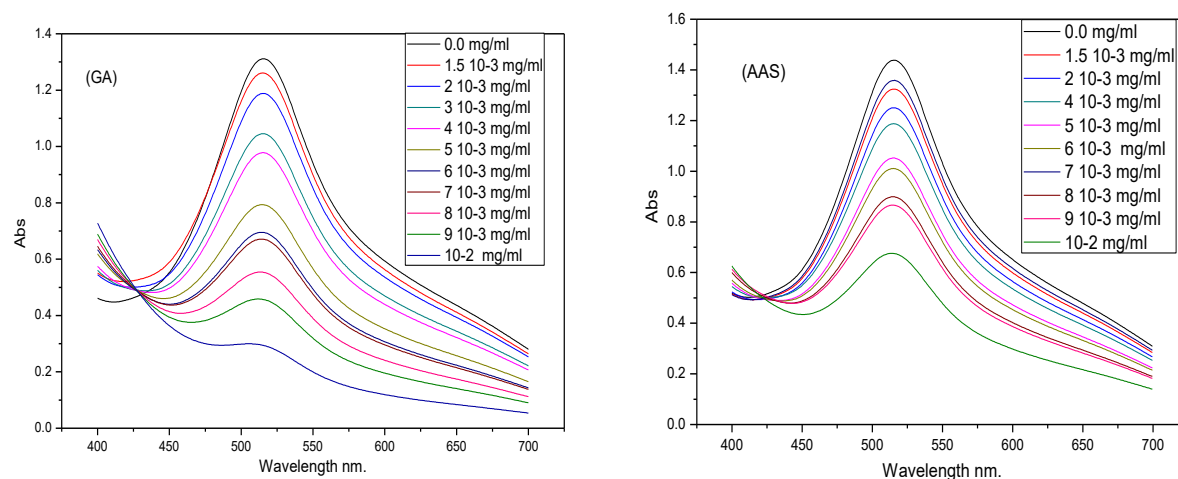
Figure V.7: Electronic absorption spectra of 0.25 mM of DPPH interaction with (GA) Gallic acid, (AAS) Ascorbic acid.

Table V.7: Values of Binding constants and binding free energy of the complexes DppH-sample extracts

Compound	Equation	R^2	K	ΔG	P
Unpeeled					
DppH-Kondor	Y = -0.031x + 2.4387	0.9089	78.6266	-10.822	0.012
DppH-Bartina	Y = -0.06x + 1.7288	0.9289	28.8133	-08.332	0.008
DppH-Spunta	Y = -0.0061x +0.7297	0.9102	119.0375	-11.849	0.012
DppH-Kuroda	Y = -0.0273x +1.3812	0.9804	50.5934	-09.728	0.001
Standard					
Ascorbic acid	Y =-6.2892x+ 358.56	0.988	50.0120	-10.024	0.000
Gallic acid	y = -4.6163x + 387.09	0.9243	83.8523	-10.98	0.001

V.2.6.2) Electrochemistry assay

All the voltammograms obtained from cyclic voltammetry assay (CV) for the four samples in fixed pH=3.6 (acetate buffer, 300mM). This assay have the same principle as FRAP assay that's why this pH value is chosen. The CVs present one oxidation peak in range from +0.87 V to +1.02 V (Figure V.8), also for ascorbic acid had a peak at +0.54 V (Figure V.8) and two oxidation peaks at +0.4V and +0.7V for gallic acid (Figure III.9). The electrochemical behavior observed for the unpeeled potatoes extracts with oxidation potential value more positive than gallic acid, these peaks indicate that the sample extracts contain flavonoids compounds showed low or very low activity in the FRAP assay according to Firuzi and Lacanna [13].
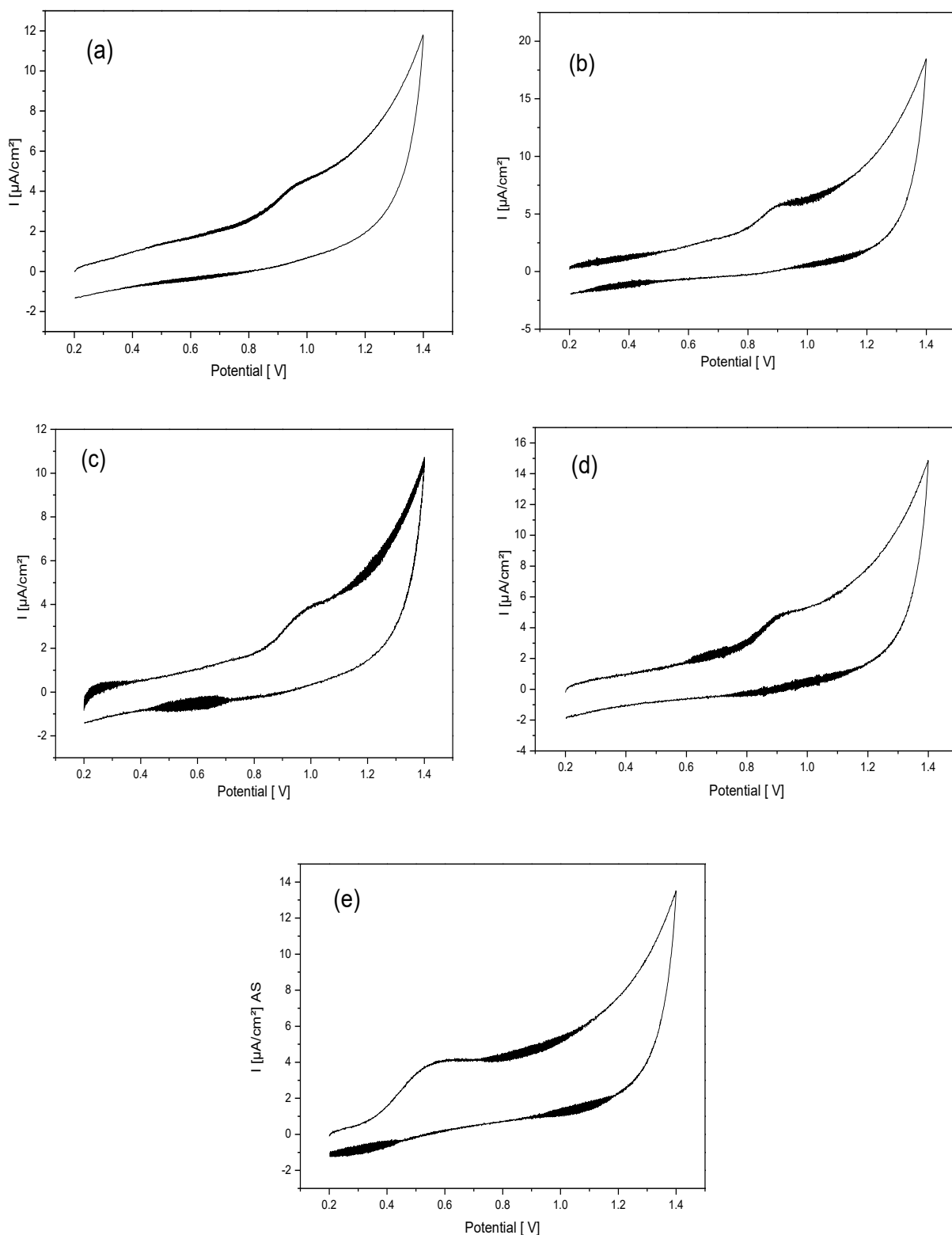
Figure V.8: Voltammograms obtained for unpeeled extract of; (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda and (e) for Ascorbic acid by cyclic voltammetry in pH=3.6 acetate buffer

Table V.8: shows The oxidation of samples extract studied by cyclic voltammetry showed capacity antioxidant, expressed in terms of gallic acids (AG) equivalent antioxidant capacity obtained from an electrochemical assay in ethanolic extract of four samples with

values ranged from 53.4813 mg EAG/g extract to 34.5025 mg EAG/g extract. On the other hand, ascorbic acid showed a higher capacity antioxidant with 637.4624 mg EAG/g extract. This capacity related to the potential peaks which determined the antioxidant power of sample extracts, according to Firuzi and Lacanna [13] the flavonoids which have potential peaks at range from +0.44 to +0.71 V this groups were active antioxidants in the FRAP assays.

Table V.8: Determination of the Antioxidant capacity of four kinds of potatoes by CV.

Sample	Antioxidant capacity CV[a]	Eap [b] (V)
Unpeeled		
Kondor	53.4813 ± 0.254	+0.88
Bartina	44.9876 ± 0.325	+0.89
Spunta	36.4515 ± 0.481	+0.95
Kuroda	34.5025 ± 0.341	+0.90
Ascorbic acid	637.4624 ± 0.117	+0.54

a capacity antioxidant expressed as milligrams of gallic acid equivalent per gram of extract

b Anodic oxidation potential vs GCE

V.2.6.2.1) Electrochemistry behavior

Figure V.9 shows cyclic voltammograms for unpeeled Kondor extracts in DMF/0.1 TBFP solvent, the observed behavior of the extract of all samples the whole roots at potential range (+200V to +1400V), present one oxidation peak recorded between + 1.029V to +1.066V (Table V.9, Fig V.9). The cyclic voltammograms of all samples extracts depends on the chemical structures, the observed electrochemical behavior of all samples extracts showed similarly in both solvent cell, aqueous buffer acetate PH 3.6, 0.3 mM (Figure V. 8, Table V. 8) and organic solvent (DMF) with a shifts to positive potential side up from 12.10 to 21.13 % in case of unpeeled extracts of Spunta and Kondor sample respectively, in DMF solution. It is worth noting that all cyclic appearance under the same patterns indicating the producibility of the resistant of the solution the phenomena.
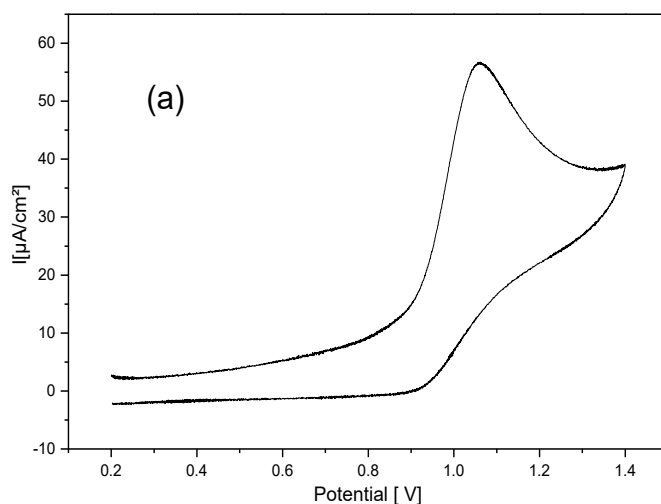
Figure V.9: Voltammograms obtained for unpeeled Kondor sample by cyclic voltammetry in DMF

Table V.9: Determination of the oxidation peaks of four kinds of potatoes by CV in DMF.

Samples	Eap * (V)
Unpeeled	
Kondor	+ 1.066
Bartina	+ 1.047
Spunta	+ 1.065
Kuroda	+ 1.029

* Anodic oxidation potential vs GCE

V.2.6.2.2) Electrochemistry assay $O_2^{\cdot-}$ radical scavenging activity

The quantification of antioxidant in the extract is made by calculating the inhibition concentration (IC_{50}) value, radical scavenging activity was plotted against different concentrations of extract samples, the effect of different concentrations is shown in (Figure III.11, and 12) for $O_2^{\cdot-}$ radical scavenging activity All the voltammograms of superoxide anion radical Figure III.11, 12 showed one electron reversible process, having well developed and clear oxidation and reduction peaks. On the level of anodic current the decreasing of peaks according to the addition of sample extracts.

The equation obtained from the linear calibration graph figure V.10. In the studied concentration range of samples extracts are presented in Table V.10, where y stand for the value of the oxidation peak current density of $O_2^{\cdot-}$ and x represents the value of the concentration of ethanolic extracts, expressed as mg/mL. The Kondor extracts have a less IC_{50}

with 1.03, and for Kuroda sample has the highest IC_{50} with 1.819 mg/mL. All the IC_{50} values were considered as very statistically significant with $p<0.005$.
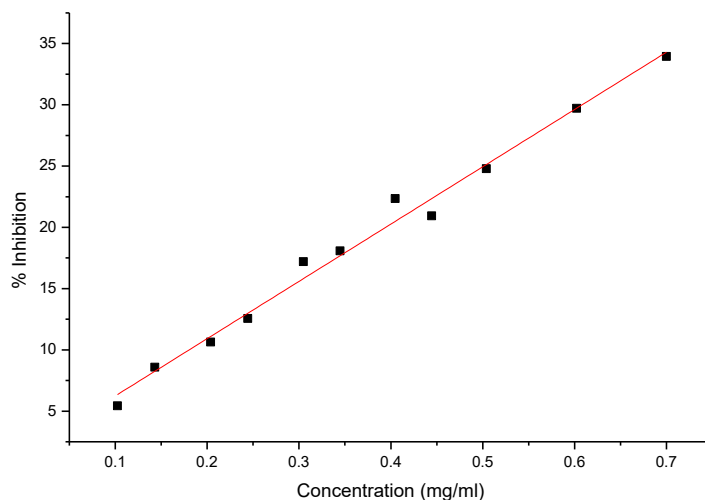
Figure V.10: The percent $O_2^{\cdot-}$ radical inhibition as a function of the concentration of unpeeled Kondor

Table V.10: IC_{50} values of sample extracts using $O_2^{\cdot-}$ radical scavenging activity

