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elaboration of this work.

GUEDIRI IMANE.

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List of Abbreviations

Abs	Absorbance
AEAC	Ascorbic acid equivalent antioxidant capacity
CV	Cyclic voltammetry
D	Diffusion coefficient
DMF	N, N-dimethylformamide
DPPH	1,1-diphenyl 1-2-picrylhydrazyl
Ep	Potential peak
Epa	Anodic oxidation potential
Epc	Cathodic oxidation potential
FRAP	Ferric-reducing antioxidant power
GAE	Gallic acid equivalent
HPLC	High-performance liquid chromatography
Ipa	Anodic potential peak
Ipc	Cathodic potential peak
TAC	Total antioxidant capacity
TBFB	Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4)
TFC	Total flavonoids content
TPC	Total phenolics content
TPTZ	2,4,6-tris(2-pyridyl)-1,3,5-s-triazine
UV	Ultraviolet
$\Delta G°_{ads}$	Standard free adsorption energy
Ba	Tafel anodic slope
Bc	Tafel cathodic slope
Cdl	Double layer capacity
E_{corr}	Corrosion potential
I_{corr}	Corrosion current density
IE%	Corrosion inhibition efficiency
R_{ct}	Charge Transfer Resistance
θ	Coverage rate

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General Introduction

General introduction

Plants have always held a very important place in the life of humans. All known civilizations have used plants either wild or cultivated for food, defence, clothing and health care [1] and they have always been used as medicines. Herbal medicines are considered less toxic and milder than pharmaceutical drugs. Pharmaceutical industries are increasingly interested in the ethnobotanical study of plants [2].

These medicinal plants contain many active compounds, some of which are derived from secondary metabolism. They already produce 70% of our drugs, so about 170,000 bioactive molecules have been identified from plants [3].

Indeed, secondary metabolites are the subject of considerable research. For example, polyphenols form a very diverse group of molecules, several of which are widely used therapeutically as antioxidants to fight against the harmful effects of oxygen at the origin of a large number of diseases [4].

The evaluation of phytotherapeutic properties, such as antibacterial and antioxidant activity is considered to be very important and useful, especially for plants, which are widely used in folk medicine [5].

The antioxidant properties of plant extracts have been extensively studied. Oxidative stress, which occurs when there is an imbalance between the production of free radicals and antioxidant enzymes, is related to the onset of diseases such as Alzheimer's, arteriosclerosis and cancer. One way to prevent this oxidative stress, which damages and destroys cells, is to seek an additional supply of antioxidant compounds. The active substances in extracts from various plant sources continue to be of great interest as a supplement in complementary medicine. Most of these extracts contain vitamins, flavonoids and other polyphenols [6]

Algeria, a country known for its natural resources, has a singularly rich and varied flora. There are about 3000 species of plants of which 15% are endemic and belong to several botanical families [7].Among the medicinal plants that make up the plant cover is the Solanaceae family, ". This family is known for its disinfectant and astringent antiseptic properties (diarrhoea, dysentery) as well as its hypoglycemic effect. It is also recognized in the treatment of diseases of the urinary and respiratory tracts [8].Thus; it has therapeutic properties that are used in traditional medicine.

In this context, the overall objective of this work is study in vitro the different polarity extracts of *Solanum nigrum* L. plant growing spontaneously in the Farms of the El-Oued (El-Debila) region. For the realization of this study, we will divide it into three essential parts:

The first part contains bibliographical research presented in two chapters; the first is a general background and description of the *Solanum nigrum* L. plant, while the second is a devoted study on polyphenols, their biosynthesis, classification, chemical properties, as well as a focus on oxidative stress, free radicals, antioxidants in nature and their mechanism of action .

The second part is the experimental one which is constituted of one chapter that presents the methods and techniques used to carry out this work including ethnobotanical study; the method of extraction of samples studied of *Solanum nigrum* L., their total polyphenol contents, as well as the evaluation of total antioxidant activities using spectro-photometric methods: the inhibition of the free radical ($DPPH^{\cdot}$) and the determination of the antioxidant reducing ferric power (FRAP) and evaluation of the total antioxidant capacity by Phosphomolybdenum method (TAC). Electrochemical methods (cyclic voltammetry (CV)) are adopted to evaluate the antioxidant capacity and the inhibition of the superoxide anion ($O2^{\cdot-}$).

The third part is devoted to the presentation and discussion of the obtained results in four chapters. The first is allotted to the study of the ethnobotanical survey. In the second chapter, the phytochemical tests are carried out and estimated the levels of total polyphenols also chromatographic analyses of phenolic compounds are presented through HPLC. The third chapter deals with the study and the assessment of the antioxidants, and in the last chapter will be evaluated the inhibition of corrosion of steel in acidic medium without and with the addition of extracts using the electrochemical technique. Finally, we complete this research work with a general conclusion giving evaluation and a summary of the main findings as well as the perspectives that will constitute the objectives of a future work.

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Chapter I

Solanum nigrum L. Plant

I. Presentation of the plant

I.1. Solanaceae families

Solanaceae are a cosmopolitan family which are herbaceous and flowering plants, shrubs, trees or vines with alternating leaves, simple and without stipules [1]. Furthermore, Solanaceae family includes a number of important agricultural crops of essential fruits and vegetables such as potatoes, tomatoes, eggplants, paprika, peppers, green and red peppers and Cape gooseberries l. It also consists of tobacco (Nicotiana spp.) which is one of the world's most damaging and economically important plants, as well as ornamental plants such as the Petunia, Schizanthus and Lycium species, also many other toxic and medicinal plants as belladonna or black nightshade (Atropa belladona L.), stramonium (Datura stramonium L.) and black chicken (Hyoscyamus niger L) [2, 3]. Although many species are toxic plants, there are many plants of this family that are used byhumans, and are important sources of food, spices and medicine.

Solanaceae family comprises about 90 genera and between 2000 and 3000 species. It is widely distributed in the tropical and temperate regions of the world, with centers of diversity in Central and South America and Australia [4].

I.2. Botanical characteristics of the family

In the botanist field, Solanaceae can be recognized by these few fundamental characteristics: regular flower, corolla with 5 fused petals, 5 stamens, ovaries with 2 carpels. They are mainly annual herbaceous plants, more rarely biennial (henbane), perennial (potato) or perennial forming addressed, climbing, sarment and spinescent shrubs or sub-shrubs. The leaves are generally alternating, simple, without stipulations. Some genera or species have compound-pinny leaves (potato, tomato) [5].

Inflorescences are generally bipolar cymes, disturbed by abortion phenomena leading to helical or umbeliform bipolar cymes (potato). Lonely flowers are also often found .

Cultivated Solanaceae have a high diversity of species, but also geographical origins, production methods, useful organs and consumption patterns. Some produce tubers (potatoes), fruits that are used as food (tomatoes, eggplants, peppers, etc.). Other species, rich in active ingredients that can be toxic, poisonous, are of great importance in pharmacy and medicine [5].

All solanaceous plants contain a toxic amount of solanine, a steroid alkaloid. Ripe berries are the least toxic parts of these plants, but their ingestion can be fatal under certain circumstances. Solanine is also found in potato sprouts and green spots. Although food species also contain alkaloids in leaves, stems and roots, edible parts (potato tubers, fruits ofaubergines and peppers) are devoid of them [6].

I.2.1. Solanum genus

Within Solanaceae family, Solanum genus is one of the most important and largest and most complex genera of flowering plant, comprising of about 1500 species, many of which contains several economically important corps, most notably are potatoes (S. tuberosum L.), aubergines or aubergines (S. melongena L.) and lulo or naranjilla (S. quitoense Lam.); plants used in horticulture include winter cherries (S. pseudocapsicum L.) and jasmine night nightshade (S. jasminoides Paxt.); species grown for drug use include S. dulcamara L. and S. viarum Dun...,both used as sources of corticosteroids. Generally, the species is distributed throughout the world. It is mainly found intropical and warm temperate regions and in sea level to altitudes above 3500 meters, obviously, in centers of diversity in the southern hemisphere, particularly in South America. Other speciation centers are found in Australia and in Africa, with relatively few species and less diversified in Europe and Asia [3, 6]. The generic name Solanum is generally considered to be derived from the Latin Solamen and refers to the calming or sedative effects associated with many species [4].

I.2.1.1. *Solanum nigrum* history

Solanum nigrum L. is one of the largest and the most variable groups of species in the genus Solanum, commonly known as Makoi or black nightshade and Black Berried too, *Solanum nigrum* L. a medicinal plant native to Eurasia, and introduced later in America, Australia and South Africa; is an annual herbaceous plant usually grows as a weed in moist habitats in different kinds of soils, including dry, stony, shallow, or deep soils [7]. This herb is considered to be troublesome weeds of agriculture, but in many developing countries they constitute a minor food crop, with the shoots and berries not only being used as vegetables and fruits, but also for various medicinal and local uses. and is supposed to have various biological properties [4].

I .2.1.2. Scientific classification of *Solanum nigrum* plant

Field: Biota **Reign:** Plantae **Sub-Region:** Viridaeplantae **Infra-Region:** Streptophyta **Class:** Equisetopsida **Sub-Class:** Magnoliidae **Superorder:** Asteranae **Order:** Solanales **Family:** Solanaceae **Sub-Family:** Solanoideae **Tribe:** Solaneae **Genus:** Solanum L. **Species:** *Solanum nigrum* L. **Sub-Species:** Solanum nigrum subsp. nigrum L.	 **Figure I.1.** *Solanum nigrum* L. plant

I.2.1.3. Botanical description

Solanum nigrum L. is an annual herb that is usually 25-100 cm in height [8], pubescent with simple hairs species belonging to Solanum section and occurs wildly in crop fields and wastelands which are characterized by the following:

- ✓ **Leaves:** are uniformly oval in shape and, the bases are cuneate, 4-10 and 3-7 cm wide, pubescent, coarsely and slightly cogged, the apex is obtuse [9].
- ✓ **Stems:** are semi-climbing, green and round to angular and covered with curved multicellular hairs sometimes tipped with a glandular head [8, 10].
- ✓ **Flowers**: are4 to 18 mm in diameter, short, small pedicellate and five widely spread petals which surround yellow prominent bright anthers.

The calyx of flower consists of ovate sepal lobes that are 1.2 to 2.5 mm long and a star-shaped corolla of five white lobes that are 1.8 to 7.5 mm long [10].

- ✓ **Fruit:** commonly called berries or grain are 6-10mm in diameter, are broadly ovoid and have dull purple to black or yellowish-green berries. when Berries ripe, they may remain on the plant or fall to the ground [9].

I.2.1.4. Properties and uses of *Solanum nigrum*

I.2.1.4.1. Beneficial properties

A) *Solanum nigrum* as sources of food:

Different parts of *Solanum nigrum* L. plants are excessively used as vegetables throughout the world and have provided a food source since oldtimes, In china, *S. nigrum*Lplants are consumed in times of famine[11]. Also used the plant in sauces and soups and leaves can be stewed or boiled and used as a relish. according Bailey (1881) [12] the herbage of forms of '*S. nigrum*' was considered to be a very valuable culinary vegetable in the Mauritius, and was imported to Australia by immigrants in the 1852 gold rush for use as a vegetable.

In Malawi (Southern Africa) add potash (which is a filtrate obtained from ashes of dried amaranth or bean plants) or soda, groundnut paste and salt to the water in which the *Solanum nigrum* leaves are boiled. Thismethod addstaste and flavor to the diets.

In western Kenya, *Solanum nigrum* L. leaves are used as a meat substitute. They are cooked in milk. The product of this cooking is pressed and left to dry for a few days until it becomes solid and turns a blackish color. We cut slices that are reputed to be high in protein and serve them with manioc and fresh vegetables. [13] This cooking in milk that is then expelled must make the toxic principles disappear to get rid of these toxic principles.

In general, leaves are used as vegetables for spinach in Australia, Cameroon, Ethiopia, Nigeria, Somalia, Tanzania, Uganda, South America, Crete and Greece. In Greece, they can be one of the vegetables of Horta, salads of vegetables cooked and drizzled with olive oil, seasoned with lemon and served warm or cold with feta cheese.

B) Nutritional value

Indeed several studies were performed to investigate the nutritive value of black nightshades. The table I.1 shows the various constituents of *Solanum nigrum* L. plant as

nutritious vegetables. Among numerous researches, leaves can provide considerable quantity of protein and amino acids, minerals including calcium, iron and phosphorus, vitamins A and C, fat and fiber. Moreover appreciable amounts of methionine, an amino acid scarce in other vegetables [14, 15]. In addition, the fruit can apparently highly yield of iron, calcium and vitamin B [14], and appreciable amounts of vitamin C and carotene[16]. Also seeds contain vitamin C and carotene [16].

The nutrient values may vary depending on the soil's fertility, plant age and type [17]. for example, In Imbamba study (1973), found that the leaf protein content of '*S. nigrum*L*'* was dependent on the age of the plant. Furthermore, the application of nitrogen increases the amount of ascorbic acid and protein while decreasing the calcium content in the leaves [17].

Mathooko and Imungi (1994) observed the values of available ascorbic acid dependency on the method of cooking where ascorbic acid content decreased with both an increase in the cooking time and in the volume of water used for cooking.

Another study [18] on the nutritional value of *Solanum nigrum* L. leaves was carried out before and after cooking. The analysis shows that the leaves of this plant are valuable dietary supplements in terms of crude protein, fat, fiber, total sugars, minerals and vitamins. Here are the results before cooking: 91.5% relative humidity; 17g/100g fat, 26.345g/100g protein, 7.8g/100g crude fibre, 0.209% citric acid equivalent, 0.373mg% vitamin A, 0.26mg% vitamin B6, 13.464mg% vitamin C,12.9% crude ash, 29.16g/100g sugar, 0.16g% calcium, 1.005 iron, 0.446g% magnesium and 0.057g% phosphorus. And the ones after cooking are: 85.5% moisture, 9.6g/100g fat, 12.85g/100g protein, 7.4g/100g crude fibre, 0.199% citric acid equivalent, 0.187mg% vitamin A, 0.33mg% vitamin B6 , 12.012mg% vitamin C , 0.05% crude ash, 16.19g/100g sugar, 0.08g% calcium, 0.585iron, 0.242g% magnesium and 0.023g% phosphorus.

Malaisse and Parent (1985) recorded nutritional data wherethey analyzed the leaves of 'S. nigrum' that are used as a wild vegetable by the Kibemba in the Zambezian Woodland Area in southern Central Africa. They found that 22 g of dry weight yielded energy levels of 1232 Kj and 295 calories.

All these results prove that this plantis important food supplements in human nutrition.

Table I.1. Nutritive value of vegetable black nightshades

Nutrient per 100-edbile portion	Range of values
Water (%)	83-91[17, 19-21]
Crude protein (g)	2.8-5.8[17, 19, 21, 22]
Crude fibre (g)	0.6-1.4[17, 19, 22, 23]
Fat (g)	0.8[23]
Carbohydrate (g)	3.3-5.0[21, 23]
Calories (kcal)	38[23]
Etheral extract (g)	38-44[19, 21, 22]
Total ash (g)	3.3-8.8[21, 22]
Iron (mg)	1.0-4.2[19, 22, 23]
Calcium (mg)	99-442[19, 22, 23]
Phosphorus (mg)	75[23]
Beta-carotene (mg)	1.7-11.6[17, 19, 23]
Ascorbic acid (mg)	20-158[19, 23, 24]
Oxalate (mg)	58.8-98.5[17, 22]
Nitrate-N (mg)	29-400[17]
Total phenolics (mg)	68.3-73.4[17, 25]

C) Midicinal uses

The section Solanum has a largely used medicinally over the world dating back to ancient times. *Solanum nigrum* is one of the various species that belongs to this section it has long history of medicinal use since earliest times. Dioscorides was one of the first to record their medicinal properties. Since then, this species has be widely acclaimed for its medicinal effects in every country [4].

For many centuries, Gerard's Herbal of 1636 reported that the "Nightshade is used for those infirmities that need cooling and binding" via the great British herbalsand that it was good for heart burning and panic of the head, and heat of the stomachtoo [4].

Later, in othre report to Culpeper's Herbal of 1649, the *Solanum nigrum* plant was known for its use to cool or decrease hot inflammations either externally or internally [4].

In Europe, 'S. nigrum' has been used traditionally to treat convulsions, in "Bohemia" [26] leaves were placed in babies' cradles to promote well sleep.

Moreover, the Arabs are used the bruised fresh leaves burns and ulcers to ease pain and reduce inflammation.

In North America, the Houmas Indians used drink from boiled roots of the plant as a remedy forbabies with worms, while using crushed green leaves mixed with a grease to make poultices for sores [27].

Solanum nigrum L. is widely known in India as cure disease. It is used as an enema from plantto childrenfor abdominal upsets. Also, the grain part recorded as possess tonic, useful in heart diseases and diuretic and are also cathartic properties and as a household to treat fevers, diarrhoea, ulcers and eye troubles. The seeds are reported used as remedy for dysuria and gonorrhoe a diseases [28].

Moreover, there are studies in India on *Solanum nigrum* L. plant were carried out and they revealed different therapeutic properties such as antiseptic and antidysenteric properties, diruetic, and laxtive proprties; also decoction is used as antispasmodic and narcotic [28].

The fresh extract of the plant appeared to be efficacious to treat cirrohosis of the liver. The extract of leave is effective against Staphylococcus aurens and Escherichia coli. Also,the extracts depress blood pressure[28]. Another study in Pakistan was conducted form aerial part of plant then the results showed that the plant could be antiulcerogenic [29].

In addition, the leaves of the plant are regarded as a febrifugal or detoxicant drug. In China, the dried aerial parts of plants are used as a diuretic and for infections of the urinary system, antihypertensive and anticancer agent. Chinese used fresh leaves to cure wounds[26].

Sajio in Japan notedthat immature fruit of ‘S. nigrum’consists steroidal glycosides that could be solasonine, solamargine, diosgenin and solasodinewhich show abundant anticancer activity [30].

In Africa, the *Solanum nigrum* plant is widely used in conventional medicine in different countries (Kenya,Zimbabwe, The Zulus,Tanzania,Cameroon, etc). African used diverse part from black nightshade as a remedy against various diseases such as stomach ulcers ,abdominal upsets, aching teeth, Tonsilliti, Malaria, eye-disease.heart pains [4].

Recently, there are some reports which have recorded form herbal extracts of *‘S. Nigrum*L*’*attribute anti-tumor and anticancer effects[31];and the investigations persist that this plant proved its importance and value as medicinal plant.

I.2.1.4.2. Harmful properties

A. As Weeds of agriculture

Solanum nigrum L. is regarded among the world's worst being a harmful weed of various crops as banana, barley, cereals, corn, coffee, field bean, garlic, lima bean, onion, cotton, pea, pepper, pineapple, potato, sorghum, soybean, sugar beet, sugarcane, tobacco, tea, tomato, wheat and vegetables. In addition, the plant can also pollute commercial crops via staining from the juice released by the grains rupturing among harvesting as soyabean and navy bean, field bean, lima bean, hence this decreases both the quality andeconomic return to farmers. Moreover, this plant can even stain wool when sheep grazelands existing in these weeds. Many of the reports have documented that *Solanum nigrum* L. are controllable either by cultivation methods or by appropriate herbicides[4].

B. Toxicity

Predominately species belonging to Solanum genus are known for its poisonous to both humans and cattle. Many of the reports of their toxic effects have been attributed to the alkaloid Solanine. Other reports informed that the amounts of toxicity varies with climate, season and soil type , part of the plant, stage of growth [32].

A chemical survey of the whole plant explained the presence of highest concentrations of Solanine in black nightshade are in the immature fruit [10].

Several effects of Solanine poisoning in animals and humans including vomiting , dark-colored ,diarrhea, nausea, salivation, abdominal pain,fever, constipation, sweating and nervous effects including drowsiness, apathy, weakness or paralysis, circulatory and respiratory depression, unconsciousness, and death [4].

Moreover; the plant consist high amount of nitrate nitrogen (NO3-N) which associated with plant can be due to N03-N toxicity in livestock. When the plant is flaworing,the levels of NO3-N reach a peak and then decrease. Hence; intense nitrate toxicity can result death. Also, chronic toxicity leads to decline in milk yield and impaired vitamin A and iodine nutrition, staggering gait, muscle tremors, increase urination, difficulty of breathing, then collapse and coma, with or without convulsions. Indeed, the actual symptoms of poisoning after intake black nightshade plants could be for reason either Solanine or N03-N or both and it is quite probable that boiling wreaks any toxicity attended in these species that prove their use as

vegetables in cooking, boiling as well as repeated boiling lead to soluble toxin being discarde [33].

I.2.1.5. Chemicals identified in the species

A number of phytochemical surveys of various taxa from different geographical regions throughout the world were indicate presence the steroidal alkaloid solasodine and of solasodine-like alkaloids in most species belonging to the genus Solanum [34].

Glycoalkaloids compounds were identified in the black night shade such as solanine, solasonine, solamargine and chaconine. Solanine is presence in different parts of the whole plant with various amountsthe highest level is found in in ripe berries. However, the level of solanine decreases inripe plant.In addition, it is modified through soil type and climate. Researches reveal that the alkaloidal content of plant parts changes during development of *Solanum nigrum* L [4].

Moreover; other studies demonstrate that the steroidal alkaloid solasodine washigher in the leaves, Steroidal sapogenins identified as diogenin and tigogenin both present invegetative parts and the unripe grain. Also solamargine getshigher during flowering[4].

The major identified organic acids in *Solanum nigrum*L are Acetic acid, tartaric acid, malic acid and citric acid acids [35]as well as ascorbic Acid were determined in fresh leaves (1mg/100g) in *Solanum nigrum* L. plant [16], Quercitin represents the most potent natural antioxidants [35].

Mineral analysis indicated the magnitude of presence in the order Mg>K>Ca>Fe>Na>Mn>Zn in the leaves and Mg>K>Fe>Ca>Na>Mn>Zn in the seeds. also Vitamin content reveale the order of magnitude as Vit C>Vit B>Folic acid>Vit E>Vit A in both the leaves and seeds.

Recently, in new study researchers isolate from whole plant of *Solanum nigrum* L two new bioflavonoids,(8-hydroxy-3'-β-D-galactosyl-isoflavone)-2'-8''-(4'''-hydroxy-flavone) biflavone (2), 2',3',5-trihydroxy-5''-methoxy-3''-O- α-glucosyl-3-4'''-O-biflavone (3) by guided fractionation activity from ethyl acetate and n-BuOH extract of *Solanum nigrum* L, respectively [36].

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Chapter II

Polyphenols Compounds

II. Polyphenols Compounds

II.1. Generalities

Polyphenols are secondary metabolites synthesized by all plants. They are present in tissue vacuoles. They participate in the defense reactions against various biotic or abiotic stresses (pathogens, UV radiation ...) and contribute to the organoleptic quality of food from plants (color, astringency, aroma, bitterness). Their qualitative and quantitative distribution in the plant varies according to species, organs, tissues or different stages of development. They are characterized by the presence of phenolic groups (presence of one or more hydroxyl functions on a benzene cycle) in their structure [1].Plants consumed by human provide more than 8000 phenolic compounds which are classified into different families according to the nature of their carbon structure [2]. We distinguish:

- ✓ Phenolic acids (C6-C1 and C6-C3)
- ✓ Flavonoids (C6-C3-C6)
- ✓ Lignans (C6-C3-C6)
- ✓ Stilbenes (C6-C2-C6).

Table II.1. The main classes of phenolic compounds

Carbon structure	Class	Example	Origin (example)
C6	Simple phenols	Catechol	
C6-C3	Hydroxybenzoic acids	p-Hydroxybenzoic	Spices, strawberry
C6-C3	Hydroxycinnamic acids	Caffeic acid, acid ferulic	Potato, apple
	Coumarins	Scopoletine,esculetin,	Citrus
C6-C2-C6	Silenes	Resveratrol	Vine
C6-C3-C6	Flavonoids		
	- Flavonols	Kampherol, quercetin	Fruits, vegetables,
	- Anthocyanins	Cyanidine, pelargonidine	flowers
	- Flavanols	Catechin, epicatechin	Flowers, red fruits
	- Flavanones	Naringenin	Apple, grape
	- Isoflavonols	Daidzein	Citrus

(C6-C3)2	Lignans	Pinoresinol	Pine
(C6-C3)n	Lignines		Wood, fruit core
(C15)n	Tannins		Red grape, khaki

II.2. Biosynthesis Polyphenols

They are synthesized by two biosynthetic pathways:

- ✓ **That of shikimic acid**, which leads after transamination and deamination to cinnamic acids and their numerous derivatives such as benzoic acids or simple phenols.
- ✓ **That from acetate**, which leads to poly ß-coesters (polyacetates) of variable length leading by cyclisation to polycyclic compounds such as 1,8-dihydroxyanthraquinones or naphthoquinones [3, 4].

In addition, the structural diversity of polyphenolic compounds due to this double biosynthetic origin, is further enhanced by the possibility of simultaneous participation of both pathways in the development of compounds of mixed origin, such as flavonoids [5].

II.3. Classification of polyphenols

II.3.1. Flavonoids

II.3.1.1. Generalities

Flavonoids are considered as almost universal pigments of plants, they are responsible for the colouringof flowers, fruits and sometimes leaves [3].

These molecules are the most abundant polyphenols in our nutrition and more than 4000 compounds have been identified [6]. They are particularly present in the epidermis of leaves as well as in the skin of fruits. They have a common C6-C3-C6 structure. Two aromatic cycles (A and B) are linked by a chain of 3 carbons forming an oxygen heterocycle (C) [7]. Flavonoids are subdivided into subclasses according to the structure of the heterocycle C. A distinction is then made between 4-oxoflavonoids (flavones, isoflavones, flavonols and flavanones, flavanols) and proanthocyanidins (condensed tannins), anthocyanidins as well as more minority compounds, chalcones and dihydrochalcones[8].

There are many compounds in each subclass according to the substitutions of the aromatic cycles. Most flavonoids are glycosylated, which increase their solubility in water [8].

II.3.1.2. Biosynthesis

Flavonoids all have the same basic structural element because they derive from a common biosynthetic origin. The A-cycle is formed from three molecules of malonyl-coenzyme A (malonyl-CoA), derived from glucose metabolism. Cycles B and C are also derived from glucose metabolism, but via shikimatethen via phenylalanine which is converted into ρ-coumarate and then ρ-coumaroyl-CoA. The ρcoumaroylCoA and the 3 malonylCoA condense in a single enzymatic step to form a chalcone, the 4, 2',4', 6'-tetrahydroxychalcone. The C cycle is formed by cyclisation of the chalcone, a reaction catalysed by the chalcone-isomerase which induces a stereospecific closure of the cycle leading to a single 2(S)-flavanone: naringenin. This cycle then hydrates to form the different classes of flavonoids [9].

II.3.2. Tannins

Tannins are water-soluble phenolic compounds with a molecular weight between 500 and 3000 Dalton, and having, in addition to the usual properties of phenols, the capacity to precipitate alkaloids, gelatin and proteins [10].

Two groups based on structural differences are usually distinguished in higher plants: hydrolysable tannins and non-hydrolysable tannins [11] .

II.3.2.1. Hydrolysable tannins

They consist of a carbohydrate molecule on which is esterified gallic acid or one of its derivatives (ellagic acid, m-digallic acid). Hence, the name pyrogalliques and ellagitanins which are sometimes given to them. They are easily hydrolyzed by chemical or enzymatic means. Gallic and ellagitannins are characteristic of dicotyledonous angiosperms [2].

II.3.2.2. Condensed tannins (proanthocyanidins)

Condensed tannins or proanthocyanidols result from the polymerisation of elementary flavanes molecules (flavanes ol-3, flavane ol-4, flavanediol -3,4). They are also referred to as "catechic" tannins. Proanthocyanidols have been isolated or identified in all groups of plants, including gymnosperms and ferns [2].

II.4. Polyphenols in plants

Location and interest: At the scale of the cell, phenolic compounds are mainly distributed in two compartments: the vacuoles and the wall. In the vacuoles, polyphenols are conjugated with sugars or organic acids that increase their solubility and limit their toxicity for the cell. At the wall level, we find mainly lignin and flavonoids linked to parietal structures. Phenolic compounds are synthesized in the cytosol. Some of the enzymes involved in the biosynthesis of phenylpropanoids are bound to the membranes of the endoplasmic reticulum, where they are organized into metabolites [12].

At the tissue level, the location of polyphenols is related to their role in the plant and can be very characteristic. Within the leaves themselves the distribution of compounds is variable, for example anthocyanins and flavonoids are predominantly present in the epidermis. At the level of the whole plant, it should be noted that some compounds are only accumulated in well-defined organs. In apples, for example, phenolic compounds are involved in the colouring of the skin via the anthocyanins, and in the organoleptic quality of the flesh, particularly for bitterness or astringency [12].

Phenolic compounds play an important role in plant metabolism but they can also react in the interactions of plants with their biological and physical environment (relations with bacteria, fungi, insects, UV resistance). All categories of phenolic compounds are involved in resistance mechanisms[13]. They ensure communication between cells, between plants, between plants and animals [14]

II.5. Chemical properties of polyphenols

The chemical properties of polyphenols are essentially linked to those of the phenolic nuclei, particularly the electron-with drawing (-M) and donor (+M) mesomeric substituents. The conjugation of one of the two free pairs of the oxygen atom with the cycle reflects the (+M) effect of the OH group. This phenomenon increases electronic delocalization and produces a partial negative charge on the C2, C4, C6 atoms (Figure II.1)[15].

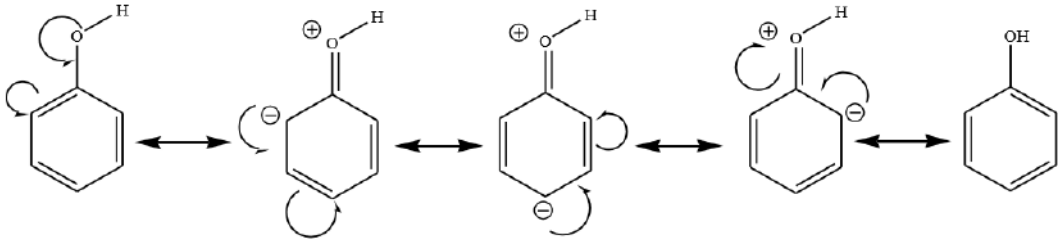

Figure II.1. Mesomeric forms of phenol

It can be seen that a negative charge appears in the ortho and para positions of the phenol (figure II.1), and these are therefore the positions likely to receive an electrophile[16]. From these basic characteristics the following different arise.

II.6. Physico-chemical properties:

II.6.1. Nucleophily

The nucleophily of phenolic compounds is carried by the oxygen atom and the carbon atoms in ortho and para of the OH group (following the (+M) effect). This property is at the origin of the reactions of aromatic electrophilic substituents (alkylation, acylation, etc.) region-selective of the ortho and para positions. The 1,3-dihydroxy (resorcinol) and 1,3,5-trihydroxy (phloroglucinol) type substituents allow an accumulation of electron density on the C2, C4 and C6 summits (all ortho or para of the OH groups), thus accentuating the nucleophilic character.

The A cycle of the flavanols has two C6 and C8 centres which are highly nucleophilic because they are ortho and para to three OH or OR groups with (+M) effect. The A cycle is also activated by the saturated C4 carbon group. This nucleophilicity allows aromatic electrophilic substitution reactions [15].

II.6.2) Reducing properties

The ionisation potential (IP) of a molecule is the minimum energy required to remove an electron from it. The more an aromatic compound is substituted by electron donor groups, the lower its IP is and the greater is its reducing character. It can then undergo mono-electron oxidation which leads to the corresponding radical. In the case of a phenol ArOH, the radical-cation formed is a strong acid which deprotonates immediately to lead to a phenoxyl or aryloxylArO radical [15].

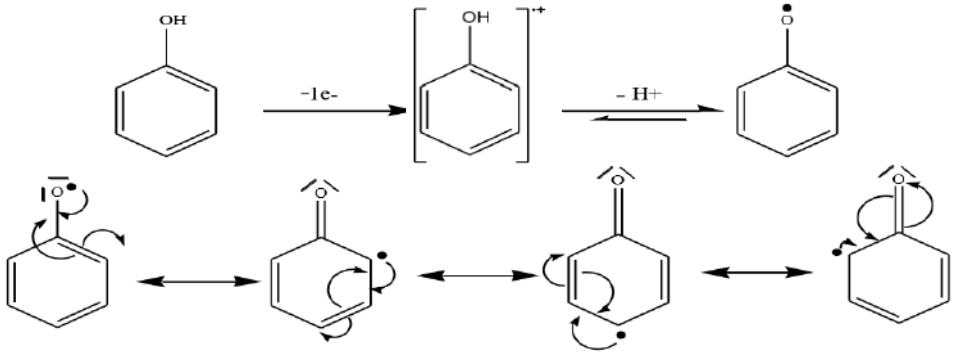

Figure II.2. Single-electron oxidation of a phenol and mesomeric forms of the aryloxyl radical formed

The aryloxyl radical (ArO.) can be formed directly by transfer of phenolic hydrogen (Figure II.2) to a high energy radical such as oxyl (RO.) and peroxyl.(ROO.) formed, for example, during the autoxidation of lipids. These H-atom and/or electron transfer reactions with conversion of a highly reactive radical into a resonance-stabilized aryloxyl radical are one of the main mechanisms of antioxidant action of phenols. The ability of phenol to transfer an H atom can be quantified by the homolytic dissociation energy of the OH bond (bond dissociation energy, BDE). The lower the BDE of a phenol, the stronger is its hydrogen-donating character[15]

II.6.3. Polarisability

The polarisability of phenols enables them to develop strong molecular dispersion interactions (attractive component of Vander Waals interactions) with other polarisable compounds. This phenomenon results from the coupling between the electronic fluctuations of two neighbouring molecules. Thus, in aqueous solution, the interaction of the apolar benzene cycle of the phenol with another polarizable entity such as a second aromatic cycle is promoted by the hydrophobic effect [15]

II.6.4. Hydrogen bond

Phenols are hydrogen bond donors (H bond) due to the acidic nature of the proton of the OH group. They are also H-bond acceptors. In fact, only the free pair of the O atom that is not conjugated with the cycle is capable of accepting an H-bond from a donor. Thus, a phenol is capable of giving an H bond and receiving only one. Note that these H bonds are mutually reinforcing (cooperativity). For example, by giving an H bond, the phenol lengthens its OH bond. This state of predissociation accentuates the electron density on the O centre and thus its H bond acceptor character [15].

II.6.5. Acidity

The heterolytic cleavage of the OH bond (deprotonation) leads to the formation of a phenate ion in which the electronic delocalization from the O atom to the ring (+M effect) is strongly increased (figure II.1). This phenomenon and the strong solvation of the phenate anion by formation of H bond with water explain the weak acid properties of phenols in water. The characteristic properties of phenols (nucleophilicity, reductive character, polarisability) are amplified during the formation of the corresponding phenate anions. The OH groups in the

para and ortho position of the phenolic nuclei of polyphenols have a reinforced acidic character, which allows dissociation of at least

II.7. Oxidative stress

II.7.1. Definition of oxidative stress

Oxidative stress is defined as an imbalance in the oxidant-antioxidant balance in favour of oxidants. It develops when the free radicals in oxidizing molecules are produced faster than they can be neutralized by the body. Free radicals are most often formed by electron gain from molecular oxygen. It regains its stability by participating in chemical reactions whose consequences are the oxidation of membrane lipids, the oxidation of amino acids that make up proteins, the oxidation of carbohydrates and the oxidation of nucleic acids .

Generally, excess free radicals contribute to oxidative stress through a series of chain reactions [17].

II.8. Free radicals

II.8.1. Definition

A free radical is a molecule or atom having one or more unpaired electrons. All free radicals and their precursors are often referred to as reactive oxygen species (ROS) [18].

Free radicals are electrically neutral or charged (ionic) and include the hydrogen atom, hydroxyl radical, superoxide anion, hydrogen peroxide (hydrogen peroxide). Free radicals are very unstable chemical species, their structure includes a single electron which seeks to pair by attacking and damaging neighbouring molecules. The term "reactive oxygen derivatives" is not restrictive, it includes free radicals of oxygen itself, but also some reactive non-radical oxygen derivatives whose toxicity is important such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) [17] .

II.8.2. The different types of free radicals

Among the oxidizing compounds formed after reduction of the oxygen we are breathing,we distinguish:

A. Primary free radicals

They derive directly from O_2 by a reduction reaction [19].

B. Secondary free radicals

They are formed by the reaction of primary free radicals on cellular biochemical compounds[19]

C. Active oxygen

Are molecules that do not possess an unpaired electron but have strong oxidizing power because they can give rise to free radicals [19].

Primary and secondary free radicals and active oxygen species are grouped together as Reactive Oxygen Species (ROS) .

✓ **The superoxide anion**

The superoxide anion is the most common species generated by the cell by reduction of an oxygen molecule. This reaction seems to be catalyzed mainly by NADPH oxidasesmembranes[20]. $O_2^{\bullet}$ can be also formed in certain cellular organelles such as peroxisomes via the conversion of hypoxanthine to xanthine and then to uric acid, catalysed by xanthine oxidase and mitochondria where 2% to 5% of the consumed oxygen is converted into superoxide radicals [21].

$$O_2^{-} + O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$$

✓ **The hydroxyl radical**

The most important of the products is the hydroxyl radical ($OH^{\cdot}$). It is a highly reactive oxygen species that comes from the coexistence of the superoxide anion and hydrogen peroxide. Hydrogen peroxide reacts with iron (ferrous form) and produces oxidized iron (ferric form) and the hydroxyl radical.

That's Fenton's reaction:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^{-} + OH^{\bullet}$$

Then the ferric iron is reduced to ferrous iron mainly by the superoxide anion .

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2$$

All these reactions together form Haber Weiss' reaction .

$$H_2O_2 + O_2^{\bullet -} \rightarrow O_2 + OH^- + OH^{\bullet}$$

- ✓ **Nitric oxide**

Nitrogen oxide NO- is mainly produced by an enzyme system, NOsynthase, which converts arginine to citrulline in the presence of NADPH.

$$\text{L- Arginine} + O_2 \rightarrow \text{L-Citrulline} + NO^{\bullet}$$

- ✓ **Nitric dioxide $NO_2^{\bullet}$**

It is formed from the reaction of the pyroxyl radical with NO. Nitric dioxide is a powerful trigger for lipid peroxidation because of its ability to remove a hydrogen atom from a double bond in polyunsaturated fatty acids .

- ✓ **Peroxynitrite**

Nitric oxide in conjunction with a superoxide ion will lead to the formation of peroxynitrite ($ONOO^-$) which is highly cytotoxic. This reaction is mainly found in the blood vessels.

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^-$$ [22]

II.8.3. Sources and formation of free radicals

Free radicals can be of endogenous origin through different physiological mechanisms in the body, but also of exogenous origin, caused by several chemical and physical sources.

II.8.3.1. Endogenous origin

At low doses, ROSs are very useful to the body and play important roles in various physiological mechanisms such as:

-The respiratory chain.

-The immune response.

-The transduction of cellular signals.

-NADPH oxidases.

-Nitric oxide synthases.

-Other endogenous sources.

II.8.3.2. Exogenous origin

Environmental factors can contribute to the formation of radical entities. A significant production of ROS is observed during heavy metal intoxication (cadmium, mercury, arsenic) or in irradiation phenomena causing DNA damage. In addition, tobacco smoke, alcohol or even certain drugs (xenobiotics) can be a source of free radicals through oxidation of these compounds at the level of cytochrome P450 [21].

II.9. Consequences of oxidative stress

ROSs become harmful and toxic to the body at excessive doses. This overproduction beyond the antioxidant capacities of biological systems gives rise to oxidative stress, which is involved in the development of several diseases ranging from arteriosclerosis to cancer, inflammatory diseases, ischemia and the aging process. These ROSs attack mainly membrane lipids, but also proteins and nucleic acids [22].

II.10. Antioxidant defense system

Cells possess endogenous enzymatic and non-enzymatic defense mechanisms that are generally sufficient to reverse oxidative stress resulting from aerobic metabolism, known as antioxidants [23].

II.10.1. Definition

An antioxidant can be defined as any substance that is capable, at relatively low concentrations, of competing with other oxidizable substrates and thereby delaying or preventing the oxidation of these substrates [24].The organism possesses a set of highly effective antioxidant defense systems to decrease the concentration of oxidant species in the body. Antioxidants are enzymatic or non-enzymatic systems [25].

II.10.2. The principles of antioxidants

II.10.2.1. Endogenous antioxidants

The physiological production of ROS is regulated by defense systems composed of enzymes (superoxidedismutase, catalase,hemeoxygenase, peroxidoxin, etc), small antioxidant molecules (glutathione, uric acid, bilirubin, ubiquinone, etc) and proteins (transferrin, ferritin, etc). A secondary defense system composed of phospholipases, DNA endonucleases, ligases and macroxy-proteinases prevents the accumulation of oxidized lipids, DNA and proteins in the cell and participates in the elimination of their toxic fragments [26].

II.10.2.2. Exogenous antioxidants

A. **Medication:** They are an important source of antioxidants. Currently, therapeutic classes such as non-steroidal anti-inflammatory drugs, antihyperlipoproteinemics, beta-blockers and other antihypertensive drugs have been evaluated for their antioxidant properties .

B. **Natural Antioxidants**

- ✓ **Vitamin C or ascorbic acid:** This is a powerful reducer. It plays an important role in the regeneration of vitamin E .
- ✓ **Vitamin E or tocopherol:** Prevents the peroxidation of membrane lipids in vivo by capturing peroxide radicals.
- ✓ **Flavonoids:** The relationships between the structures and antioxidant activities of flavonoids and phenolic compounds have shown that the antioxidant activity was determined by the position and degree of hydroxylation .
- ✓ **Tannins:** These tannins are proton donors to the lipid free radicals produced during peroxidation .
- ✓ **Phenols:** Phenolic acids, such as rosmarinic acid, are highly antioxidant and anti-inflammatory and may have antiviral properties [27].

II.11. Therapeutic interests of polyphenols

The main characteristic of polyphenols is that they are very powerful antioxidant agents. Indeed, they are able to trap free radicals and activate other antioxidants present in the body. This same antioxidant activity allows polyphenols to regulate good/bad free radicals, such as nitric oxide, which promotes good blood circulation, coordinates immune system activity with that of the brain, and modulates communication between brain cells [28]. The antioxidant activity of polyphenols can affect blood lipid transporters, particularly the "bad"

cholesterol transporter (LDL or low-density lipoproteins). Polyphenols thus prevent the formation of oxidized LDL, a formation that takes place during various pathological states characterized by oxidative stress [29]. They help fight inflammation and reduce the fragility of capillaries, reduce the effects of diabetes and protect the skin from ultraviolet rays by reducing damage caused by solar rays [30]. Numerous epidemiological studies show that a food rich in polyphenols reduces the risk of chronic diseases[31],the most important of which are cited below.

II.11.1. Polyphenols and cancer

Among the interesting biological properties of polyphenols is the prevention of cancer. Indeed, a number of in vitro and in vivo research studies have shown that polyphenols could be used as agents for the prevention of various cancerous diseases.[32]. Numerous studies have shown that three types of cancer (breast, prostate and digestive) can be strongly influenced by nutrition, especially the intake of lipids and antioxidants, and that organ oilcould due to its polyphenol content, contribute to the prevention of certain cancers such as prostate cancer [33]. More recent research has described the anti-carcinogenic activities of curcumin, resveratrol, and epigallocatechin-3-gallate (EGCG) for the treatment of cervical cancer [34]. The inhibitory effects of green and black tea in the treatment of cancer have been extensively studied. Tea polyphenols of the flavan-3-ol type are potent bioactive compounds that interfere with the initiation, development and progression of cancer through critical processes [35, 36]. They have the ability to interrupt or reverse the process of carcinogenesis by acting on intracellular signaling network molecules involved in cancer initiation and/or promotion to stop or reverse the phase of cancer progression. Polyphenols can also trigger apoptosis in cancer cells through the modulation of a number of key elements in the cell signal [37].

II.11.2. Polyphenols and cardiovascular diseases

Various epidemiological studies have shown that there is an inverse correlation between the consumption of polyphenol-rich foods and the risk of developing cardiovascular disease,[38, 39]. At the level of arteries, these molecules prevent the oxidation of low density lipoproteins (LDL) thus avoiding atherosclerosis (thickening of the arteries which contribute to reducing blood flow and can lead to asphyxiation of irrigated tissues). Polyphenols also inhibit the platelet aggregation involved in the phenomenon of thrombosis, which induces occlusion of the arteries. Thus, by preventing arthrosclerosis and the risk of thrombosis, these

compounds limit the risk of myocardial infarction [28]. According to epidemiological studies, an increased intake of flavonoids from fruits and vegetables is associated with a decreased risk of developing cardiovascular disease. The mechanisms for this observation are unclear, but the evidence suggests that flavonoids exert their effects by reducing cardiovascular risk factors. Recent evidence suggests that some polyphenols in purified form, including resveratrol, berberine and naringenin, have beneficial effects on dyslipidemia in human or animal models. Treatment with naringenin reduced atherosclerosis by correcting dyslipidemia [40].

II.11.3. Polyphenols and inflammation

Inflammation is the body's main response to aggression and is precisely regulated to limit possible damage to body structures. However, inappropriate regulation of thisphenomenon can lead to a chronic inflammatory state. Most chronic conditions have an inflammatory component. This is the case with obesity, type II diabetes, cardiovascular disease and cancer. The various studies carried out on the protective effects of polyphenols in these pathological contexts have shown that they reduce the markers of inflammation and act on numerous molecular targets at the centre of inflammation signaling pathways. Numerous studies have shown that polyphenols and their metabolites also act as modulators of inflammatory signaling pathways. Studies in healthy humans have shown that a diet rich in fruits and vegetables is inversely correlated with plasma markers of inflammation, and that consumption of anthocyanins is associated with decreased levels of circulating cytokines [41].

Recent research has shown that flavonoids, particularly flavonols, can prevent muscle pain by accelerating tissue repair at the molecular level. Specifically, they inhibit the NOS enzyme responsible for the synthesis of nitric oxide, which is a chemical trigger for inflammation. Other studies confirm the inhibitory action of these flavonoids, more specifically luteolin, apigenin, catechin on the cyclo-oxygenase enzyme synthetic molecules strongly involved in the inflammatory process [42].

II.11.4. Polyphenols and neurodegenerative diseases

Neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease represent a growing problem related to pathologies of cerebral ageing, mainly because there is an increase in the prevalence of both Alzheimer's disease and Parkinson's disease with age. These and other neurodegenerative diseases appear to be triggered by multi-factorial events

including neuro-inflammation, increased oxidative stress, iron and/or depletion of endogenous antioxidants. In addition, regular dietary intake of flavonoid-rich foods and/or beverages has been associated with a 50% reduction in the risk of dementia, preservation of cognitive performance with age, delayed onset of Alzheimer's disease and reduced risk of developing Parkinson's disease. Flavonoids may act to protect the brain in a number of ways, including protecting vulnerable neurons, enhancing existing neuronal function or stimulating neuronal regeneration [43]. Numerous dietary intervention studies conducted in humans or animals with foods or beverages derived from grapes, tea or berries such as blueberries have been shown to improve memory and cognition. However, it would seem that the antioxidant properties of flavonoids contained in these foods alone are not sufficient to explain their beneficial effects at the brain level, especially since the concentration of compounds found at this level is relatively low (Spencer 2008a). It has thus been suggestedthat polyphenols may act by protecting vulnerable neurons, stimulating neuronal function and blood flow, and promoting neurogenesis [41].

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Chapter III

Materials and Methods

III. Introduction

This work was carried out in the laboratory of valorisation and technology of the Saharan Resources (VTRS) of the Faculty of Technology at Echahid Hamma Lakhdar University of El Oued. All methods and protocols that are used to evaluate the studied plant (*Solanum nigrum* L.) are detailed in this chapter.

III.1. Plant Material

The three parts (leave and stem and fruit) of *Solanum nigrum* L. were collected on October 2017 from the area of Debila (EL-Oued, Algeria). It is a medicinal species that belongs to the family of *Solanaecae*.

a) Presentation of the study area (El Oued) [1]

The district of El Oued is located in the south-east of Algeria. It has an area of 44 586.80 Km². The length of its border with Tunisia is about 300 km. It is covered by the large Erg: Oriental on 2/3 of its territory. The wilaya of El Oued is delimited to:

- The north, by the wilayas of Tebessa and Khenchela.
- The north and to the northwest by the wilaya of Biskra.
- The south and to the south-east by the wilaya of Ouargla.
- The east by Tunisia.

Figure III.1. Map of Algeria representing the Wilaya of El-Oued (Integrated Agricultural and Rural Information Management System of Algeria)

b) Collection site

The plant samples were taken from a site in the El-Oued "El Debila" region located at 33 ° 30 '23 "North and 6 ° 56' 17" East (FigureIII.2)[2].

Figure III.2. Geographical location of the study area

c) Drying technique

Before the drying process, the three parts were cleaned from insects, coarse parts and gravel. Then, they were divided into small parts to facilitate the drying process. After that, they were stirred twice a day without exposure to the sun for a long time. Once the drying period is over, the crushing of the plant begins so that to prevent it from being rotten [3, 4].

III.2. Ethnobotanical survey

The term "ethnobotany" was coined by Hargsberger, in 1895 [5], as he was lecturing for the archaeologists in Philadelphia. This concept refers the study of plants used by primitive populations to shed light on the distribution and the diffusion of these plants in the past and suggest other uses better adapted to the current times .

Ethnobotany is an interpretative and associative discipline that seeks, uses, links and interprets the facts of interrelationships between Human Societies and Plants in order to understand and explain the birth and progress of civilizations from their beginnings to the use and processing of the plants themselves in the Primitive or evolved societies [6] .

III.2.1. Methodology

In order to give an account of the value of *Solanum nigrum* L. represented in the region of EL-Oued, this ethnobotanical study is carried out using a questionnaire from the

survey which is divided into two parts to collect information about the following topics which concern the people, and the medicinal plant.

During this survey, 207 people between the ages of 18 and more then60 (90 men and 117 women) from different regions, EL Oued, Guemar, Taleb Larbie and Debila were consulted. **(The survey sheet is presented in the Appendix).**

III.3. Phytochemical analysis [7]:

One of the essential aims of a phytochemical test is to detect the different families of secondary metabolites existing in the studied part of the plant by qualitative characterization reactions[8]. Qualitative phytochemistry based on color reactions or precipitation by specific chemical reagents performed on the extracts [9].

III.3.1. Preparation of Extracts:

3gms of each powder plant (leaves, stems, fruit) was taken into separate 100 ml conical flasks and 50 ml of each solvent (Ethanol, Distilled water, Chloroform, Petroleum ether) were added separately. These flasks were allowed to stand for 2hrs and filtered using Watmann No.1 filter paper. The obtained filtrates were used for the screening of secondary metabolites following standard procedures with some modifications [10-13].

a) Tests for Alkaloids

√ **Mayer's test**: 1ml of each extract was added with a few drops of Mayer's reagent (5g KI and 1.36g from $HgCl_2$, then dissolved it in 100ml of Distilled water). Formation of yellowish white color precipitate is a sign the presence of alkaloids

√ **Wagner's test**: 1ml of each extract was mixed with equal volumes of Wagner's reagent (2g KI and 1.27g I2 were dissolved in 100ml of Distilled water). Formation of reddish brown precipitate indicates the presence of alkaloids.

√ **$FeCl_3$ test**: 1ml of all the extracts was added few drops of ferric chloride solution. Deposition of yellow precipitate indicates the presence of alkaloids.

b) Tests for Flavonoids:

√ **Shinoda's test**: To 1ml of all the extracts was added a little of magnesium and add carefully a few drops of concentration Chlorohydride acid along the walls of the tube. Appearance of red color indicates the presence of flavonoids.

√ **The Base reagent test NaOH:** 1ml of each extract are put in test tubes and then added to the sodium hydroxide solution. The color change of the extract to yellow indicates the presence of flavonoids.

c) Tests for Tannins: 0.5 ml of each extract was mixed with 1 ml of distilled water then treated with few drops of FeCl3. Formation of green color precipitation indicates the presence of tannins.

d) Test for Coumarins: 2 ml of all the extracts were taken in separate tubes then add to it 3 ml Sodium hydroxide solution NaOH (10%). The appearance of yellow indicates the presence of Coumarins (Phenolic compounds).

e) Test for sterols and triterpenoids:

√ **Salkowski test:** To 1 ml of all the extracts, 5ml of chloroform was added to the above mixture. 1ml of conc. H_2SO_4 was added carefully along the walls of the tube and mixed together. The formation of ScarletRed color in the lower layer indicates the presence of sterols.

f) liebermann-Burchard test: To 1 ml of all the extracts, few drops of acetic anhydride solution were added to this mixture, a few drops of Conc. H2SO4 were added carefully along the walls of the test tube. Formation of reddish brown ring indicates the presence of triterpenoids. The appearance of green color indicates the presence of sterols.

g) Test for Saponins: 5ml of each extract is taken in a test tube and shaken vigorously to obtain a stable froth. To this frothing solution, 5-6 drops of oil was added. Formation of an emulsion indicates the presence of saponins.

III.4. Extraction of phenolic compounds

In our study we use maceration extraction method. Maceration in solvent is a very old classical process.

The use of different solvents with different polarity allows the separation of compounds according to their degree of solubility in the extraction solvent. This method extraction process conducted at a room temperature, allows to extract the maximum of the components bioactives and to prevent their denaturation or probable modification, including high temperature causes inactivation of phenolic compounds, decreased extractability in the solvent, and also affects their quantification.

III.4.1. Maceration

A mass of 300 g air-dried of three parts of *Solanum nigrum* L. (leaves ,stems, fruit) were macerated three times during 24 hours at room temperature with both ethanol and distilled water solvents (80:20 V/V) in a 500 ml glass Erlenmeyer flask closed with aluminium foil .The Erlenmeyer flasks have also been completely covered with aluminum foil to prevent the degradation of photosensitive molecules .

After maceration, the mixtures were separated by filtration, they were combined and dissolved in distilled water with magnetic stirring and then refrigerated overnight (elimination of the few chlorophyll pigments) and then, they undergo a second filtration. The extracts were then dried through evaporation using the rotary evaporator at a temperature of about 45°C.

III.4.2. Extractions with solvents of increasing polarity

After second filtration, the extraction liquide-liquide is carried out using successively solvents of increasing polarity:

1. Hexan: is not an extraction solvent, it is used to eliminate the fats and chlorophyll
2. Chloroform of a lower polarity: it is a solvent that brings compounds to have a less polarity
3. Ethyl acetate of average polarity it is a solvent that brings mono-o-glycosides and aglycones together
4. n.Butanol of strong polarity: this solvent will essentially remove the rest of the di-o-glycosides, tri-o-glycosides and C-glycosides.

Furthermore, the organic phases were dried with Na_2SO_4, and were filtered via filter paper to obtain the extracts [14].

III.5. Quantitative analysis of phenolic compounds

Analyses of the sample extracts of the obtained plant by using 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, or the ability of forming such chromophores after reacting with certain reagents, and this quantification relying on Beer-Lambert law and often quantification is done as the total amount of similar compounds in the extract.

The benefits of the non-chromatographic spectrophotometric methods are simple, fast, and of a low cost, but these methods lack the specificity for individual compounds and the results are less precise [15]

The total phenol determination was performed by an adapted Singleton-Rossi method using the Folin-Siocalteu reagen[16], while the flavonoids were quantified by the direct aluminum trichloride determination using an adapted of bahorun method [17].Also Total flavanol content was perforemed by kumaran method [18]

III.5.1. Determination of total polyphenols (TPC)

a) Principle

Polyphenols are determined by the Singleton and Ross method is the most widely used for the estimation of total phenolic content (TPC) in extracts of plant by using the Folin-Ciocalte reagent.It is a solution of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) whose reduction by the action of polyphenols gives a mixture of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) salt complexes of blue color. This solution absorbs at a wavelength of λ max765 nm. Thus, the determination of TPC is done by comparing the absorbance of the solution studied with that obtained by a standard that is gallic acid [15].

b) Mode of operation

The total phenolic compounds are determined as follows , 100 µl of the appropriately diluted extract was added to 0.5 ml of freshly diluted 10-fold Folin-Ciocalteu reagent solution in distilled water that is added immediately. Then, 2 ml of 20% Na2CO3 solution is added the resulting mixture and it is incubated at room temperature for about 30 minutes away from light. The absorbance of each solution was determined at 760 nm against a blank. The readings of the optical density at 760nm, solutions thus prepared, made it possible to plot the calibration curve for gallic acid [19].

III.5.2. Determination of total flavonoids (FVT)

a) Principle

The used reagent is aluminium chloride ($AlCl_3$, 2%). The principle of the method is based on the oxidation of carbons 4 and 5 of flavonoids by this reagent(Al ions), it causes the formation of a yellow complex absorbed at a field can vary from 367 to 510 nm in different experimental procedures [20].

b) Mode of operation

Total flavanoid was determined through the method of bahorun et al (2003). A Volume of 1 ml of 2% $AlCl_3$ was mixed with 1ml of the extract. The resulting mixture was incubated for 10 minutes in the dark. The absorbance was measured at 430 nm using UV-VIS spectrophotometer. Rutin was used as standard. The results were expressed in mg of Rutin equivalents/g of extract [17].

III.5.3. Total flavanol content

a) Principle

The estimation of the content of total flavanols contained in the extracts is carried out by the method of kumaran et al.

b) Mode of operation

The content of flavanols was determined by the method described by kumaran et al(2007). 1ml Aliquots of the ethanolic solution of the extracts were mixed with 1ml of $AlCl_3$ (20mg/ml) prepared in ethanol and 3ml of soduim actate (50mg/ml) the absorbance at 440nm was recorded after 2.5h. The content of flavanols was determined by using Rutin as reference compound. The results were expressed in mg Rutin equivalent per g of extract (mg RE/g) [21].

III.6. Chromatographic study:

Chromatography consists in driving the different molecules contained in a mixture to separate them according to their elution rates. The molecules are adsorbed on a fixed support which is the stationary phase and desorbed by the eluetion. There is, therefore, a more or less rapid distribution or partition of the components of the mixture between these two phases. The different components of the mixture are recovered separately in several fractions [21].

During our study we used high performance liquid chromatography (HPLC)

III.6.1. High performance liquid chromatography

This analytical technique is based on the balance of the concentrations of the compounds to be separated between two phases in contact: the stationary phase (chromatographic column) and the moving mobile phase. The separation is based on the different retention of the components present in the column. The latter travel through it with

variable times and linked to their intrinsic properties (size, structure, etc.) and their respective affinity for the stationary and mobile phases. At the end of the column, the detector continuously measures the quantity of each of the components of the mixture.

HPLC analysis is performed using a system consisting of four main modules:

1. a pumping system.

2. a sample injection system.

3. a chromatographic column.

4. a detector [22] .

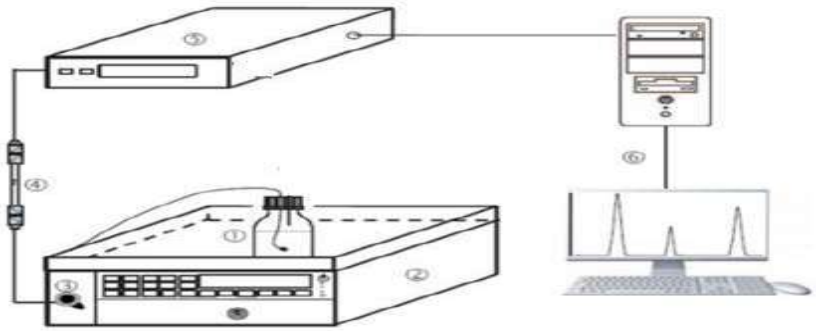

Figure III.3. Representation of the different elements composing the HPLC chain

The most commonly used method for the identification and quantification of different phenolic compounds. HPLC analysis includes determining the operating conditions that will be used during the analysis, such as the types of columns to be used, solvents, analysis time, etc. In general, the columns used to analyze polyphenols are almost exclusively reverse phase (RP) with 100 to 300 mm in length and 4.6 mm in internal diameter. Elution systems are usually binary (solvent A and solvent B): an acidified polar aqueous solvent such as aqueous acetic acid, perchloric acid, phosphoric acid or formic acid (solvent A) and another less polar organic solvent such as methanol or acetonitrile which can be acidified (solvent B). Usually, an elution gradient is used where eluent B increases over time [23].

III.6.2. Protocol of chromatography (HPLC)[25]

III.6.2.1. Samples preparation

The different polarity extracts of *Solanum nigrum* (10 mg) were dissolved in HPLC grade methanol (10 mL). The samples were filtered with 0.45 μm Millipore nylon filter disk. Then 20 μL of the mixture was analyzed in the HPLC system.

III.6.2.2. Standard Solutions

By dissolving 10 mg of distilled polyphenol in a 50 mL volumetric bottle containing a sufficient amount of methanol (HPLC grade) to dissolve the polyphenol, a stock solution of polyphenol was prepared, ranged for about 10 minutes and then brought to volume with a mobile phase.

By proper dilution of the one with the mobile phase, ordinary working standard polyphenol solutions were prepared. The column was injected with each of these solutions (20 μL) and the peak area and retention times were recorded.

III.6.2.3. Chromatographic Conditions

The chromatographic process for separation, a study of phenolic acids and flavonoids was carried out with liquid chromatography of shimadzu model prominence, thermostatic column compartment, online degasser and model SPD-20A (operating at 268 nm) UV visible detector. A Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 μm), (Shimadzu Co., Japan), was used as an analytical column.

A linear binary gradient method consisting of acetonitrile -A was used and 0.2 % acetic acid -B in water. By starting with 90% B, the gradient method was generated, then decreasing to 86 % B in min, to 83 % B in 16 min, to 81 % B in 23 min, to 77 % B in 28 min, kept at 77 % B in 28-35 min, to 60 % B in 38 min, to 90 % B in 50 min.at a 1 mL / min flow rate.

Calibration with standard gallic acid (GA), chlorogenic acid (CGA), vanillic acid (VA), caffeic acid (CA), p-coumaric acid (p-CA), vanillin (V), rutin (RU), naringin (NAR) and quercetin (QC) are used to measure separate peaks. By plotting a standard curve with the respective standards, the phenolic composition was quantified.

III.7. Study of antioxidant activity:

Two methods were adopted to evaluate the antioxidant properties of the extract. The First one is spectrophotometers methods, while the second is electrochemistry methods.

III.7.1. By spectrometric methods

Three spectrometric assays were selected to determine the antioxidant activity

III.7.1.1. DPPH radical scavenging assay

a) Principal:

From a methodological point of view, the free radical test DPPH- is recommended for compounds containing -SH, -NH and -OH groups. 2,2-diphenyl-1-picrylhydrazyle has an unpaired electron on a nitrogen atom. This radical does not form dimeric, it therefore remains in its monomeric form which is relatively stable [24]

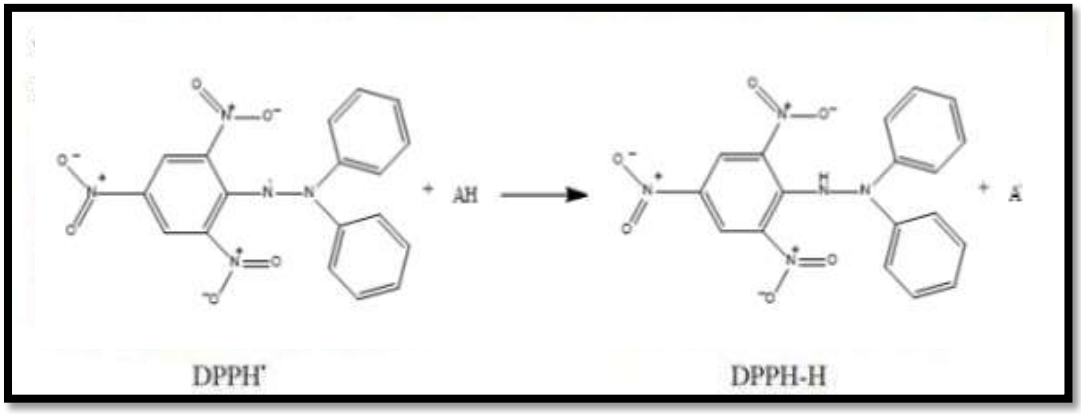

Figure III.4. Reaction mechanism in the DPPH assay between the DPPH radical species and an antioxidant (HA)

The reduction of DPPH- by an antioxidant agent to DPPH-H induces a loss of its dark violet color which will change to yellow light [25](Figure I.4). This reaction, which is carried out at room temperature to eliminate any risk of thermal degradation of thermolabile molecules [24], can be monitored spectrophotometrically by measuring the decrease in its absorbance between 515-518 nm[25]

b) Protocol :

The method described by of Ohinishi and many others with little amendments was used for the determination of scavenging activity of DPPH radical in the extract solution. A 1ml of 0.2Mm of DPPH) was prepared in methanol and it was mixed with 1ml of aqueous extract and the reaction was left in the dark at room temperature for 30 minutes. The absorbance was measured spectro-photometrically at 517 nm. Ascorbic acid was used as antioxidant standard. The results expressed as IC50 which are calculated from the curves of the variation in the percentage of inhibition I% as a function of the concentration of each extract. It should be remembered that the smaller the (IC_{50}) value, the greater the antioxidant activity of the extracts [24]

The inhibition power is expressed in % and determined by applying the following formula (III.1):

$$I\% = ((A_0 - A_S) / A_0) * 100 \quad \textbf{(III.1)}$$

Where

A_0: absorbance of the control.

A_S: Absorbance of the sample.

III.7.1.2. Ferric-reducing antioxidant power (FRAP) assay :

a) Principle

The FRAP assay corresponds to the reduction of a ferric tripyridyltriazine complex [(Fe(III)-TPTZ)2] to a ferrous tripyridyltriazine complex [(Fe(II)-TPTZ)2] by an antioxidant (AH), at a pH of 3.6 to maintain iron solubility. When the ferric complex is reduced to a ferrous complex, an intense blue coloring appears which allows it to be quantified by UV-Vis spectrophotometry at 593 nm[26].

b) Protocol

The reducing power was determined by using FRAP assay which was performed and based on the procedure described by Benzie and Strain (1996) [26]. In that respect, 100µL of the sample extract was added to 300µL distilled water then 3 ml of the FRAP reagent (25 ml of 0.3 M acetate puffer, PH = 3.6, plus 2.5ml of 10mM TPTZ in 40Mm HCL and 2.5ml of 20 Mm FeCl3) was incubated for 30min. The absorbance of the reaction mixture is 593 nm. $FeSO_4$ was used as standard with concentration varying. The results were expressed as mg Fe (II)/g of extract.

III.7.1.3. Evaluation of total antioxidant capacity (TAC) by Phosphomolybdenum method

a) Principle

During this test, hydrogen and electron were transferred from the reducing compound (extractantioxidant) to the oxidant complex (PPM). This transfer depends on the redox potential, the pH of the medium and structure of the antioxidant compound.

The test is based on the reduction of molybdenum from the oxidation (VI) stage to the oxidation (V). This reduction is materialized by the formation of a greenish complex phosphate/ Mo (V)) at an acidic pH. The decrease in the coloration of the complex is measured molybdenum (VI) in the presence of antioxidants. Unlike other tests, this test allows not only to quantify the contribution of the antioxidant activity of polyphenols but also to other antioxidant compounds such as vitamins [27].

b) Protocol

The total antioxidant capacity of the plant extracts was determined through the use of the phosphomolybdenum method of prieto et al(1999).[28]A 0.1 ml aliquot of the plant extract was combined with 1ml of the reagent solution (0.6 M sulfuric acid, 28mM Sodium phosphate, and 4mM ammonium molybdate). The reaction mixture was incubated at 95 C° for 90 min. After the samples were cooled to room temperature, the absorbance of the mixture was measured at 695nm ascorbic acid was used as standard. The antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalents per gram of dry weight.

III.7.2. By Electrochemical method

The method of voltmetric cyclic analysis on other methods is characterized by measuring current-potential curves. It takes place on a static hard electrode such as platinum and shows the complete voltmatric diagram in the form of roughly opposite cathodic and elevator peaks, which represent the reduction and oxidation processes, respectively. This is done at different registration speeds, and it may take two seconds or less to record the scheme Kortkova recently developed a new method to determine the effectiveness of antioxidants [29-32]. This method relies on returning the dissolved oxygen in the presence of antioxidants on a platinum or glass carbon electrode coated in a thin method of mercury.

The voltmetric method is considered as one of the most modern methods for measuring the antioxidant activity of phenol compounds and is an elective method of oxidation [33-35].It has been used to assess the total potency of antioxidants for real samples such as biological fluids or tissues and plant extracts [36-42].

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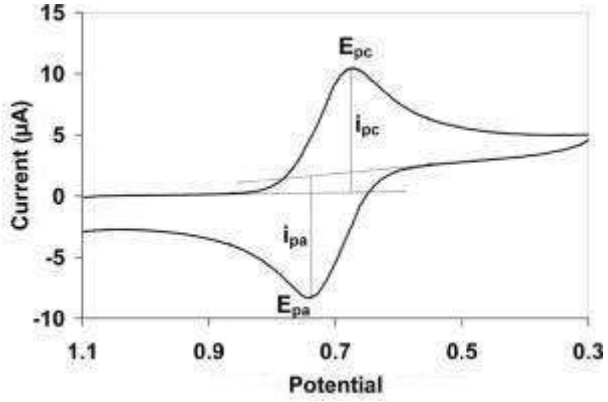

Figure III.5. Cyclic voltammogram for redox system

III.7.2.1. Evaluation of antioxidant activity by electrochemical assay

Electrochemical measurements were performed on a PGZ 301 Potentiostat (radiometer analytical SAS) associated to an electrochemical cell with a volumetric capacity of 25 mL containing a glassy carbon working electrode, a Platine wire counter electrode and a saturated calomel $Hg/Hg_2Cl_2/KCL$ electrode was used as a reference. The antioxidant capacity is measured from sample varieties in a buffer solution (dihydrogen phosphate mixture of potassium and dipotassium hydrogen phosphate) concentration of 0.2 mol.l-1 and at pH=7.2.Cyclic voltammograms were obtained by a single cycle performed at a scan rate of 100 mVs-1 and potentials range 00 to +1000 mV. ascorbic acid was used as a standard in the calculation of antioxidant capacity of the studied sample extracts of *Solanum nigrum* L. because of its wide-spreading in nature and also because of its anodic area display excellent linearity toward ascorbic acid concentration[43].

III.7.2.2. Evaluation of antioxidant activity by superoxide anion $O^{\bullet}{}_2^-$ radicals

Superoxide anion radical was created electrochemically by the reduction of commercial dioxygen in DMF commercial molecular oxygen containing 0.1M Bu_4NPF_6 at

room temperature 28± 1 °C. The studied extract samples were added to the mixture and they were generated $O_2^{\bullet -}$.

The cyclic voltammograms were recorded following the method of Le Bourvellec et al [44]. , The experiments were performed using a potentiostat/galvanostat voltalab40 PGZ301 (SAS analytical radiometer) in a 25 ml double-walled electrochemical cell, and the conventional three-electrode system was used. A vitreous carbon (GC) working electrode (SAS analytical radiometer) with a surface area of 0.013 cm^2, a platinum wire counter electrode and a Hg/Hg2Cl (3.0 M KCl) reference electrode were used. The data acquisitions were carried out with a Pentium IV microcomputer (3.0 GHz CPU and 1 Gb RAM) using the VoltaMaster4 software version 7.08 (SAS analytical radiometer). The scan rate was maintained at100 mV/s, and the potential window was -1.6 to 0.0 V. The extract samples studied were added to the superoxide O_2·- anionic radical generated in situ, and cyclic voltammograms were recorded.