Sample	Equation	R^2 value	IC_{50}	P
Unpeeled				
Kondor	Y= 1.56 + 46.8 X	0.989	1.0350	0.000
Bartina	Y= - 7.52 + 50.6 X	0.923	1.1367	0.002
Spunta	Y= - 0.134 + 27.6 X	0.988	1.8164	0.000
Kuroda	Y = - 4.23 + 29.8 X	0.987	1.8197	0.000
Standard				
Ascorbic acid	Y = -3.118 + 928.4 X	0.995	0.0572	0.000
α-tocopherol	Y = 2.10 + 251.0 X	0.845	0.1908	0.027
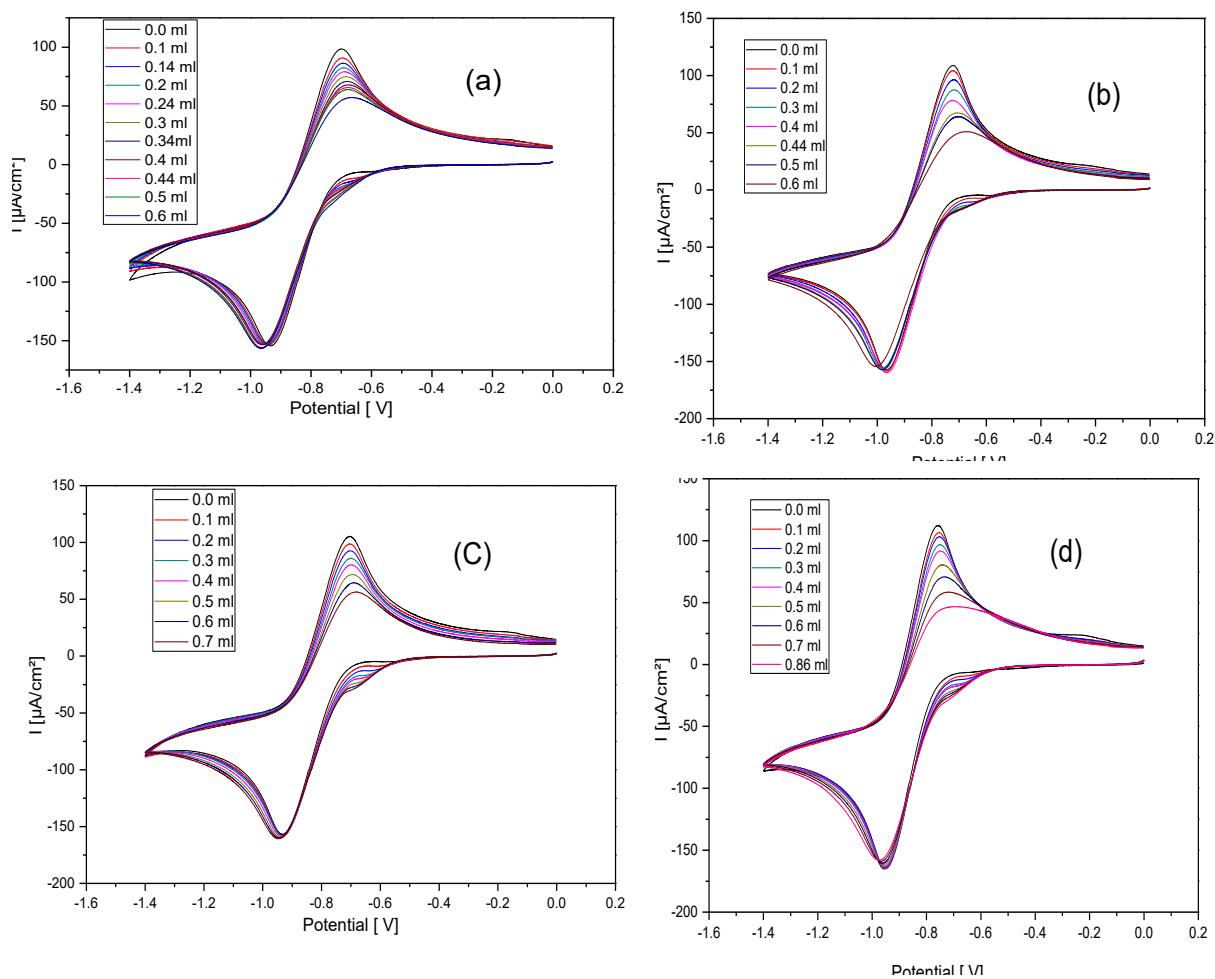
Figure V.11: Cyclic voltammograms of oxygen-saturated in the absence and presence of different concentration of sample extract Roots: (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda at scan rate 100 mV/s
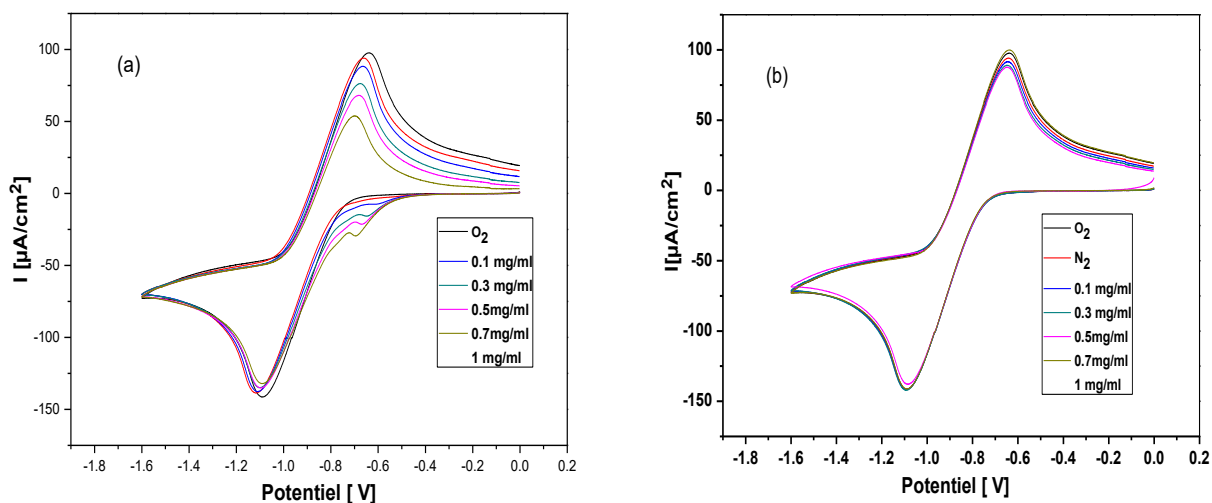
Figure V.12: Cyclic voltammograms of oxygen-saturated in the absence and presence of different concentration of standard: (a) ascorbic acid, (b) α-tocopherol at scan rate 100 mV/s

V.2.6.2.3) Ratio of binding constants (Kox/Kred)
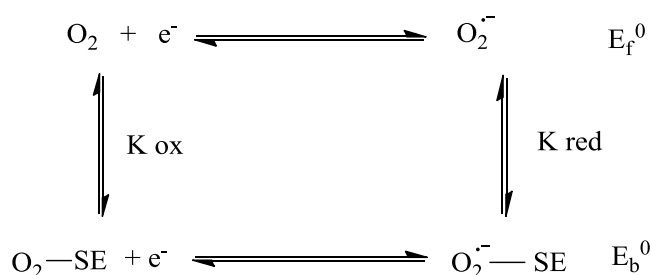
Scheme1: The redox behavior of the free radical and its bounded forms SE

The peak potential shift values additionally pointed out that $O_2^{\bullet-}$ is easier to oxidize in the presence of ethanolic extract because its reduced form $O_2^{\bullet-}$ is more strongly bound to extract than its oxidized form. For such a system, where both forms of the $O_2/\ O_2^{\bullet-}$ redox couple interact with the potential antioxidant compound, scheme 2 can be applied [16]. Based on the process presented in scheme 2, the following equation is obtained [17],

$$E_b^0 - E_f^0 = 0.059 \, Log \, \frac{K_{red}}{K_{ox}} \qquad (V.2)$$

Where E_b^0 and E_f^0 are the formal potentials of the $O_2/\ O_2^{\bullet-}$ redox couple in the free and bound forms, respectively. E^0 and ΔE^0 are calculated using the following formulas (3) and (4)

$$E^0 = \left| \frac{E_{Pc} + E_{Pa}}{2} \right| \qquad (V.3)$$

$$\Delta E^0 = E^0(O_2^{\bullet-} - SE) - E^0(O_2^{\bullet-}) = E_b^0 - E_f^0 \qquad (V.4)$$

Figure V.11 and 12 shows the CV's behavior of oxygen-saturated DMF/0.1 TBFP in the potential window of -1.4 to 0.0 V at a glassy carbon electrode in the absence and presence of 0.4 mL sample extracts, the free $O_2/O_2^{\bullet-}$ redox couple exhibits a single oxidation peak at -0.72 V and a single reduction peak at -0.74 V. These Figures also shows the effect of the addition of extract on the oxidation peak current of $O_2/O_2^{\bullet-}$.

The CV technique was also helpful to determine the mechanism of free radical scavenging, the observed changes in anodic peak current of $O/O_2^{\bullet-}$ is caused by the addition of different content of ethanolic extracts, this decrease in anodic peak can be used for the calculation of the binding constant, where the shift in peak potential values can be employed for the determination of the mode of interaction, in the same manner, used for study of binding of drug molecules to DNA [14,15].

By adding 0.4 ml of sample extracts in solution of DMF, the peak potential was shifted by ΔE, the peak potential was displaced by ΔEf^0 mV to more negative for complex $O_2^{\cdot-}$-Kondor and more positive for the rest of the complexes which associated with the decrease in oxidation peak current densities $\Delta Ipa\%$ which confirms the interaction of ethanolic extracts with $O_2^{\cdot-}$ (Table V.11 and 12) The significant decrease in oxidation peak current densities can be attributed to the diminution in $O_2^{\cdot-}$ concentration due to the formation of sample extract complex.

Table V.11: Shifts in peak potential and a decrease in anodic peak current of $O_2^{\cdot-}$ bound forms of Roots extract samples.

| Sample	Ipa (µA)	Epa (V)	Epc (V)	Ef0 (V)	ΔEf0(mV)	ΔIpa%	Kox/Kred
$O_2^{\cdot-}$-	134.2672	-0.7068	-0.7247	-0.7157	-	-	-
$O_2^{\cdot-}$ −Kondor	104.2637	-0.678	-0.7566	-0.7173	-1.5574	22.3460	0.94
$O_2^{\cdot-}$-	137.3784	-0.7251	-0.7714	-0.7482	-	-	-
$O_2^{\cdot-}$-Bartina	118.844	-0.7251	-0.7624	-0.7438	4.4776	13.4914	1.19
$O_2^{\cdot-}$-	137.5848	-0.7149	-0.7438	-0.7294	-	-	-
$O_2^{\cdot-}$-Spunta	114.627	-0.7021	-0.7477	-0.7249	4.4776	16.6863	1.19
$O_2^{\cdot-}$-	142.4359	-0.7613	-0.7800	-0.7706	-	-	-
$O_2^{\cdot-}$-Kuroda	123.7957	-0.7519	-0.7617	-0.7568	13.8221	13.0867	1.71
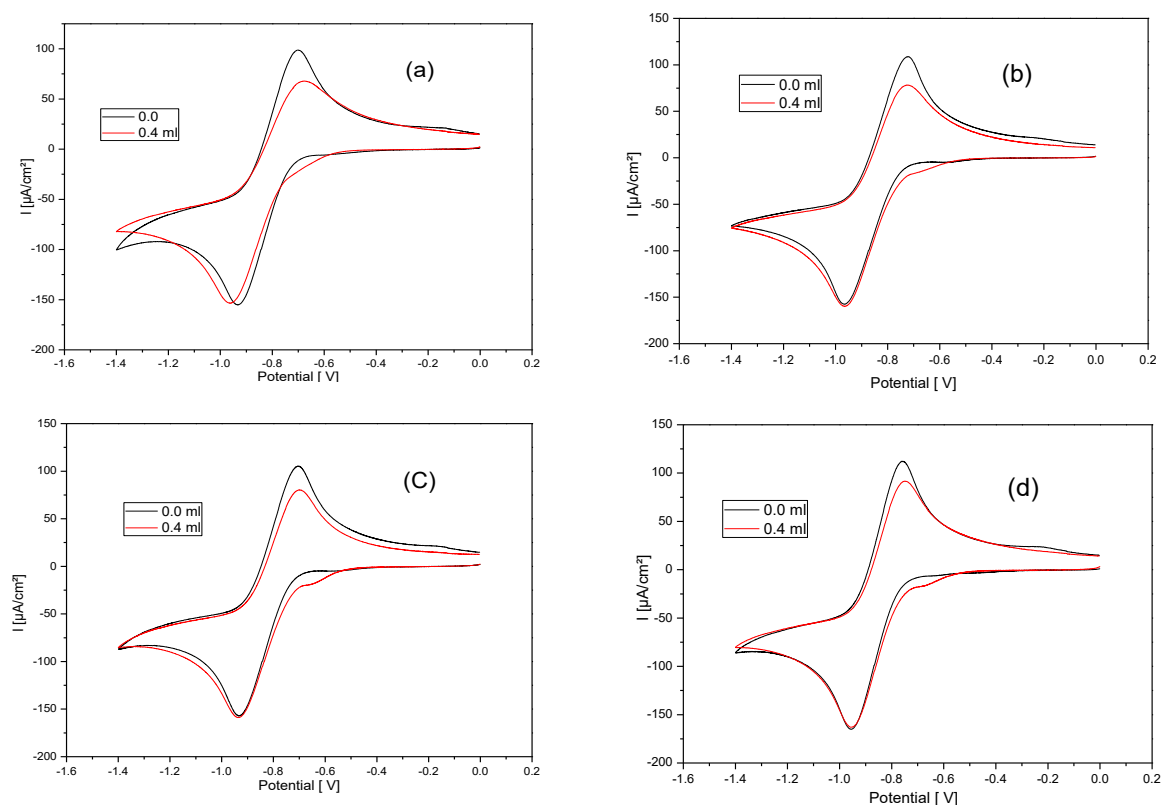
Figure V.13: Cyclic voltammograms of oxygen-saturated in the absence and presence of 0.4 ml sample extract Roots (a) Kondor, (b) Bartina, (c) Spunta, (d) Kuroda at scan rate 100 mV/s

**Table V.12:** Shifts in peak potential and a decrease in anodic peak current of $O_2^{\cdot-}$ bound forms of Standard samples.