III.8. Study of the inhibition activity of *Solanum nigrum* extracts on steel X52

In this part, we have exploited the inhibitory action of n.BuOH extracts of *Solanum nigrum* plant against corrosion of mild steel (X52) in $H_2SO_4$0.5M medium with different concentrations of inhibitors, using the usual electrochemical methodsthe study of the corrosion phenomenon such as the potentiodynamic polarization method and electrochemical impedance spectroscopy (EIS).

III.8.1. Experimental Procedures

III.8.1.1. Inhibitors tested

n.BuOH extracts of the different parts of the plant, leaves, stem and fruit have been selected as inhibitors to evaluate their inhibitory effect on corrosion of mild steel (X52) in $H_2SO_4$0.5M

III.8.1.2. Preparation of the material used

The studied material is steel X52 whose composition is given in table III.1 is a non-alloy steel that is generally used in the manufacture of molding, and small tooling parts mild steel is called X52 with a composition (by weight %), was used as a working electrode, which was obtained from a cylindrical bar steel. The surface area is 0.0707 cm^2, The sample was polished with abrasive paper (number2000-4000 categories). The working electrode was

degreased with acetone then copiously washed with distilled water and finally dried at room temperature.

The steel samples are prepared, before immersion in the solutions, by polishing under water with abrasive paper. These samples are then rinsed with distilled water, degreased with acetone and air-dried.ambient temperature before use.

Table III.1. Chemical composition of carbon steel X52 in % by mass.

Element	C	P	Si	Mn	Cr	Cu	Al	Ni	Fe
Mass (%)	0. 76	0.012	0.026	0.192	0.050	0.135	0.023	0.050	balance

III.8.1.3. Preparation of solutions

The corrosive solution is a 0.5M sulfuric acid solution (with or without inhibitor)obtained by dilution from 98% concentrated commercial acid (Biochem mark)The range of concentrations used for the inhibitor varies from0.5 to 1.75mg/ml.This range of concentrations was determined after studying the solubility of the inhibitor in corrosive medium.

III.8.1.4. The electrochemical cell

The electrochemical experiments are carried out in an electrochemical cell of 25 ml capacity, equipped with conventional montage with three electrodes:

- A platinum electrode as auxiliary electrode
- A saturated calomel electrode Hg/ Hg2Cl2/ KCl as reference electrode
- The working electrode in order to minimize the influence of the ohmic drop.

Considering X52 steel as working electrode which is in the form of acylindrical of 0.0707 cm^2, the electrolyte is an acid solution maintained at constant temperature.

III.8.1.5. Polarization curves

The electrochemical measurements are carried out using a montage consisting of a potentiostat-galvanostat PGZ 301 type VOLTA LAB and Radiometer model, controlled by an analysis software " VoltaMaster4 ." In the potentiodynamic method, the potential applied to the sample varies from continuously from -800 to -300 mV vs ECS, with a scan rate of 30 $mV.sec^{-1}$we opted for a relatively low scan speed in order to maintain anquasi-stationary mode .

Before these curves are drawn (cathodic then anodic), the working electrode is maintained at its abandonment potential for half hour .

The current intensity is measured between the working electrode and the counter-electrode of platinum .The plotting of the intensity-potential curves allows the determination of the potential of corrosion (E_{corr}), polarization resistance (R_P), corrosion current density (I_{corr}), the cathodic (Bc) and anodic (Ba) slopes of Tafel and consequently the inhibition efficiency in the operating conditions used.

III.8.1.6. Electrochemical impedance spectroscopy

Electrochemical impedance measurements are performed after 30 min of immersion in sulfuric acid medium H_2SO_4 0.5M at laboratory temperature .

The electrochemical system and the operating conditions used are the same as those used polarization curves .

The amplitude of the sinusoidal tension applied to the polarization potential is 10 mV peak-to-peak, at frequencies between 100 kHz and 100 mHz, with 5 points per decade . Determined measurements at the abandonment potential are automatically processed by the Volta Master 4 software. From impedance diagrams made at corrosion potential (E_{corr}), we reach charge transfer resistances (R_{ct}), double layer capacities (C_{dl}) and thus at the inhibition efficiency under the used operation conditions.

III.8.1.7. Studies of adsorption isotherms

Adsorption plays an important role in the inhibition of metallic corrosion by organic inhibitors. Adsorption isotherms are often used to demonstrate the performance of organic adsorbent-type inhibitors and thus important for determining the mechanisms of organic electrochemical reactions. The establishment of isotherms that describe the adsorption behavior of a corrosion inhibitor is an important part of this study because they can provide important clues about the nature of the interaction of the inhibitor / metal. Three types of adsorption isotherms are generally all covered isothermal adsorption data. Adsorption isotherms more frequently used are Langmuir, Temkin and Frumkin isotherms having the following equation between the surface area and concentration [45, 46].

•Langmuir : $C/\theta = Cet + C$ **(III.2)**

•Temkin : $\exp(-2a\theta) = KC$ **(III.3)**

Frumkin: $(\theta / (1-\theta)) \exp(-2a\theta) = KC$ **(III.4)**

III.8.1.7.1. Measures of electrochemical parameters: formulas used

- IE% inhibitory efficacy is determined from the following relations:

✓ From the polarization curves :

$$IE\ (\%) = ((I_{corr} - I_{corr(inh)})/I_{corr})\ 100 \qquad \textbf{(III.5)}$$

Where :

I_{corr} and $I_{corr(inh)}$ represent respectively the corrosion current densities determined by extrapolation of the Tafel cathode lines to the corrosion potential without and with addition of the inhibitor.

✓ From electrochemical impedance measures :

$$IE\ (\%) = ((R_{ctinh} - R_{ctcorr})/R_{ctinh})\ 100 \qquad \textbf{(III.6)}$$

Where :

R_{ctinh} and R_{ctinh} represent respectively the values of the resistance of charge transfer in presence and absence of the inhibitor .

The values of the charge-transfer resistance are calculated from the difference impedance at high and low frequencies on the real axis [47].

- The capacity of the double layer C_{dl} :The value of the double-layer capacity is obtained by the equation:

$$C_{dl} = \frac{1}{w_0 R_{ct}} \text{ with } w_0 = 2\pi f_0 \qquad \textbf{(III.7)}$$

.f_0 being the frequency for which -Zimg reaches a maximum on the Nyquist diagram

This frequency of interruption makes it possible to define the different time constants of the circuit.

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Chapter IV

Ethnobotanic study

IV. Introduction

The ethnobotanical investigation is the first link in a scientific process that allows the researcher to move from the traditional knowledge of the use of a plant to its valuation. The knowledge and the valorization of the plants used by the populations contribute to the sustainable management of local plant diversity. The study of traditional knowledge is all the more urgent because this knowledge and practices are eroded through cultural exchange or lost forever. Ethnobotany is indeed an interface domain par excellence, since it deals with the practical use of plants[1].

IV.1. Aim

This chapter focuses on an ethnobotanical survey on medicinal plant *Solanum nigrum* L; which was taken from arid region of Debila (Eloued) in order to identify the benefits and the therapeutic habits of the local population of this plant. Moreover, in this ethnobotanical studies, a good approach appears to understand the uses and impacts of the environment in a given region and the socio-cultural and economic perceptions of plant resources by the local populations.

IV.2. Calculated ethnobotanical indices

- ✓ **Response rate (or Relative citation frequencies):**

After counting the survey forms, the response rate by type of use is as follows is expressed by the formula used by DOSSOU et al [2] as follows :

$$F = 100.S/N \qquad \textbf{(VI.7)}$$

F: Calculated response rate;

S: Number of people who gave a positive response (Yes) for the use of the organ concerned.

S: Number of persons who provided a response in relation to a given use.

N: Total number of people interviewed.

IV.3. Results and interpretation

Ethnobotanical studies within ethnic groups involve the search for the information on the use of plants, techniques of use, names, therapy, origins, etc. Direct survey is the most important and satisfactory source of information [4].

a. According to sex

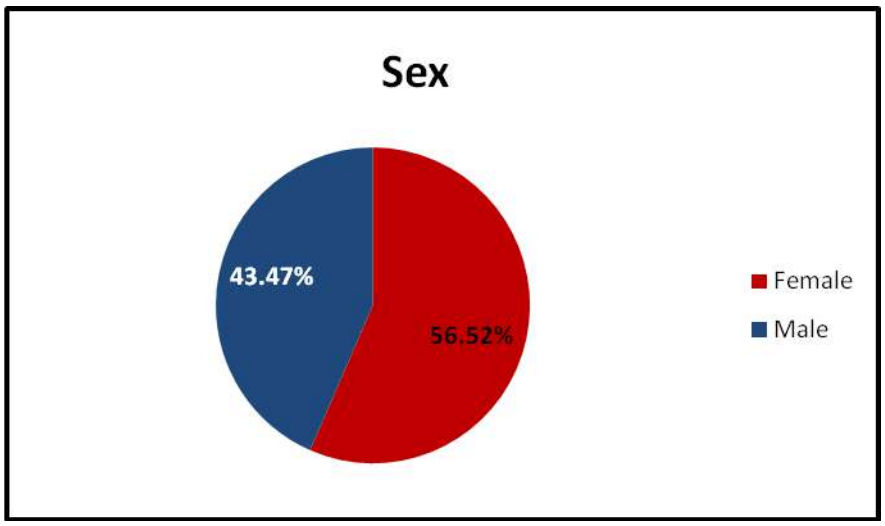


Figure IV.1. Descriptive statistical representation of the results of the ethnobotanical survey according to sex

In the study area, men and women are concerned with traditional medicine (FigureVI.1). However, men have a less knowledge of the medicinal species of *Solanum nigrum* L. compared to women (56.52% against 43.47%)

b. According to the Age

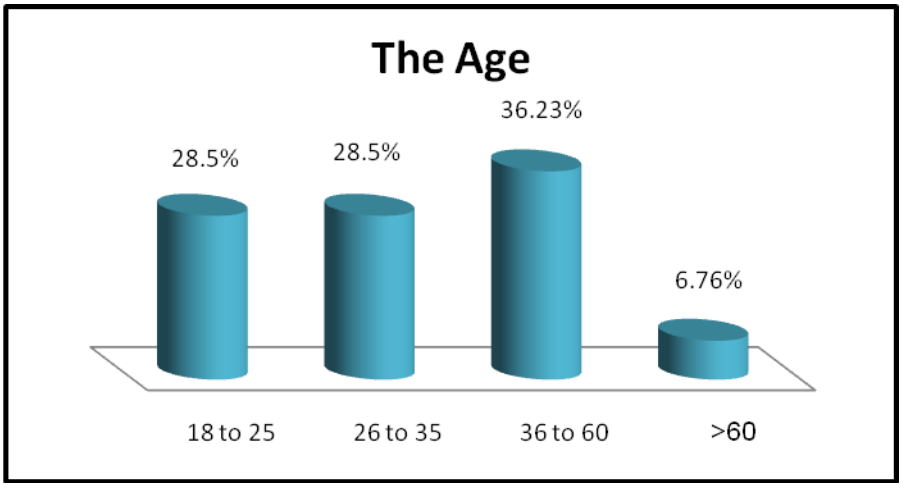


Figure VI.2. Descriptive statistical representation of ethnobotanical survey results by age

The use of medicinal plants (FigureVI.2) in the El-OUED area is widespread in all age groups, with a predominance in people aged 36 to 60 years (36.23%).However, for the age group of 26 to 35 years, there is a rate of (28.5%), and for the age group of 18 to 25 years (28.5%) else, then people above 60 years old (6.76%).

c. According Use field:

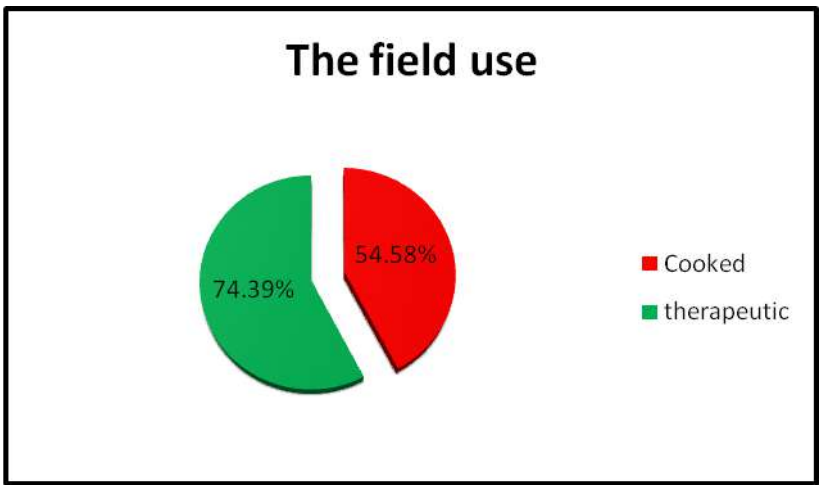


Figure VI.3. Descriptive statistical representation of ethnobotanical survey results according use field

In our survey we found that the person who used the plant for therapeutic was (74.39%) and the person who used it for cooking was (54.58%).

d. According the part used:

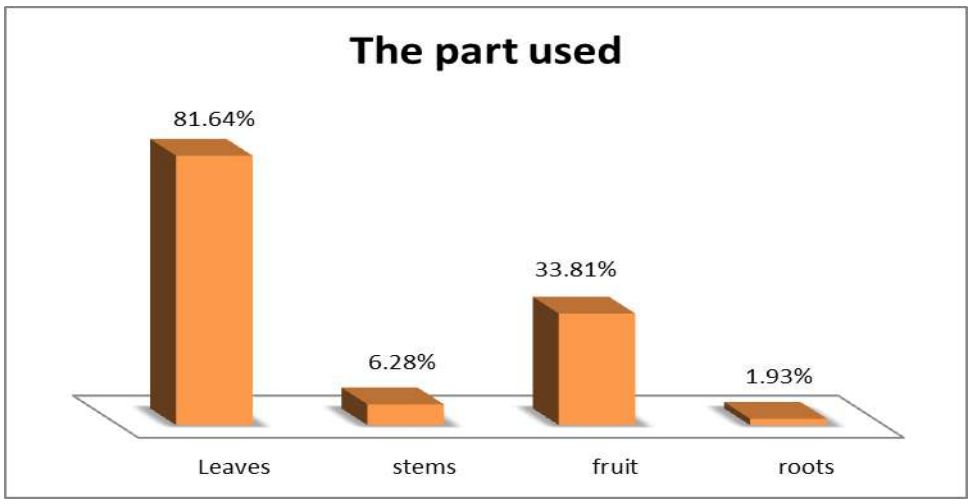
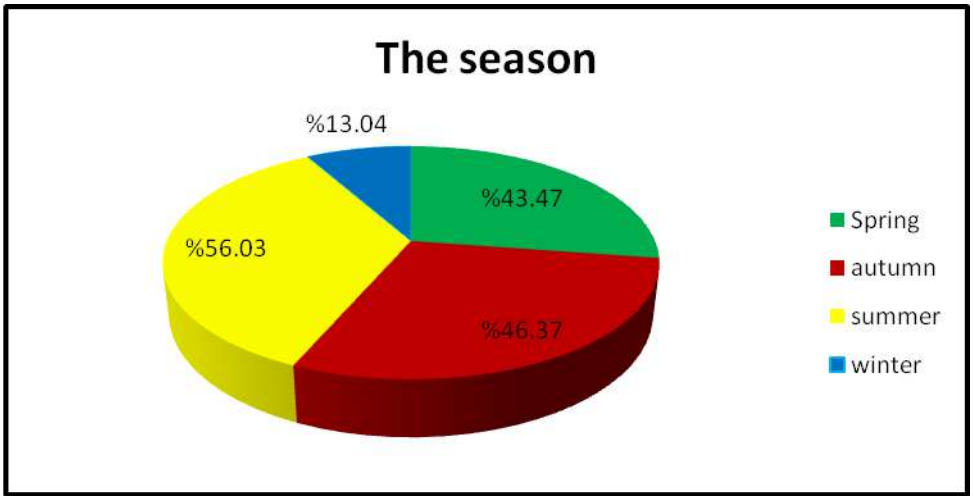


Figure VI.4. Descriptive statistical representation of ethnobotanical survey results according part used.

The active compounds can be located in different parts of the medicinal plants

(leaves, flowers, roots , fruit, stems ...). In the study area, the leaves are the most used part of the plants medicinal with a rate of 81.64%, followed by the fruit 33.81%, then come the stems with 6.28%, roots with a utilization rate of 1.93% (Figure VI.4).

e. According to season:



FigureVI.5. Descriptive statistical representation of ethnobotanical survey results according to use in season.

Depending on this survey, the season in which the plant is abundant is summer by rate 56.03%, and then the autumn season 46.37%; then 43.47% for spring, and the lowest rate 13.04% for winter season

f. According the mode of preparation

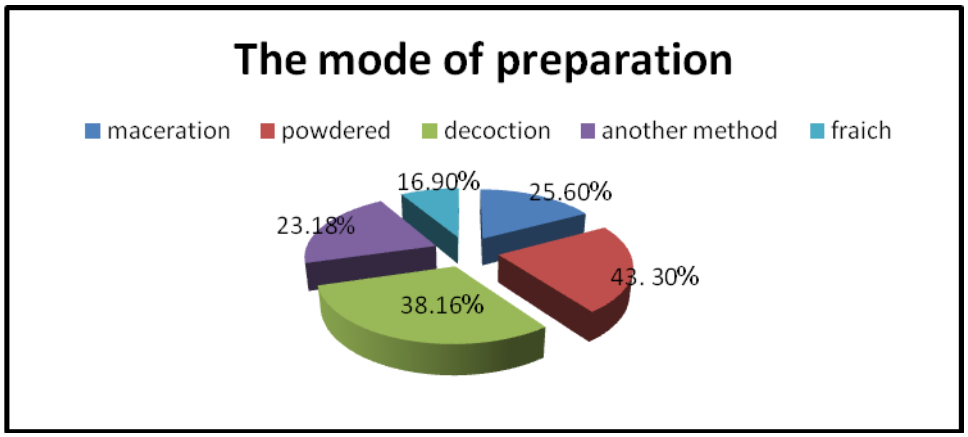


Figure I.6. Descriptive statistical representation of ethnobotanical survey results according the mode of preparation

According to the survey, the method of preparation of the plant varies according to the efficiency of the mode towards the plant. The method of decoction is the most dominant mode (38.16%). This percentage shows that the local population has confidence in this type of preparation and finds it suitable. The powdered preparation is indicated with a rate of 30.43%. Maceration 25.60% and 23.18% for another method of preparation by Fraich (16.90%).

g. According to the treated diseases:

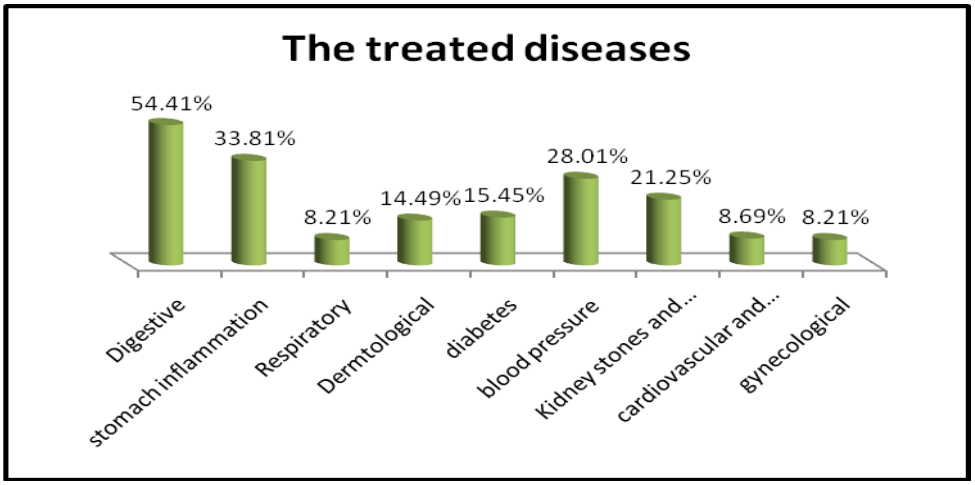


Figure VI.7. Descriptive statistical representation of ethnobotanical survey results according to the treated diseases

The obtained results show that *Solanum nigrum* L. is used in traditional medicine involved in the treatment of digestive diseases with a percentage of 54.41%, stomach inflammation 33.81% blood pressure 28.01% and diabetes 15.45% ,and then 14.49 % dermatological 8.21% respiratory diseases (Figure I.7).

Moreover according to the ethnobotanical survey, there are other treated diseases using medicinal plant *Solanum nigrum* L., in which a percentage of kidney stones and urinary tract (21.25%), cardiovascular and Cholesterol (8.69%) gynecological (8.21%).

IV.4. Discussion

According to Agbogidi (2010) [3], ethnobotanical studies appear to be a good approach for understanding in a given region, the uses as well as socio-cultural and economic perceptions of plant resources by local populations.

In this regard, an ethnobotanical study on the medicinal plant *Solanum nigrum* L. was undertaken in the El-Oued area and was the theme of our study.

The female sex is dominant in the study area with a percentage of 56.52% which reflects the distribution of both sexes in our society (high number of females). In addition, this can be explained by the use of medicinal plants by women in other filed than therapy and by their responsibility as mothers as they are the ones who give first aid especially for their children.

The findings show that 36 to 60 year-olds of 39.74% have more knowledge of medicinal plants compared to other age groups. These results confirm the results of other ethnobotanical work done nationally by Henka & Nesba[4], which displayed that people aged 40 to 50 are more familiar with traditional herbal knowledge.

The decoction remains the most used form 38.16%, these data indicate that the activity of Active Principle is more effective in this mode of use.

The therapeutic use of medicinal plants is very present in some countries of the world, especially the developing countries, in the absence of a modern medical system [5].

Solanum nigrum L. is traditionally used in different countries of the world as a remedy for treating various ailments such as pain, cough, cold, asthma, skin diseases and liver problem[6], inflammation fever and enteric diseases. It possess many activities like antitumorigenic, antioxidant, anti-inflammatory, hepato-protective, diuretic, and antipyretic agent, antibacterial, mycotic infection, cytotoxicity, anti-convulsant, antiulcerogenic. It is also used against sexually transmitted diseases [7].

As for its use for digestive diseases (54.41%), inflammations of the stomach 33.81%"almost all the properties of the plant" have declared some herbalists, and this is proven for *Solanum nigrum* L. in El Oued area, and that it is due to several researches which affirm that, following research by Saravanan et al [8] indicated that the plant methanolic extracts of *Solanum nigrum* L have potential drug against gastro protective and Ulcer [8]. For another study of Mallika et al about the fruit of SN, the results further suggest that SNE was found to possess antiulcerogenic as well as ulcer healing properties, which might also be due to its antisecretory activity[9]. According to Jainu M et al, The results indicate that *Solanum nigrum* berries may exert its gastroprotective effect by a free radical scavenging action. *Solanum nigrum* L. berries may have considerable therapeutic potential in the treatment of gastric diseases [10].

The ethanolic extract of the dried fruit of *Solanum nigrum* L. was assessed for antidiarrhoeal activity [7].

In India and Tamil Nadu, Fresh leaves cooked with onion bulbs and cumin seeds or leaf juice can also be taken orally for stomachache and for treating stomach ulcer[11]also Himalayan region where *Solanum nigrum* L. is used for indigestion.

(28.01%) said that they were treating high blood pressure. According to the literature reviewer[11], the leaves of *S. nigrum* could be a good source of natural antioxidant with considerable therapeutic effect in the management of metabolic diseases such as diabetes, obesity, hyperlipidemia, atherosclerosis, hypertension and cancer

15.45% of investigators reported that they prescribe *Solanum nigrum* L. for diabetes. These results are consistent with the study carried out by Nazoora Saleh Aali (2010)[6], who states that the *Solanum nigrum* L. has the anti- diabetic property also. The blood sugar levels of the control rates remained almost static during 7 days when an oral dose of (250mg/kg b. wt.) of *Solanum nigrum* L. was administered daily for 5 days and 7 days, there was a successive decrease in the blood sugar level.

Thus, it can be concluded that *Solanum nigrum* L. also has the anti- diabetic property [12].

Another study has indicated that aqueous extracts of leaf and fruit possess significant hypoglycemic effect in dose dependent manner, followed by hydroalcoholic extracts. The stem extract of S. nigrum has no profound effects[13].

In India, people use *Solanum nigrum* L. to treat diabetes as traditional medicinal plants [7].

8.69% treatment cardiovascular and Cholesterol have been reported based on the results in the study of Amartya et al[14]. It is proposed that, steroidal saponins of S. nigrum and S. xanthocarpum, if properly formulated, can be used as an antihyperlipidaemic agent with beneficial to the antioxidant and hepatoprotective functions.

CONCLUSION

The ethnobotanical study was carried out in El Oued region has allowed us to demonstrate the different uses of the *Solanum nigrum* L. plant and several diseases that local population are using to treat by this plant. In addition, we noted three organs (leaves, stems and fruit) that are mostly used in the study area.

This work will continue in next In vitro chapter to investigate the benefits of this plant and enhancement development of the Saharan regions

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Chapter V

Phytochemicals Analysis

V. Introduction

Bioactive substances are responsible for therapeutic properties of the plant in folk medicine. They have actions in the body that may promote good health. They are being studied in the prevention of disease. The Phytochemicals analysis plays an important role in detecting the presence of bioactive compounds of plants that can be a good nature source of phenolic and flavonoid compounds in the diet and in therapeutic section.

V.1. Aim

Based on the results of the previous chapter, three parts (leaves, stems, and fruit) of *Solanum nigrum* L. which are the most used in the studied area have been selected and a method study for the detection of active substances was conducted along with a quantitative study to assess these active substances.

V.2. Phytochemical tests

The results of the preliminary phytochemical screening tests are presented in the tables below.

Table V.1. Preliminary Phytochemical Results of leaves

Extracts / Phytochemical test		Ethanol extract	Distilled water extract	Chloroform extract	Petroleumether extract
Tests for Flavonoids	Mayer's test	+	+	–	+
	Wagner's test	++	–	+	+++
	$FeCl_3$ test	+	–	–	++
Tests for Alkaloids	Shinoda's test	–	++	–	–
	NaOH test	+	+	–	–
Tests for Tannins		+++	+	+	–
Test for Coumarins		+	+	–	–

Test for Sterols and Terpenoids	Sterols	Salkowski Test	+++	+	–	+
	Sterols	Liebermann-Burchard Test	+	–	+	+
	Terpenoids		–	–	–	–
Test for Saponins			–	–	+++	–

(-) absent, (+) present, (++) mediumpresent, (+++) strongly present.

The table above shows test results on Preliminary phytochemical screening of leaves' extracts, the phytochemical analysis revealed the presence of flavonoids, sterols in all the extracts ,and alkaloids, tanins except Petroleum ether extract, then were found Coumarins only in the Chloroform Petroleumether extracts, further presence saponins only in the Chloroform extract, triterpanoids were absence in all extracts.

Table V.2. Preliminary Phytochemical Results of stems

Extracts / **Phytochemical test**		**Ethanol extract**	**Distilled water extract**	**Chloroform extract**	**Petroleumether extract**
Tests for Flavonoids	Mayer's test	+	–	–	–
	Wagner's test	–	–	+	+
	FeCl3 test	–	–	–	–
Tests for Alkaloids	Shinoda's test	–	–	–	–
	NaOH test	+	++	+	–
Tests for Tannins		+	–	+	–
Test for Coumarins		+	+++	–	–

Test for Sterols and Terpenoids	Sterols	Salkowski Test	+	+	–	–
	Sterols	Liebermann-Burchard test	+	–	–	+
	Terpenoids		+	–	–	–
Test for Saponins			–	–	+++	–

(-) absent, (+) present, (++) medium present, (+++) strongly present.

The table above displays the results of phytochemical analysis of stem part which consists of different family flavanoids in all the extracts except distilled water and alkaloids without Petroleumether. Further presence tanins in the ethanol and Chloroform extract; the presence of Coumarins is only in the Chloroform Petroleumether extracts, sterol appeared in all extract only on the Chloroform extract; saponins disappeared from all extracts except in the Chloroform extract, triterpanoids were absent in all extracts only in ethanol extract.

TableV.3. Preliminary Phytochemical Results of fruit

Extracts / **Phytochemical test**		**Ethanol extract**	**Distilled water extract**	**Chloroform extract**	**Petroleumether extract**
Tests for Flavonoids	Mayer's test	++	++	–	–
	Wagner's test	–	–	+++	+
	FeCl3 test	–	–	+	–
Tests for Alkaloids	Shinoda's test	+	++	–	–
	NaOH test	++	+	+++	_
Tests for Tannins		++	++	_	_

Test for Coumarins			++	++	_	_
Test for Sterols and Terpenoids	Sterols	Salkowski Test	+++	_	_	_
	Sterols	Liebermann-Burchard test	+++	_	+	++
	Terpenoids		+++	_	_	++
Test for Saponins			_	_	+++	_

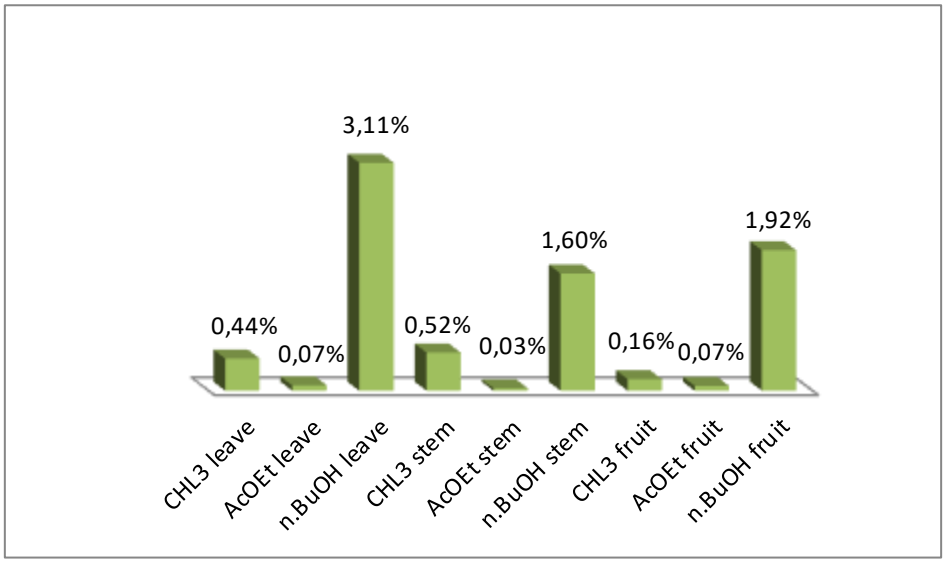
(-) absent, (+) present, (++) medium present, (+++) strongly present.

Preliminary Phytochemical results of fruit part of the table above shows a group of family which are flavanoids in all the extracts and alkaloids only Petroleum ether. Furthermore, the presence of Coumarins and tanins in all the extracts only the Petroleumether and Chloroform extract; the sterol were absent in all the extracts except distilled water, triterpanoids were absent in all extracts only in ethanol and Petroleumether extract; saponins have also disappeared in all the extracts except in the Chloroform extract.

V.3. Extraction yield:

Extraction yields are calculated according to the formula below:

$$yield\,\% = \frac{\text{extracted residue mass}}{\text{initial plant mass}} * 100 \qquad \textbf{(V.1)}$$



Figuer V.1. Extraction yield

The obtained results from the extracts show that there are differences in the extraction rates with the different solvents. The best yield is observed with n.BuOH for three parts (n.BuOH leaves, n.BuOH fruit, n.BuOH stem),3.11% and 1.92% and 1.60% respectively, followed by $CHCL_3$part ($CHCL_3$ stem , $CHCL_3$ leaves, $CHCL_3$ fruit) 0.52% and 0.44% and 0.16% respectively, then the lowest yield for AcOEt extract (AcOEt leaves and AcOEt fruit with the same amount 0.07%, then AcOEt stem,0.03%)

V.4. Quantitative analysis of phenolic compounds

Three Quantitative Phytochemical tests were carried out for different polarity extracts:

V.4.1. Determination of polyphenol contents

The total polyphenol content of the various extracts was determined through using the method using the Folin-Ciocalteu reagent. The curve shows a linearity of absorbance as a function of concentration. The amounts of the corresponding extract polyphenols were reported in mg gallic acid equivalent per gram of extract (mg GAE/g) and it is determined by the type equation : $y = 3.7143x - 0.0914$, $R^2 = 0.9951$.

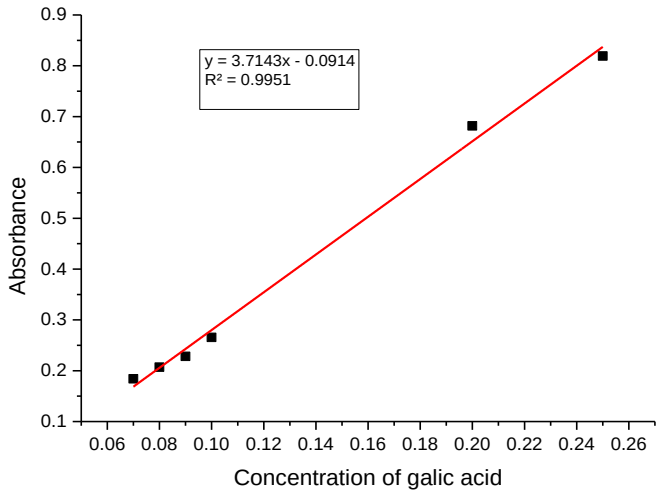


Figure V.2. Calibration curve of polyphenol.

Table V.4. Total polyphenol contents

Polyphenol Content (mg GAE/g)	$CHCl_3$ extract	ETACO extract	n.BuOHextract
Leaves	64.531± 0.008	99.902±0.012	39.498±0.003
Stems	66.427±0.001	63.73565± 0.001	21.186±0.002
fruit	52.697±0.003	87.683±0.0005	70.645±0.003

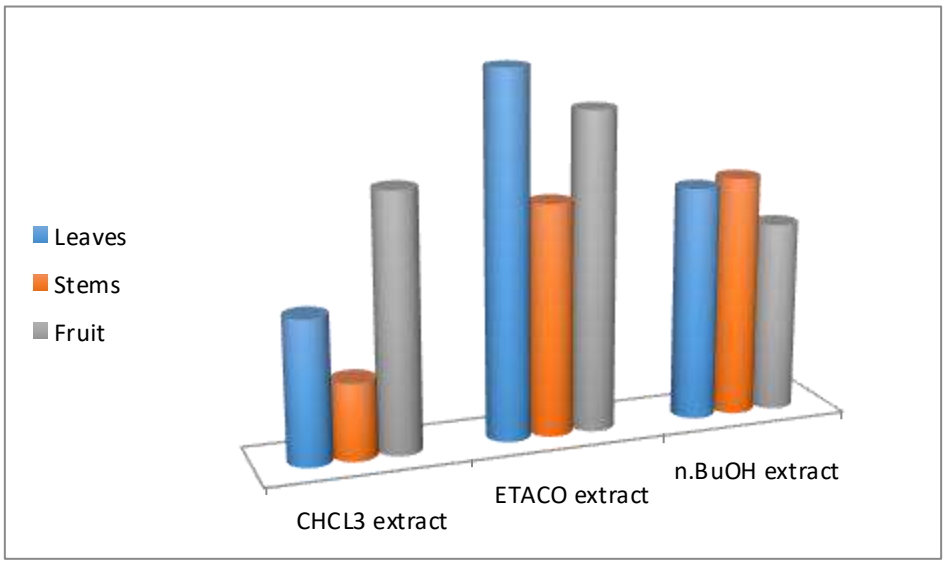


Figure V.3. Total polyphenol contents

TPC in various extracts of three parts of *Solanum nigrum* L. showed different results ranged from21.186 to 99.902 mg GAE/g. Moreover, Chloroform had the highest phenolic content (66.427 mg GAE/g) for stem part. (Table VI.4). Then, ethyl acetate had the highest phenolic content for two parts leaves (99.902mg GAE/g) and fruit (87.683mg GAE/g).