Sample	Ipa (μA)	Epa(V)	Epc(V)	Ef0 (V)	ΔEf0 (mV)	ΔIpa%	Kox/Kred
$O_2^{\cdot-}$-	129.5016	-0.7825	-0.6462	-0.7143	-	-	-
$O_2^{\cdot-}$-Ascorbic acid	120.8858	-0.7798	-0.6676	-0.7237	-9.35	6.6530	0.69
$O_2^{\cdot-}$-	127.5129	-0.6453	-0.7752	-0.7102	-	-	-
$O_2^{\cdot-}$-α-tocopherol	121.1147	-0.6458	-0.7707	-0.7080	2.25	5.0177	1.09
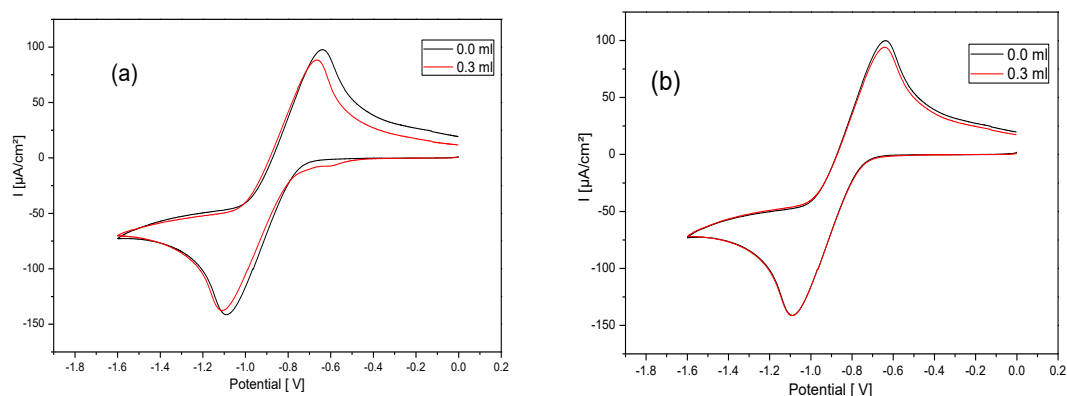
Figure V.14: Cyclic voltammograms of oxygen-saturated in the absence and presence of 0.3 ml sample extract (a) Ascorbic acid, (b) α-tocopherol at scan rate 100 mV/s

V.2.6.2.4) Thermodynamic properties

To quantify the results, the strength of interaction, between superoxide anion radical and the probable antioxidant in the extract, was estimated in terms of binding constant Kb. Based on the decrease in peak current, the binding constant (Kb) was calculated using following equation Feng et al [18].

$$\log\left[\frac{1}{[AO]}\right] = \log K_b \; + \; \log\left[\frac{I_p}{I_{po} - I_p}\right] \qquad (5)$$

Where, Ipo and Ip are the peak currents of superoxide anion radical in the absence and presence of additives, respectively, [AO] is the concentration of the antioxidant. As [AO] is not known, therefore, this term was replaced by the volume of the extracts (ΔVext). It is noticeable that the volume of the solution containing $O_2^{.-}$ is fixed thus the addition of volume increments of the extract is proportional to the addition of more number of moles (i.e. concentration) of the compound(s) according to Safeer et al [19]. Another thermodynamic parameter, standard Gibbs free energy (ΔG°) was calculated using the measured Kb.

The obtained values from CV's voltammograms showed in Figure V.13 and 14 indicated that the binding constant (K_b) ranged as the follows:

$O_2^{.-}$- α-tocopherol < $O_2^{.-}$-Spunta < $O_2^{.-}$ Kondor < $O_2^{.-}$-Ascorbic acid < $O_2^{.-}$-Kuroda < $O_2^{.-}$-Bartina
The values of free energy varied in the same order as of the binding constant. The negative values of ΔG indicated the spontaneity of $O_2^{.-}$ − SE interaction. The data obtained in Table V.13 were very statistically significant with p<0.002

Table V.13: Values of Binding constants and binding free energy of the complexes $O_2^{\cdot-}$-sample extracts

Compound	Equation	R^2	K	ΔG	P
Unpeeled					
$O_2^{\cdot-}$-Kondor	y = 0.9086x + 2.8778	0.990	07.5474x102	-16.42	0.000
$O_2^{\cdot-}$-Bartina	y = 0.4818x + 3.1119	0.956	13.1522x102	-17.80	0.001
$O_2^{\cdot-}$-Spunta	y = 0.8952x + 2.8012	0.986	06.3270x102	-15.99	0.000
$O_2^{\cdot-}$-Kuroda	y = 0.576x + 2.9473	0.986	08.8572x102	-16.82	0.000
Standard					
$O_2^{\cdot-}$-Ascorbic acid	y = 0.5923x + 2.8376	0.996	06.8801x102	-16.19	0.000
$O_2^{\cdot-}$-α-tocopherol	y = 1.15x + 2.035	0.959	01.0839x102	-11.61	0.001

V.2.6.2.5) Diffusion coefficients

The redox process of the four sample extracts as a function to scan rates presented in Figure V.15 and 16. The voltammogram contained a couple of a clear stable oxidation and reduction peaks attributed to the electrochemical behavior of the redox $O_2/O_2^{\cdot-}$ couple. In addition, demonstrate clearly the reversibility of the oxidation reaction of the redox couple $O_2/O_2^{\cdot-}$ as the oxidation peak potentials are not significantly affected by the variation in the scan rate.

The diffusion coefficients of the free radical $O_2^{\cdot-}$ and its $O_2^{\cdot-}$ bounded forms with ethanolic extract were calculated from the following Randles–Sevcik equation [20].

$$i = 2.69 \times 10^5 n^{\frac{3}{2}} SCD^{\frac{1}{2}} v^{\frac{1}{2}} \qquad (6)$$

Where i presents the oxidation peak current density, S presents the surface of the working electrode (cm^2), C is the bulk concentration ($mol.cm^{-3}$) of the electroactive species, D present the diffusion coefficient (cm^2/s) and v is the scan rate (V/s). The linearity of the plot $ip_a = f\left(v^{\frac{1}{2}}\right)$ of the four adducts. Further demonstrates that the kinetic of the oxidation reaction is limited by the diffusion process. The diffusion coefficients were determined from the slopes of the plot ipa vs the square root of the scan rate. Values are given in Table V.14.

Table V.14 shows that the diffusion coefficient of complexes $O_2^{\cdot-} - SE$ is lower than that of the free $O_2^{\cdot-}$ radical. The diffusion coefficients of four adducts are very close because sample complexes have the same contents the slight difference can only be attributed to the amount of the phytochemical compound.
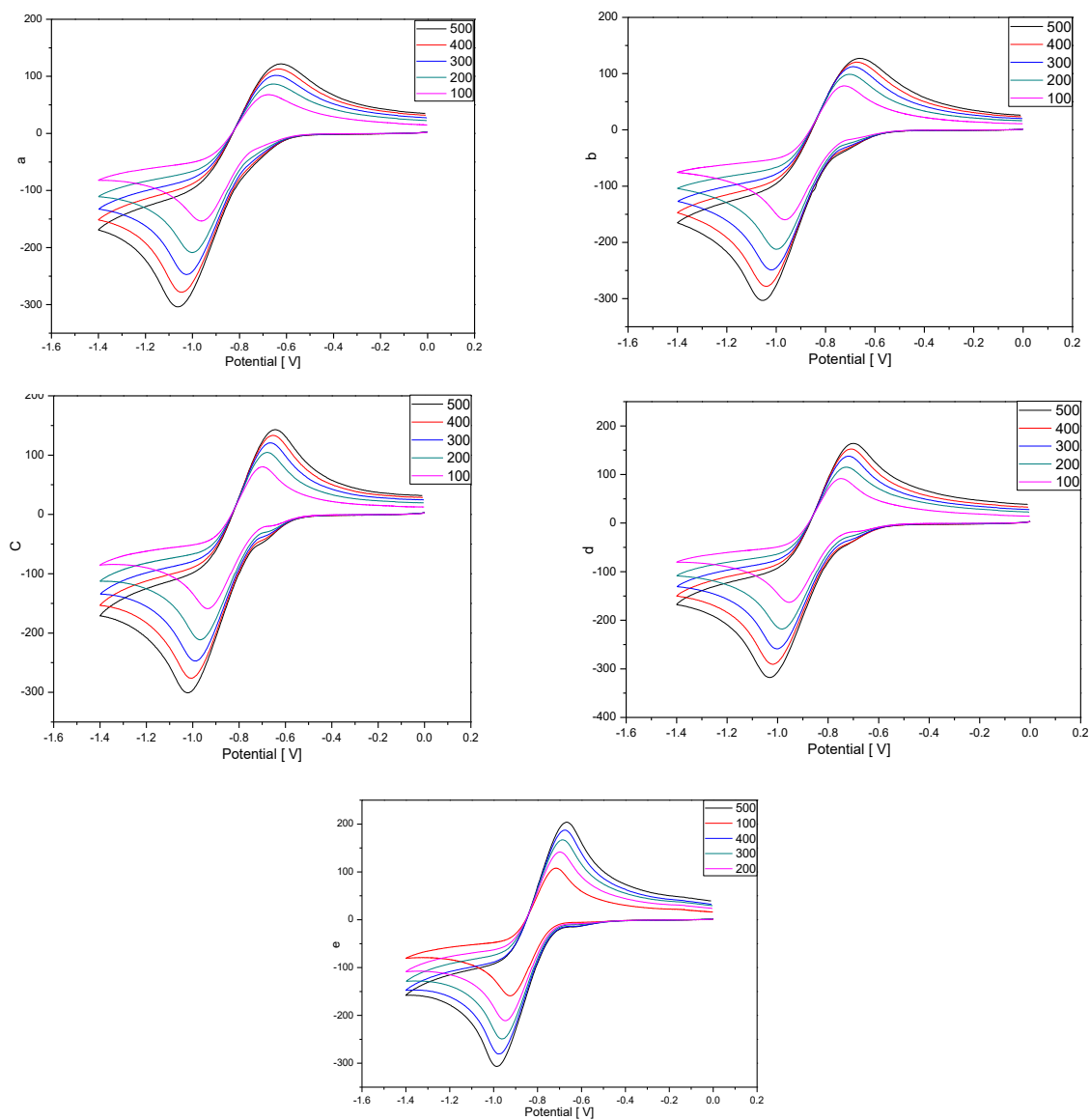
Figure V.15: Succession of cyclic voltammograms at GC electrode in oxygen-saturated DMF/0.1 TBFP at different scan rates ranging from 100 to 500 mV/s , unpeeled (a) $O_2^{\cdot-}$ -Kondor, (b) $O_2^{\cdot-}$-Bartina, (c) $O_2^{\cdot-}$-Spunta, (d) $O_2^{\cdot-}$-Kuroda and (e) for $O_2^{\cdot-}$
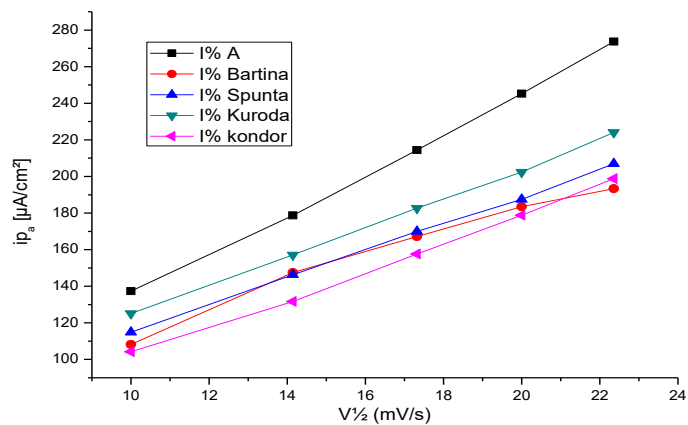
Figure V.16: ipa versus V1/2 plots of oxygen-saturated DMF A in absence of 0.4 ml of unpeeled extract

Table V.14: Diffusion coefficient values

Sample	Equation	R^2 values	D (cm^2/s)
Roots			
O•2−	y = 11.046x + 24.721	0.998	1.87x10-6
O•2−-Kondor	y = 7.6929x + 25.282	0.997	9.09 x10-7
O•2−-Bartina	y = 6.8567x + 44.991	0.978	7.22 x10-7
O•2−-Spunta	y = 7.3768x + 41.425	0.999	8.36 x10-7
O•2−-Kuroda	y = 7.941x+ 45.149	0.999	9.68 x10-7

V.2.7) Discussion

As discussed in chapter II.5, potatoes contain a number of phytochemicals compounds, which have an antioxidant and antibacterial activity. This function had a role in disease prevention. The purpose of this experiment was to assess the relative phenolic and flavonoids content on the potential antioxidant capacity of four different potatoes varieties.

Based on the results which identified the cultivars with the greatest total phenolics content and flavonoids content, all the four potatoes varieties show great levels of phenolic and flavonoids content. Kondor, in particular, had much greater in phenolics content (1.01-2.16) and flavonoids content (1.06-2.29) times greater than other varieties. Similar to finding in HPLC analysis, extraction from Kondor root sample has been shown to exhibit variable degrees of Quercetin acid with a high content 10.91 µg/mg and Caffeic acid with 0.53 µg/mg. These acids present only in Kondor root variety.

The difference of genetic and cultivars condition caused variable effects amongst phytochemical compounds in potatoe samples. The major trends appeared to include a general difference in the actual quantity of the phytochemical compounds that are present in potatoes sample, the other trends include a disappeared of some compounds such as vanillin disappeared in Spunta and Kuroda samples, for the rutine was present only in Kuroda sample.

As expected, due to the high phytochemical content. Kondor variety has the greatest in vitro antioxidant capacity. Two techniques used to measure antioxidant capacity were spectrophotometers (FRAP, and DppH) and electrochemical (total antioxidant activity and O2). Results of the FRAP and CV assays from different technique with the same principle measure directly the antioxidant activity, showed that the four potato varieties have an antioxidant potential. Kondor also displayed the greatest protection against radicals DppH and O_2, then the other four potato cultivars.

Finally, Kondor has a great potential for future development as a marketable food product. Kondor production has also the potential to contribute in useful ways to the development goals in health, more than Bartina, Kuroda and Spunta other potato varieties.

V.3) Experiment Two: Kondor tuber analysis

V.3.1) Aim

The phytochemical content of the tuber of Kondor will then be further quantified to investigate; (a) to what extent of the phenolic and flavonoids content, (b) HPLC analysis, (c) antioxidant activity by the two techniques mentioned above and another spectrophotometer assay. Except where stated, analyses were carried out on Kondor variety in two cases: the first one is the whole tuber (unpeeled) and the second is the tuber flesh (peeled).

V.3.2) Phytochemical contents :

The Table V.19 shows the total phenolic contents (TPC) and the total flavonoid contents (TFC) of Kondor variety in two cases the whole root and the flesh. The tuber samples flesh showed the lower amount of TPC and TFC than the whole tuber sample.

Table V.19: Determination of total Phenolics contents and total flavonoids of the unpeeled and the peeled.

Kondor Sample	Total Polyphenols (mg AG* / g of extract)	Total flavonoids (mg rutin / g of extract)
unpeeled	19.2144 ± 0.096	25.0350 ± 0.125
peeled	14.6496 ± 0.102	14.6878 ± 0.073

As previously shown in the first experiment, with regards to the quantity of total phenolic in different potato tubers, the presence of these compounds contributors with the order: Kondor (unpeeled) > Bartina (unpeeled) > Kondor (peeled) > Spunta (unpeeled) > Kuroda (unpeeled).

A return to the total flavonoids content in all five extract samples, these compounds decreasing in contribution was: Kondor (unpeeled) > Bartina (unpeeled) > Spunta (unpeeled) > Kondor (peeled) > Kuroda (unpeeled).

However, these results showed that the extract of peeled Kondor has a great value in phytochemical content when compared to the whole tuber component in all varieties.

V.3.3) HPLC analysis

The chromatographic profile of extract of the unpeeled sample and the peeled sample of the variety Kondor represented in Level of the figures (V.1 and V.17). The results of quantitative analyses of phenolic compounds Identified are represented in the Table V.20. The analysis of these results shows that in The extract of the variety Kondor in case of peeled, the ascorbic acid (0.226 µg/mg), the Gallic acid (5,764 µg/mg), the Chlorogenic acid (1.845 µg/mg). For the caffeic acid, Vanillin and Quercetin present only in the unpeeled sample. But from the point of view of quantity, the extract of the whole tuber (unpeeled) of Kondor is richness than the extract of the peeled.

Table V.20: Constituents content analyzed by HPLC

Sample extract	Kondor	
	unpeeled	peeled
Ascorbic acid [µg/mg]	0.1896	0.2266
Gallic acid [µg/mg]	5.2946	5.7646
Chlorogenic acid [µg/mg]	3.0520	1.8450
Caffeic acid [µg/mg]	0.5322	-
Quercetin [µg/mg]	10.9197	-
Vanillin [µg/mg]	0.0596	-

The HPLC analysis of all five extract samples presented in tables V.20 and V.4. When compared to the proximate composition of three bioactive compounds found in all potato tubers. Gallic acid is mostly concentrated in all four varieties (7.80 - 5.29 µg/mg) greater in the unpeeled sample and Kondor peeled sample; or Chlorogenic acid (3.05 - 0.75 µg/mg); followed by Ascorbic acid 0.31 - 0.02 µg/mg.
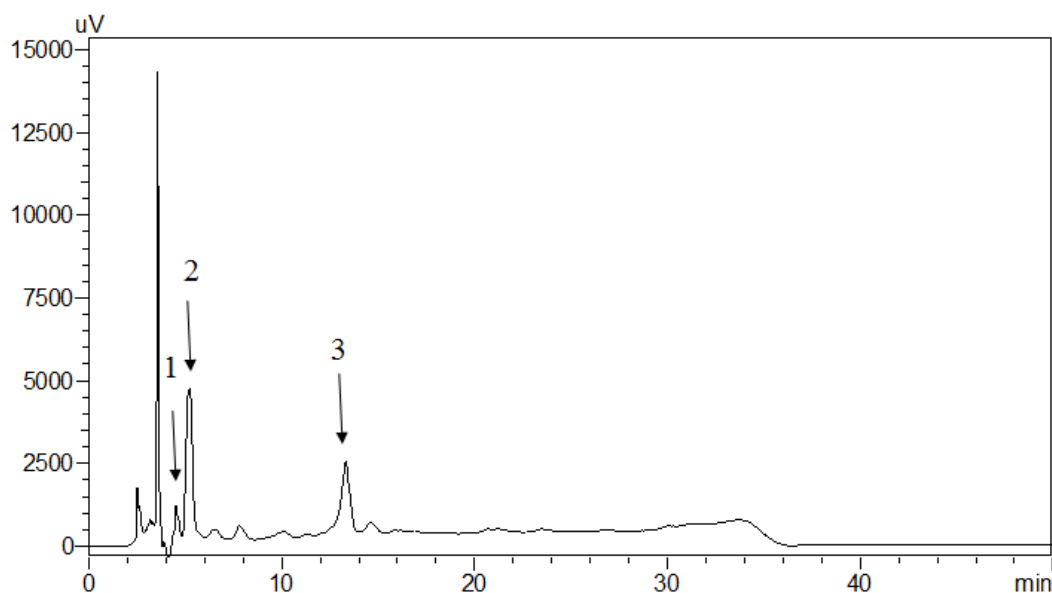
Figure V.17: Chromatograms of leaf extract;(1)Ascorbic acid;(2) Gallic acid; (3) Chlorogenic acid.

V.3.4) Spectrophotometers assays

The phytochemical analysis of bioactive compounds in Kondor variety unpeeled and peeled samples showed a comprehensive identification of phenolics and flavonoids content, but rather presents an idea of the array of phytochemical compounds. As there are many phenolic compounds with different properties and characteristics. Hence one particular assay may be better at detecting a certain type of antioxidant compound than another and therefore using more one assay enables a researcher to pick up a wide range of oxidative compounds.

V.3.4.1) FRAP assay

Ferric reducing antioxidant power (FRAP) is based on the ability of the antioxidant to reduce Fe3+ to Fe2+, according to Rodriquez & Hadley, the ethanolic extracts of potato tubers showed marked hydrogen-donating activity using reducing power in the Fe(III) Æ Fe (II) reaction in FRAP assay. [9]

Based on the FRAP assay (Table V.21), the results indicated that the unpeeled samples had a reducing antioxidant power more than the peeled sample, with a value 38.8032 and 23.2443 mg Fe(II)/ g of extract respectively. However, the results mentioned above (Table V.5) shows three of potato unpeeled Kondor, Bartina, and Spunta had higher values of reducing antioxidant power than Kondor peeled sample, but less than Gallic acid and ascorbic acid.

Table V.21: Determination of reducing the antioxidant power of four kinds of potatoes.

Sample	FRAP (mg Fe(II)/ g of extract)
unpeeled	38.8032 ± 0.043
peeled	23.2443 ± 0.019
Ascorbic acid	2512.985 ± 0.020
Gallic acid	7778.2511 ± 0.388

V.3.4.2) Total antioxidant capacity

Several assays have been introduced for the measurement of the antioxidant activity of plant extracts including total antioxidant capacity by the phosphomolybdenum method. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green Mo(V) complexes with a maximal absorption at 695 nm [21]. Using this method, the result indicated that both the peeled and unpeeled extracts of Kondor variety have an antioxidant capacity. The unpeeled extract displayed the higher antioxidant capacity than the peeled extract with value 47.08 and 27.87 mg ascorbic acid equivalent/g extract respectively. Overall, the two samples had lower antioxidant activity than the Gallic acid (Table V.22).

Table V.22: Determination of the Total antioxidant capacity of Kondor.

Sample	Total antioxidant capacity
unpeeled	47.0873 ± 0.043
peeled	27.8784 ± 0.019
GA	656.54263 ± 0.035

V.3.4.3) β-carotene bleaching assay

In the β-carotene-linoleic acid coupled oxidation model system, the linoleic acid-free radical (LOO·) formed attacks the highly unsaturated β-carotene molecules and in the absence of an antioxidant rapidly bleaches the typically orange color of β-carotene which is monitored spectrophotometrically at 450 nm. The extracts reduced the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [22]. The antioxidant activities of Kondor peeled and unpeeled extracts were

determined after 120 min reaction time (Table V.23), and compared with a well known natural antioxidant, gallic acid, and ascorbic acid.

However, the results indicated that the extracts ranked in the following order: unpeeled extract (68.42% ± 0.007)> peeled extract (53.33% ± 0.006). It is clear that the two extracts are capable of inhibiting β-carotene bleaching and the activity depends on the amount of extract. The unpeeled extract showed the highest level of activity at 4 mg/ml and also demonstrated a significant increase in activity compared to the peeled extract. In a comparison of the β-carotene bleaching activity, gallic acid and ascorbic acid showed the highest antioxidative activity at 0.08 mg/ml with values 62.64% ± 0.013 and 16.51% ± 0.007 respectively.

Table V.23: Determination of antioxidant activity % of sample extracts using β-carotene bleaching

Sample extraction	Concentration (mg/ml)	Antioxidant activity %
Unpeeled	0.75	03.2967 ± 0.018
	1.5	28.1250 ± 0.014
	4	68.4210 ± 0.007
Peeled	0.75	08.4337 ± 0.005
	1.5	15.2941 ± 0.019
	4	53.3334 ± 0.006
Ascorbic acid	0.03	04.6204 ± 0.005
	0.06	13.7500 ± 0.008
	0.08	16.5178 ± 0.007
Gallic acid	0.03	21.2477 ± 0.003
	0.06	42.5287 ± 0.005
	0.08	62.6436 ± 0.013

Additionally, the inhibition of the antioxidant activity of β-carotene oxidation also expressed as IC_{50} (Table V.24). The IC_{50} value of each extract is deducted from the equations of the curves of the variation in the percentage of inhibition I% as a function of the concentration of each extract as shown in Figure V.18 and Annexe (A)

The IC_{50} scavenging activity of the Kondor unpeeled and peeled extracts were 2.85 mg/ml, 3.90 mg/ml respectively. Also for the gallic acid and ascorbic acid showed the highest

value 0.066 mg/ml and 0.21 mg/ml. All the results have a very statistically significant with p<0.005, the order of antioxidant activity towards β-carotene oxidation was :

Gallic acid > Ascorbic acid > Unpeeled > Peeled.
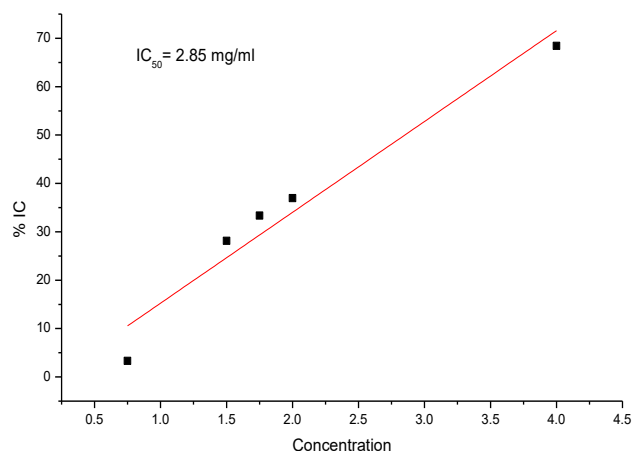
Figure V.18: Antioxidant activity (%) of unpeeled Kondor assayed by β-carotene– linoleate bleaching.

Table V.24: IC_{50}% values of sample extracts using β-carotene bleaching

Sample extraction	Equation	R^2	IC_{50}%	P value
Unpeeled	Y = 18.779x - 3.531	0.954	2.85	0.004
Peeled	Y= 13.231x - 1.649	0.993	3.90	0.000
Ascorbic acid	Y= 241.54x - 2.069	0.918	0.21	0.003
Gallic acid	Y= 784.8x - 2.2963	0.993	0.066	0.000

V.3.4.3) DPPH

DPPH scavenging activity (%) had increased with the increased concentration of the extract in all samples (Figure V.19). The stable DPPH free radical accepts an electron or hydrogen radical from donors to form a stable molecule which could be seen as color reduction.

V.3.4.3.1) Calculation of radical inhibition

Kondor extract was the strongest electron or hydrogen donor due to the highest DPPH scavenging activity. The IC_{50} of DPPH scavenging activity of the Kondor unpeeled and peeled extracts were 1.21 mg/ml, 2.80 mg/ml respectively. Also for the Gallic acid and

ascorbic acid showed the highest value 0.0069 mg/ml and 0.01 mg/ml. All the results have a very statistically significant with p<0.000 (table V.24)

Table V.24: IC50 values of sample extracts using DPPH

Sample	DppH			
	Equation	R^2 value	IC50*	P
unpeeled	Y = 3.33 + 38.3 X	0.994	1.21	0.000
peeled	Y = - 3.47 + 25.7 X	0.991	2.80	0.000
Ascorbic acid	Y= 0.94 + 4720X	0.972	0.010	0.000
Gallic acid	Y=-6.78 + 8154X	0.982	0.0069	0.000

* inhibition expressed as mg/ml

V.3.4.3.2) Calculation of the binding constant

The electronic spectra of complexes extract–DppH were recorded in the range 200-900 nm, which showed intense absorption at range 515-518 nm The determination of binding constant Kb of extract–DppH complex can be calculated from the inhibition of absorbance in UV-spectra after the addition of sample extract (Figure V.19).

As mention above: A plot of Ao /(A-Ao) to 1/[extract] has shown linearity which is suggestive of 1:1 complex formation of both extracts with DppH. The intercept to slope ratio of this plot gives the value of binding constant "Kb " (Annexe A). Kb values were calculated given in Table V.25

UV spectra of DppH showed a hypochromic effect upon addition of sample extract the intensity decreased rapidly with increasing concentration of the extract were recorded in Figure V.20. In the absence of sample extract the DppH displayed one absorbance peak at 517 nm, this peak intensity exhibited hypochromism up from 56% to 77% for 2 mg/ml concentration of sample extract.

The values of binding constant "Kb " were further used to calculate standard Gibbs free energy "ΔG" of extract–DppH complex, using the following equation;

$$\Delta G = -RT\ln K_b$$

Free energy changes of extracts values, Table V.25, and showed spontaneous of their binding with DppH. The p-value of the results was <0.05 which a significant statistically.
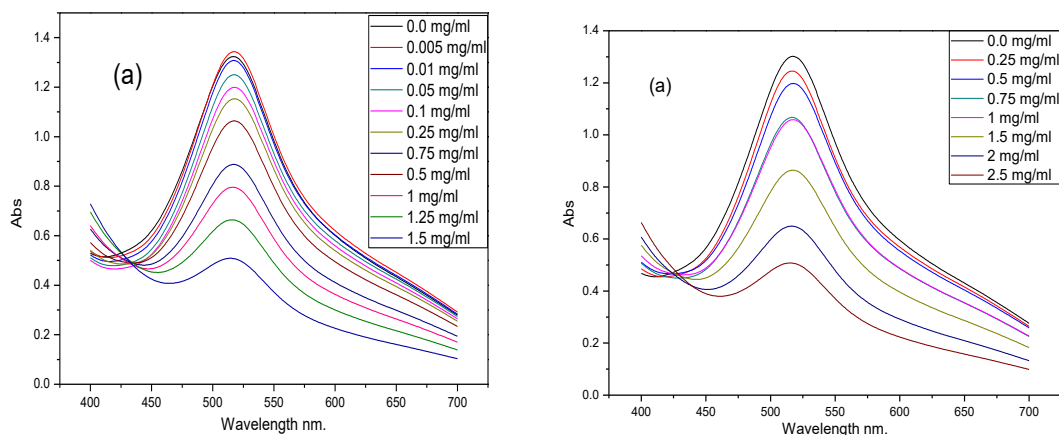
Figure V.19: Electronic absorption spectra of 0.25 mM of DPPH interaction with of (a) Unpeeled and (b) Peeled

Table V.25: Values of Binding constants and binding free energy of the complexes DppH-sample extracts

Compound	Equation	R^2	K	ΔG	P
unpeeled					
DppH-Kondor	Y = -0.031x + 2.4387	0.9089	78.6266	-10.822	0.012
peeled					
DppH-Kondor	Y = -0.0289x +1.9914	0.9129	68.9065	-10.494	0.011

V.3.5) Electrochemistry assay

Table V.26: shows The oxidation of Kondor samples extract studied by cyclic voltammetry showed capacity antioxidant, expressed in terms of gallic acids (AG) equivalent antioxidant capacity obtained from an electrochemical assay in ethanolic extract of two cases, the unpeeled, and the peeled. This capacity related to the potential peaks which determined the antioxidant power of sample extracts, this potential peak show almost the same value but the peeled sample has lower antioxidant capacity than the unpeeled.