V.4.2. Determination of flavonoid content

The determination of flavonoids was carried out using the aluminium trichloride ($AlCl_3$) method and the standard is rutin. The flavonoid content is expressed in milligram rutin equivalent per gram of plant extract (mg RE/g). The flavonoid content of extracts was obtained from the calibration curve following a type equation: $y = 11.787x + 0.0991$, $R^2 = 0.9912$.

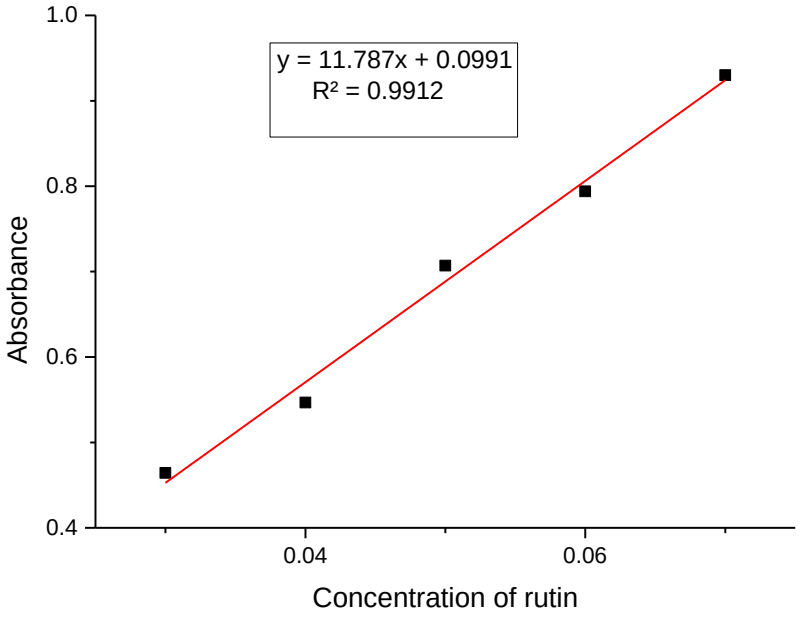


Figure V.4. Calibration curve of flavanoid.

Table V.5. Flavanoid contents

Flavanoid Content(mg RE/g)	$CHCl_3$extract	ETACO extract	n.BuOH extract
Leaves	52.195± 0.005	71.935± 0.020	19.769±0.022
Stems	12.165±0.009	23.591±0.023	3.322±0.009
fruit	30.438±0.008	18.105±0.0025	6.458±0.0103

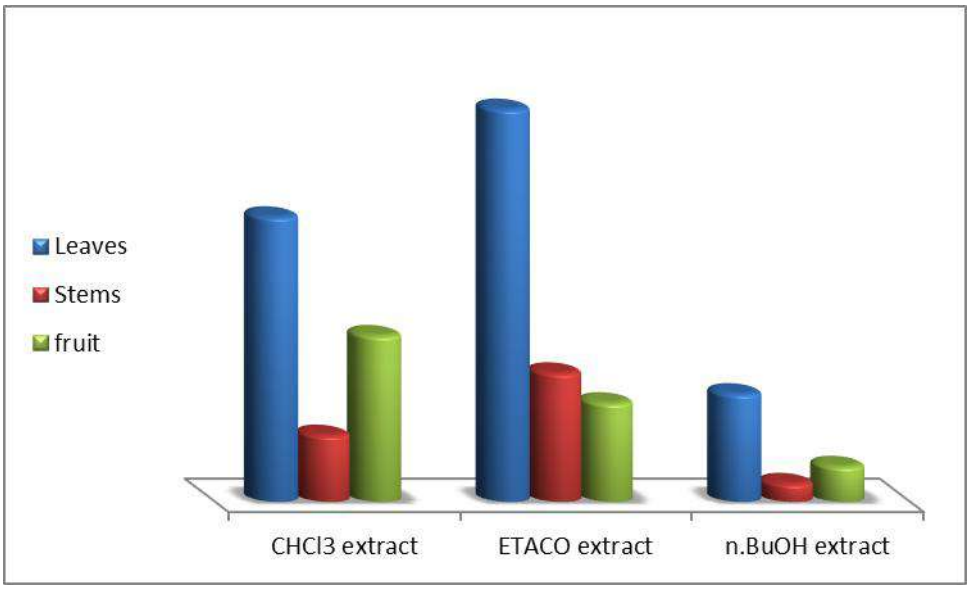


Figure V.5. Flavanoid contents

The amount of TFC from *solanum nigrum* L. ranged from 3.322 to 71. 935 mg RE/g of extract (TableV.5). The overall results indicate that the highest amount of TFC was obtained from different parts of *solanum nigrum* L. was for Ethyl acetate extract of leaves also acetate extract was the highest in stem part (23.591mg RE/g) and the largest amount in fruit part for Chloroform extract (30.438mg RE/g).

V.4.3. Determination of Flavanol:

The determination of flavanol was carried out by the method of Kumaran et al.

The flavanol content in various extracts was demonstrated in terms of rutin equivalent using the standard curve equation y = = 3.4583x + 0.0198, R^2 = 0.9991

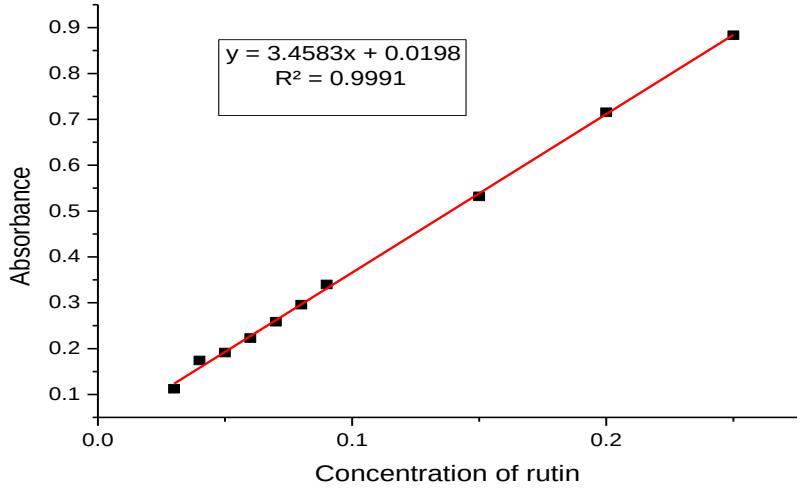


Figure V.6. Calibration curve of flavanols

Table V.6. Flavanol contents

Flavanol content (mgRE/g)	$CHCl_3$ extract	ETACO extract	n.BuOH extract
Leaves	105.398± 0.012	174.72±0.027	115.008±0.019
Stems	25.111± 0.006	76.752±0.007	23.754±0.003
Fruit	71.59±0.019	56.675±0.002	22.968±0.018

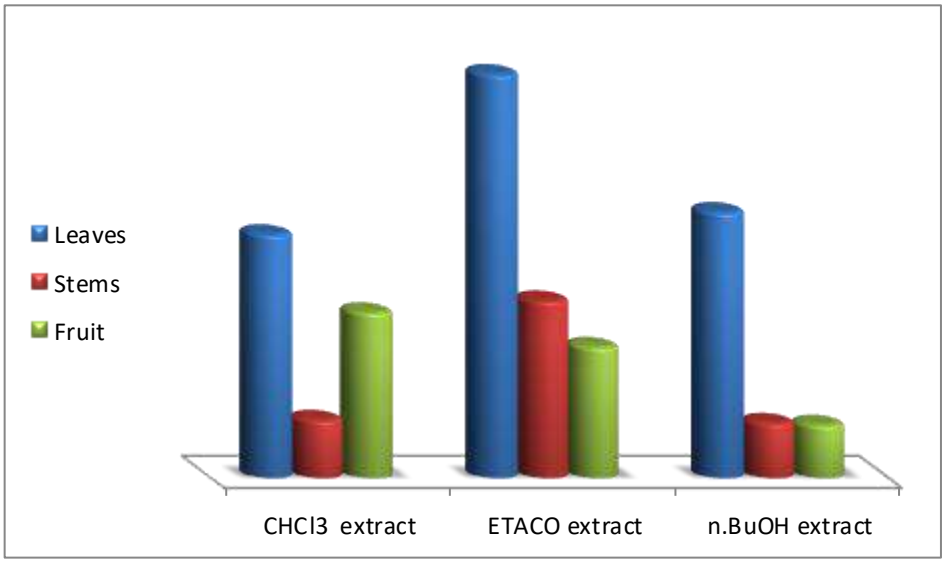


Table V.7. Flavanol contents

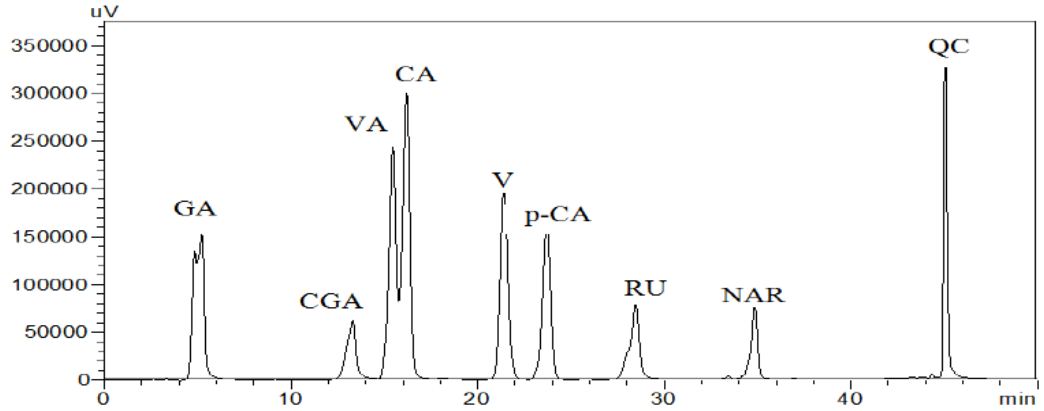
The flavanol contents values vary considerably between the extracts of the different solvants. The amount of different extracts of Three part of *Solanum nigrum* L. ranged from 22.968 to 174.72mg RE/g(Table V.6). As noted also , ETACO extract of leave and stem part had the highest quantity of flavanol (174.72mg RE/g,76.752mg RE/g) respectively, for fruit part the chloroform had the highest content 71.59 mg RE/g.

V.5. Chromatographic analysis of phenolic compounds by HPLC

V.5.1. Chromatograms of standards

Figure (V.5) shows the chromatogram profiles of the mixture of standards used. These analyses are recorded at a wave length of 268 nm. The mixture consists of a solution that contains nine standards:

Gallic acid (GA), chlorogenic acid (CGA), p-Coumaric Acid(p-CA), Naringin (NAR),caffeic acid(CA), Vanilic Acid(VA),quercetin(QC), vanillin(V), and rutin (RU). The identification and then quantification of phenolics compounds in the tested extracts are made by comparison of their time of retention and peak areas are obtained with those of the standards.



FigureV.8. High-performance liquid chromatography (HPLC) chromatogram of phenolic compounds. Identified compounds are: GA—gallic acid. CGA —Chlorogenic acid. VA—Vanillic acid. CA—Caffeic acid. V—Vanillin. p-CA—p-coumaric acid. RU—Rutin. NAR—naringin and QC—quercetin.

Table (V.7) summarizes the retention time, correlation coefficient and calibration curve equation for each phenolic compound.

Table V.7. Retention time, correlation coefficient and the curve equation calibration standards

Compound	Rt (min)	Calibration curve	R^2
Gallic Acid	5.29	Y = 23616X − 7232	0.9986
Chlorogenic Acid	13.392	Y = 39775X − 1881	0.9963
Vanillic Acid	15.531	Y = 65077X + 33	0.9885
Caffiec Acid	16.277	Y = 72328X − 52	0.9992
Vanillin	21.46	Y = 58930X + 64	0.9933
p-Coumaric Acid	23.817	Y = 157538X + 122	0.9996
Rutin	28.37	Y = 28144X − 24	0.9854
Naringin	34.788	Y = 19379X − 212	0.9922
Quercetin	45.047	Y = 45378X + 177	0.9988

V.5.2. Analysis of leaves extracts:

The results of the qualitative and quantitative analyses of the phenolic compounds identified in the samples extracts of the leaves of *solanum nigrum* L. are grouped in table (V.8). The chromatographic profiles are shown in Figure (V.6).

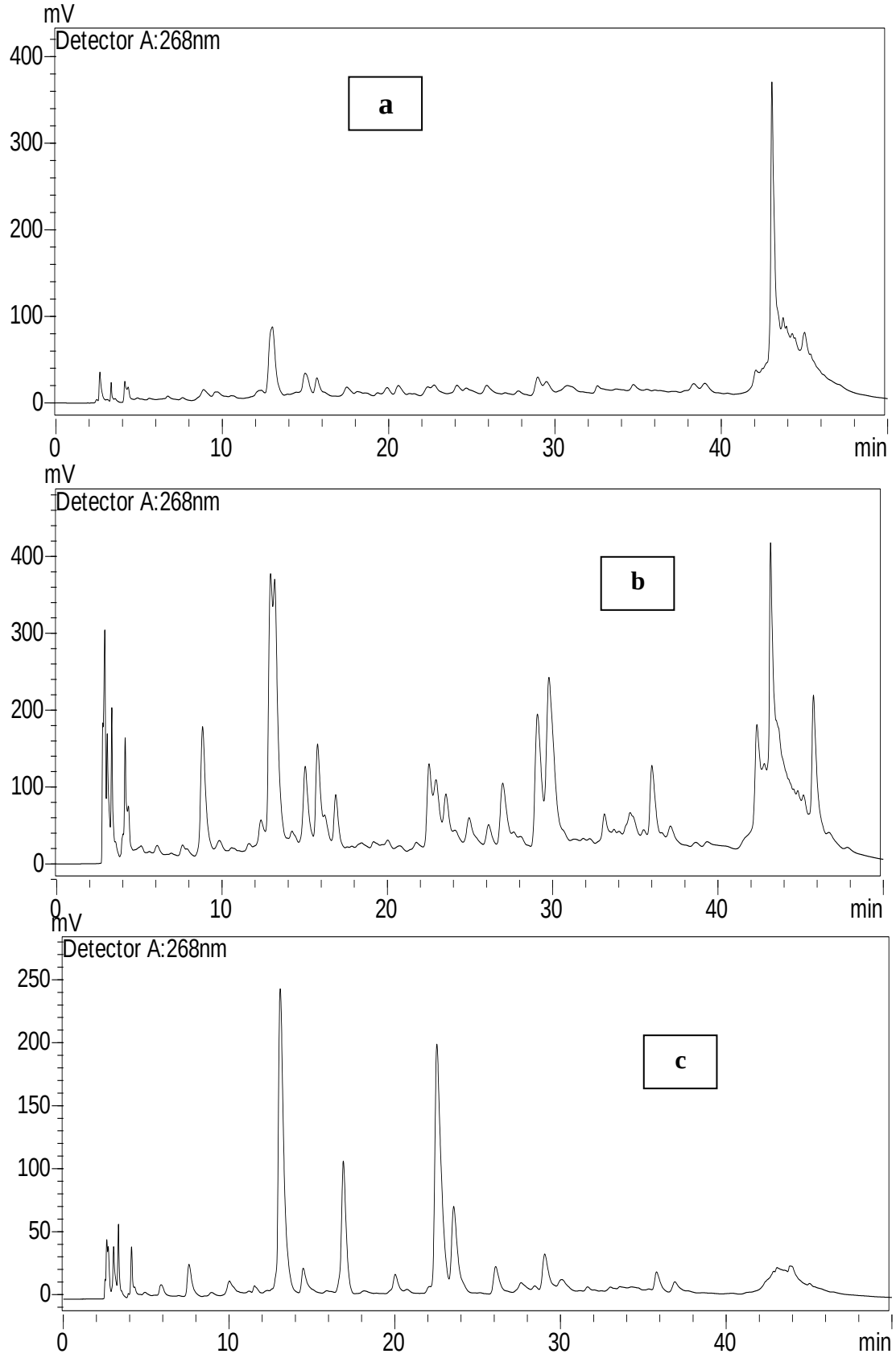


Figure V.9. HPLC chromatogram profile of leave extracts.(a) Chloroformextract (b) EtACOextract (c) n.BuOHextract

It is noted that the active compounds are present in significant quantities in all extracts. Thus, the analysis of leaf extracts showed that Chlorogenic acid is the most dominating compound phenolic in all the studied extracts, Chlorogenic acid levels range from 24912.651µ/g (Chloroform extract) to 75302.201µ/g (EtACO extract).EtACO extract is rich by phenolic compounds, also it contains all the compounds used as standards. The buotanolic extract of leaves shows significant level of Chlorogenic acid (60992.079 µ/g) then Chloroform extract has considerable concentration of Chlorogenic acid(24912.651 µ/g).

Table V.8. Quantitative and qualitative analysis of Leave extract of *Solanum nigrum* L.

samples / standards	Concentration (µg /g)		
	Chloroform extract	**EtACO extract**	**n.BuOH extract**
Gallic Acid	549.829	1305.323	211.642
Chlorogenic Acid	24912.651	75302.201	60992.079
Vanillic Acid	2971.655	10449.381	123.539
Caffiec Acid	272.85942	3234.825	5710.387
Vanillin	747.721	3960.709	188.661
p-Coumaric Acid	2897.710	9808.966	9675.413
Rutin	5546.809	8359.557	1797.541
Naringin	8116.115	32063.955	4729.697
Quercetin	10026.096	8277.918	2855.903

V.5.3. Analysis of stem extracts:

Chromatographic profiles of samples extracts from stem part of *Solanum nigrum* L. represented at figure (V.7). The results of the qualitative analyses and quantitative levels to identified phenolic compounds are shown in Table (V.10).

Table V.9. Quantitative and qualitative analysis of stem extract of *Solanum nigrum* L.

Samples / Standards	Concentration (µg /g)		
	Chloroform extract	**EtACO extract**	**n.BuOH extract**
Gallic Acid	1053.817	1054.293	486.966
Chlorogenic Acid	3968.179	2839.076	2719.769
Vanilic Acid	1816.807	1340.833	1330.473
Caffiec Acid	1185.856	544.857	676.444
Vanilin	1793.650	705.867	1407.072
p-Coumaric Acid	1472.213	1551.469	905.275
Rutin	3336.227	2725.312	3146.645
Naringin	1424.500	5708.860	4083.131
Quercetin	10580.320	514.416	1108.114

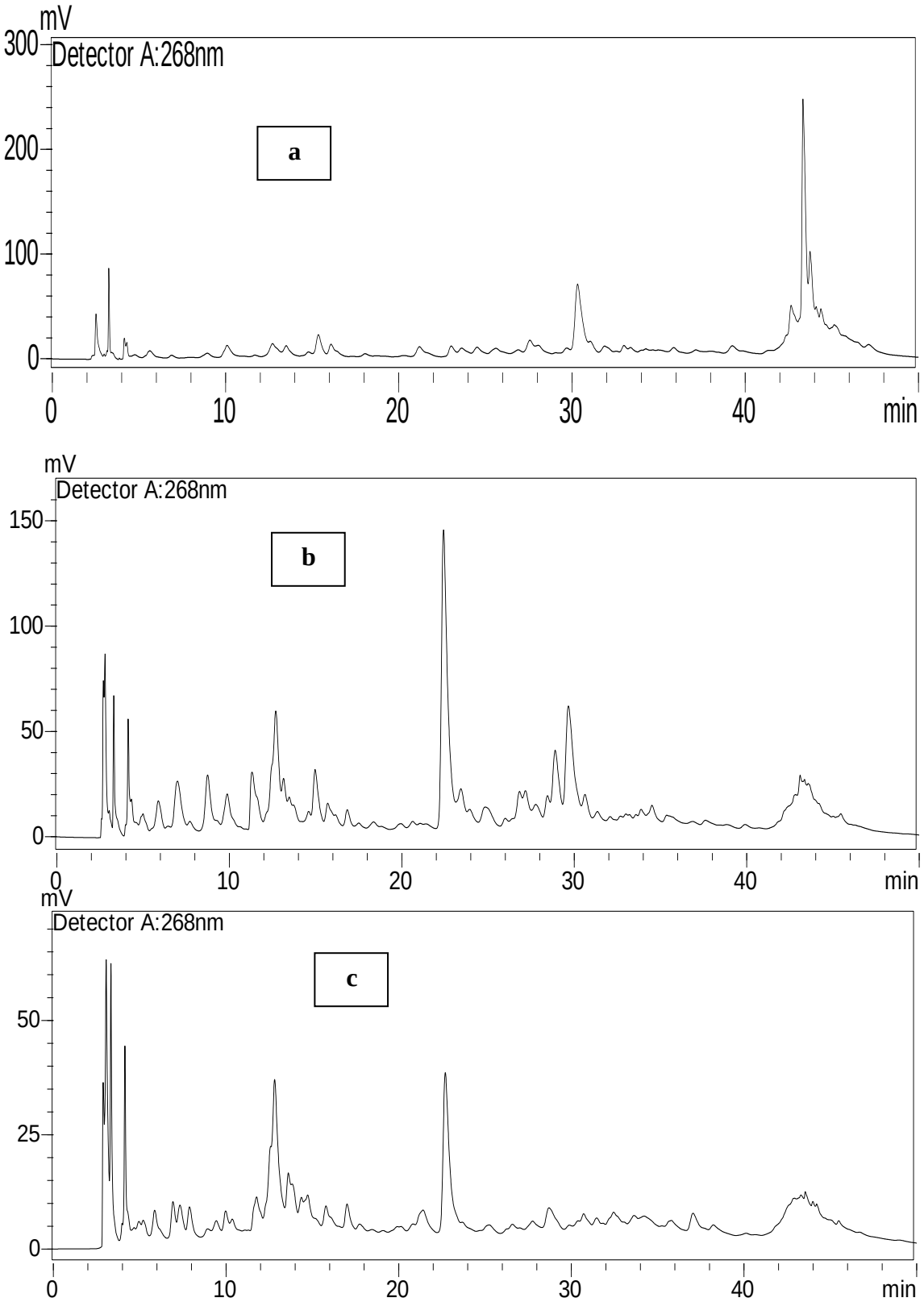


Figure V.10. HPLC chromatogram profile of stem extracts.(a)Chloroform extract (b) EtACO extract (c) n.BuOH extract

Analysis of these results shows that quercetin in Chloroform extract is the compound that has the highest amount which is 10580.320 µ/g, while in EtACO extract also The Naringin compound has the most representative value 5708.860 µ/g. Moreover, the Naring compound abundantly available (4083.131µ/g) in buotanolic extract.

Overall, according to the obtained results, the stem part has considerable quantities of standard compounds.

V.5.4. Analysis of fruit extracts:

Chromatograms of qualitative and quantitative analyses of phenolics' compounds of extracts of fruit part are shown in Figure (V.8), and the individual quantitative assessment to identified compounds is shown in Table (V.11).

Table V.10. Quantitative and qualitative analysis of fruit extract of *Solanum nigrum* L.

Samples / Standards	Concentration (µg /g)		
	Chloroform extract	**EtACO extract**	**n.BuOH extract**
Gallic Acid	364.817	1533.216	658.166
Chlorogenic Acid	9072.854	240701.610	41747.722
Vanilic Acid	4628.387	8533.352	132.384
Caffiec Acid	1282.547	1875.769	81.909
Vanilin	478.812	897.444	132.937
p-Coumaric Acid	1274.334	13477.171	4772.122
Rutin	1612.521	6000.483	1857.305
Naringin	6276.649	26439.671	1125.125
Quercetin	8293.904	3552.276	845.541

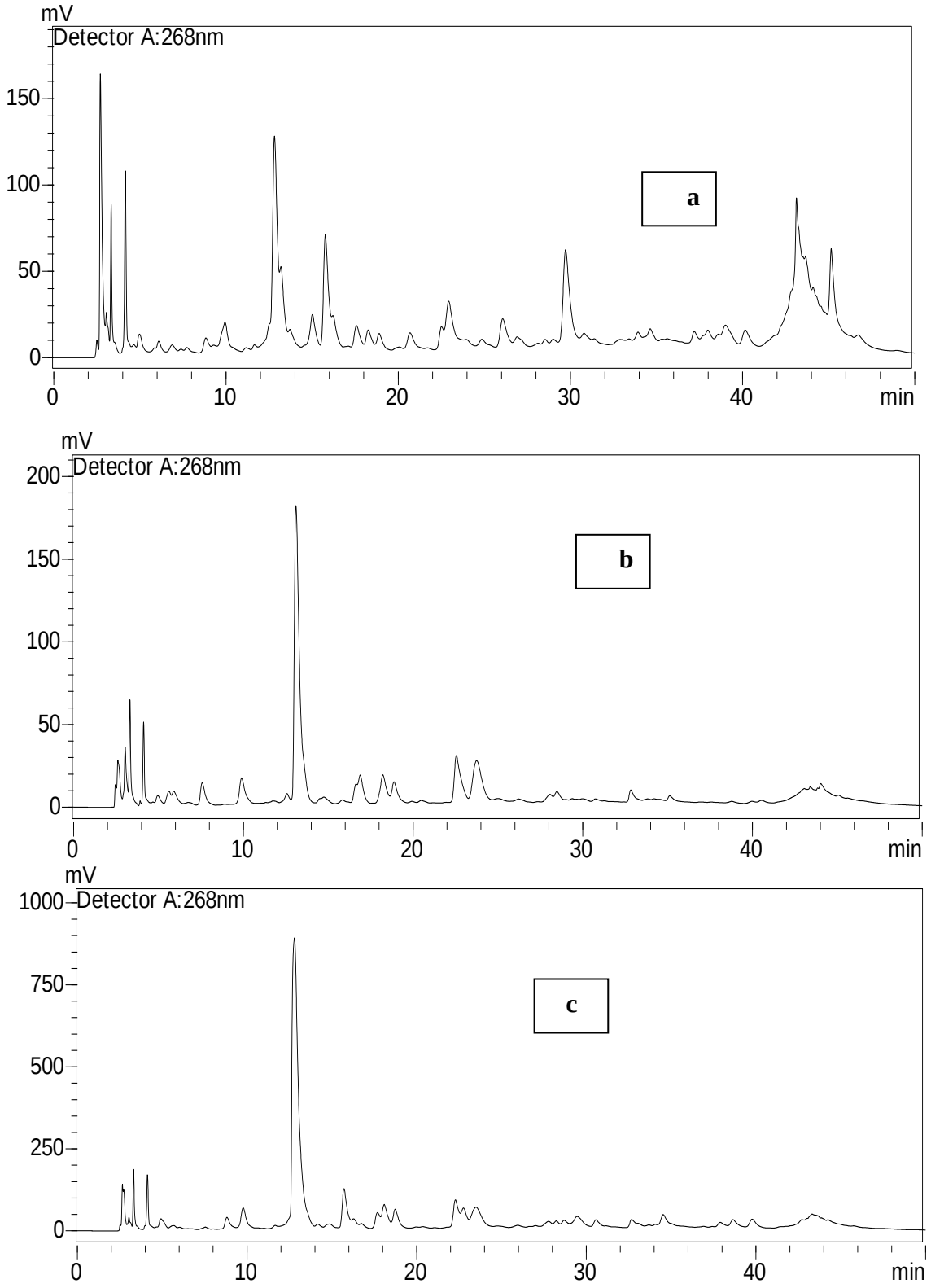


Figure V.11. HPLC chromatogram profile of fruit extracts (a) Chloroform extract (b) EtACO extract (c) n.BuOH extract

Through the table V.11, it was noted that the majority compound in all extracts is Chlorogenic acid that is estimated 41747.722µ/g in buotanol extract followed by EtACO extract 240701.610µ/g, then chloroform extract 9072.854 µ/g. According to the findings, it can be seen that the different extracts contain all the compounds used as standards with a considerable concentration.

V.6. Discussion

The results of the phytochemical analysis carried out on the different solvants of different parts of *Solanum nigrum* L. plant showed the presence of certain active compounds, alkaloids, flavonoids, terpenoids, tannins, saponins Coumarins and sterol. These results are consistent with the literature [1, 2].The various results above refer to selective solvents and maybe due to the interaction of the phyto constituents with the solvent system and solvent natural such as polarity .

Regarding the yield, the use of different solvents with different polarity allows the separation of compounds according to their degree of solubility in the extraction solvent.

According to the phytochemical investigation, the leaves part of Solanum nigrum L. have the highest amount of phenolic compounds that lead to susceptible to give credibility to largest uses of this part from local population. In addition, the ethyl acetate has the highest amount of phenolic compounds that explain the majority of bioactive substances having medium polar

In comparison with another study of *Solanum nigrum* L.[3], the amount of phenolic compound in leaf part was estimated at (2.6mg GAE/g) and (0.53mgGAE/g) in stem part and (1.47 mg GAE/g) for fruit each part prepared in ethanolic extract.

The amount of flavonoids was also found in another study[3] (2.55 mgQE/g, 1.32mg QE/g, 0.62mgQE/g) in leaf ,stem ,fruit respectively were also obtained from ethanolic extract.

Therefore, it has been noted that the amount of phenols and flavonoids obtained in this study is higher than other studies due to the distribution of secondary metabolites that may change during plant growth or it could be related to climatic conditions (high temperature, sun exposure, drought, salinity), which stimulate the biosynthesis of secondary metabolites such as polyphenols[4, 5].

According to the HPLC results *Soulanum nigrum* L plant is the richest of all the compounds used as standards such as Chlorogenic Acid, Naringin, Quercetin, p-Coumaric

Acid and these phenolic compounds have substantial biological activities and they might be responsible for the beneficial effects of the plant.

V.7. Conclusion

In this chapter *Solanum nigrum* L. contains different bioactive substances that have different pharmacological properties and which protect the plant from damage. Hence, *Solanum nigrum* L. can serve as a source of natural antioxidants and natural source of active compounds for developing therapeutic section. In the following chapter, we presented and discussed the results of antioxidant activities in the studied extracts

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Chapter VI

Antioxiodant activity

VI. Introduction

Extracts are mixtures of several compounds, with different groups, functions, polarities and chemical behaviors. This chemical complexity of the extracts could lead to results that are dispersed depending on the used test. Therefore, an approach with multiple assays to evaluate the antioxidant potential of extracts would be more informative and even necessary

VI.1. Aim

Demonstration in vitro antioxidant activity of the different extracts of *Solanum nigrum* L. plant was carried out by two methods: spectrometric assays and electrochemical assays in addition to attempting to confirm the therapeutic effect of this plant.

VI.2. By spectrphotometric method

VI.2.1. DPPH radical scavenging assay:

The DPPH test is one of the most widely used tests to determine the antiradical activity of plant extracts [1]. DPPH is a free radical allowing us to determine the scavening potential of our extracts to its sensitivity to detect active components at low concentration[2] .

Antiradical activity was estimated spectrophotometrically by following the reduction of DPPH at 517nm [3]. This reduction capacity is determined by a decrease in absorbance induced by antiradical substances [4]

In this test ascorbic acid is used as the standard, the obtained results (percentage of inhibition I %) are represented in the calibration curve (Figure VI.1), having the equation: $y=11274x-28.616$ with a correlation coefficient $R^2 = 0.9915$

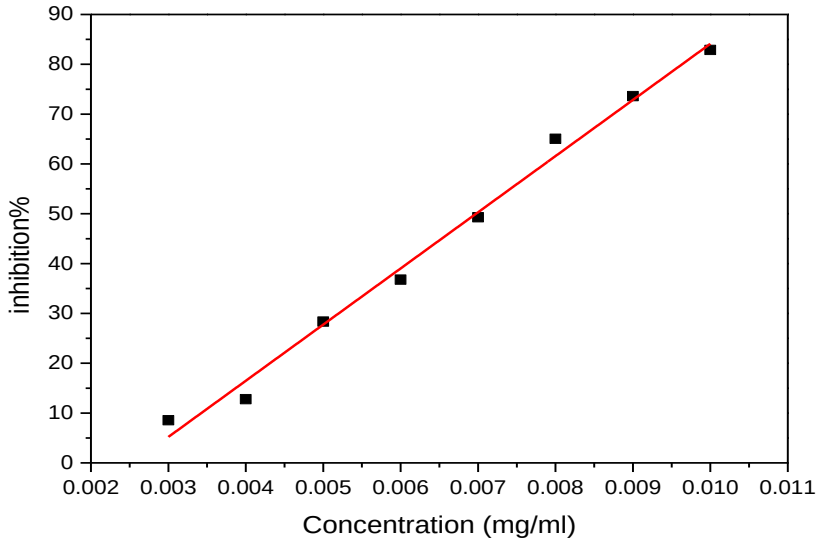


Figure VI.1. Calibration curve of ascorbic acid

To characterize the antioxidant power, we have introduced the IC50 parameter.

IC_{50}: it defines the effective concentration of the substrate that causes the 50% reduction of DPPH in solution.

The IC50 values of the different extracts were estimated through using the linear regression curve: y = ax + b. The IC50 values, presented in table (VI.1)

- **Calculation of IC50**

The antioxidant capacity of our different extracts is determined from IC50, parameters commonly used to measure antioxidant activity. It is the concentration of extract necessary to reduce 50% of the DPPH radical in a defined period of time. A low value of IC50 corresponds to a higher antioxidant activity of the extract [5]. The IC50 are therefore calculated from the graphs shown in Figure (VI.2).