Table V.26: Determination of the Antioxidant capacity of Kondor variety by CV.

Sample	Antioxidant capacity CV[a]	Eap[b] (V)
unpeeled	53.4813 ± 0.254	+0.88
peeled	30.5753 ± 0.540	+0.87

[a] capacity antioxidant expressed as milligrams of gallic acid equivalent per gram of extract

[b] Anodic oxidation potential vs GCE

V.3.5.2) Electrochemistry assay $O_2^{\cdot-}$ radical scavenging activity

The quantification of antioxidant in the extract is made by calculating the inhibition concentration (IC_{50}) value, radical scavenging activity was plotted against different concentrations of extract samples, the effect of different concentrations is shown in (Figure V.21) for $O_2^{\cdot-}$ radical scavenging activity All the voltammograms of superoxide anion radical Figure V.20 showed one electron reversible process, having well developed and clear oxidation and reduction peaks. On the level of anodic current the decreasing of peaks according to the addition of sample extracts.

The equation obtained from the linear calibration graph (Annexe) in the studied concentration range of samples extracts are presented in Table V.27, where y stand for the value of the oxidation peak current density of $O_2^{\cdot-}$ and x represents the value of the concentration of ethanolic extracts, expressed as mg/mL. The unpeeled Kondor extracts have a less IC50 with 1.03 mg/mL than peeled with 2.02 mg/mL. All the IC_{50} values were considered as very statistically significant with $p<0.000$.

Table V.27: IC50 values of Kondor sample extracts using $O_2^{\cdot-}$ radical scavenging activity

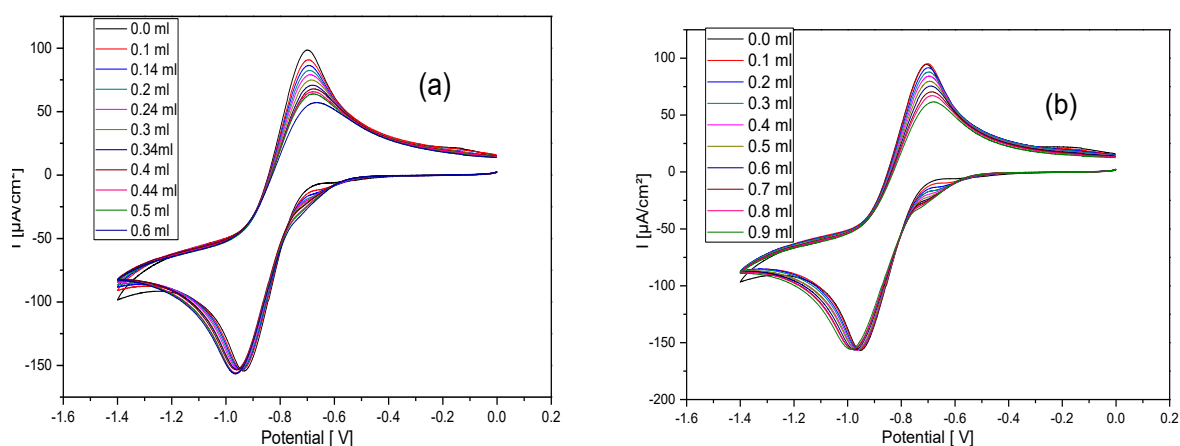| Sample	Equation	R^2 value	IC50	P
unpeeled	Y= 1.56 + 46.8 X	0.989	1.0350	0.000
peeled	Y = - 4.16 + 26.7 X	0.948	2.0284	0.000

Figure V.20: Cyclic voltammograms of oxygen-saturated in the absence and presence of different concentration of Kondor sample extract: (a) unpeeled, (b) peeled at scan rate 100 mV/s

V.3.5.2.1) Ratio of binding constants (Kox/Kred)

Figure V.21 shows the CV's behavior of oxygen-saturated DMF/0.1 TBFP in the potential window of -1.4 to 0.0 V at a glassy carbon electrode in the absence and presence of 0.4 mL sample extracts, the free $O2/O_2^{\cdot-}$ redox couple exhibits a single oxidation peak at -0.72 V and a single reduction peak at -0.74 V. These Figures also shows the effect of the addition of extract on the oxidation peak current of $O2/O_2^{\cdot-}$.

The CV technique was also helpful to determine the mechanism of free radical scavenging, the observed changes in anodic peak current of $O/O_2^{\cdot-}$ is caused by the addition of different content of ethanolic extracts, this decrease in anodic peak can be used for the calculation of the binding constant, where the shift in peak potential values can be employed for the determination of the mode of interaction, in the same manner, used for study of binding of drug molecules to DNA [14,15].

By adding 0.4 ml of sample extracts in a solution of DMF, the peak potential was shifted by ΔE, the peak potential was displaced by ΔEf^0 mV to more negative for complex $O_2^{\cdot-}$-Kondor in two cases unpeeled and peeled complexes which associated with the decrease in oxidation peak current densities $\Delta Ipa\%$ which confirms the interaction of ethanolic extracts with $O_2^{\cdot-}$ (Table V.28) The significant decrease in oxidation peak current densities can be attributed to the diminution in $O_2^{\cdot-}$ concentration due to the formation of sample extract complex.

Table V.28: Shifts in peak potential and a decrease in anodic peak current of $O_2^{\cdot-}$ bound forms of Kondor extract samples.

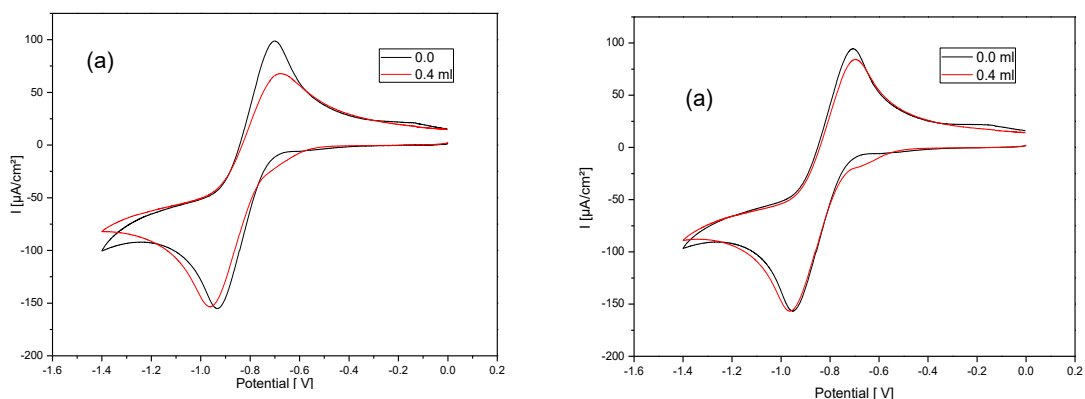| Sample	Ipa (µA)	Epa (V)	Epc (V)	Ef^0 (V)	ΔEf^0(mV)	$\Delta Ipa\%$	Kox/Kred
$O_2^{\cdot-}$-	134.2672	-0.7068	-0.7247	-0.7157	-	-	-
$O_2^{\cdot-}$-Unpeeled	104.2637	-0.678	-0.7566	-0.7173	-1.5574	22.3460	0.94
$O_2^{\cdot-}$-	129.9617	-0.7114	-0.7484	-0.7299	-	-	-
$O_2^{\cdot-}$-Peeled	121.6849	-0.7060	-0.7570	-0.7315	-1.5574	6.3686	0.94

Figure V.21: Cyclic voltammograms of oxygen-saturated in the absence and presence of 0.4 ml Kondor sample extract (a) Unpeeled (b) Peeled at scan rate 100 mV/s

V.3.5.2.2)Thermodynamic properties

The obtained values from CV's voltammograms showed in Table V.29 indicated that the binding constant (K_b) ranged as the follows:

$$O_2^{\cdot -} \text{- Peeled} < O_2^{\cdot -}\text{-Unpeeled}$$

The values of free energy varied in the same order as of the binding constant. The negative values of ΔG indicated the spontaneity of $O_2^{\cdot -} - SE$ interaction. The data obtained in Table V.29, were very statistically significant with $p<0.000$.

Table V.29: Values of Binding constants and binding free energy of the complexes $O_2^{\cdot -}$ - sample extracts

Compound	Equation	R^2	K	ΔG	P
unpeeled					
$O_2^{\cdot -}$-Kondor	y = 0.9086x + 2.8778	0.990	07.5474×10^2	-16.42	0.000
peeled					
$O_2^{\cdot -}$-Kondor	y = 0.5606x + 2.7765	0.962	05.9772×10^2	-15.85	0.000

V.3.6) Discussion

Finally, fresh Kondor tuber including the whole tuber (unpeeled) and the peeled, are each likely to be great bioactive compounds when compared with other potato varieties due to their higher content of phenolics and flavonoids as well as greater antioxidant capacity.

One of the major aims of this work was to provide a preliminary investigation on the phytochemical composition of one variety which has greater levels of phytochemical compounds and to compare these results in both the unpeeled and peeled samples.

The results of the second experiment suggest that the freshly peeled potato tubers from Kondor variety show promising potential with regards to greater potential health benefits from greater phenolics, flavonoids as well as antioxidant activity. Similar to finding in this work, other researchers have found that total phenolic compounds were greater in the tuber skin compared to the tuber flesh [23-24].

As found in this experiment, the peeled Kondor variety showed higher concentrations of phenolic and flavonoid compounds compared to unpeeled Spunta and Kuroda respectively, and thus great antioxidant activity.

In this manner, with regards to health value, since the skin of potatoes generally has greater phytochemical compounds relative to the flesh, it would be advisable to eat the whole potato tuber rather than peeling them.

V.4) Statistic analysis

The analysis of variance of the main effect plot between the variable studied and their significance was performed using GLM. To confirm that the antioxidant activity of the extracts of the potatoes back to their richness in phytochemical compounds, we tried to find a linear correlation between the antioxidant capacity values calculated by the methods measured the antioxidant activity with their polyphenol total content and flavanoids total content, also a correlation between each other

V.4.1) Main effect plot

Fig V.22, 23 and Table V.15 shows an overall view of the relative of variety on the level of phytochemical content and the effect of activity antioxidants a further analysis of variance of the main effect plot between the variable studied and their significance was using performed using GLM, Fig V.22 shows the overall effect of variety on the level total phenolic content and total flavonoid content. The average plot of TPC is valued 14.88, which presented with a line across the plot, the variety Kondor and Bartina have the higher amount of TPC with values above the average line, for the variety Spunta and Kuroda shows a lower amount below the average line. Moreover, the TFC plot three varieties Kondor, Bartina, and Spunta have a higher amount above the average line (at 20.33) than the Kuroda variety. as it can be seen there is a statistically significant difference in the amounts of TFC, TPC and varieties ($p < 0.005$- see Table V.15).

Furthermore, the effect of different varieties on their antioxidants activity can be seen in Fig V 23 for CV and FRAP assays. This plot shows two varieties Kondor and Bartina above the average line which presented at a value 42.36 and 30.68 respectively, for the other varieties Spunta and Kuroda have a low average value presented below the average line. These factors have a very significant statistically with $p < 0.000$.

Another antioxidant capacity DppH and O_2, their plot of IC_{50} average presented in Figure V.23, also these plots show two varieties Spunta and Kuroda have values above the average line, in this case, means the lowest antioxidant capacity, the Kondor, and Bartina variety have the highest antioxidant capacity which presented lower than the average line, this analysis has a statistically significant with $p<0.005$.

Table V.15: Analysis of variance for the main effect plot

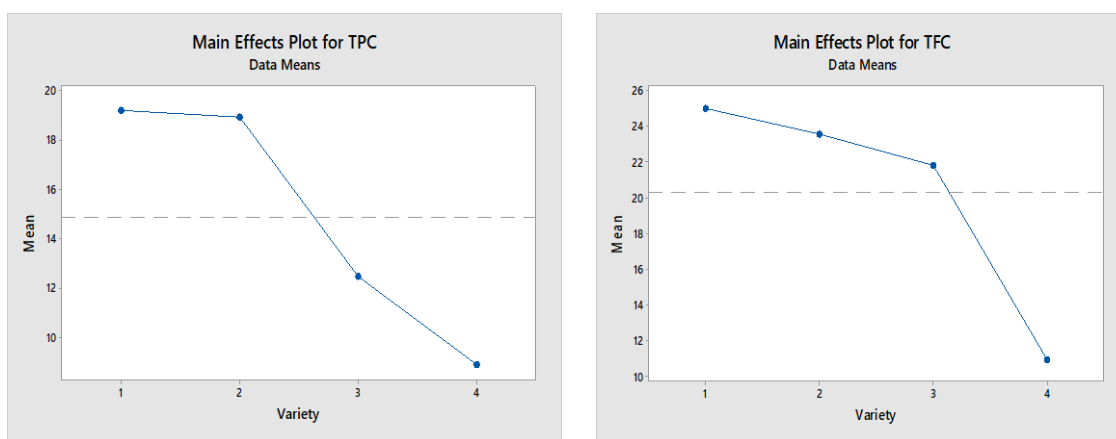| Main effect plot	P value
TPC	0.000
TFC	0.000
FRAP	0.000
CV	0.000
DppH	0.003
$O_2^{\cdot-}$	0.003