The variability of antiradical activity and antiradical potency in different polarity extracts from three part of *Solanum nigrum* L. are shown below:

✓ **Leaves part:**

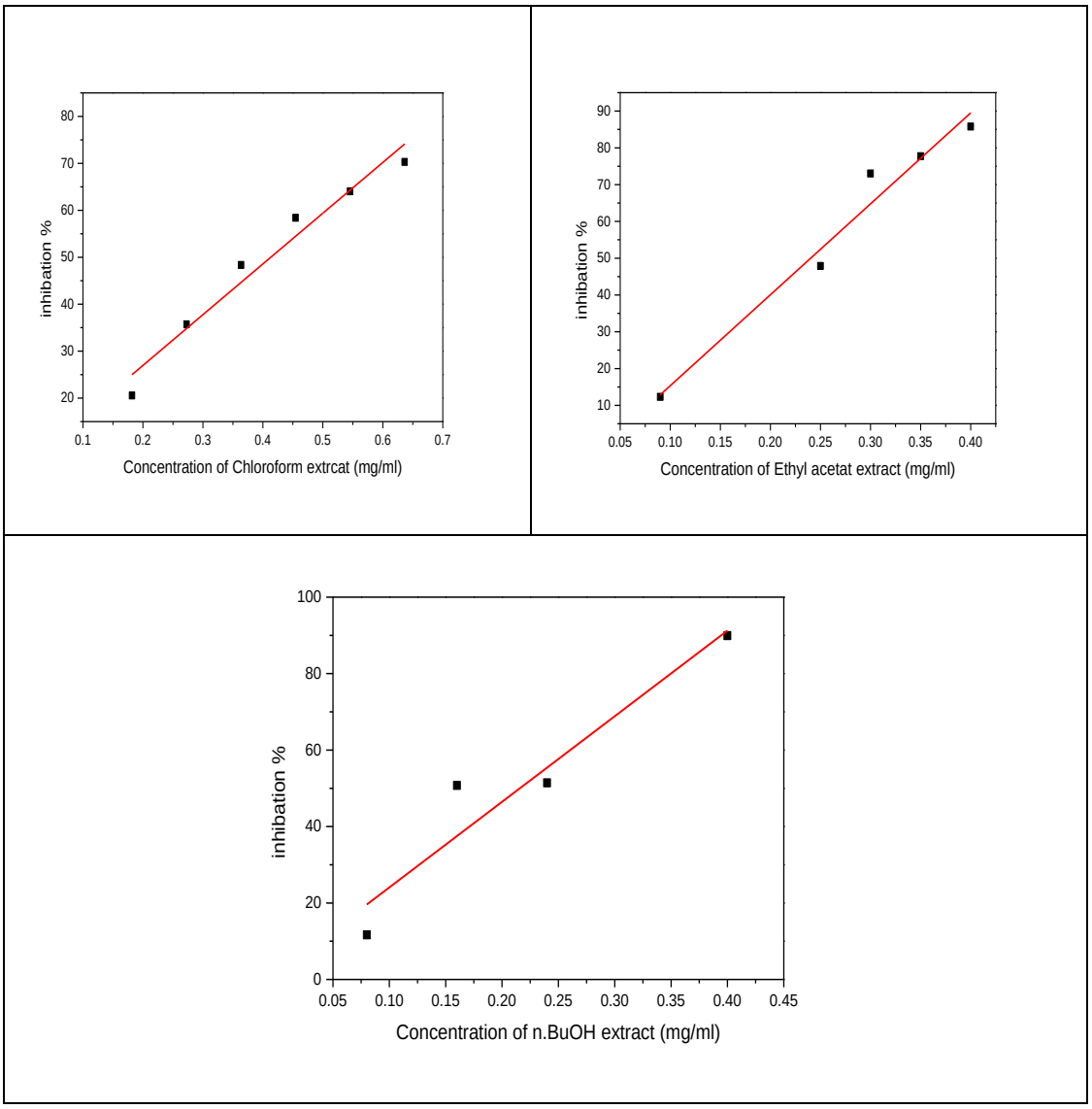


Figure VI.2. Variation in inhibition power as a function of the concentration of Leave extract

Table. VI.1. IC50 values of sample extracts of leave part using DPPH

Sample	DPPH method		
	Equation	R^2 values	IC50 (mg/mL)
$CHCL_3$ extract of leaves	y = 108.05x + 5.36	0.9633	0.413142
ETACO extract of leaves	y = 247.19x - 9.3715	0.9711	0.240186
n.BuOH extract of leaves	y = 223.92x + 1.6852	0.9164	0.204904
Ascorbic acid	y = 11274x - 28.616	0.9915	0.0069

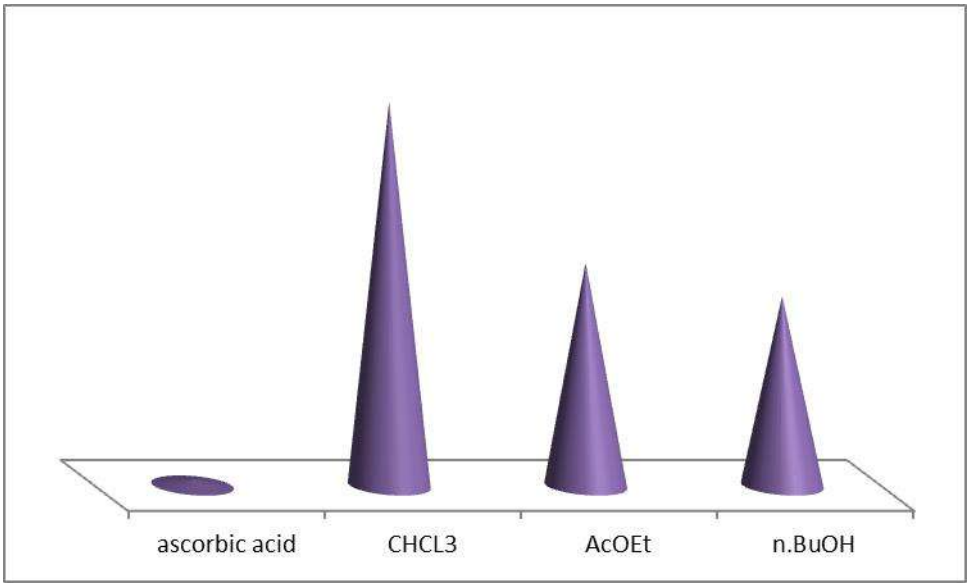


Figure VI.3. Inhibition of free radical scavenging activity of leaves

From the curves (Figure VI.2**)**, the value of percentage of inhibition of free radical scavenging activity of the three different extracts from the leaves of *Solanum nigrum* L. varied from 11.65% to 89.93%. Furthermore, the table (IV.1) exhibited the largest capacity to neutralize DPPH radicals was found for n.BuOH extract which neutralized 50% of free radicals at the concentration of 0.204mg/mL. A moderate activity was found for ETACO extract 0.240mg/ml than $CHCL_3$ extract which had the lowest activity 0.413 mg/ml. In comparison to IC50 value of ascorbic acid (0.006 mg/ml), n.BuOH extract has shown the strongest capacity for neutralization of DPPH radicals. As long as the type of antioxidants compounds is polar compound.

✓ **Stem part:**

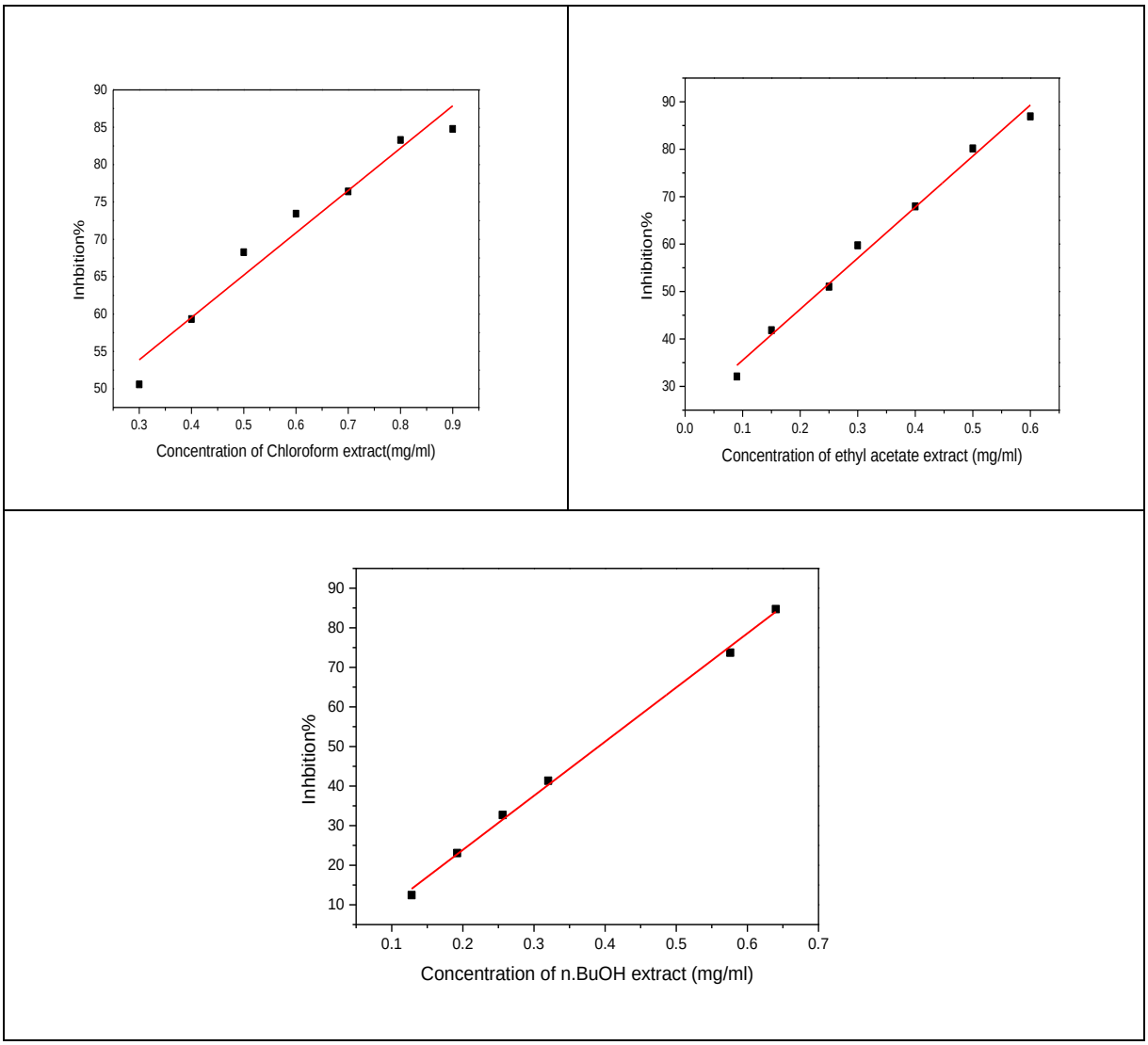


Figure VI.4. Variation in inhibition power as a function of the concentration of Stem extract

Table. VI.2. IC50 values of sample extracts of stem part using DPPH

Sample	DPPH method		
	Equation	R^2 values	IC50 (mg/mL)
$CHCl_3$ extract	y = 56.651x + 36.883	0.9598	0.2315
ETACO extract	y = 107.61x + 24.759	0.9905	0.2345
n.BuOH extract	y = 136.79x - 3.4827	0.998	0.3909
Ascorbic acid	y = 11274x - 28.616	0.9915	0.0069

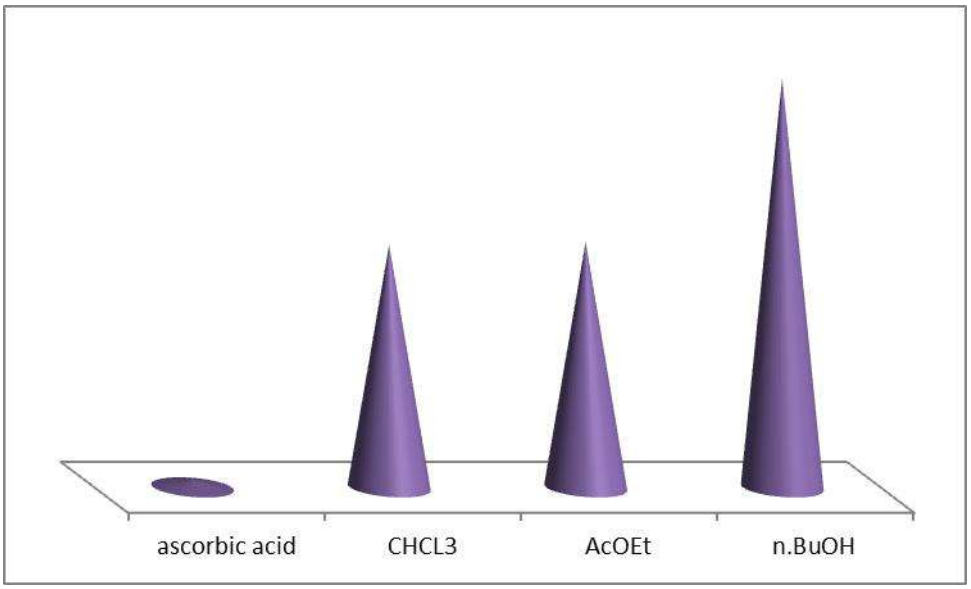


Figure VI.5. Inhibition of free radical scavenging activity of stem

The free radical scavenging activity of the three different extracts from the stem of *Solunum nigrum* L is expressed in terms of percentage of inhibition (%) and IC50 values (mg/mL) (Table .IV.2. and Figure VI.4). Parallel to examination of the antioxidant activity of these plant extracts, the value of standard compound was obtained and compared to the value of the antioxidant activity. The examination of antioxidant activities of the extracts from stem has exhibited different values which varied from 12.43% to 84.77%. Furthermore, the largest capacity to neutralize DPPH radicals was found for chloroform extract which neutralized 50% of free radicals at the concentration of 0.23 1mg/mL. A moderate and almost equal activity was found for EtOAc extract 0.234mg/ml than n.BuOH extract which had the lowest activity 0.390 mg/ml. In comparison to IC50 value of ascorbic acid, chloroform extract has shown the strongest capacity for neutralization of DPPH radicals. Hence, the type of antioxidants compounds are a polar compound.

✓ **Fruit part:**

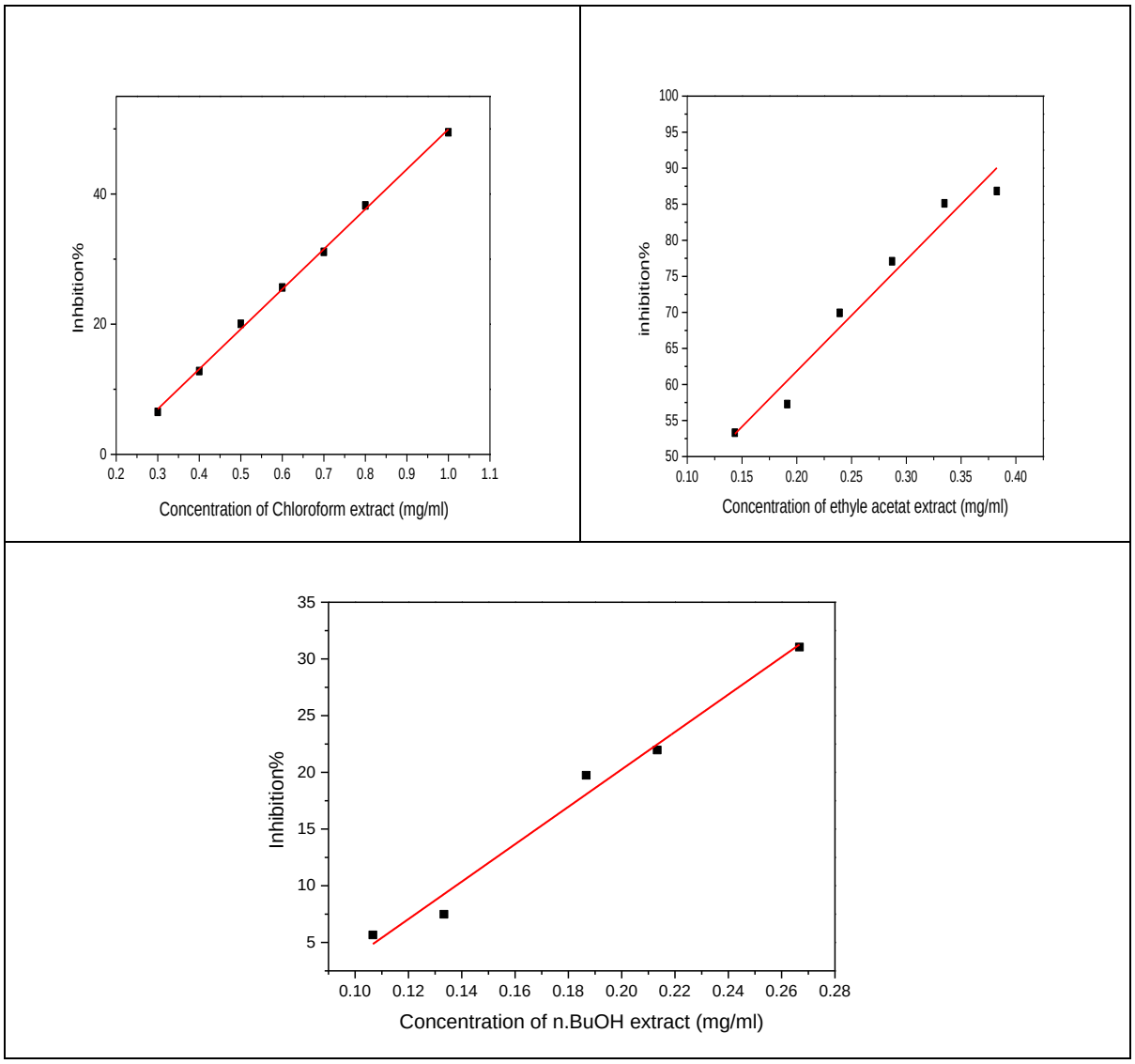


Figure VI.6. Variation in inhibition power as a function of the concentration of Fruit extract

Table. VI.3. IC50 values of sample extracts of fruit part using DPPH

Sample	DPPH method		
	Equation	**R^2 values**	**IC50 (mg/mL)**
$CHCl_3$ extract of fruit	y = 61.412x - 11.462	0.9987	1.000
ETACO extract of fruit	y = 154.32x + 31	0.9653	0.123
n.BuOH extract of fruit	y = 164.98x - 12.732	0.9846	0.380
Ascorbic acid	y = 11274x - 28.616	0.9915	0.0069

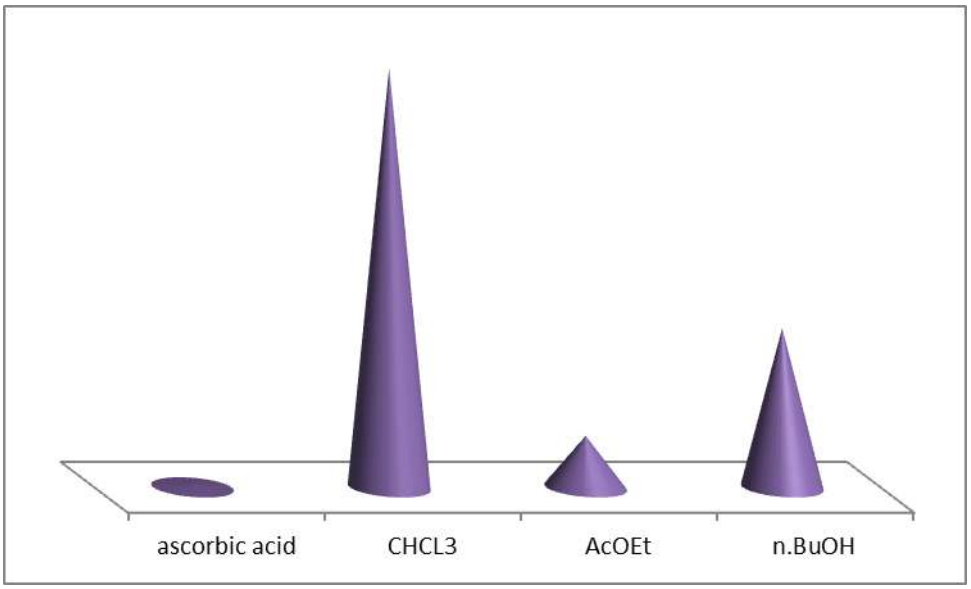


Figure VI.7. Inhibition of free radical scavenging activity of fruit

The results obtained to examine antioxidant activity of the extracts from fruit part of *Solanum nigrum* L. has exhibited different values of percentage of inhibition (%) which ranged from 5.66% to 89.93%. Moreover, the highest capacity to neutralize DPPH radicals was found for EtOAc extract, which neutralized 50% of free radicals at the concentration of 0.123mg/mL. A moderate activity was found for n.BuOH extract 0.380mg/ml than chloroform extract which had the lowest activity 1.000mg/ml. In comparison to IC50 value of ascorbic acid, EtOAc extract has shown the strongest capacity for neutralization of DPPH radicals. Thus, the type of antioxidants compounds are moderate polar compound.

VI.2.2. The reducing power of ferric ion (FRAP)

The antioxidant activity of the extracts of the studied plant was evaluated using the FRAP (Ferric reducing antioxidant power) method. This is a simple, rapid and reproducible test [5]. It is universal and can be applied in plants as well as in plasmas and in organic and aqueous extracts [6]. This method is based on the ability of polyphenols to reduce ferric iron Fe^{3+} to ferrous iron Fe^{2+}. The power of reduction is one of the antioxidant mechanisms [5].

A calibration curve is made using Ferrous sulfate ($FeSO_4$). The results are expressed in milligram (mg) Ferrous sulfate ($FeSO_4$) equivalent per gram of extract (mg $FeSO_4$/g).

The obtained equation from the linear calibration graph in the studied concentration $y = 2.977x - 0.02$ ($R^2 = 0.9951$) where y represents the value of the absorbance and x the value of standards concentration.

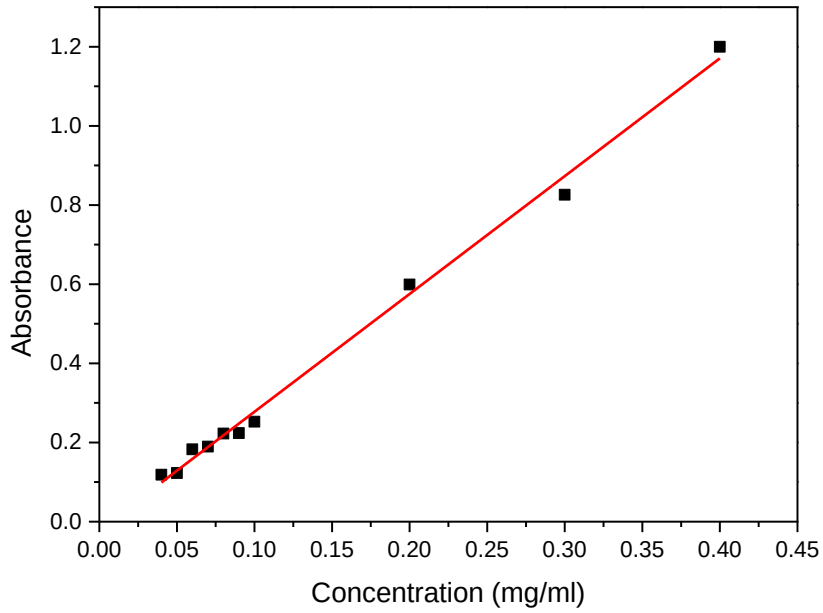


Figure VI.8. Calibration curve of Ferrous sulfate ($FeSO_4$)

The results of the reducing activity of the ferric ion of our extracts are shown in the table below:

Table VI.4. The reducing activity of the ferric ion of *Solanum nigrum* L. extracts

Sample	FRAP method (mg $EFeSO_4$/ g)		
	$CHCl_3$ extract	**ETACO extract**	**n.BuOH extract**
leaves	635.98±0.013	436.12±0.020	112.77±0.030
stem	198.41 ± 0.0030	458.29±0.0055	348.89± 0.0049
fruit	134.363± 0.001	299.51±0.002	610.45±0.001

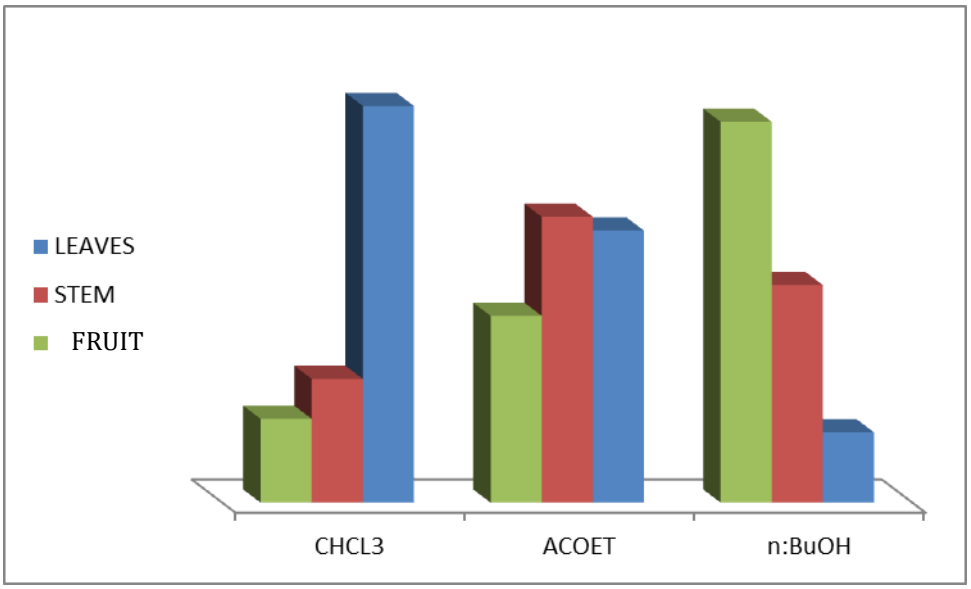


Figure VI.9. The results of FRAP method of *solanum nigrum* L.

From the findings shown in table (VI.4), it can be seen that all the tested extracts have a dose-dependent capacity to be reduced iron but with different amounts that vary from 635.98±0.013 mg $EFeSO_4$/ g to 112.77±0.030 mg $EFeSO_4$/ g. Moreover, FRAP test on figure IV.8 indicates that the chloroformic extract of the leaf part has the highest antioxidant effect of the order of 635.98±0.013 mg $EFeSO_4$/g and considerably amount of butanolic extract of fruit part 610.45±0.001 mg $EFeSO_4$/g then acetate extract of stem part 458.29±0.0055 mg $EFeSO_4$/g .

VI.2.3. Total antioxidant capacity (CAT):

Total antioxidant capacity is one of the Several assays have been introduced for the measurement of the antioxidant activity of plant extracts by 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 [7]

The total antioxidant capacity of the different extracts is expressed in number of ascorbic acid equivalents from a calibration curve ($y = 0.0952x - 0.0879$,$R^2 = 0.8571$), established using ascorbic acid as a reference. Unlike other tests, the CAT not only quantifies the antioxidant activity of polyphenols but also of other antioxidant compounds such as vitamins (C, E,.....) [8].

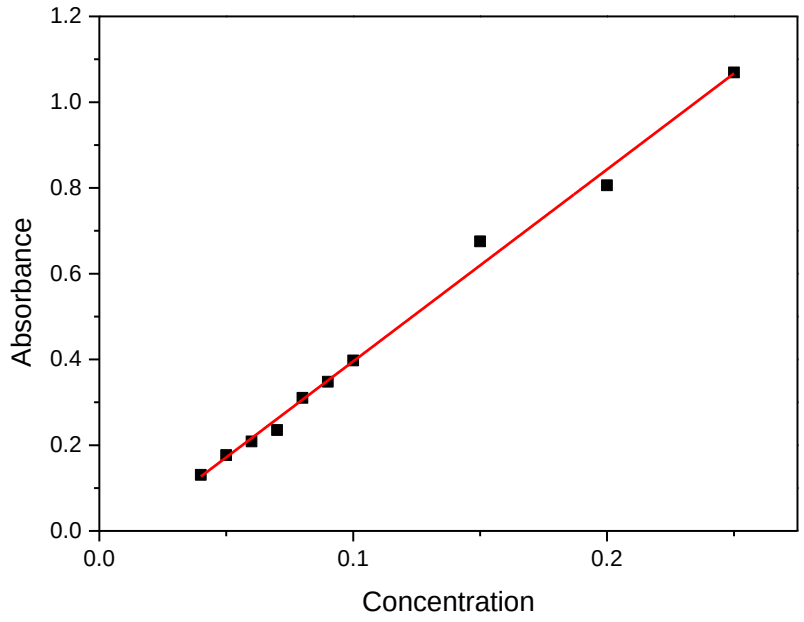


Figure VI.10. Calibration curve of ascorbic acid

The results in table (**VI.5**) show that different extracts have an important antioxidant capacity.

Table VI.5. Total antioxidant capacity of *Solanum nigrum* L. extracts

Sample	Total antioxidant capacity method (mg EAS/ g)		
	$CHCl_3$ extract	ETACO extract	n.BuOH extract
Leaves	153.36± 0.040	75.42±0.005	67.32±0.006
Stem	87.65± 0.012	65.09±0.014	93.93±0.006
fruit	138.36±0.004	63.47±0.003	89.99±0.006

The chloroform extract of leaves part has the best total antioxidant capacities153.36 mg EAS/ g followed by n.BuOH extract which has highest value 93.93±0.006 mg EAS/ g in stem part extract then $CHCl_3$ extract of fruit part has most total antioxidant capacities 138.36±0.004 mg EAS/ g.

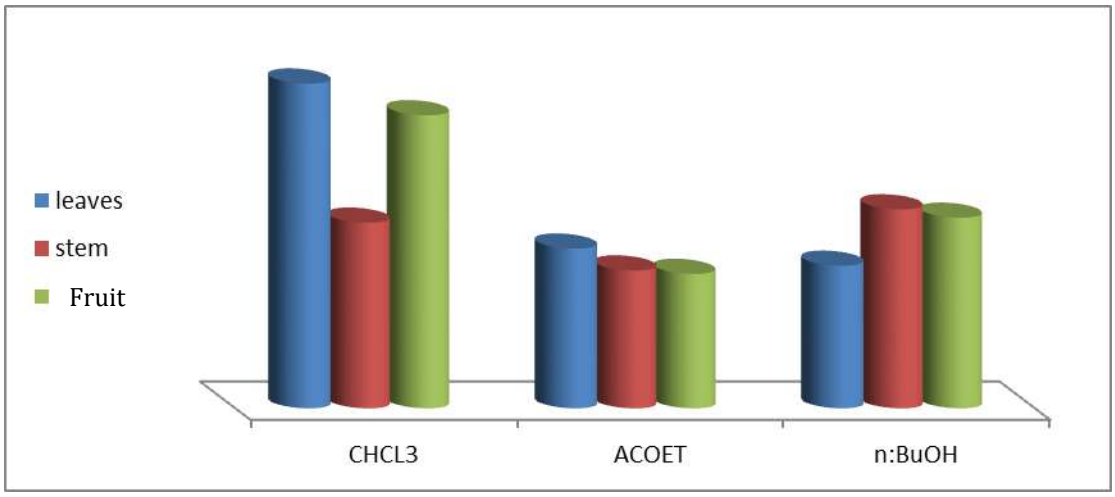


Figure VI.11. Total antioxidant capacity method of *Solanum nigrum* L. extracts

In this method, a change in the polarity of the solvent could significantly influence the antioxidant activity, as it can be seen in figure(VI.11) the chloroform extracts a polar solvent in three parts has the highest amount of antioxidant capacity followed by n.BuoH extracts polar solvent then lowest antioxidant capacity for ethyl acetat extracts which has medium polarity solvent.

VI.3. By lectrochemical method:

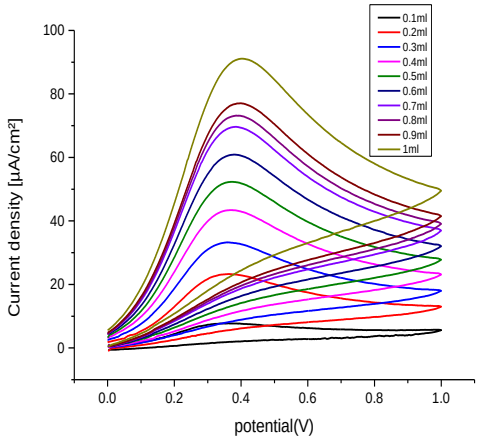
Electochemical assays is a new selected method to evaluate the antioxidant capacity of the extract. An extensive literature review has shown that there was no previous study which dealt with antioxidant activity of voltammetry cyclic assay of stem and fruit from *solanum nigrum* L.

VI.3.1. Study of total antioxidant activity

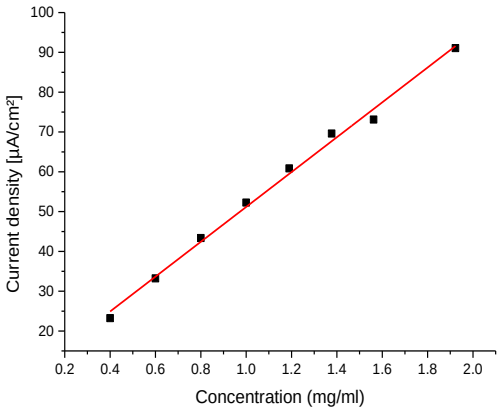
For expressing the antioxidant capacity of three study parts of different polarity extracts of *Solanum nigrum* L. plant in equivalent terms of ascorbic acid equivalent antioxidant capacity (AEAC), different concentrations of the standards of ascorbic acid were plotted versus the anodic current obtained density from different cyclic voltammograms at pH 7 in 0.2 M phosphate buffer solution as a supporting electrolyte using a 3 mm-diameter glassy carbon electrode.

Linearity was evaluated by the regression graph of the calibration curve of Ascorbic acid at different concentrations for Total antioxidant capacity. The equation obtained from the linear calibration graph in the studied concentration $Y= 43.784x + 7.3924$ ($R^2 = 0.9949$)

where y represents the value of the anodic current density and x the value of standards concentration expressed as mg/mL



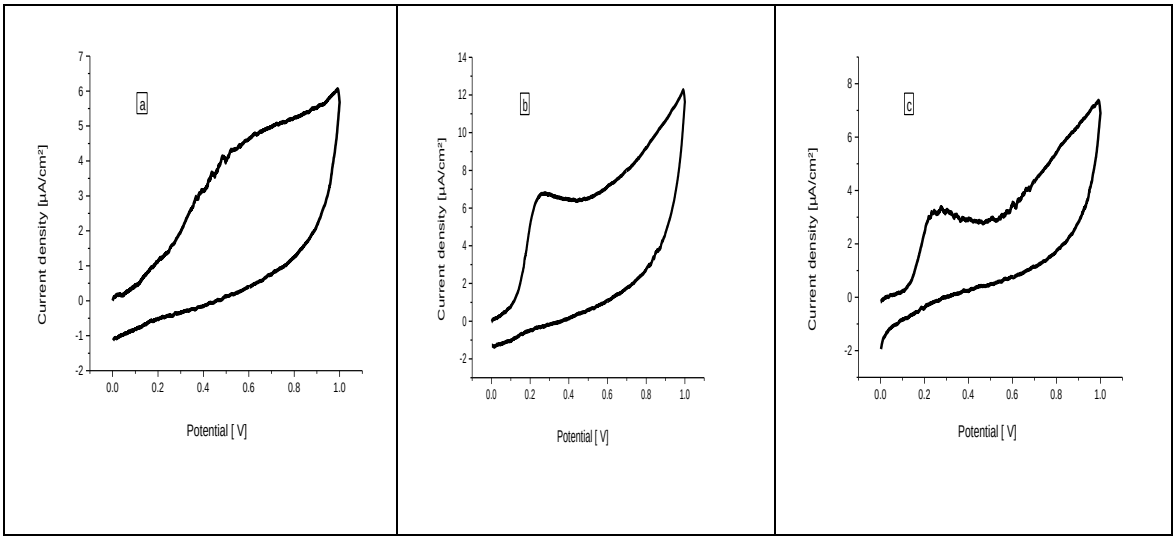
Figuer VI.12: Cyclic voltammograms for different concentrations of ascorbic acid.



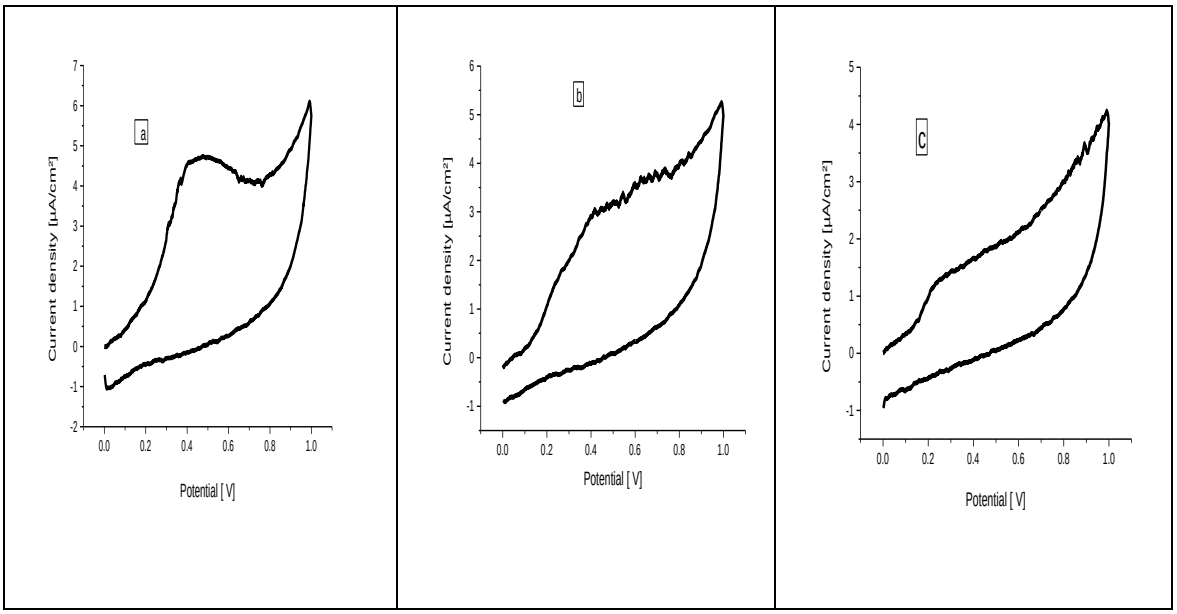
Figuer VI.13: Calibration line for ascorbic acid.