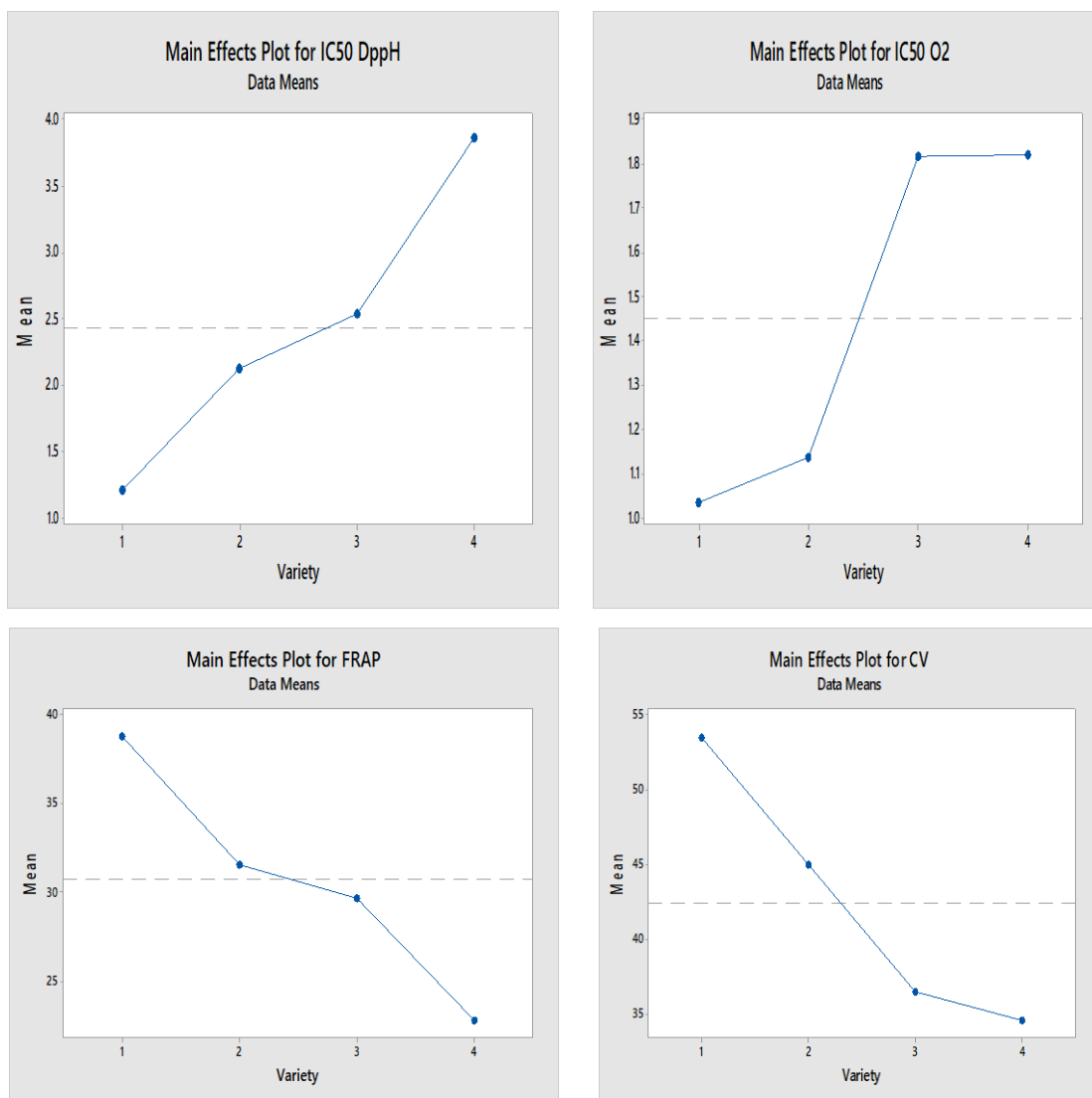**Figure V.22:** Effect of variety: 1 Kondor, 2 Bartina, 3 Spunta, 4 Kuroda, on the level of (a) TPC, (b) TFC

Figure V.23: Effect of variety: (1) Kondor, (2) Bartina, (3) Spunta, (4) Kuroda, on the level of inhibition IC_{50} of (a) DppH, (b) $O_2^{\cdot-}$

V.4.2) Linear regression

V.4.2.1) Linear regression between total polyphenol content and antioxidant activity

From these plots Figure V.24, a correlation (Table V.16) is observed between the content of total phenolic content TPC and ethanolic extracts of a different variety of potatoes and the antioxidant activity as measured by different methods with correlation indices R^2, which ranged from 0.602 to 0.825.
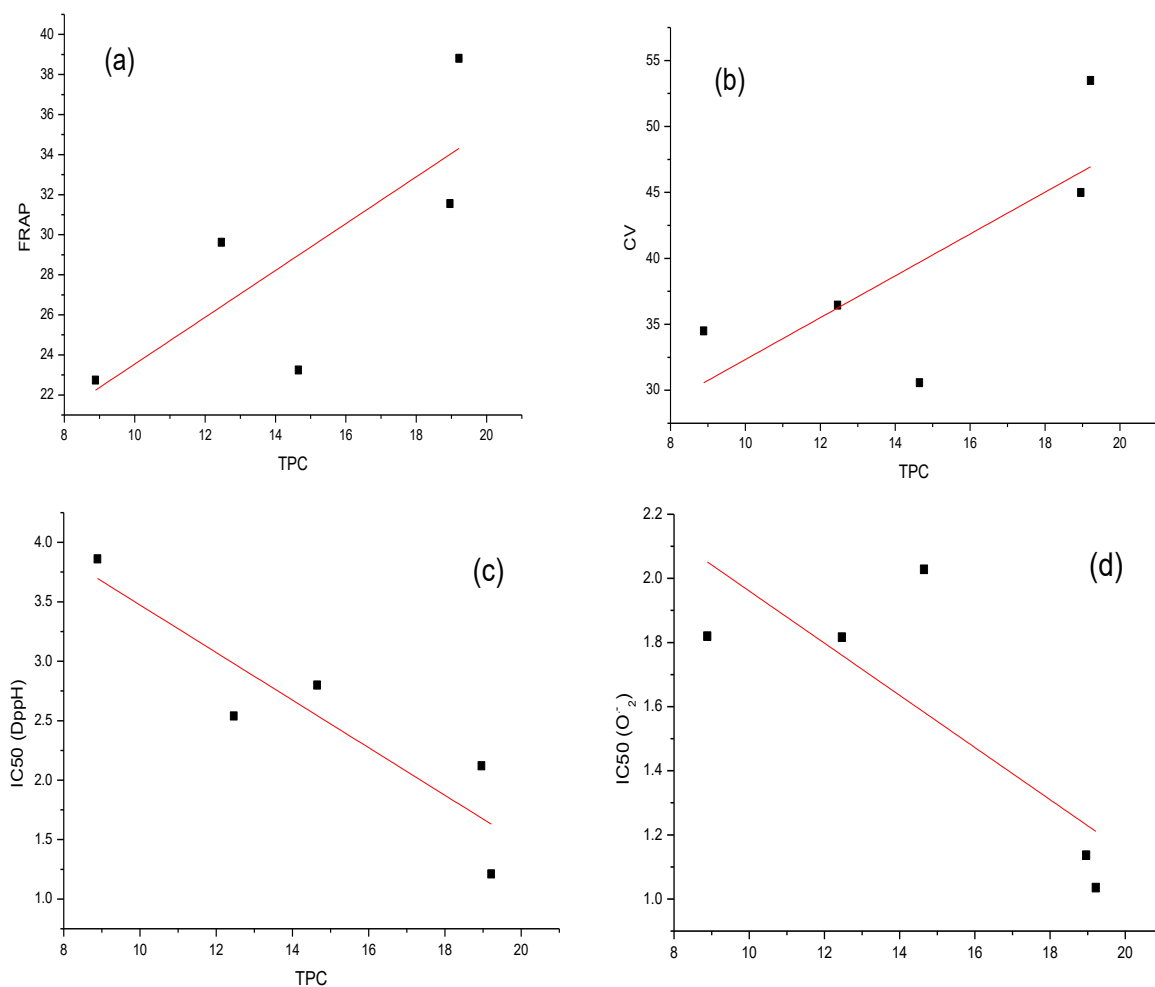
Figure V.24: Correlation graphs for (a) FRAP, (b) CV, (c) $IC_{50}DppH$, and (d) IC_{50} $O_2^{.-}$ with total phenolic content (TPC)

Table V.16: Determination of the correlation between capacity antioxidant and Total Phenolic content.

Assays	Equation	R^2
FRAP	Y= 11.85 + 1.169X	0.602
CV	Y = 16.5 + 1.586 X	0.574
IC_{50} DppH	Y= 5.474 – 0.2001X	0.825
IC_{50} $O_2^{.-}$	Y= 2.774 – 0.0814X	0.633

V.4.2.2) Linear regression between total flavonoids content and antioxidant activity

From these plots Figure V.25 a correlation (Table V.17) is observed between the total flavonoids content TFC and ethanolic extracts of a different variety of potatoes and the antioxidant activity as measured by the with correlation indices R^2. which ranged from 0.602 to 0.843. The regression coefficient values obtained for total flavonoids content and antioxidants assay was higher compared with total phenolics content.
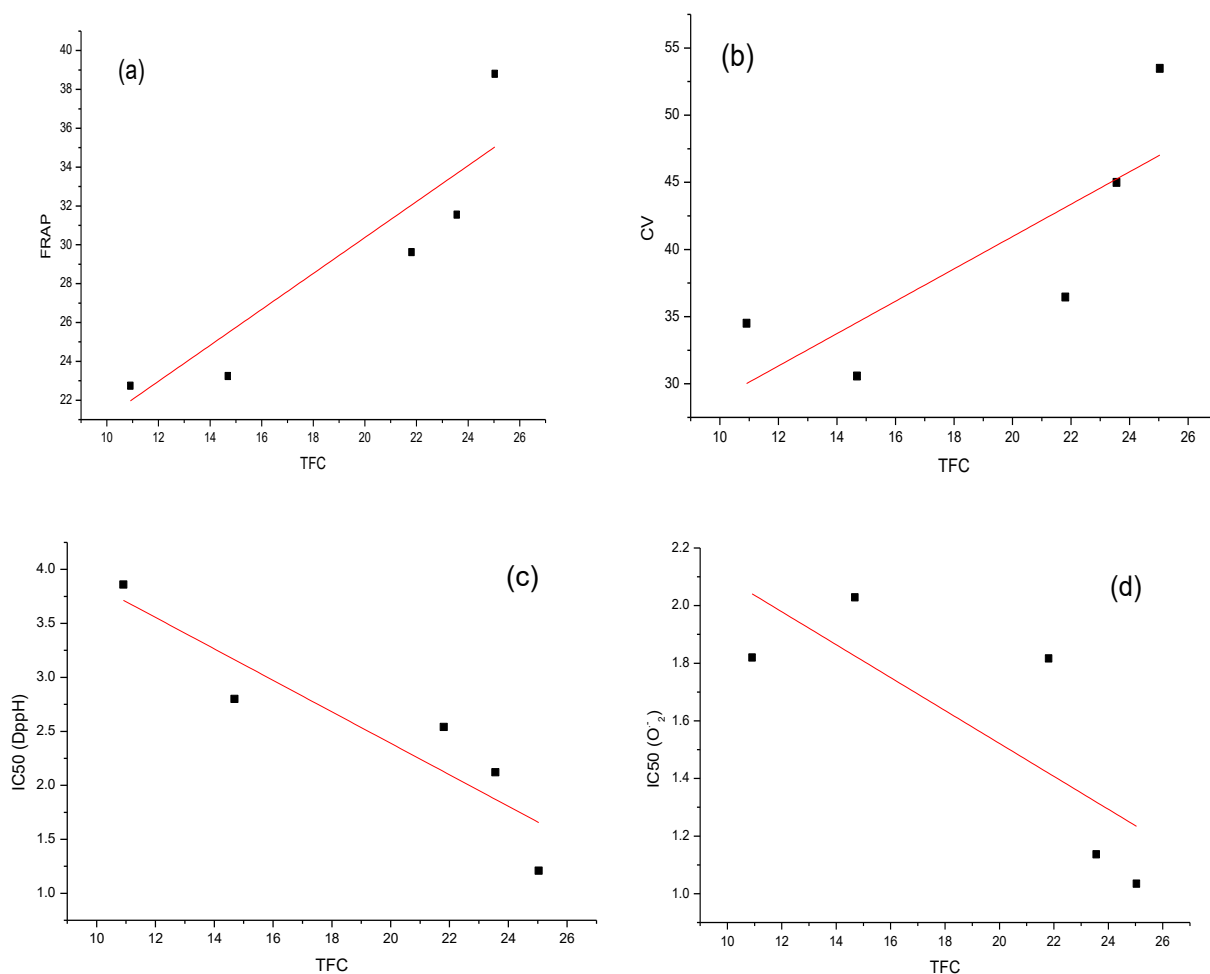

Figure V.25: Correlation graphs for (a)FRAP, (b) CV, (c) IC50 DppH, and (d) IC_{50} $O_2^{\cdot-}$ with total flavonoids content (TFC)

Table V.17: Determination of the correlation between capacity antioxidant and Total Flavanoids content.

Assays	Equation	R^2
FRAP	$Y = 10.14 + 0.992 X$	0.837
CV	$Y = 16.9 + 1.204 X$	0.638
IC_{50} DppH	$Y = 5.035 - 0.1458X$	0.843
IC_{50} O_2	$Y = 2.664 - 0.0571X$	0.602

As shown in Table V.18, significant positive correlations (R^2 = 0.547 - 0.825, the average of R^2 was 0.651) were observed between total phenolic content and FRAP, CV assays and IC_{50} values for DPPH, $O_2^{\cdot-}$, indicating the significant contribution of phenolics to these antioxidant assays. Also for the same assays, the significant positive correlations (R^2 = 0.602 - 0.843, the average of R^2 was 0.730) were observed between them and the total flavonoids contents, this R^2-value indicated that total flavonoids contents exert high antioxidant activity than total phenolic content in sample extracts.

However, among the four antioxidant assays (FRAP, CV, DppH, and $O_2^{\cdot-}$) the R^2 value of CV and FRAP show less correlation (0.547 and 0.602) for TPC respectively, this result indicated that total phenolics content exert a less capacity effect on CV and FRAP than other assays.

Finally, R^2 values ranged from 0.547 to 0.843, with average value was 0.690, these high levels indicate that the antioxidant activity of potatoes might in large of the contribution of phytochemical contents, especially flavonoids compounds.

Table V.18: The R^2 values between antioxidant activities and phytochemical content

	FRAP	CV	IC_{50}		Average
			DppH	$O_2^{\cdot-}$	
TPC	0.602	0.547	0.825	0.633	0.651
TFC	0.837	0.638	0.843	0.602	0.730
Average	0.719	0.592	0.834	0.617	0.690

V.4.2.3) Linear regression between different methods antioxidant activity

The relation between different methods was measured using different linear correlations. From these plots Figure V.26 (a), a significant correlation is observed between the antioxidants assay FRAP, and CV with correlation indices R^2 value 0.903 and equation $Y= 1.84 + 0.684\ X$.