VI.3.1.1. Cyclic voltammograms of different polarity extract

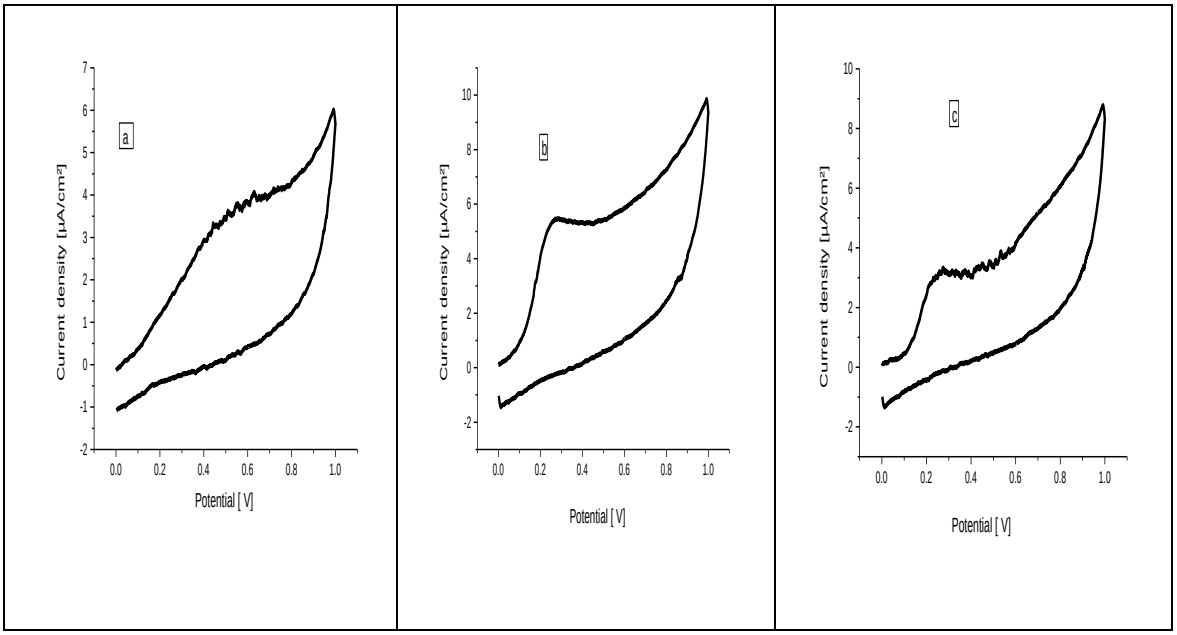
All the obtained voltammograms from cyclic voltammetry assay (CV) for three part of *Solanum nigrum*. Each voltammogram shows one oxidation peak.



Figuer VI.14. Cyclic voltammograms of leaves part of *solanum nigrum* L. (a) chloroform extract (b) ethyl acetat extract and (c) n.BuOH extract



Figuer VI.15. Cyclic voltammograms of stem part of *Solanum nigrum* L. (a) chloroform extract (b) ethyl acetat extract and (c) n.BuOH extract



Figuer VI.16. Cyclic voltammograms of fruit part of *Solanum nigrum* L. (a) chloroform extract (b) ethyl acetat extract and (c) n.BuOH extrac

Table. VI.6: Antioxidant capacities of three parts of *Solanum nigrum* L.

Samples	Antioxidant capacities (mg/g)		
	Chloroform extract	**Acetate extract**	**Butanol extract**
leaves	43.954 ±0.568	11.690±0.046	55.477±0.052
Stem	9.58 ± 0.010	0.83 ± 0.270	0.23 ± 0.286
Fruit	4.53±0.010	17.75±0.238	3.37±0.010

As it can be seen from table VI.6, the values of antioxidant activities of samples' extracts is ranged from 0.23 mg/g to 55.477 mg/g, the Butanol extract of leaves part has the highest value and then come those of Chloroform extacts for leaves and Acetate extract for fruit part .

The values of antioxidants activity by electrochemical assay are moderate and are not that considerable; According to the principle technique [9] there are not so many antioxidant compound oxidized on the surface of the working electrode, thus, the three parts of *Solanum nigrum* L. have moderate potent antioxidant capacity

VI.3.2. Evaluation of antioxidant activity by the scavenging of superoxide anion O_2

Voltammogram and calibration curve of standard antioxidant (Ascorbic Acid). The standard antioxidant with different amounts is dissolved in DMF to obtain different concentrations at each injection into the cell with which their calibration curve can be plotted. Then, the voltammograms of the standard were recorded.

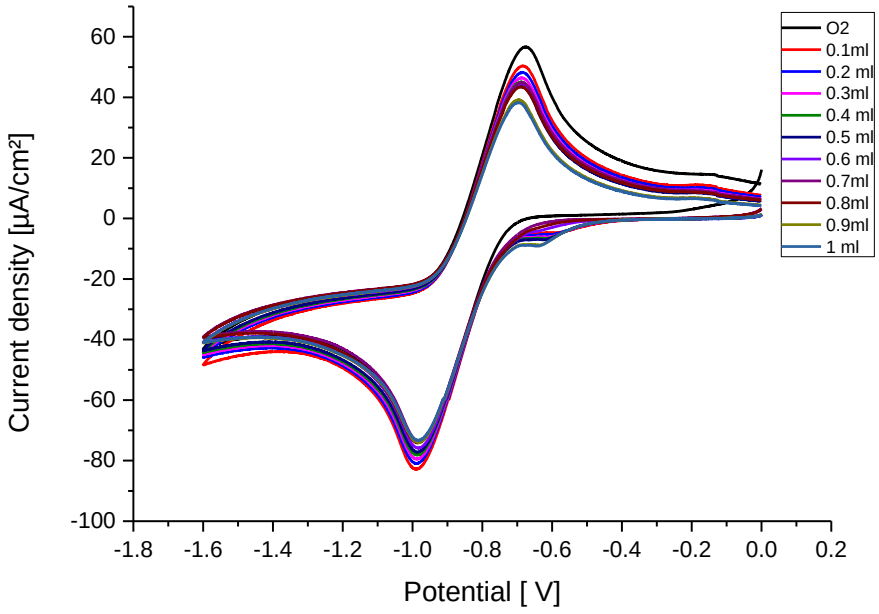


Figure VI.17: Ascorbic acid

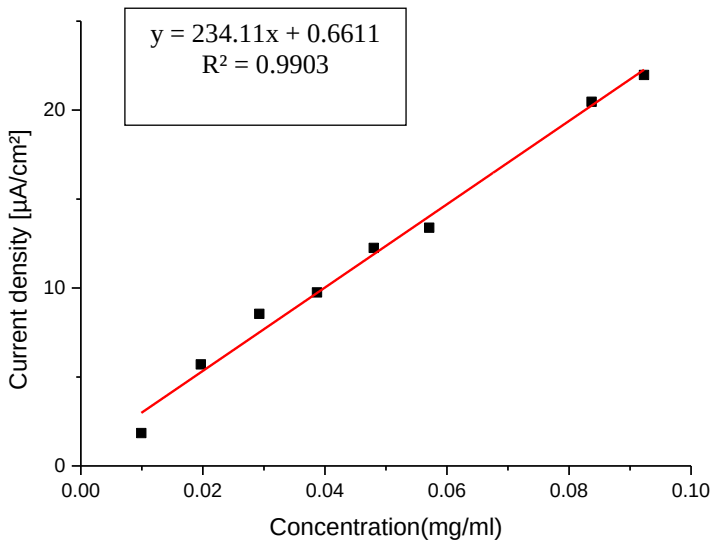
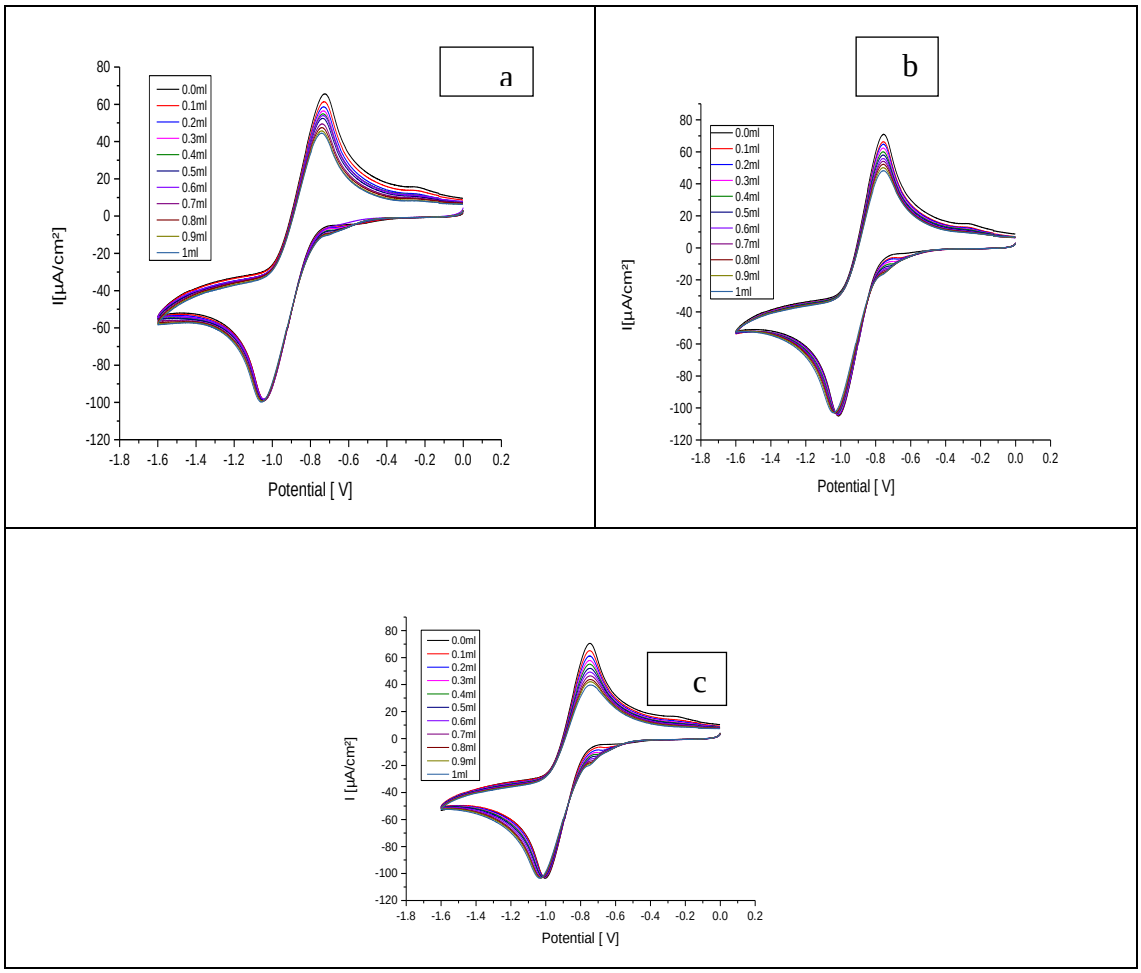


Figure VI.18: Calibration line for ascorbic acid.

VI.3.2.1. Voltamograms of the samples tested:

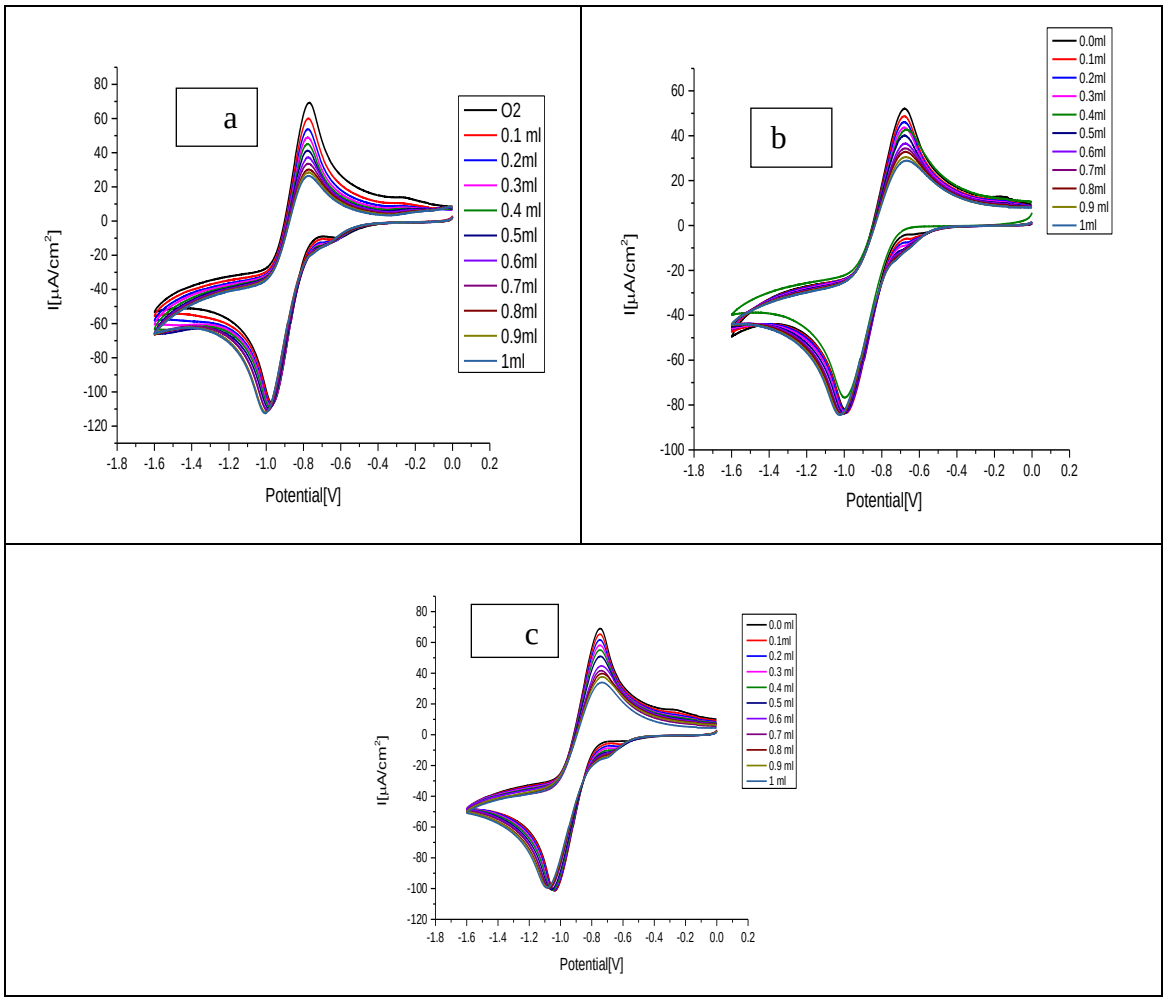
All the voltammograms recorded to reduce superoxide anion radical (Figure VI.19 , VI.20, VI.21) in the presence of the samples of extracts of three parts to evaluate the antioxidant activity for scavenging free radical, the figure shows clear oxidation and reduction peaks. At the anode current level, the peaks decreased with the addition of sample extracts, while there is no change at the level of the cathode current.

Leave part:



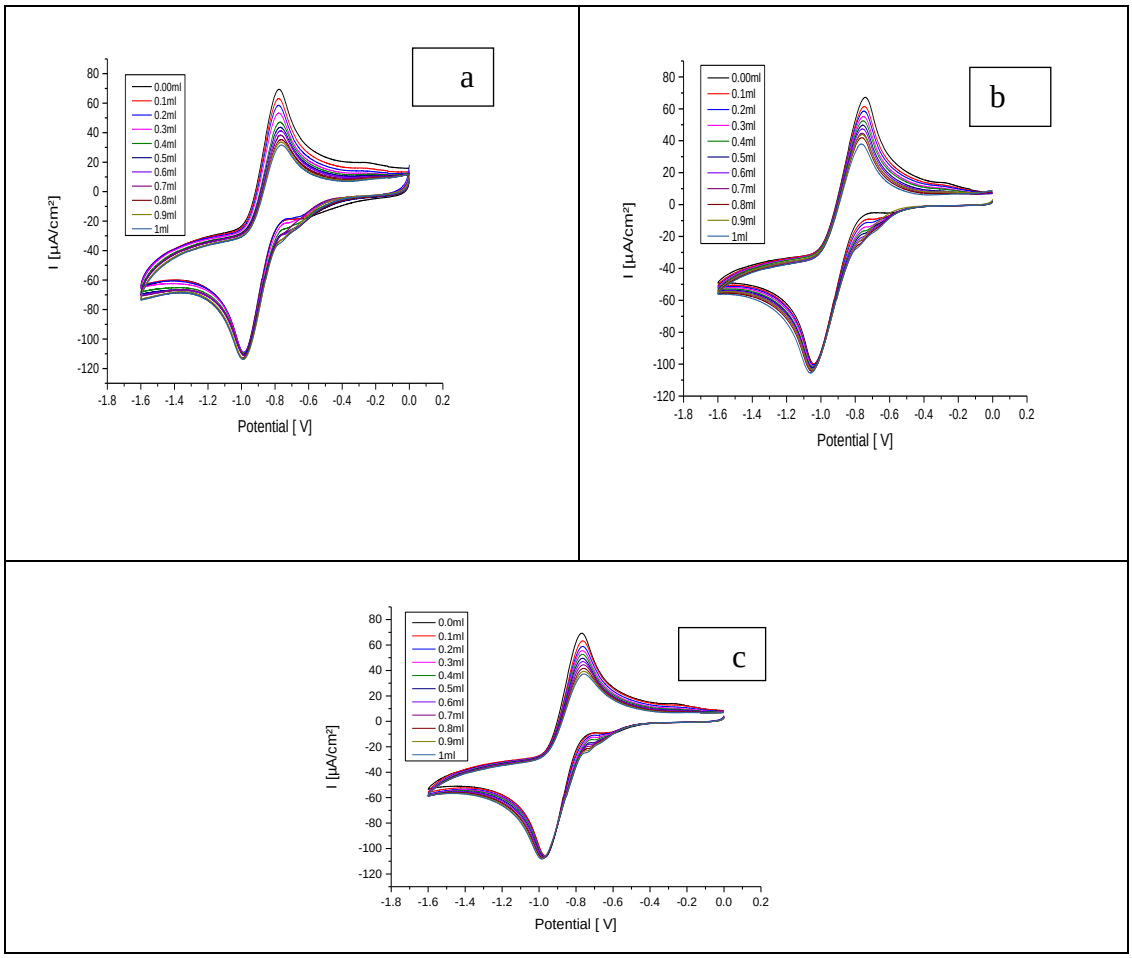
FigureVI.19. Cyclic voltammograms of oxygen saturated $O_2/O_2^{\bullet-}$ DMF/0.1 Bu4NPF6 on a GC electrode in the presence of different concentration of leaves extract (a) $CHCL_3$ extract (b) ETACO extract (c) n.BuOH extract at scan rate 100 mV.s-1, T = 28°

Stem part:



FigureVI.20. Cyclic voltammograms of oxygen saturated $O_2/O_2^{\bullet-}$ DMF/0.1 Bu4NPF6 on a GC electrode in the presence of different concentration of stem extract (a) $CHCL_3$ extract (b) ETACO extract (c) n.BuOH extract at scan rate 100 mV.s-1, T = 28°

Fruit part:



FigureVI.21. Cyclic voltammograms of oxygen saturated $O_2/O_2^{\bullet-}$ DMF/0.1 Bu4NPF6 on a GC electrode in the presence of different concentration of fruit extract (a) $CHCl_3$ extract (b) ETACO extract (c) n.BuOH extract at scan rate 100 mV.s-1, T = 28°

The quantification of the antioxidant in the extract is done by calculating the value of the inhibition concentration (IC50).

These values express the concentration of the studied extracts needed to decrease half of the free radical concentration table VI.10 .The obtained equation from the linear calibration graph figure VI.10. ,where y stands for the value of the oxidation peak current density of $O_2^{\cdot-}$ and x represents the value of the concentration of extracts, expressed in mg/mL.

Table VI.7: IC50 values of sample extracts using $O_2^{\bullet-}$ radical scavenging activity

	$O_2^{\bullet-}$ **method**			
		Equation	**R^2 values**	**IC50 (mg/mL)**
Leaf	**$CHCL_3$ extract**	y = 18.542x + 3.9169	0.9911	2.485
	ETACO extract	y = 31.398x + 2.8888	0.999	1.500
	n.BuOH extract	y = 43.873x + 4.8155	0.9961	1.029
Stem	**$CHCL_3$ extract**	y = 59.22x + 10.644	0.9901	0.664
	ETACO extract	y = 34.063x - 0.2382	0.9902	1.330
	n.BuOH extract	y = 34.063x - 0.2382	0.9902	1.474
fruit	**$CHCL_3$ extract**	y = 54.594x + 8.7947	0.9771	0.754
	ETACO extract	y = 24.402x + 5.6216	0.9877	1.818
	n.BuOH extract	y = 44.744x + 7.1784	0.9949	0.957
	Ascorbic acid	y = 234.11x + 0.6611	0.9903	0.210

Through the results obtained in the table, we note that:

All the extracts in the three parts possess the ability to inhibit free radical O_2 as compared to ascorbic acid.

Values of IC_{50} ranged from 0.66 to 2.48mg/ml, the lowest value of IC_{50} was recorded for chloroform extract of stem which corresponds to the highest antioxidant activity. Whereas, the highest value of IC_{50} was recorded for the chloroform sample of leaves.

The ability to inhibit free radicals O_2 increased in the following order:

Leaves part: n.BuOH extracts>ETACO extracts>$CHCL_3$ extracts.

Stem part: $CHCL_3$ extracts >ETACO extracts> n.BuOH extracts.

Fruit part: $CHCL_3$ extracts > n.BuOH extracts > ETACO extracts.

As a consequence:

The IC_{50} values confirm the presence of antioxidants as principal components of extracts against oxidants

The antioxidant capacity of phenolic compounds depends on the number and arrangement of hydroxyl groups in addition to the presence of substitutes electron donor at cycle structure [10].

VI.3.2.2. Voltammetric studies of $O_2^{\bullet-}$ − Extract interaction

The superoxide anion $O_2^{\bullet-}$ was generated electrochemically in dimithyl formamide using commercial oxygen of high purity from Lind-Gas Algérie and tetrabutylammonium tetrafluoroborate (Bu_4NPF_6) as a supporting electrolyte. The typical CV behavior of oxygen-saturated DMF/0.1 M Bu_4NPF_6 in the potential window of −0.0 to −1.6 V at a glassy carbon electrode in the absence and presence of 0.5 ml sample extracts.

Figures (VI.22, VI.23 ,VI.24) show a decrease in anodic peak current of the redox $O_2/O_2^{\bullet-}$ couple in the presence of sample extract and hence, the binding constant can be calculated. Moreover, the mode of interaction can be determined among exploitation of the shift in peak potential values according the same method that is employed for studying the binding of drug molecules to DNA [11, 12].

When 0.5 ml of sample extracts was added to DMF solution; the peak potential was labeled by ΔE to more negative values for $O_2^{\bullet-}$ −$CHCl_3$ extract of fruit part and to more positive values for the other samples which are shown in table (VI.8. VI.9 VI.10)

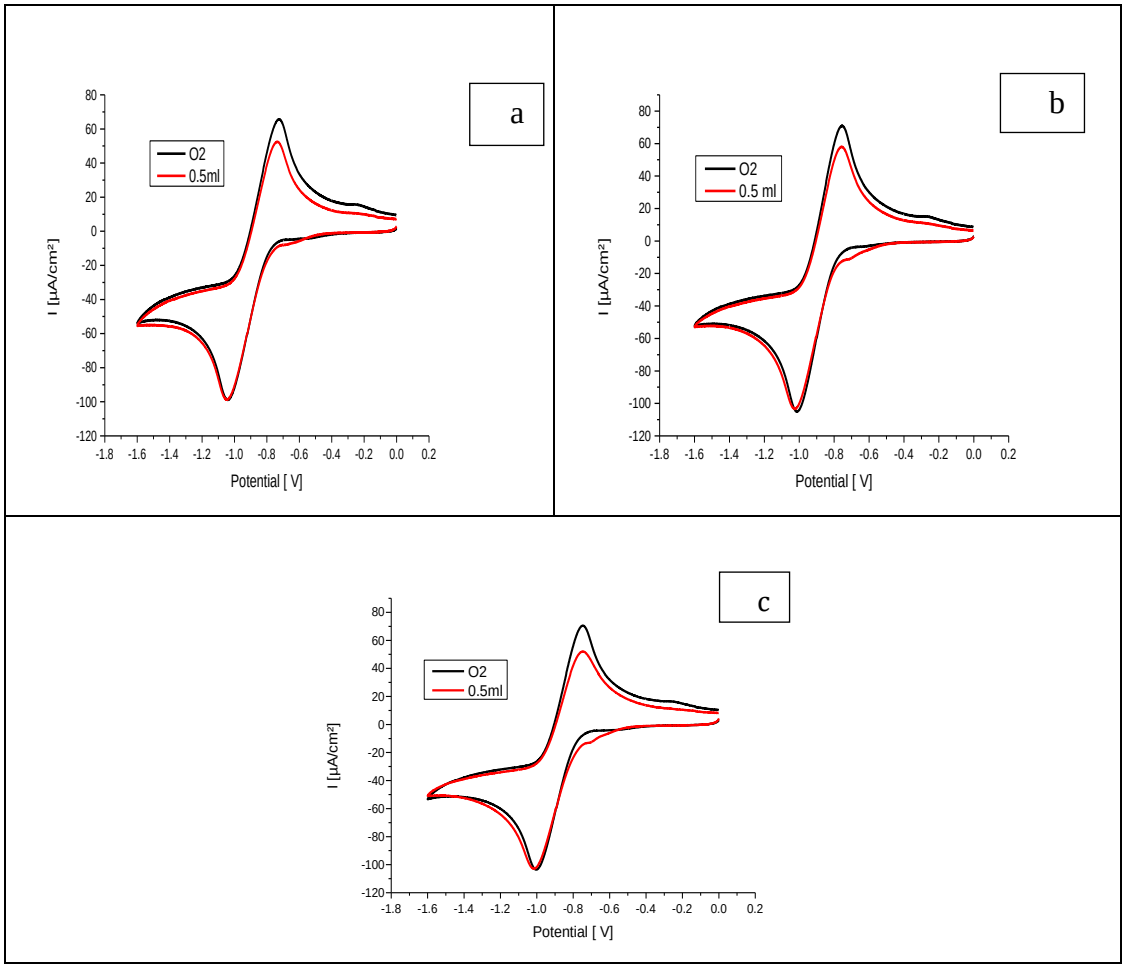
These values were associated with the decrease in anodic peak current densitis Δipa% which indicate the interaction of samples extracts with $O_2^{\bullet-}$.

Thus, the diminution in superoxide anion radical concentration leads to the formation of sample extract−$O_2^{\bullet-}$ complex.

Leaves part:

Table VI.8. Shifts in peak potential and a decrease in anodic peak current of $O_2^{\bullet-}$ – bound forms of extract samples of leaves part

Sample	Ipa (µA)	Epa (V)	Epc (V)	Ef^0 (V)	ΔE_f^0 (V)	ΔIpa %	Kox/Kred
$O_2^{\bullet-}$	90.436	-0.725	-1.041	-0.883	-	-	-
$CHCl_3$ extract	80.522	-0.737	-1.049	-0.893	0.0096	10.962	1.458
$O_2^{\bullet-}$	97.674	-0.759	-1.009	-0.884	-	-	-
ETACO extract	86.033	-0.758	-1.024	-0.891	0.006	11.917	1.305
$O_2^{\bullet-}$	95.452	-0.747	-1.003	-0.875	-	-	-
n.BuOH extract	77.88	-0.749	-1.017	-0.883	0.007	18.409	1.348



FigureVI.22. Cyclic voltammograms of oxygen saturated $O_2/O_2^{\bullet-}$ DMF/0.1 Bu4NPF6 on a GC electrode in the presence of different concentration of leaves extract (a) $CHCL_3$ extract (b) ETACO extract (c) n.BuOH extract at scan rate 100 mV.s-1, T = 28°

Stem part:

Table VI.9. Shifts in peak potential and a decrease in anodic peak current of $O_2^{\bullet-}$ – bound forms of extract samples of stem part.

Sample	Ipa (µA)	Epa (V)	Epc (V)	Ef^0 (V)	ΔEf^0 (V)	ΔIpa %	Kox/Kred
$O_2^{\bullet-}$	93.825	-0.778	-0.985	-0.881	-	-	-
$CHCl_3$ extract	71.993	-0.768	-0.966	-0.867	0.014	23.268	1.772
$O_2^{\bullet-}$	72.607	-0.678	-0.987	-0.832	-	-	-
ETACO extract	60.732	-0.680	-1.001	-0.841	0.008	16.355	1.375
$O_2^{\bullet-}$	94.226	-0.746	-1.038	-0.891	-	-	-
n.BuOH extract	79.407	-0.745	-1.06	-0.903	0.0106	15.727	1.516

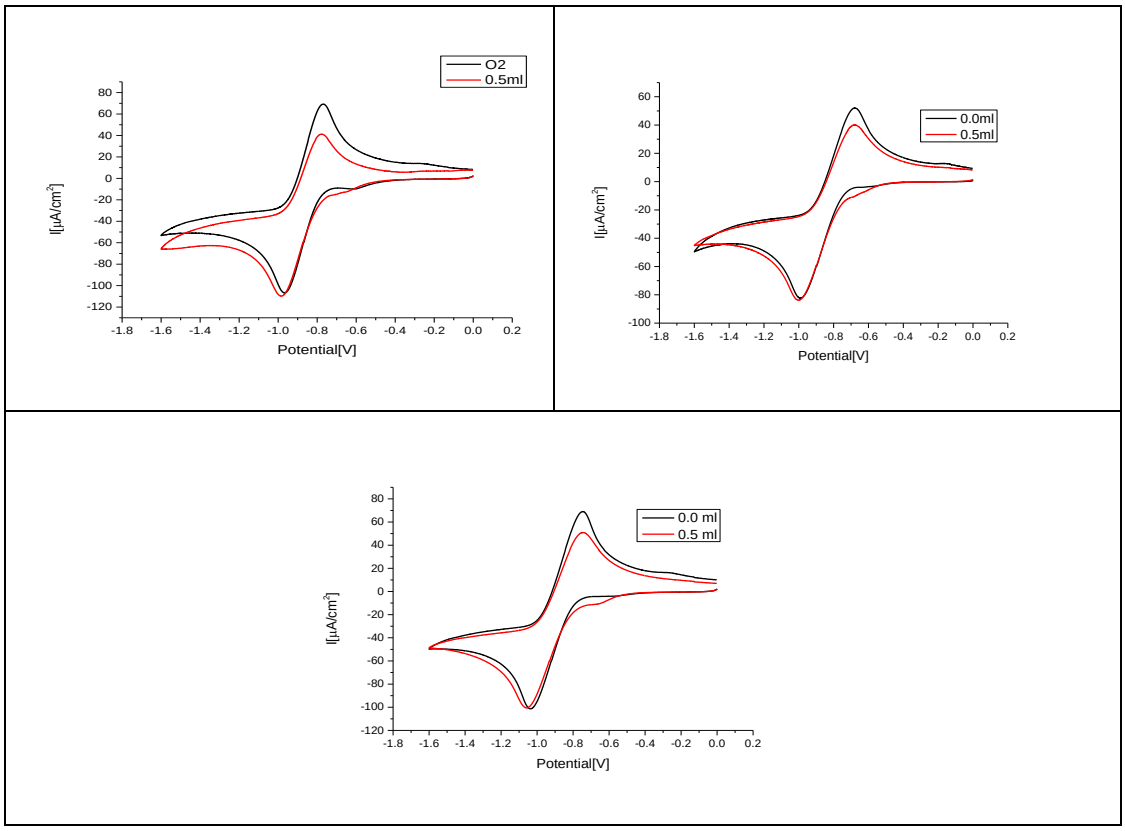


Figure.VI.23. Cyclic voltammograms of oxygensaturated DMF/0.1 Bu_4NPF_6 on a GC electrode in the absence (black line) and in presence of 0.5 ml Sample Extract of stem part (a) $CHCL_3$ extract, (b) ETACO extract, (c) n.BuOH extract, at Scan rate of 100 mV.s-1, T = 28°

Fruit part

Table VI.10. Shifts in peak potential and a decrease in anodic peak current of $O_2^{\bullet-}$ – bound forms of extract samples of fruit part.