However, for the inhibitory assays $O_2^{\cdot-}$, and DppH (Figure V.26.b), the equation obtained from the linear regression $Y=0.670 + 0.358\ X$ and the coefficient value $R^2 =0.595$. This higher regression coefficient indicated a strong correlation between these assays.
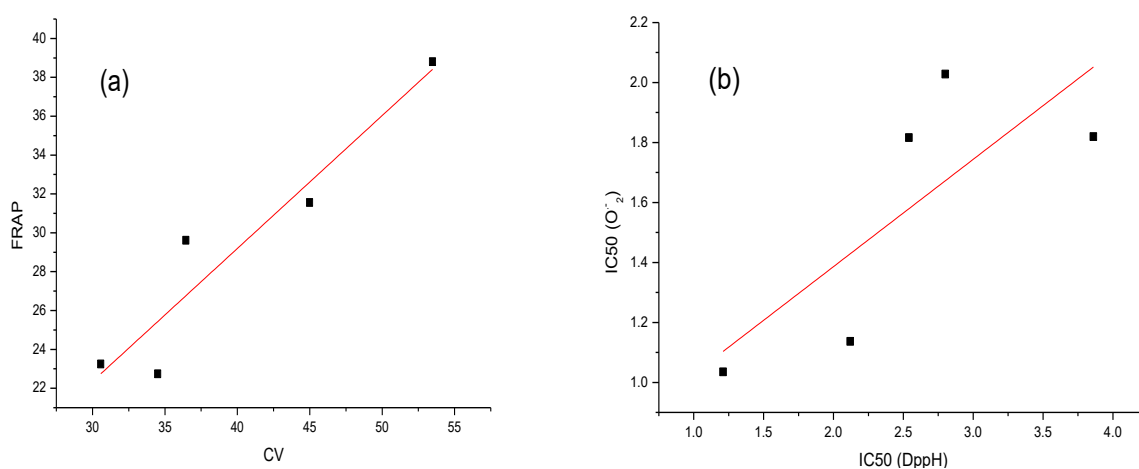

Figure V.26: Correlation graphs for (a) FRAP and CV, (b) IC_{50} between DppH and $O_2^{\cdot-}$

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General conclusion

This study theme sets as the main objective the evaluation in vitro of the phytochemical content and antioxidant activity of four different varieties of potatoes *Solanum tuberosum L* (Bartina, Kondor, Kuroda, and Spunta). Until now, no studies concerning the antioxidant activity of the potato of the El Oued region have been conducted. We think that the present study is the first to investigate and evaluate the bioactive compounds

For this concern, we choose two methods for extracting the higher level of bioactive compounds from potato. These techniques are ultrasonic-assisted extraction combines with maceration and Soxhlet extraction. The obtained results indicated that the amount extracted from both total phenolic and flavonoids contents is remarkably improved when using UAE-M. The yield of extraction also improved when maceration is used prior to ultrasonic-assisted extraction. The most efficient method for extraction of TPC and TFC is UAE-M with 30 % of amplitude, ethanol as solvent and maceration time of 24 hours, all obtained results are confirmed by HPLC analysis.

The phytochemical investigation of four varieties detected Alkaloids, Coumarins, Phytosterols, Carbohydrates, phenolic, and Flavonoids. The presence of phenolic content was lower when compared with flavonoids content in the whole roots and the flesh. All the results indicate that roots have a higher level of bioactive compounds more than the flesh. Overall the sample Kondor showed the highest amount of total phenolic content and total flavonoids content than other samples. This difference in phytochemical content is clearly shown in HPLC analysis which is greatly influenced by cultivar condition and their variety, also, reflect on their antioxidants activity.

The antioxidant capacity of ethanolic extract was evaluated and compared using two techniques: spectrophotometers and electrochemical assays. For four different varieties of the Solanum tuberosum L.

According to the spectrophotometers assays, the antioxidants capacity was calculated with Ferric reducing antioxidant power assay (FRAP), and the oxidation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method expressed as IC_{50}. All the results show that the ethanolic extract of the whole roots of the Kondor variety had a higher antioxidant

capacity than the other ethanolic extract in order was; Kondor > Bartina > Spunta > Kuroda. This antioxidants capacity was less active than Gallic and Ascorbic acids.

For the electrochemical assay, we used the cyclic voltammetry technique to evaluate the antioxidant capacity of ethanolic extract, the redox potentials of these compounds used to calculate the antioxidant capacity in an aqueous solvent (acetate buffer). The redox potentials also measured in an organic solvent to compare their electrochemical behavior. In this mid, the antioxidant property of phenolics extracted were evaluated by the inhibitory effect of superoxide anion radicals $O_2^{\cdot-}$ and expressed as IC_{50}. The obtained data shows value ranged as the follows; Kondor > Bartina > Spunta > Kuroda, for the whole root.

The thermodynamic properties quantify the strength of interaction between radicals and the probe antioxidant in the ethanolic extract. These properties were estimated in terms of binding constant Kb, based on the decrease in peak current and UV-spectra absorbance for $O_2^{\cdot-}$ and DppH respectively. This constant allows calculating the standard Gibbs free energy (ΔG°). Their negative values indicated the spontaneity of interaction between radicals and extracts.

The analysis of variance was determined by the main effect plot and linear correlation between the variable studied. All the results show statistically significant, positive correlations were observed between total phenolic content, total flavonoids content and antioxidant capacity measured with different assays. This correlation determined with indices R^2 which ranged from medium to high levels. the total flavonoids content has exerted less antioxidant activity than total phenolic content in sample extracts.

The evaluation and the quantification of antioxidants content and antioxidants activity in vitro, for all four varieties, show that the whole roots of variety Kondor has a high level of bioactive compounds more than other varieties. Based on this results, the phytochemical analysis was carried out on Kondor for two cases the whole root and the flesh, and the information obtained has led to show that the whole roots have a higher amount in phytochemical content than the flesh for this variety.

Kondor has great potential for future development as a marketable food product. Thus we advise to cultivate this variety more than Bartina, Kuroda and Spunta other potatoes varieties,

This study scientifically valids the traditional use of these plants and reveals their interest in the framework of an exploitation in biotechnology. For the suite, it would be interesting to isolate and characterize the phenolic compounds of the extracts of four varieties selected and other. Also, would be very desirable, for a better understanding of the mode of action of the polyphenolic derivatives, to evaluate in vitro and in vivo the antioxidant activity of each of these compounds taken separately. What would then highlight the active principle of plant extracts and/or a possible synergy between the various phenolic compounds?

Annexe
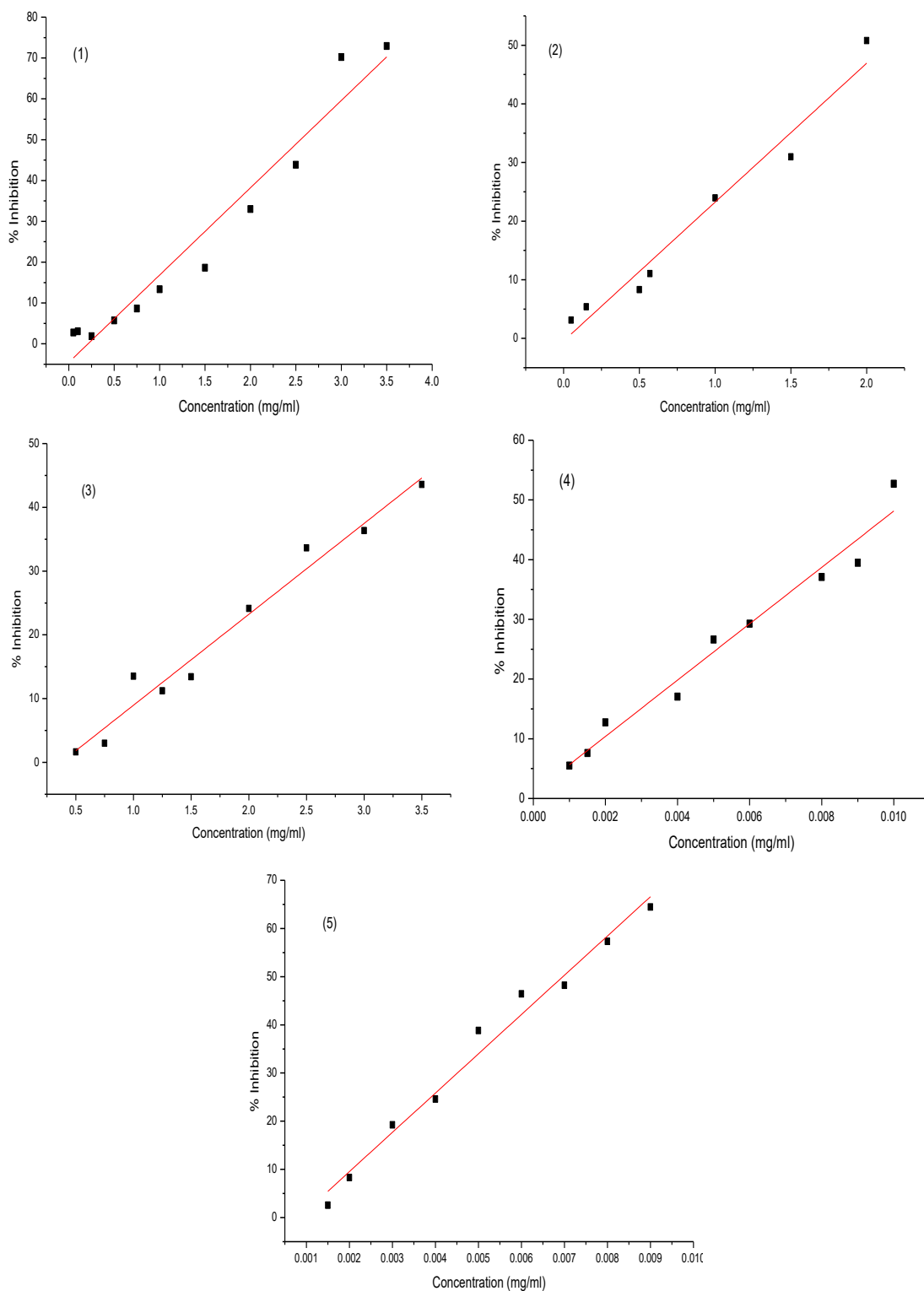
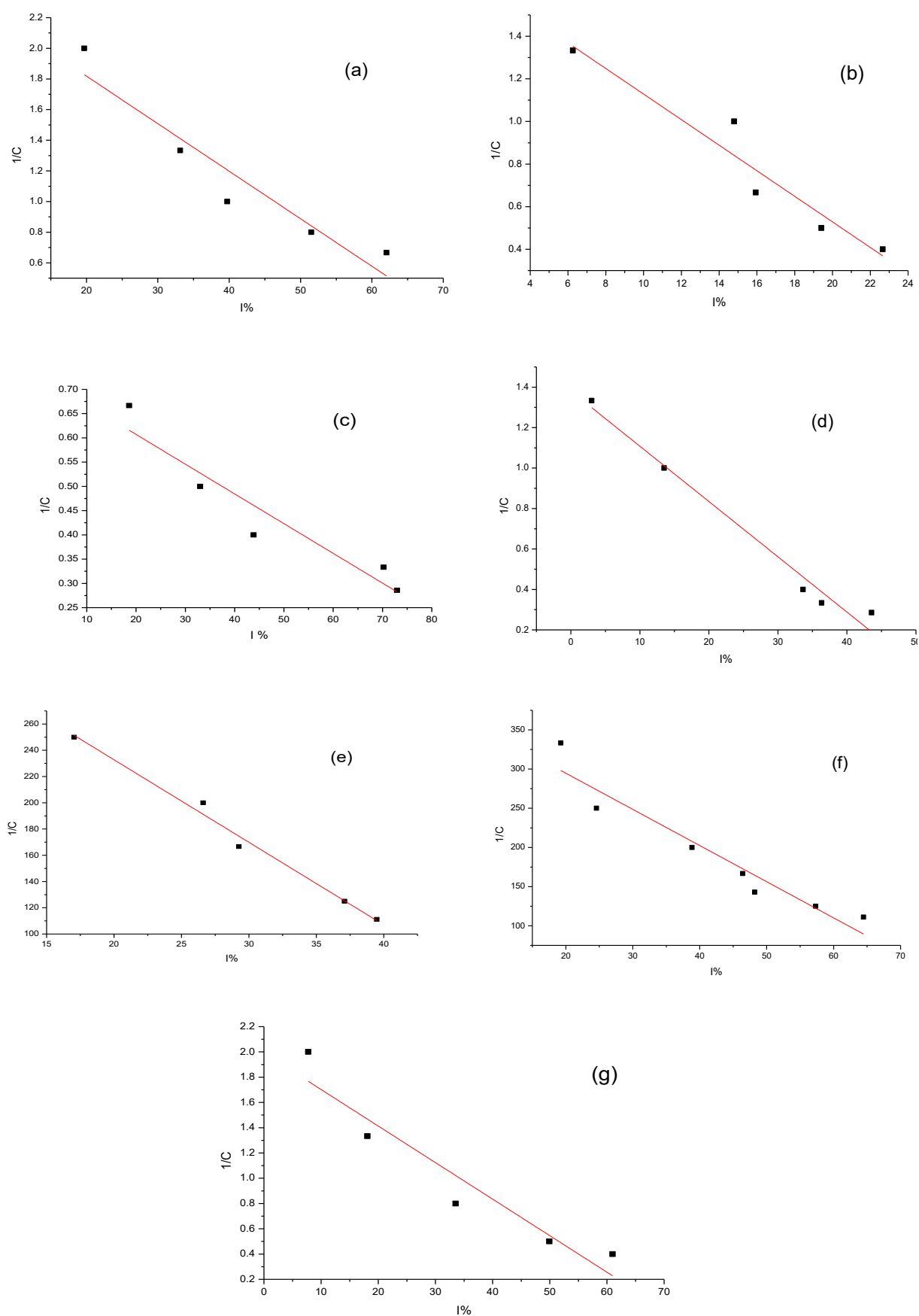**Figure 1:** The percent DPPH radical inhibition of (1) Spunta (2) Bartina (3) Kuroda (4) Ascorbic acid (5) Gallic acid

Figure 2: A plot of Ao /(A-Ao) to 1/[extract] for DppH radical (a) Kondor (b) Bartina (c)