Sample	Ipa (µA)	Epa (V)	Epc (V)	Ef^0 (V)	ΔEf^0 (V)	ΔIpa %	Kox/Kred
$O_2^{\bullet-}$	92.667	-0.779	-0.981	-0.880	-	-	-
$CHCl_3$ extract	69.182	-0.772	-0.985	-0.878	-0.001	25.343	1.074
$O_2^{\bullet-}$	92.093	-0.742	-1.043	-0.892	-	-	-
ETACO extract	77.441	-0.756	-1.047	-0.901	0.009	15.909	1.430
$O_2^{\bullet-}$	93.08746	-0.765	-0.965	-0.865	-	-	-
n.BuOH extract	74.940	-0.766	-0.969	-0.868	0.003	19.490	1.124

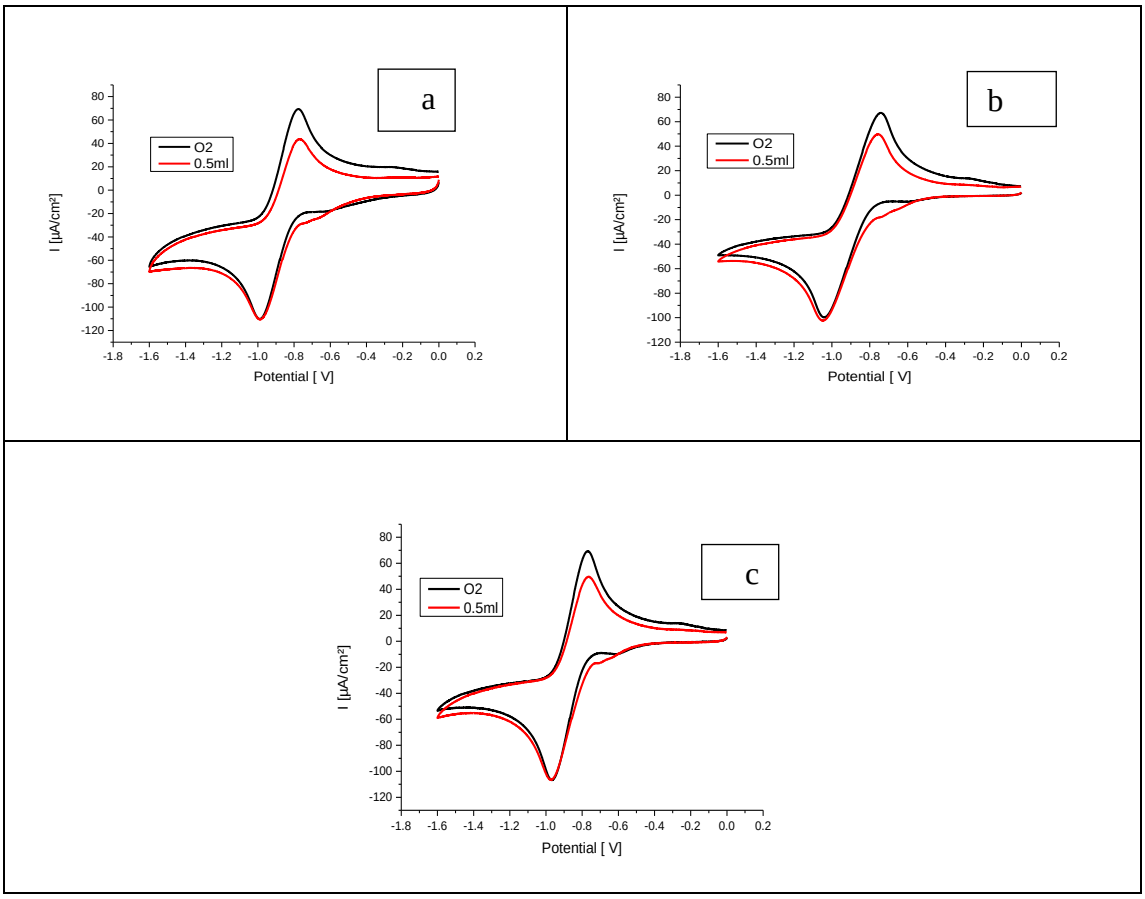
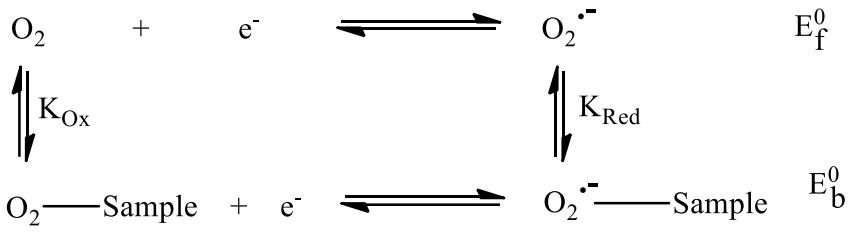


Figure VI.24. Cyclic voltammograms of oxygensaturated DMF/0.1 Bu_4NPF_6 on a GC electrode in the absence (black line) and in the presence of 0.5 ml Sample Extract of fruit part (a) $CHCL_3$ extract, (b) ETACO extract, (c) n.BuOH extract, at Scan rate 100 mV.s-^{1}, T = 28°

VI.3.2.3. Ratio of binding constants (Kox/Kred)

Scheme.VI.1: The redox behavior of the free radical and its bounded forms SE



The presence of samples extracts pointed out that $O_2^{\bullet -}$ is easier to oxidize among the peak potential shift values because its reduction form $O_2^{\bullet -}$ is strongly bound to extract than its oxidized form. Scheme VI.1 illustrates both forms of the $O_2/O_2^{\bullet -}$ redox couple interaction with the potential antioxidant compound [13]. The following obtained equation depends on the process presented in scheme VI.1 [14].

$$E_b^{\,0} - E_f^{\,0} = 0.059 \mathrm{Log} \frac{K_{ox}}{K_{Red}} \qquad \textbf{(VI. 1)}$$

Where $E_b^{\,0}$ and $E_f^{\,0}$ are the formal potentials of the redox couple in free and bound forms, respectively and Δ are calculated using the following formulas (VI.2) and (VI.3)

$$E^{\,0} = \left| \frac{E_{pc} + E_{pa}}{2} \right| \qquad \textbf{(VI.2)}$$

$$\Delta E^{\,0} = E^{\,0}\left(O_2^{\bullet -} - SE\right) - E^{\,0}\left(O_2^{\bullet -}\right) = E_b^{\,0} - E_f^{\,0} \qquad \textbf{(VI.3)}$$

VI.3.2.4. Binding constant and free energy

The addition of different concentrations of sample extract in DMF to a solution of $O_2^{\bullet -}$ which leads to a decrease in peak current . The reduction in peak current can be attributed to the diminution in free superoxide anion radical concentration due to the formation of sample extract$-O_2^{\bullet -}$ complex. The decrease can be employed to calculate the binding constant (Kb) by using following equation of Feng (VI.4) [15] .

$$Log\left[\frac{1}{[AO]}\right] = LogK_b + Log\left[\frac{I_p}{I_{P0} - I_P}\right] \qquad \textbf{(VI.4)}$$

Ip0 and Ip represents the peak currents of superoxide anion radical in the absence and the presence of additives respectively,[AO] is the concentration of the antioxidant.

As [AO] is unknown,this term was replaced by the volume of the extracts (ΔV_{ext}).It is noticea ble that the volume of the solution containing $O_2^{\bullet-}$ is fixed and hence, 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 [16]. Another thermodynamic parameter, standard Gibbs free energy

(ΔG°) was calculated using the measured Kb. The obtained values from CV's

voltammograms showed in table VI.11,VI.12,VI.13 indicated that the binding

constant is in the following order:

Leaves part:

$O_2^{\bullet-}$ n.BuOH extract > $O_2^{\bullet-}$ $CHCL_3$ extract > $O_2^{\bullet-}$ ETACO extract

Stem part:

$O_2^{\bullet-}$ ETACO extract> $O_2^{\bullet-}$ $CHCL_3$ extract > $O_2^{\bullet-}$ n.BuOH extract

Fruit part:

$O_2^{\bullet-}$ $CHCL_3$ extract > $O_2^{\bullet-}$ n.BuOH extract > $O_2^{\bullet-}$ ETACO extract

The values of free energy are varied in the same order as of the binding constant.

The negative values of ΔG indicated the spontaneity of $O_2^{\bullet-}$ −sample extract interaction.

Table VI.11. Values of binding constants and binding free energies of leaf part

Sample	Equation	R^2	K[L.mol^{-1}]	ΔG([KJ.mol^{-1})
$O_2^{\bullet-}$ $CHCl_3$ extract	y=0.9343x+2.4432	0.9744	277.4598	-13.9481
$O_2^{\bullet-}$ ETACO extract	y=1.3601x+2.1627	0.9912	145.4454	-12.3467
$O_2^{\bullet-}$ n.BuOH extract	y=1.2307x + 2.525	0.9841	334.9654	-14.4151

Table VI.12. Values of binding constants and binding free energies of stem part

Sample	Equation	R^2	K [L.mol^{-1}]	ΔG([KJ.mol^{-1})
$O_2^{\bullet-}$ $CHCl_3$ extract	y=1.2307x + 2.525	0.9841	549.1614	-15.640
$O_2^{\bullet-}$ ETACO extract	y=-1.2116x+4.7942	0.9875	62258.69	-27.3698
$O_2^{\bullet-}$ n.BuOH extract	y= 0.8821x + 2.7046	0.9827	506.524	-15.4404

Table VI.13.Values of binding constants and binding free energies of fruit part

Sample	Equation	R^2	K [L.mol^{-1}]	ΔG ([KJ.mol^{-1})
$O_2^{\bullet-}$ $CHCl_3$ extract	y=1.0886x + 2.7602	0.9958	575.705	-15.7578
$O_2^{\bullet-}$ ETACO extract	y = 1.2278x + 2.396	0.9894	248.8857	-13.6786
$O_2^{\bullet-}$ n.BuOH extract	y = 1.0369x +2.6544	0.9939	451.2321	-15.1538

VI.3.2.5) Diffusion coefficients

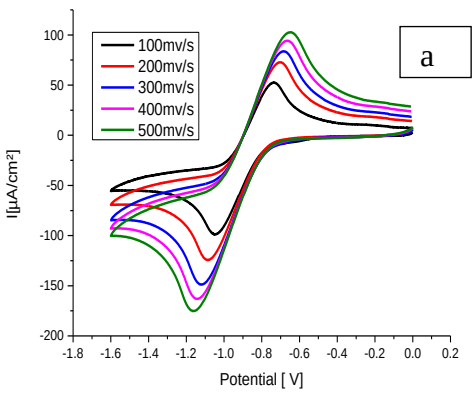
The electrochemical behavior of sample extracts at various scan rates is shown in Figuer. IV.25. The voltammogram contained a couple of well-defined stable redox peaks attributed to the redox process of $O_2/O_2^{\bullet-}$ redox couple (Figuer. IV.25). The diffusion coefficients of the free and $O_2^{\bullet-}$ bound forms of samples extracts were determined using the following Randles–Sevcik equation [17].

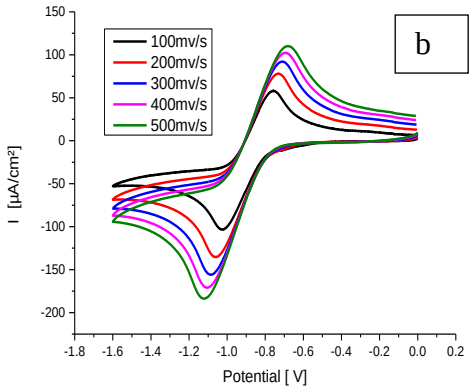
$$i = 2.69\times10^5 n^{\frac{3}{2}} SCD^{\frac{1}{2}} v^{\frac{1}{2}} \qquad \textbf{(VI.5)}$$

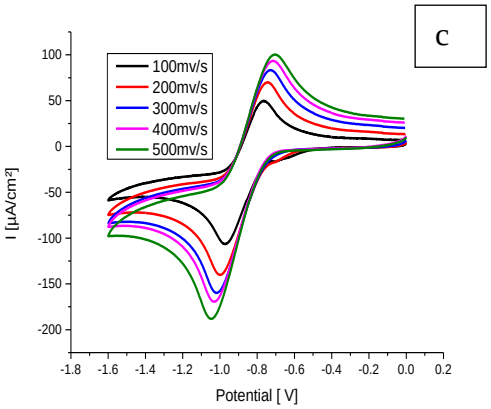
Where i represents the oxidation peak current density (A), S represents the surface of the working electrode (cm^2), C is the bulk concentration (mol cm^{-3}) of the electroactive species, D is the diffusion coefficient (cm^2 s^{-1}), and v is the scan rate (Vs^{-1}) (Figuer. IV.26). The linearity of the relation ipa = f ($v^{\frac{1}{2}}$) for the whole extracts samples suggests that the redox process is kinetically controlled by the diffusion step. The diffusion coefficients were determined from the slopes of Randles–Sevcik plots. The values are summarized in table IV.14.

The diffusion coefficients of complexes $O_2^{\bullet-}$ −Sample extract is small when compared to free $O_2^{\bullet-}$ which is expected of $O_2^{\bullet-}$ −SE complex formation. The reason for the diminution in the diffusion coefficient of $O_2^{\bullet-}$ in the presence of sample extract is due to the large phytochemical compound weight of the formed complex.

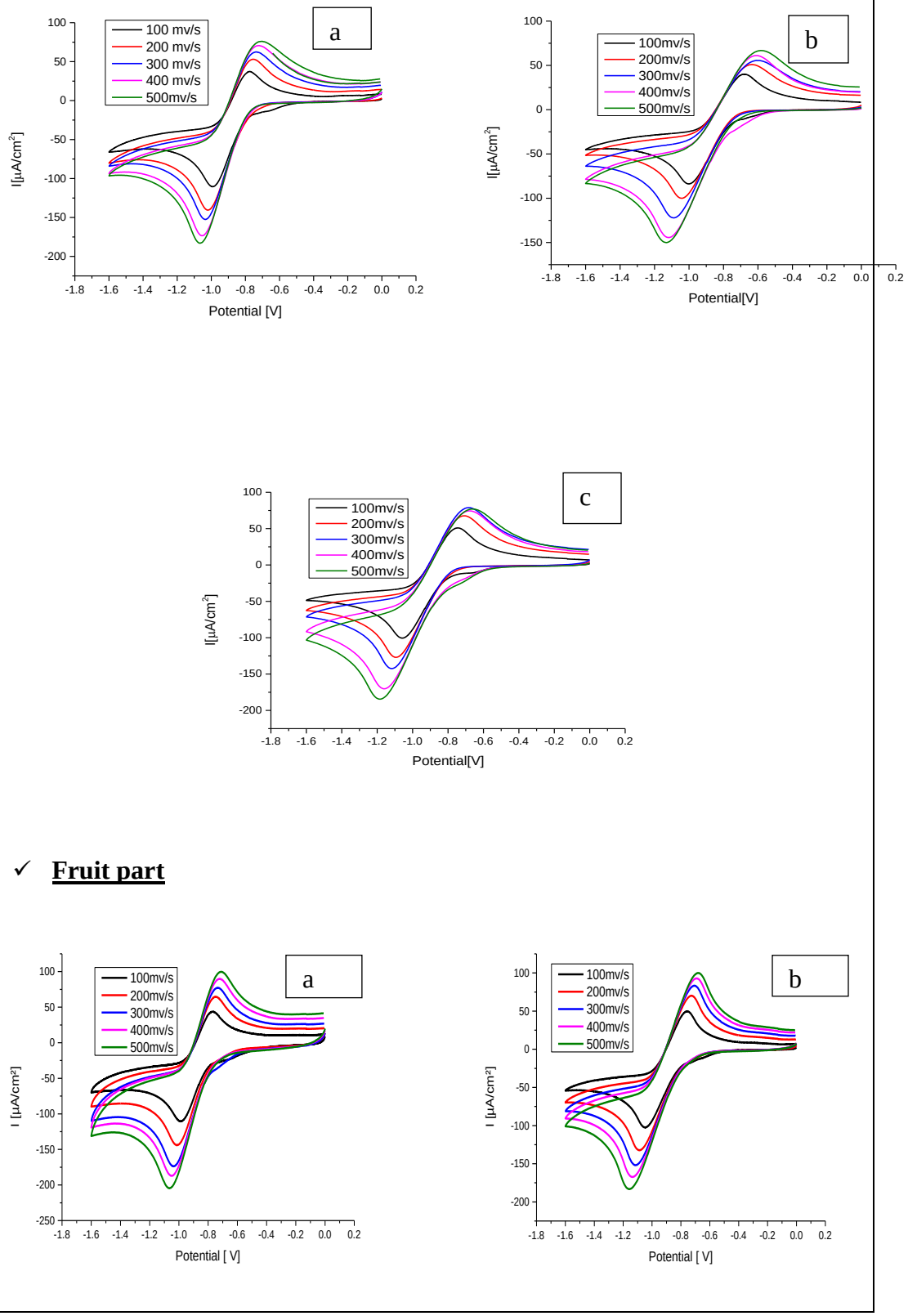
✓ **Leaves part**







✓ **Stem part**


a
100 mv/s
200 mv/s
300 mv/s
400 mv/s
500mv/s
I[µA/cm²]
Potential [V]
b
100mv/s
200mv/s
300mv/s
400mv/s
500mv/s
I[µA/cm²]
Potential[V]
c
100mv/s
200mv/s
300mv/s
400mv/s
500mv/s
I[µA/cm²]
Potential[V]

✓ **Fruit part**

a
100mv/s
200mv/s
300mv/s
400mv/s
500mv/s
I [µA/cm²]
Potential [V]
b
100mv/s
200mv/s
300mv/s
400mv/s
500mv/s
I [µA/cm²]
Potential [V]

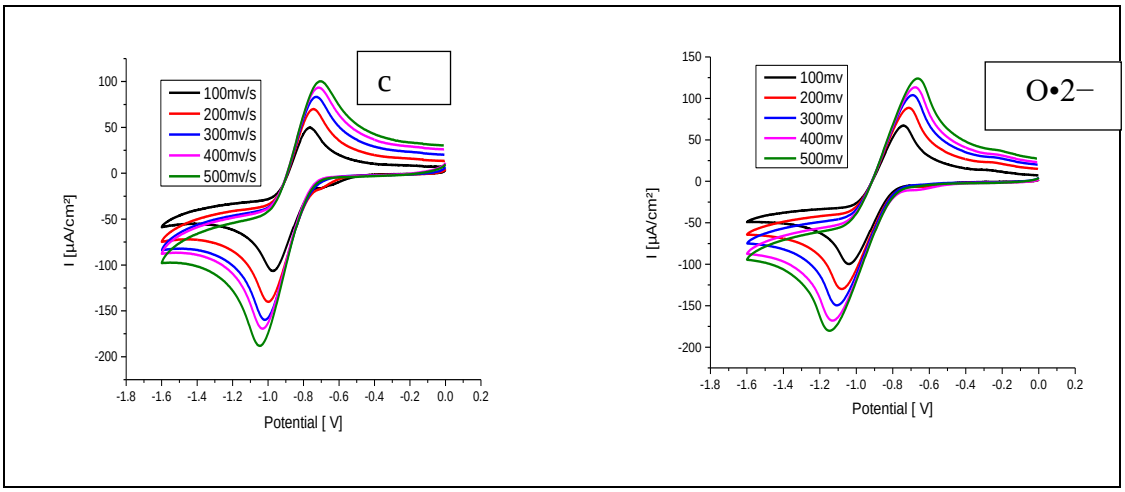


Figure VI.25. Succession of cyclic voltammograms at GC electrode in oxygen saturated DMF/0.1 Bu4NPF6 at different scan rates ranging from 100 to 500 mV/s , (a) −CHCL3 extract, (b) −ETACO extract, (c) −n.BuOH extract , (d) for O•2− for three parts of *solanum nigrum* L.

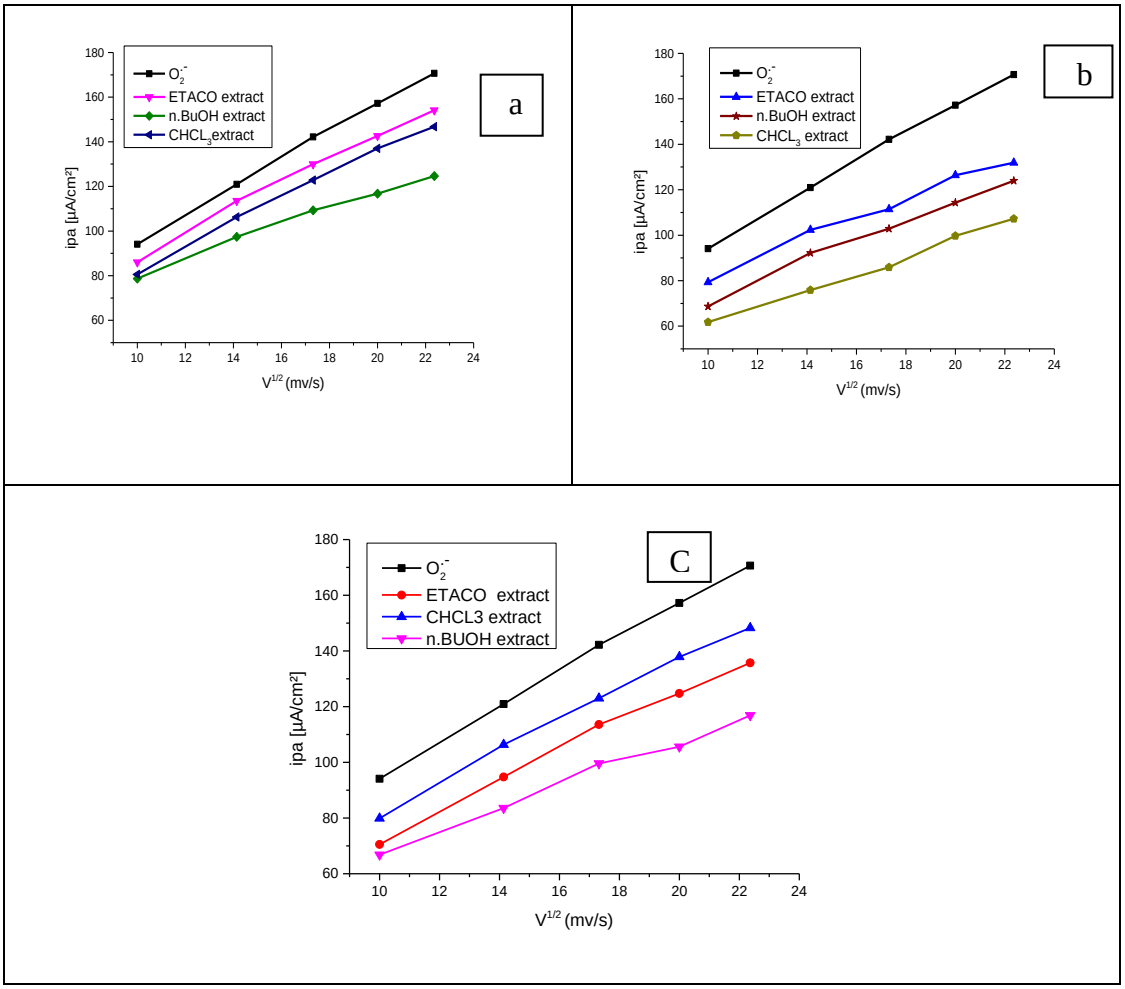


Figure VI.26. ipa versus v1/2 plots of oxygen-saturated DMF in the absence and in the presence of 0.5 ml of the extract with (a) Leave part, (b) stem part (c)fruit part

Table VI.14. Diffusion coefficient values

	Sample	Equation	R^2	D (cm^2/s)
leaves	**$CHCl_3$ extract**	y = 0.1852x - 5.2158	0.9955	$5.26666x10^{-10}$
	ETACO extract	y = 0.1823x - 6.0685	0.9937	$5.10301x10^{-10}$
	n.BuoH extract	y=0.2695x - 11.626	0.9911	$1.11525x10^{-09}$
stem	**$CHCl_3$ extract**	y = 0.1881x - 3.5254	0.9948	$2.89325x10^{-9}$
	ETACO extract	y = 0.1799x - 4.6532	0.9962	$2.64649x10^{-9}$
	n.BuoH extract	y = 0.2474x - 6.6169	0.9921	$5.00505x10^{-9}$
fruit	**$CHCl_3$ extract**	y = 0.2265x - 5.979	0.991	$4.195\ x10^{-9}$
	ETACO extract	y = 0.266x - 6.1388	0.9933	$5.785x10^{-9}$
	n.BuoH extract	y = 0.2306x - 8.6685	0.9847	$4.348\ x10^{-9}$
	$O_2^{\bullet -}$	y = 0.1607x - 5.2507	0.9986	$2.111\ x10^{-9}$

VI.4. Discussion

Indeed, [18]MICHEL (2011) shows that extraction conditions can influence the antioxidant capacity of extracts. Thus, antioxidant activity depends not only on the concentration, but also on the structure and nature of antioxidants[19]. This may explain this variation in the results obtained.

Moreover, based on the above findings, the FRAP test gave a good result at the leaves' part and acetate extract have the highest amount of antioxidants and these results are in concordance with phytochemical investigation results which the leave part has a high level of phenolic compounds. Furthermore, according the FRAP assay, the experimental study enhances the ethenobotanical study because the most used part by local population is the leave's part.

The total antioxidant capacity (CAT) by spectrometric method showed that the leave part contains the most antioxidants. Moreover, this result is compatible with the results of the ethenobotanical study.

According to other tests that were determined in our experimental study, two rest parts have the highest antioxidant effects; the fruit part with DPPH assay and stem part for

electrochemical methods, hence the fruit and stem parts also have considerable values of antioxidants in *Solanum nigrum* L plant.

The various results in other tests indicate the antioxidant activity varies considerably depending on different polarity extracts of each part and chemical structure properties of bioactive compounds [20]. Moreover, according to principle technique of each method, it is possible that the powerful antioxidant activity of extracts is due to the presence of substances with free hydroxyl groups. In this context, flavonoids have an ideal structure for free radical scavenging as they have a number of hydroxyl groups acting as hydrogen donors [21].Through the literature research, it is well established that antioxidant activity is positively correlated with the structure of polyphenols. Generally, polyphenols with a high number of hydroxyl groups have the highest antioxidant activity [22] due to their ability to give more atoms to stabilize free radicals [23].

Overall, the antioxidant power and their biological virtues contribute to the prevention of degenerative and cardiovascular diseases and these results emphasize the importance of therapeutic effect of *Solanum nigrum* L. plant in our arid zone.

VI.5. Conclusion

In conclusion, due to its antioxidant properties, our plant contributes to the protection against the harmful effects of free radicals, thus justifying its traditional use against different diseases. Subsequent studies, allowing a purification of the compounds (fractionation) will have to be carried out in order to confirm the first obtained results and to deepen the structural elucidations initiated by means of other analytical techniques (LC-MS, NMR) and other tests, so as to finally lead to the identification of the bioactive molecules present in this plant.

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Chapter VII

Corrosion study by extracts plant

VII. Introduction

Different synthetic-organic and inorganic compounds have been used as inhibitors to protect metals from corrosion. However, they are expensive and have dangerous effects. Current research is increasingly moving towards the use of the so-called green inhibitors. The non-toxic and biodegradable nature of natural products has led to their use as green inhibitors. Plant extracts obtained by simple and low-cost procedures are considered as an incredibly rich source of naturally synthesized chemical compounds that are biodegradable in nature .

VII.1. Aim

In the following study, we investigated the corrosion-inhibition power of mild steel in acidic medium in the absence and presence of butanol extracts of each of the three parts (leaves, stems, fruit) of *solanum nigrum* L. plant by using electrochemical technique.

During this experimental study, the considered electrochemical system metal / solution is composed of:

-A mild steel electrode X52

-a 0.5M sulfuric acid medium .

The study of the corrosion phenomenon such as the potentiodynamic polarization method and electrochemical impedance spectroscopy (EIS) were performed. Then, we calculated and commented thermodynamic value of the processΔG) from adsorption isotherms.

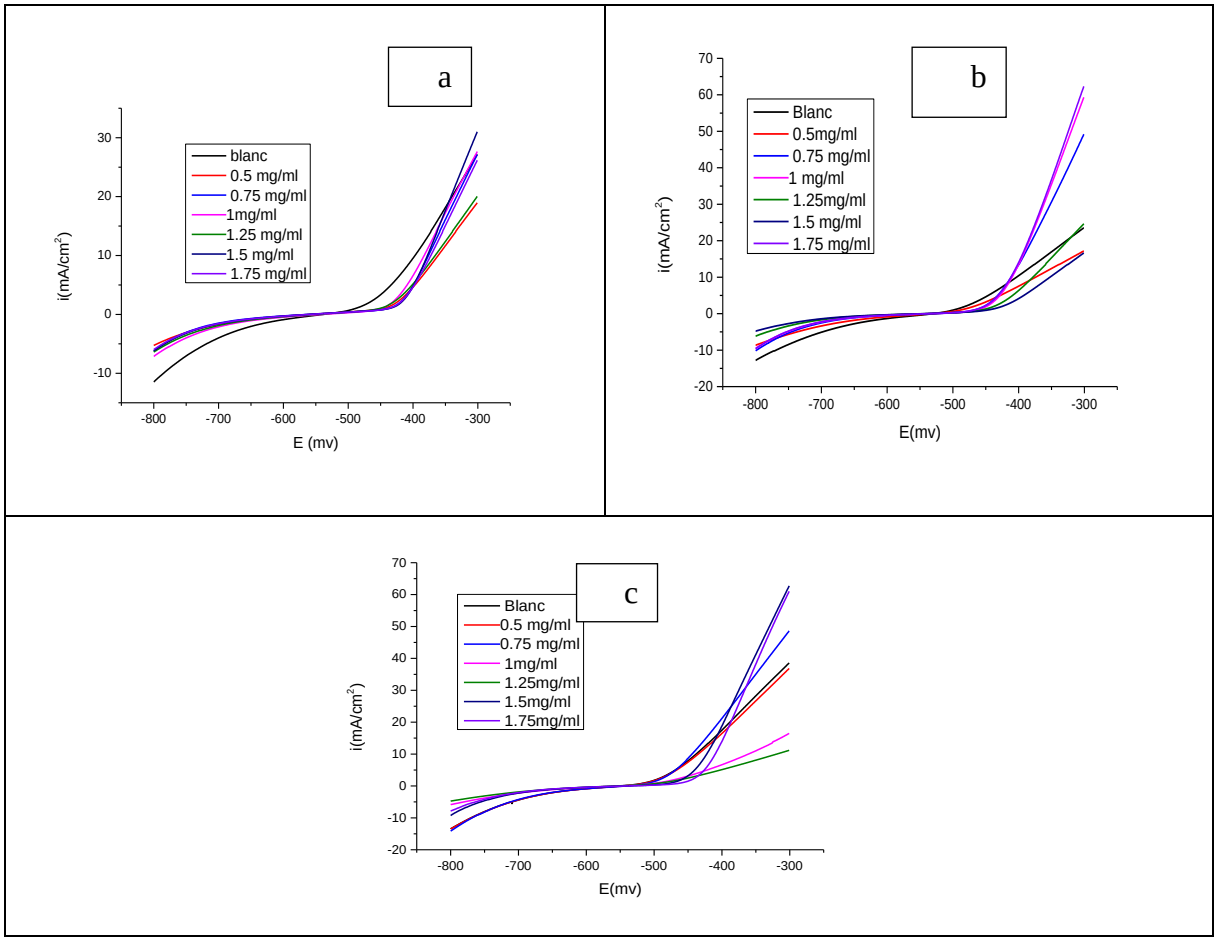
VII.2. Polarization curves

This method is consistent and enables us to determine the activity of a inhibitor by determining the corrosion current in the absence and presence of butanol extract of the leaves/stems and fruit of *Solanum nigrum* L. plant at different concentrations through changing the potential difference of the working electrode, then measuring the density of the current passing through it, and this method depends on slowing down the corrosion stage that is at the steel/acid interface by plotting the polarization curves.

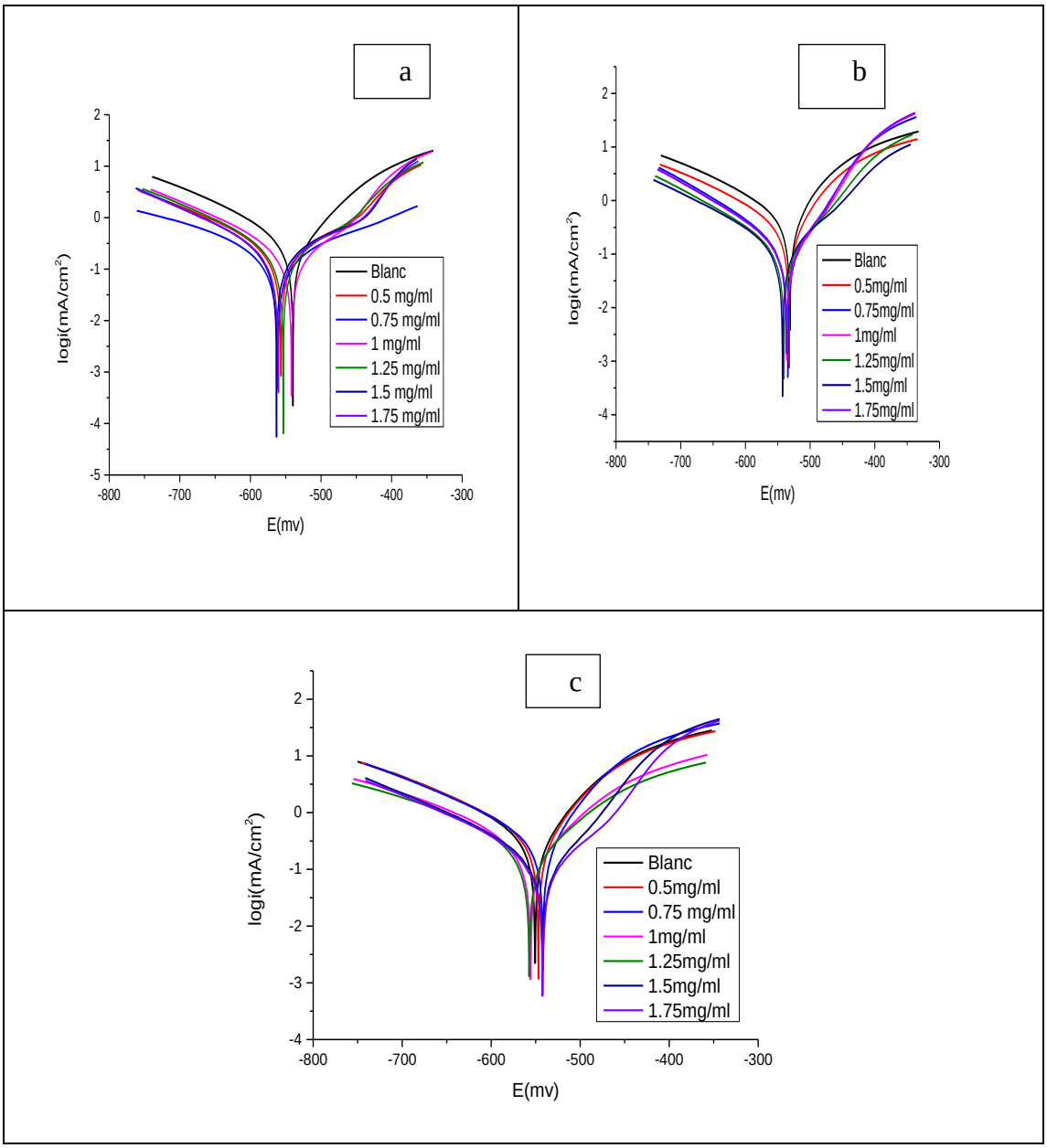
i = f(E) and Log i = f (E) for Tafel curves. It is worth mentioning that the work was carried out at a laboratory temperature

VII.2.1. Study of inhibitors concentration influence of mild steel in H_2SO_4 0.5M:

30 minutes after immersion of working electrode X52in (H_2SO_4, 0.5M) medium, and by adding inhibitors, the polarization was obtained and Tafel curves of these inhibitors allow us to know several electrochemical data:



Figuer VII.1. Polarization curves of X52 in (H_2SO_4,0.5M) medium for (a) n.BuOH extract of Leaves(b) n.BuOH extract of stem (c) n.BuOH extract of fruit



Figuer VII.2. Tafel curves of X52 in (H_2SO_4,0.5M) medium for (a)n.BuOH extract of Leaves(b)n.BuOHof extract stem (c) n.BuOH extract of fruit

After obtaining the Tefal curve which provides several important electrochemical parameters, and it is represented by:

E_{cor}: Corrosion potential (mV) **R_p:** Polarization resistance ($\Omega.cm^2$)

I_{cor} : Current density of corrosion (mA/cm^2) **B_a :** The anodicslope of Tafel(mV)

B_c : The cathodic slope of Tafel (mV) **V:**Corrosion speed (mm / y)

The elctrochemical amounts and the inhibition efficiency IE % are calculated according the formula (7.V), and the surface coverage ratio (θ) was obtained.