Spunta (d) Kuroda (e) Ascorbic acid (f) Gallic acid (g) Peeled Kondor

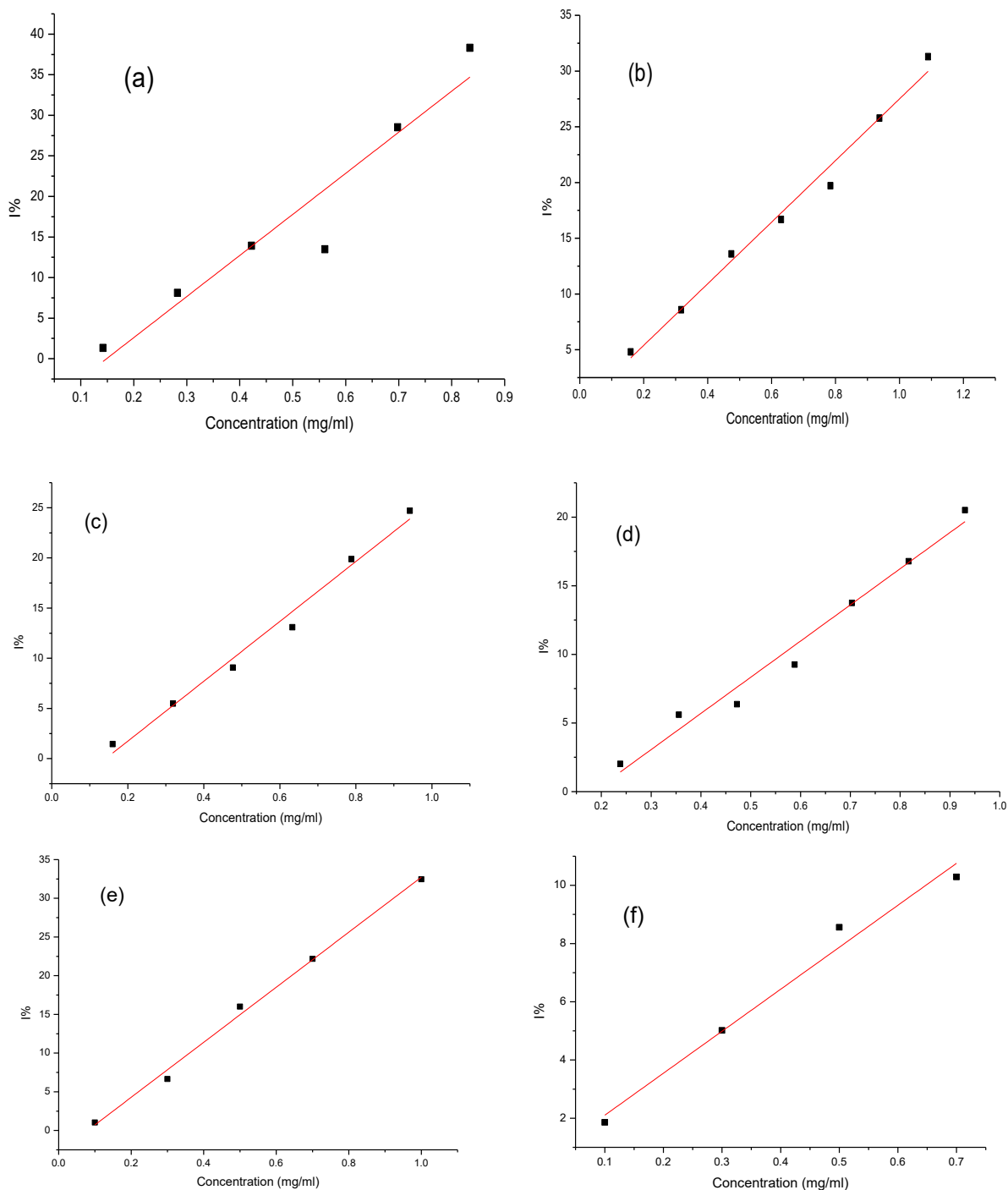**Figure 3:** The percent $O_2^{\cdot-}$ radical inhibition as a function of the concentration of (a) Bartina(b) Spunta (c) Kuroda (d)peeled Kondor (e) Ascorbic acid (f) α-tocopherol
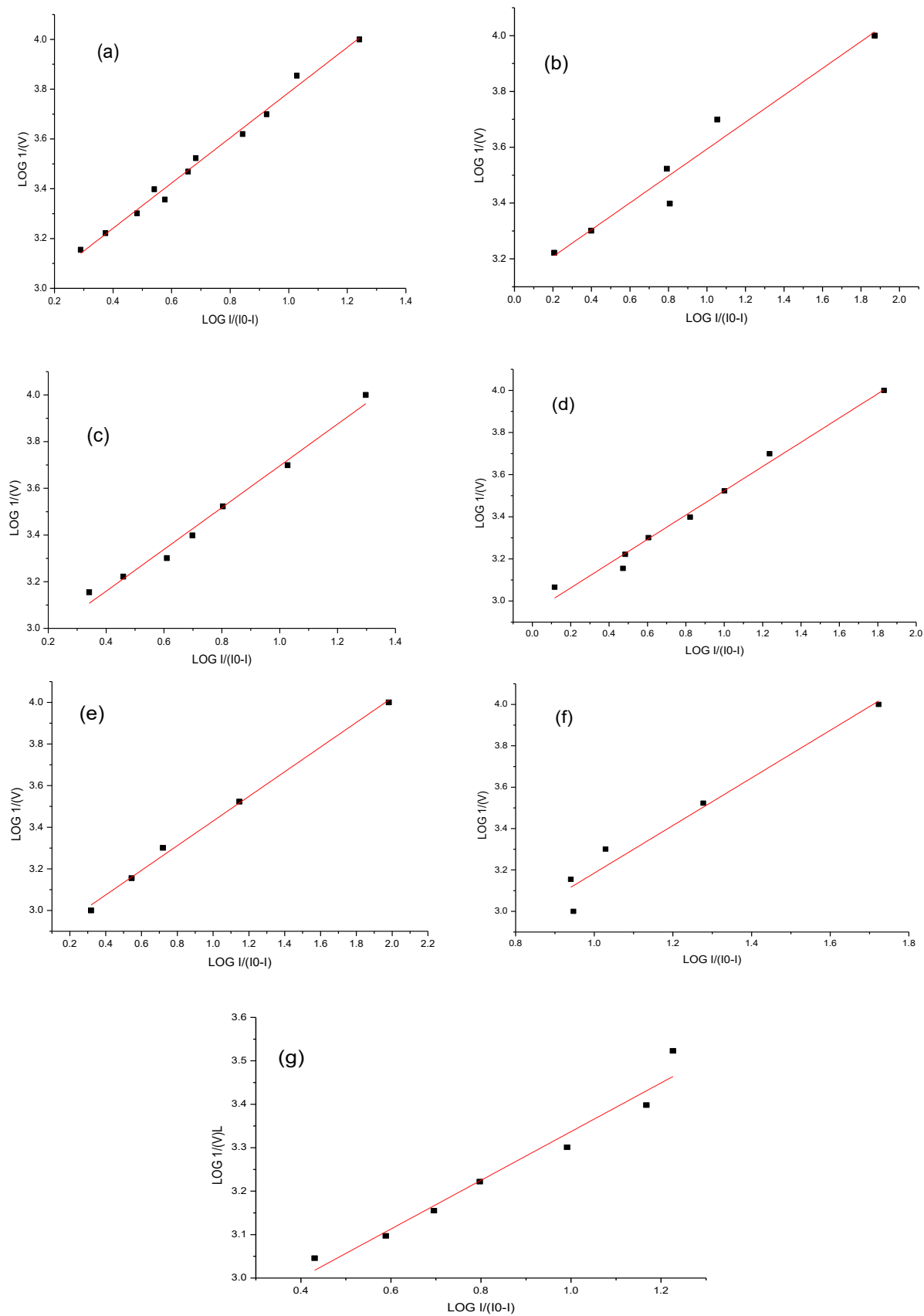
Figure 4: A plot of $\log\left[\frac{1}{[AO]}\right]$ to $\log\left[\frac{I_p}{I_{po}-I_p}\right]$ for $O_2^{\cdot-}$ (a) Kondor (b) Bartina (c) Spunta (d) Kuroda (e) Ascorbic acid (f) α-tocopherol (g) Peeled Kondor
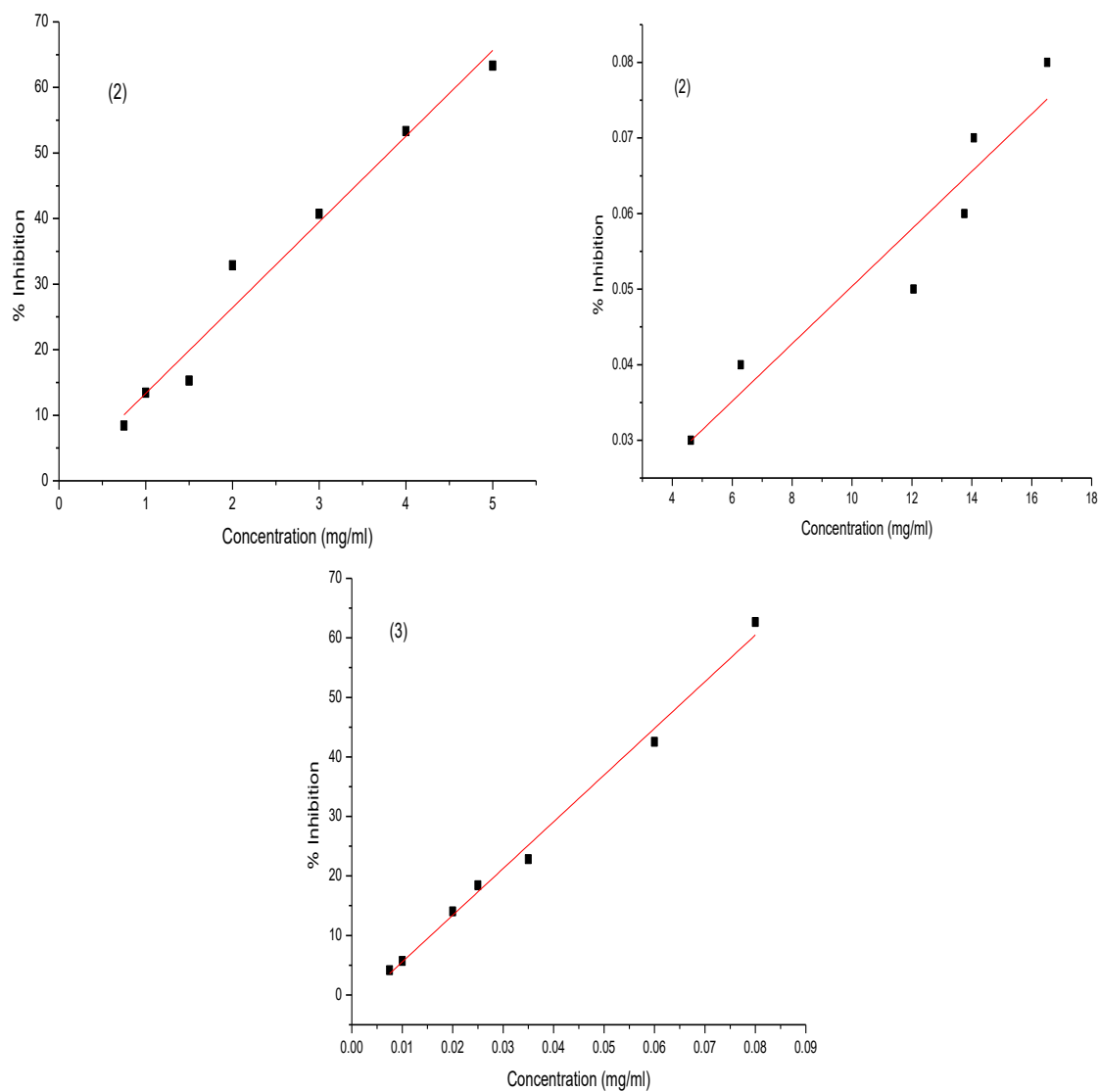
143

Figure 5: Antioxidant activity (%) of (1) peeled Kondor (2) Ascorbic acid (3) Gallic acid assayed by β-carotene–linoleate bleaching.
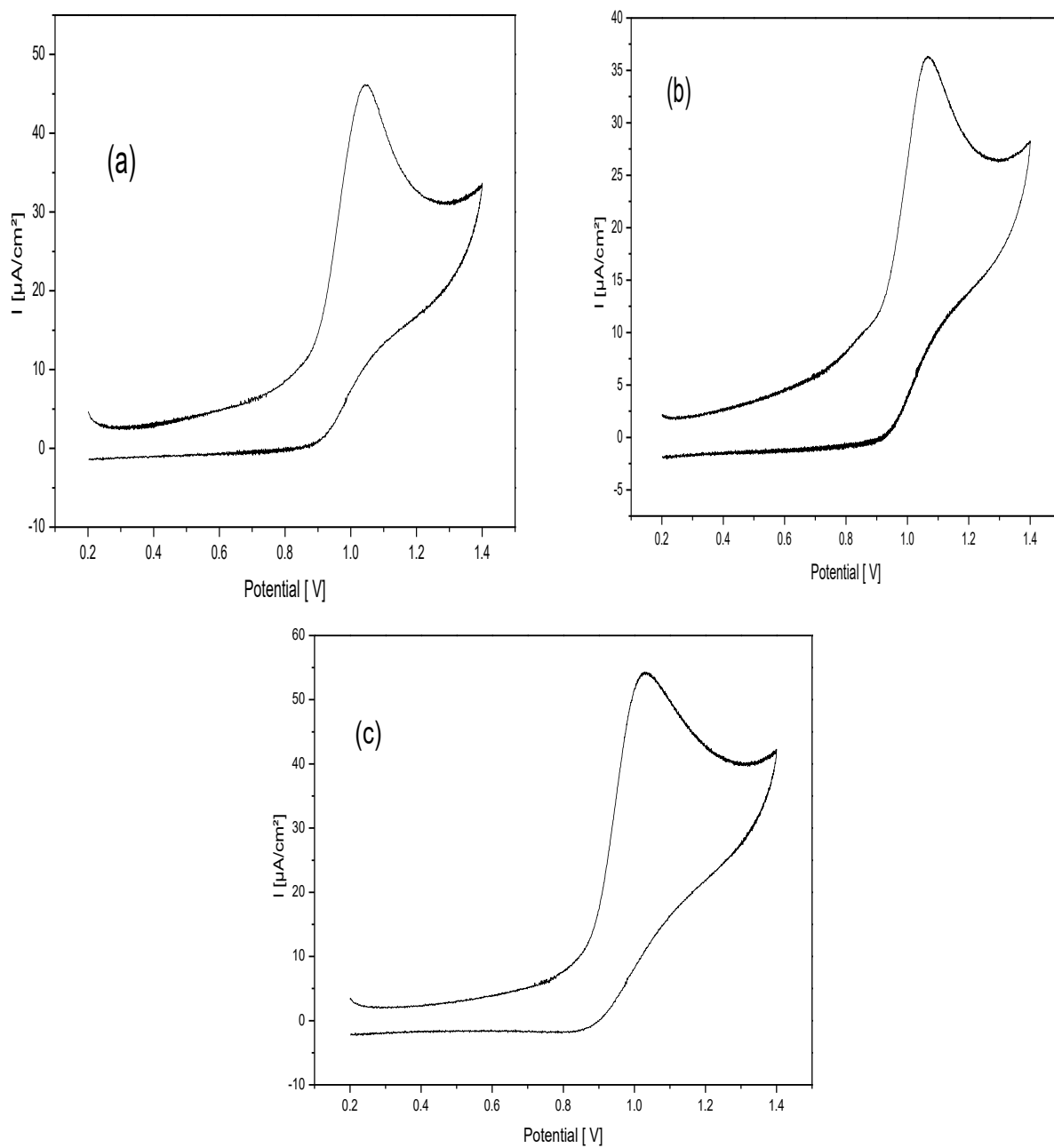
Figure 6: Voltammograms obtained for (a) Bartina, (b) Spunta and (c) Kuroda unpeeled
samples by cyclic voltammetry in DMF