Calculating through the (V.8) formula determines the absence and presence of inhibitors in different concentrations that are listed in the table.

$$IE\ (\%) = ((I_{corr} - I_{corr\ (inh)})/I_{corr})\ 100 \quad \textbf{(1.VII)}$$

$$\theta = ((I_{corr} - I_{corr\ (inh)})/I_{corr}) \quad \textbf{(2.VII)}$$

Table VII.1. Electrochemical parameters and steel corrosion inhibition efficiency in H2SO4 0.5M with and without the addition of the n.BuOH extract of leave at different concentrations

C(mg/ ml)	E(i=0) (mV)	Rp ($\Omega.cm^2$)	I_{corr} (mA/cm^2)	Ba (mV)	Bc (mV)	V_{corr} (mm /y)	IE (%)	θ
Blanc	-539.8	70.66	0.611	114.3	-197.3	7.148	-	-
0.5	-556.7	132.31	0.294	126.1	-190.6	3.44	51.881	0.5188
0.75	-561.7	198.66	0.276	245.1	-287.2	3.234	54.761	0.547
1	-542	143.03	0.268	100.8	-177.6	3.136	56.135	0.561
1.25	-553.7	135.09	0.260	116.5	171.6	3.045	57.395	0.573
1.5	-542	140.26	0.226	207	-162	2.649	62.925	0.629
1.75	-560.5	151.65	0.222	112.4	-166	2.59	63.678	0.636

Table VII.2. Electrochemical parameters and steel corrosion inhibition efficiency in H2SO4 0.5M with and without the addition of the n.BuOH extract of stem at different concentrations

C(mg/ ml)	E(i=0) (mV)	Rp ($\Omega.cm^2$)	I_{corr} (mA/cm^2)	Ba (mV)	Bc (mV)	V_{corr} (mm /y)	IE (%)	θ
Blanc	-531.3	44.9	0.823	107.5	-214.5	9.63	-	-
0.5	-532.6	72.06	0.525	103.9	-208.7	6.143	36.215	0.362
0.75	-534.6	175.38	0.191	71.1	-148.7	2.239	76.742	0.767
1	-536.4	186.77	0.177	71.7	-148.8	2.08	78.394	0.783
1.25	-540.5	173.03	0.167	88.4	-160.7	1.963	79.608	0.796
1.5	-542.7	170.46	0.166	101.3	-170.5	1.941	79.839	0.798
1.75	-536.7	157.95	0.164	69.5	-145	1.925	80.009	0.800

Table VII.3. Electrochemical parameters and steel corrosion inhibition efficiency in H2SO4 0.5M with and without the addition of the n.BuOH extract of fruit at different concentrations

C(mg/ ml)	E(i=0) (mV)	Rp ($\Omega.cm^2$)	I_{corr} (mA/cm^2)	Ba (mV)	Bc (mV)	V_{corr} (mm /y)	IE (%)	θ
Blanc	-550.7	57.33	0.693	93.9	-187.5	8.106	-	-
0.5	-546.9	55.32	0.582	84.9	-176.2	6.809	16.000	0.160
0.75	-542	60.64	0.484	72	-168.8	5.667	30.082	0.300
1	-555.7	100.7	0.418	120.4	-200.4	4.894	39.619	0.396
1.25	-557.2	112.54	0.38	130	-207.9	4.444	45.173	0.451
1.5	-542	156.26	0.216	71.4	-157.2	2.535	68.720	0.687
1.75	-542.2	175.82	0.167	73.7	-144.9	1.963	75.775	0.757

According the results from table VII.1 we observe that The addition of extracts to the corrosive medium leads to a decrease corrosion speed compared to absence inhibitor where the value is 7.148(mm/y) for n.BuOH leaves extract, and minimum amount for it is (2.59mm/y) in concentration 1.75mg/ml with maximum efficiency inhibition 63.678%, also a high corrosion speed for n.BuOH leaves extract is 3.44(mm /y) in 0. 5mg/ml concentration by a 51.881% inhibition efficiency.

Regarding n.BuOH stem extract the value of corrosion speed without adding extract is 9.63 (mm /y), and a lower amount for it is (1.925mm/y) in concentration 1.75mg/ml with a high efficiency inhibition of 80.009 %, and maximum corrosion speed is 6.143 (mm /y) in 0. 5 mg/ml concentration with an inhibition efficiency of 36.215%.

For n.BuOH fruit extract, the value of corrosion speed with an absent extract is 8.106 (mm /y), and a least value for it is (1.963mm/y) in concentration of 1.75mg/ml with an upper efficiency inhibition of 75.775%, and superior corrosion speed of 6.809 (mm /y) in 0. 5mg/ml concentration by an inhibition efficiency of 16%.

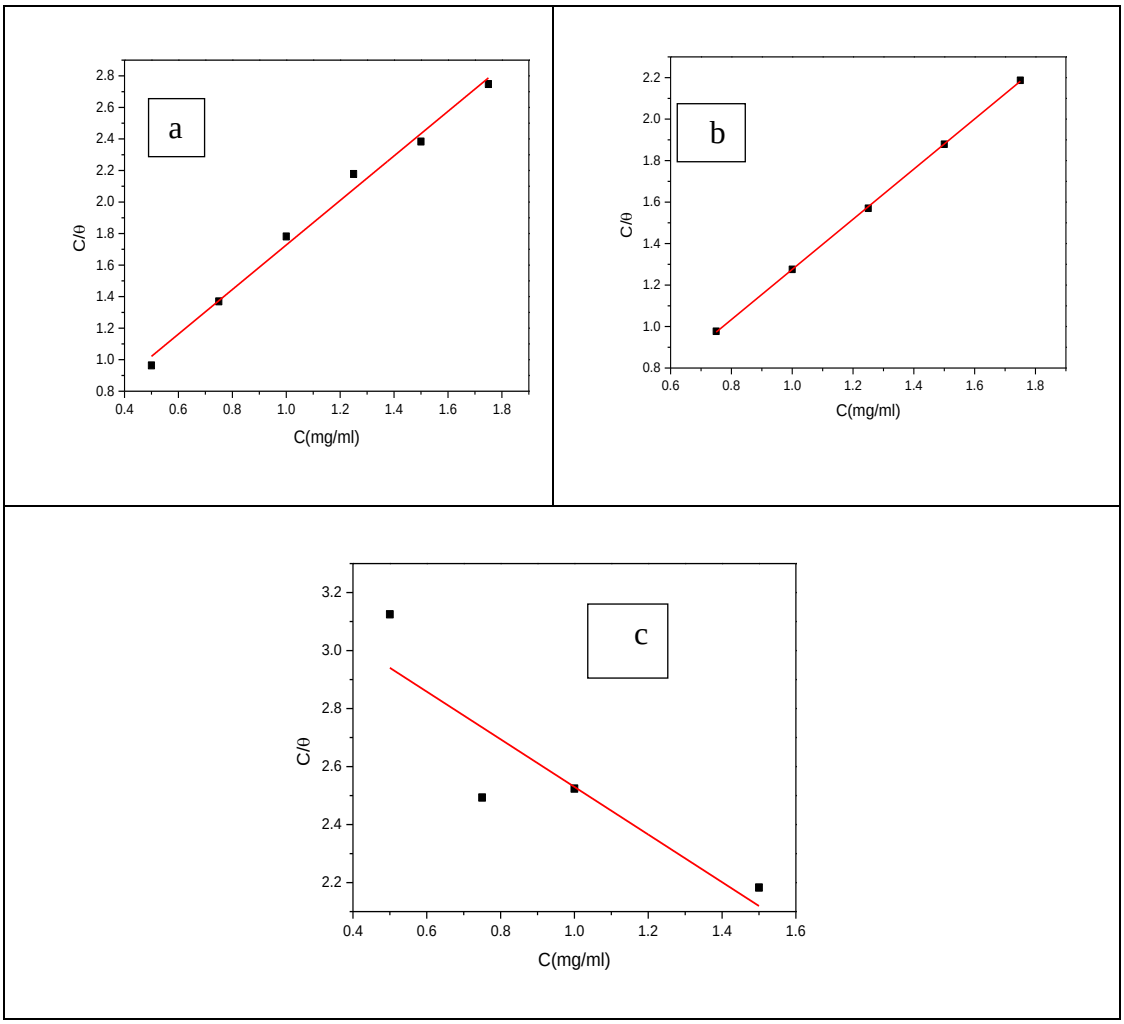
In addition, it has been noticed that there is a decrease in corrosion current in three extract parts n.BuOH leaves, n.BuOH stem, n.BuOH fruit that it was 0.611(mA/cm^2); 0.823(mA/cm^2);0.693(mA/cm^2) respectively before the addition of inhibitors, then the values have become 0.222(mA/cm^2),0.164(mA/cm^2), 0.167(mA/cm^2) respectively:

VII.3. Adsorption isotherms

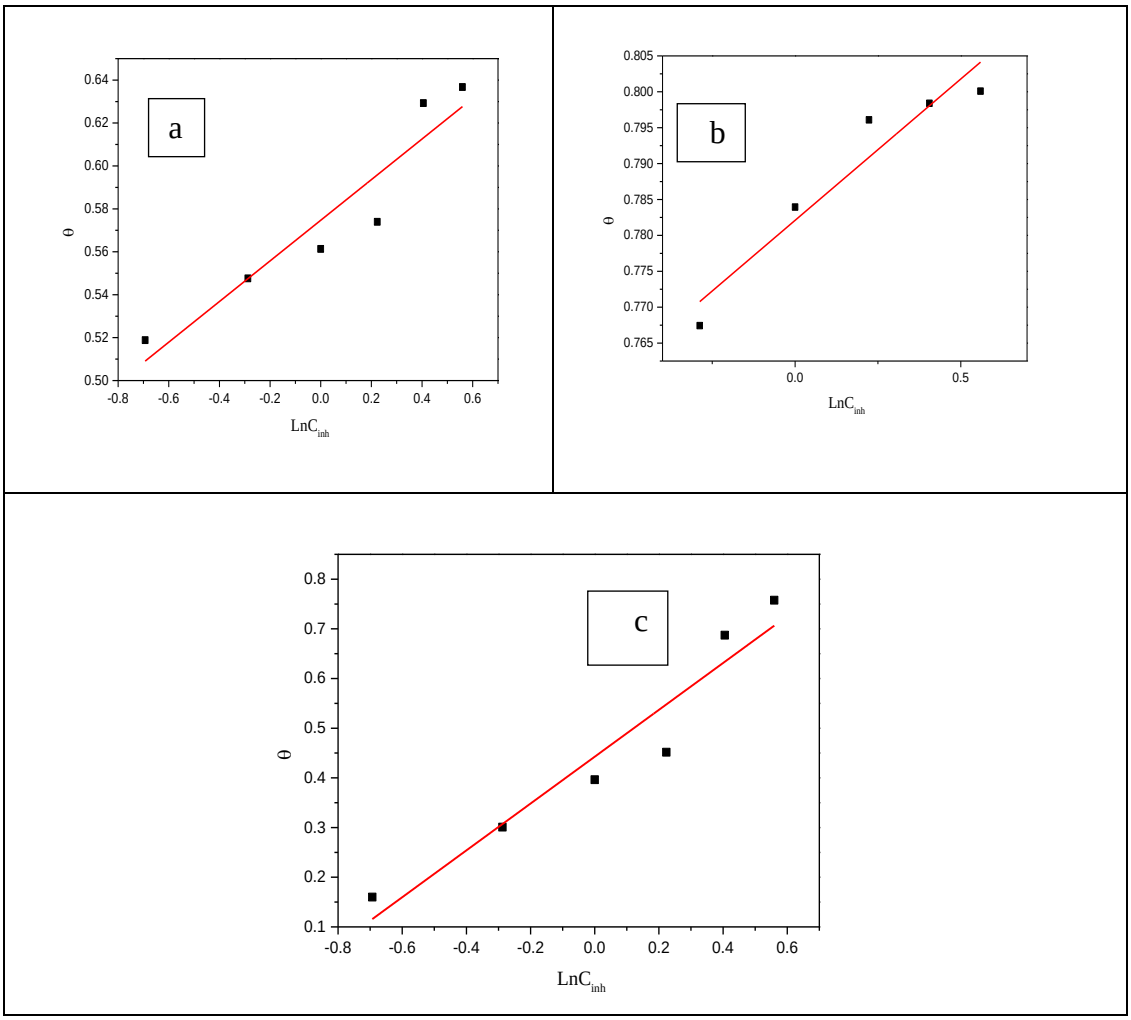
The inhibition of metal corrosion by extract of a plant that contains different chemical families is explained by the adsorption of its organic compounds.

The physical or chemical nature of the adsorption depends on several parameters, as follows, the nature of the metal and its charge, the chemical structure of the organic molecules as well as the type of the electrolyte.

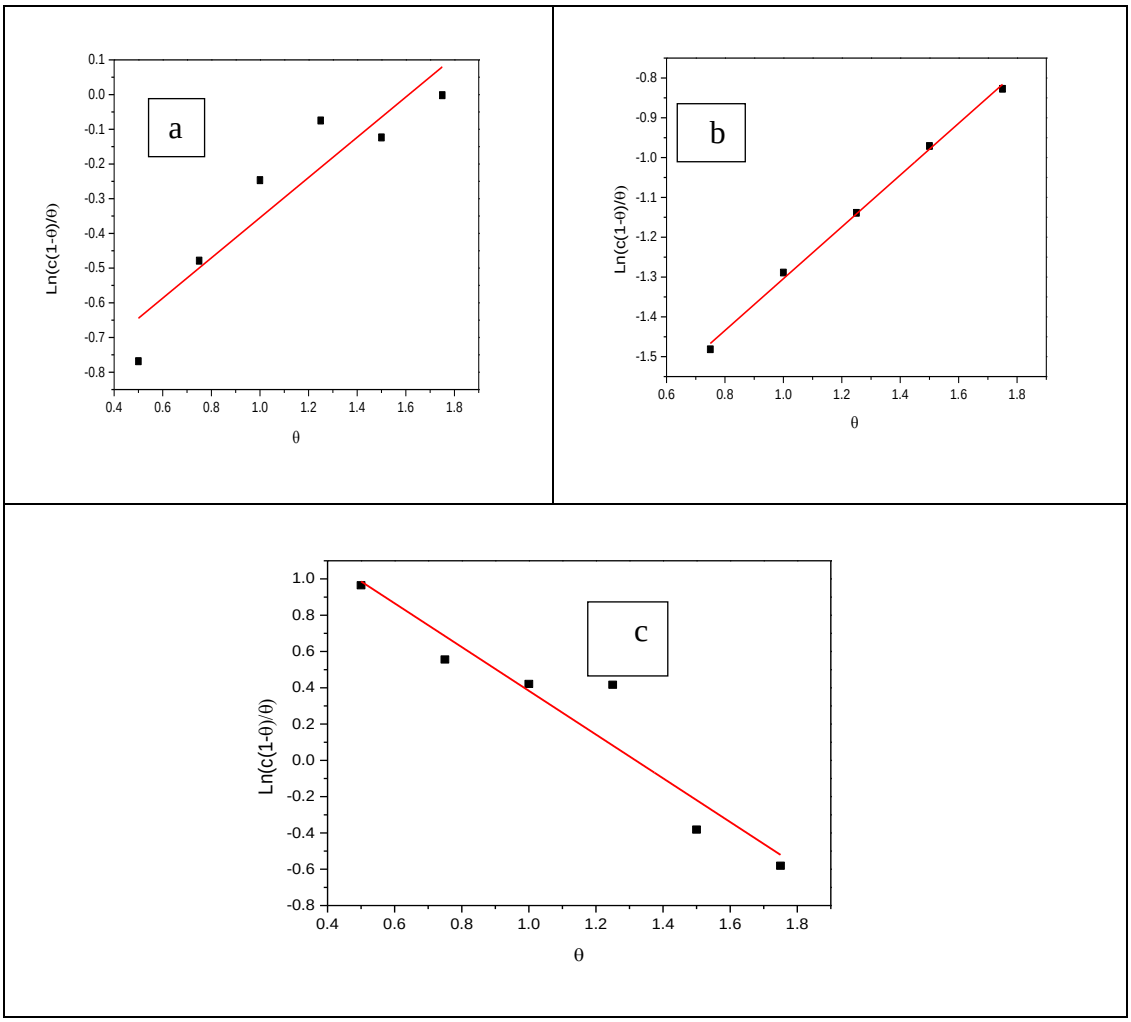
In order to understand the mechanism of corrosion inhibition, the behavior of adsorption of the organic adsorbate on the metal surface must be known. The degree of surface coverage (θ) was evaluated based on the results of measures of behavior electrodeposition of mild steel in the aggressive solution of (H_2SO_4,0.5M) in the presence and in absence of the extracts carried out at laboratory's temperature. The values of the surface coverage (θ) for the three extracts are given in Table V.2. The data were tested graphically by adapting to various isotherms such as Langmuir, Frumkin and Temkin.



Figuer VII.3. Adsorption isotherms Langmuir of mild steel for inhibitors:(a)n.BuOH extract of Leaves (b)n.BuOHof extract stem (c) n.BuOH extractof fruit



Figuer VII.4. Adsorption isotherms Temkin of mild steel for inhibitors:(a)n.BuOH extract of Leaves (b)n.BuOH of extract stem (c) n.BuOH extractof fruit



Figuer VII.5. Adsorption isotherms Frumkin of mild steel for inhibitors:(a)n.BuOH extract of Leaves (b)n.BuOHof extract stem (c) n.BuOH extractof fruit

Table VII.4. Correlation coefficient R^2 for each inhibitor adsorption

	adsorption isotherms		
Inhibitors	Langmuir	Temkin	Frumkin
n.BuOH leave	0.98883	0.87322	0.83511
n.BuOH stem	0.99985	0.89709	0.99675
n.BuOH fruit	0.90922	0.6885	0.885527

The plotting of Langmuir curves shows that these variations are linear with a value of the regression coefficient very close to unity (of the order of 0.999). This shows that the adsorption of the extracts from each of the three parts of the plant on the steel surface in H_2SO_4 0.5M medium obeys the Langmuir adsorption isotherm.

Table VII.5. Activation parameters of the steel X52, 0.5M H2SO4 of presence inhibitors

Inhibitors	$k_{ads}(M^{-1})$	ΔG_{ads} (kJ.mol^{-1})
n.BuOH leaves extract	3176.822	-29.9291
n.BuOH stem extract	15085.23	-33.7888
n.BuOH fruit extract	2259.121	-29.0845

The values of the adsorption constants of the different parts of the plant, which are obtained by extrapolation of the straight lines of the Langmuir adsorption isotherms with regard to the axis C/θ. In addition, the adsorption constant K is related to the standard free energy of adsorption ($\Delta G°_{ads}$) by the following equation :

$$\Delta G_{ads} = - RT \ln (55.5 \times Kads) \quad \textbf{(3.VII)}$$

The values of the standard free energy of adsorption $\Delta G°_{ads}$ is calculated from the formula (II.9) for the different parts of the plant which are: -29.9291kJ/mol for n.BuOH leavesextract,-33.7888kJ/mol for n.BuOH stem extract and -29.0845kJ/mol for n.BuOH fruit extract . Examination of these values shows that they are negative and close to - 40 kJ/mol.

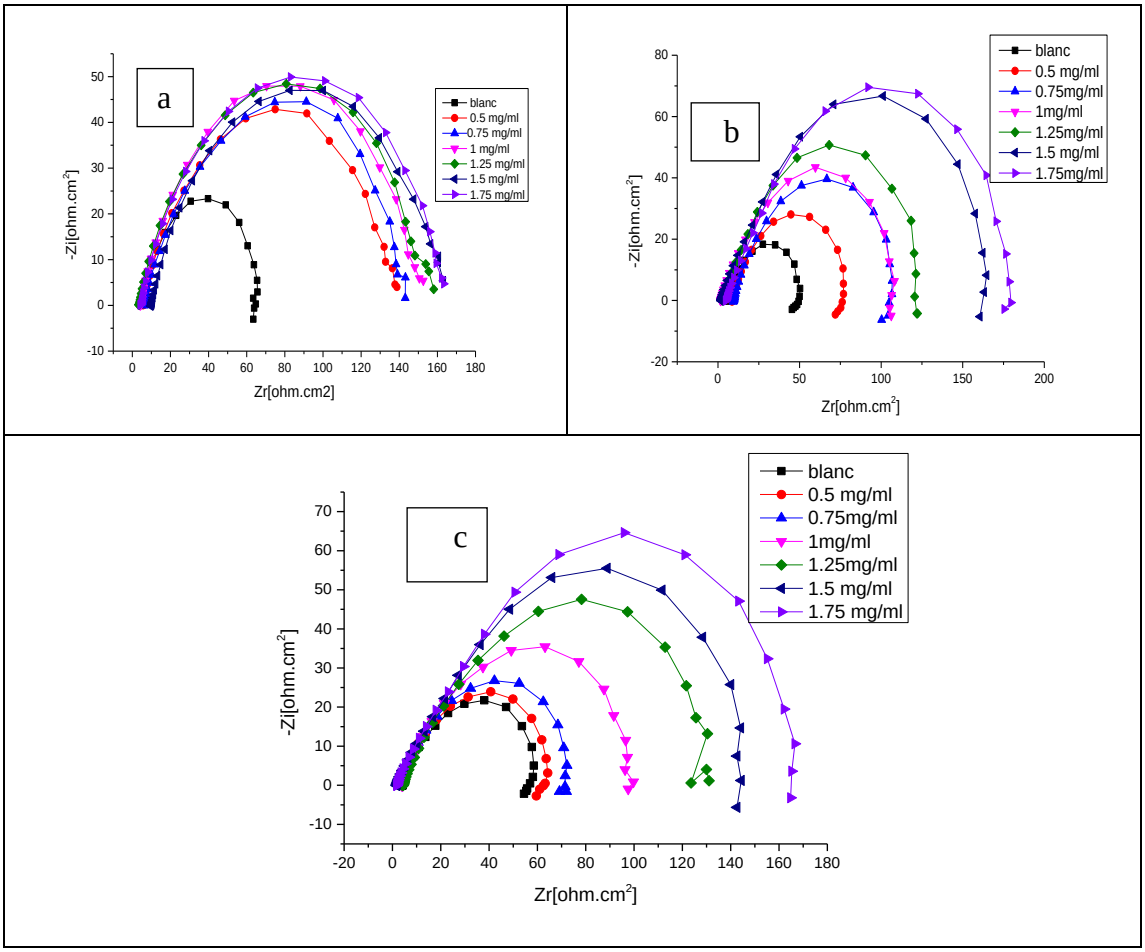
VII.4. Electrochemical impedances pectroscopy

Electrochemical Impedance Spectroscopy (EIS) is an advanced technique for the study of corrosion mechanisms and adsorption phenomenon. The behavior of X52 steel in 0.5M H_2SO_4 was studied by EIS in the absence and presence of extracts. The obtained impedance diagrams are made up of a single capacitive buckle which is not a perfect semicircle, and this is attributed to the frequency dispersion of the interfacial impedance [1, 2], generally due to the heterogeneity of the electrode surface. This heterogeneity can result from the rugosity, the impurities, dislocations, inhibitor adsorption, and layer formation porous [3, 4]. This type of diagram is generally interpreted as a mechanism of charge transfer on a heterogeneous and irregular surface.

Impedance diagrams for Nyquist curves are analyzed by fitting to obtain the equivalent circuit model shown in Fig. IV.4. The Nyquist curve contains flattened semicircles with a center below the real axis which is evident from the figures.

VII.4.1. Study Influence of inhibitors concentration of mild steel in H2SO4 0.5M medium:

Nyquist plots obtained for mild steel in acid medium in the absence and presence of *solanum nigrum* L. plant at various concentrations of extracts are shown in figures (VII.6)



Figuer VII.6. Nyquist curves of X52 in (H_2SO_4,0.5M) medium for (a) n.BuOH extract of Leaves (b) n.BuOH of extract stem (c) n.BuOH extract of fruit

Various electrochemical parameters are obtained from the EIS technique are listed in Tables 3 and 4.R_{ct} is the charge-transfer resistance, and C_{dl} a double-layer capacitor (IE %) inhibition efficiency are calculated following formulas(III.7) (III.6) respectively

Table VII.6. Electrochemical parameters from Nyquist curve of steel X52 in H2SO4 0.5M with and without the addition of the n.BuOH extract of leave at different concentrations

C(mg/ ml)	R_{ct}(Ω. cm^{-2})	F(Hz)	C_{dl} (µF cm^{-2})	IE%
Blanc	61.378	10	259.434	
0.5	134.494	10	118.396	54.363
0.75	137.29	6.3	184.103	55.293
1	146.925	10	108.378	58.224
1.25	153.359	10	103.832	59.977
1.5	161.004	10	98.901	61.877
1.75	162.868	6.3	155.190	62.314

Table VII.7. Electrochemical parameters from nyquist curve of steel X52 in H2SO4 0.5M with and without the addition of the n.BuOH extract of stem at different concentrations

C(mg/ ml)	R_{ct}(Ω. cm-2)	F(Hz)	C_{dl} (µF cm^{-2})	IE%
Blanc	43.689	6.329	575.881	-
0.5	67.389	10	236.293	35.168
0.75	101.176	4	393.462	56.818
1	95.709	15.8	105.300	54.352
1.25	128.125	4	310.703	65.901
1.5	160.491	4	248.044	72.777
1.75	175.947	4	226.255	75.169

Table VII.8. Electrochemical parameters from Nyquist curve of steel X52 in H2SO4 0.5M with and without the addition of the n.BuOH extract of fruit at different concentrations

C(mg/ ml)	R_{ct}(Ω. cm-2)	F(Hz)	C_{dl} (µF cm^{-2})	IE%
Blanc	52.472	6.329	479.487	-
0.5	59.492	6.329	422.908	11.799
0.75	68.419	6.329	367.729	23.307
1	94.51	4	421.213	44.479
1.25	126.764	4	314.039	58.606
1.5	142.212	2.5	447.882	63.102
1.75	163.59	2.5	389.353	67.924

Following the obtained result in tables above the n.BuOH leave extract the inhibition efficiency reach maximum 62.314 % at 1.75 mg/ml concentration with charge transfer resistance of (R_{ct})162.868(Ω. cm^{-2}), where n.BuOH stem extract is the highest value of inhibition efficiency is 75.169% at 1.75 mg/ml concentration with charge transfer resistance (R_{ct})175.947 (Ω. cm^{-2}), and for n.BuOH fruit extract the maximum value of inhibition efficiency is 67.924 % at 1.75 mg/ml concentration with charge transfer resistance(R_{ct})163.59 (Ω. cm^{-2}),thus the inhibition efficiency and charge transfer resistance increases with the concentration of the inhibitor

The addition of inhibition leads to the decrease of the capacity of the double layer C_{dl} which was 259.434(µF cm^{-2}) without an addition and a decrease to 98.901(µF cm^{-2}) at 1.5 mg/ml concentration in n.BuOH leave extract. Also in n.BuOH stem extract the value of C_{dl} was 575.881 (µF cm^{-2}) without addition and decrease to 105.300 (µF cm^{2}) at 1mg/ml concentration, and the value of C_{dl} was 575.881 (µF cm^{-2}) without addition and decrease to479.487 (µF cm^{-2}) in absent inhibitor decrease to 314.039(µF cm^{-2}) at 1.25 mg/ml concentration for n.BuOH fruit extract.

VII.5. Discussion

From tafel curves, the addition of n.BuOH leaves and n.BuOH stem extracts leads to displacement the corrosion potential towards the anodic potential. This indicates that extracts is cathodic inhibitors. Moreover, the addition of n.BuOH fruit extract led to displacement the corrosion potential to the anodic and cathodic potential which indicates that the extract is mixed inhibitor.

The decrease in corrosion current in three extract parts is due to the decrease in the transfer of electrons between the steel and the acid, which returns to the formation of a protective layer on the surface of the electrode and thus [4] greater diffusion of particles giving a very large surface area coverage this is due to the chemical nature of inhibitors at these concentrations.

The values of the anode (Ba) and cathode (Bc) of tafel slops vary the tendency of the surface of the mild steel. This indicates that the presence of the inhibitor acts on the mechanism of the H^+ reduction and oxidation reaction [5]

The adsorption of the extracts from each of the three parts of the plant on the steel surface in H_2SO_4 0.5M medium obeys the Langmuir adsorption isotherm. That indicates the formation a single layer on the surface of the metal and blocks most of the active centers according to the Langmuir model.

The high values of K are a characteristic of a high chemical adsorption of the inhibitor on the surface of the steel [6].

The negative of $\Delta G°_{ads}$ sign indicates the spontaneity of the adsorption process and stability of the double layer adsorbed on the metal surface.

Moreover, several researchers [7-11] have shown that values of $\Delta G°_{ads}$ close to or greater than -20 kJ.mol-1 are generally related to interactions electrostatics between the charged molecules and the charges of the metal (physisorption). On the other hand, values of $\Delta G°ads$ close to or below - 40 kJ/mol corresponding to a charge transfer between the inhibitor molecules and the metal surface with formation of covalent bonds (chemisorption) .

In our case, the calculated values of $\Delta G°ads$ are close to - 40 kJ mol-1 and this shows that the molecules of the extracts are chemisorbed in the metal surface.

Thus, it can be said that the study of adsorption is an interesting approach in order to propose the probable corrosion inhibition mechanism of steel in a solution of sulfuricacid.

The phytochemical screening of the extracts of the plant under investigation has allowed the identification of several chemical families (polyphenols, alkaloids, saponosides, acids, etc.). In the considered acidic medium, the molecules belonging to these families of compounds heterocyclic molecules can be adsorbed as neutral molecules through a chemisorptions involving the sharing of free electron doublets of heteroatoms (nitrogen in the alkaloids, oxygen and hydroxyl group - OH in the tannins and the flavonoids...) existing in the molecule and the metal. The adsorption of the molecules in the extract can also occur as a result of the interactions of the π electrons of the aromatic nucleus of the molecule and steel [12] .

The inhibitory efficacy of the extracts is the result of a synergy obtained by compounds with several functional groups: this is the synergistic effect of the intra-molecular, as it may be due to an intermolecular synergy [13].

The decrease in Cdl is due to the adsorption of the inhibitor on the surface of the steel which reduces the dielectric constant of the medium ε, and/or to increase the thickness of the electric double layer d ($C_{dl} = \frac{\varepsilon\varepsilon_0}{d}$)

And its value increases according to the gradual replacement of water molecules with the organic compound particles to form a cohesive layer on the surface of the metal [14, 15]

From figures (V 27.), (26.V), (25.V) and (38.V), by adding the inhibitors the increasing semicircles, which are circles have the same shape at each concentration and this proves that the of type of transition is charge and high-frequency loops with low semicircles. This is known as the phenomenon of frequency dispersion due to inequality.[16] and The surface roughness Also, it is the result of the formation of a double layer on the surface, indicating that the phenomenon of electronic transition is responsible for the corrosion mechanism.

Adhesion of the inhibitor on the surface of the electrode leads to blocking or preventing electronic transition from the metal to the solution. This leads to an increase in inhibition and coverage.

VII.6. Conclusion

The study consists in determining the inhibition efficiency of the extracts of a plant . (*solanum nigrum* L.) against corrosion of mild steel in acidic environment H_2SO_4 0.5M by electrochemical methods (polarization curves, polarization resistances and measuring of electrochemical impedance). Indeed, the BuOH extracts of the different parts of solanum nigrum L. were tested. The three extracts effectively inhibit the corrosion of steel whose effectiveness of the extracts of the stem part is better than that of the leaves and fruit extracts.

In this perspective, it would be more appropriate to study the corrosion inhibition of steel by adopting other techniques such as the gravimetric method in order to be able to compare it to the electrochemical methods. Finally, this study contributes considerably to valorization of natural products as biodegradable inhibitor. These proposals present an important economic interest at a time when synthesized organic inhibitors are toxic and very expensive.

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General Conclusion

General Conclusion

Medicinal plants have been used for thousands of years to treat various diseases because they are natural and safe source of human health[1].Currently, most scientists and researchers focus on the importance of medicinal plants as they contain several active substances (phenols, flavonoids, etc) that carrying various biological properties such as antioxidants. Thus, natural antioxidants may be useful in the treatment and prophylaxis of chronic diseases

This research work is concerned with the study of the different phases of aqueous and organic extraction of *Solanum nigrum* L. plant, that has grown spontaneously in our arid zone (El-Oued). The research investigates its importance as well as antioxidant activities of bioactive substances of three extracts , i.e. ($CHCl_3$,AcOEt, *n*-BuOH) of three parts *Solanum nigrum* L.(leaves, stem, fruit) of Solanaceae family

Ethnobotanical survey was carried out in El-Oued and result at show that *Solanum nigrum* L. has important in folk medicine, the people they use it as remedy for several diseases.

The phytochemical investigation of plant detected presence various families, includes: alkaloids, saponins, tanins, Coumarins, Terpenoids, Flavnoids.

Quantification by spectrophotometric methods has allowed us to determine the levels of total phenols using the Folin-Ciocalteu reagent, flavonoids and flavanols by aluminium trichloride. Our results show that most classes of phenolic compounds exist in concentrations considerable in the various extracts.

Qualitative analysis by High Performance Liquid Chromatography revealed the presence of the principal phenolic compounds, including Chlorogenic Acid; Naringin and p-Coumaric Acid and, Quercetin, gallic acid.

The antioxidant capacity of different polarity extract was evaluated through the use of two techniques: spectrophotometers and electrochemical assays.

Depending on the spectrophotometers assays, the antioxidants capacity was calculated

with oxidation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method expressed as IC50 and Ferric reducing antioxidant power assay (FRAP) and Total antioxidant capacity (TAC).

The cyclic voltammetry technique was used based on the electrochemical assay in order to determine the antioxidant ability of different extracts, the redox potential of these compounds was used to measure the antioxidant capacity of in aqueous solvents (phosphate buffer). Redox potentials were also calculated in an organic solvent so as to compare their electrochemical behavior. At this time, the antioxidant properties of phenolic extracted were assessed by the inhibitory effect of superoxide anion radicals and expressed as IC50.

The thermodynamic properties quantify the strength of the extract's interaction between radicals and the probe antioxidant. These properties, based on the decrease in peak current, were calculated in terms of the binding constant Kb. This constant allows the standard free energy of Gibbs to be measured (ΔG°). The spontaneity of interaction between radicals and extracts was indicated by their negative values.

Generally, spectrochemical and electrochemical techniques to detect antioxidants activity provided us with good results and considerable amount of antioxidants in *Solanum nigrum* L plant and that confirm therapeutic use from local population in corrosion part of the thesis is a contribution to the use of a new type of metal corrosion inhibitors called green inhibitors.

The study consists in determine the inhibition efficiency of extracts of a plant. (*Solanum nigrum* L.) against corrosion of mild steel in acidic environment H_2SO_4 0.5M by electrochemical methods (polarization curves, polarization resistances and measurements of electrochemical impedance).

As the results obtained in vitro in this work are only a first step in the search for substances of natural source and which are biologically active, we can put forward certain perspectives to be considered in this line of research and thus complete our project which is about:

-Phytochemical studies: isolation, characterization, and identification of metabolites of *Solanum nigrum* L. using more efficient identification techniques (chromatography coupled with spectroscopic techniques such as mass and NMR).

-The determination of the minimal inhibitory (MIC), bactericidal (BMC) and fungicidal (CMF) concentrations of the extracts of this plant.

-The realization of in vivo biological tests in order to determine the presence of certain active principles.

- study the corrosion inhibition of steel by adopting other techniques such as the gravimetric method in order to be able to compare it to the electrochemical methods.

Appendix

بطـــــاقة استبيـــــان

كلية علوم المادة — جامعة قاصدي مرباح

التاريخ:.....

الجنس: ذكر ☐ أنثى ☐

الفئة العمرية:

18 -25 ☐ 26-35 ☐ 36-60 ☐ أكثر من 60 ☐

منطقة السكن: .....

1. هل تعرف نبات عنب الذيب؟

.....

2. ما هي استعمالاته:

طبي علاجي ☐ الطهي ☐

3. ما هو الجزء تستخدمه في النبتة؟

أوراق ☐ السيقان ☐ ثمار ☐ جذور ☐

4. ما هو الفصل الذي تتوفر فيه النبتة؟

الربيع ☐ الخريف ☐ الصيف ☐ الشتاء ☐

5. ما هي الأمراض التي يتم علاجها بنبات عنب الذيب؟:

هضمية ☐ معدية ☐ تنفسية ☐ تجميلية للبشرة ☐

سكر ☐ ضغط الدم ☐

☒ أمراض أخرى إن وجدت:

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6. كيف يتم استخدامها في العلاج؟

منقوع ☐ مسحوق ☐ مغلى ☐ بطريقة أخرى ☐

☒ ما هي الطريقة الأخرى:

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7. ما هي الفوائد والأضرار لنبات عنب الذيب؟

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8. هل لديك معلومات إضافية على النبتة؟

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Abstract

Abstract

Solanum nigrum L. is a medicinal plant that is originated from Asia. It has been known since ancient times as it has benefits in traditional folk medicine. To appreciate the importance of this plant that grows randomly in our arid region of El Oued we studied different polar extracts of three parts of this plant (leaves, stem, and fruits).

An investigative study was carried out on *Solanum nigrum* L, and we found that most of the population of the region uses this plant for therapeutic purposes and that the part that is used most frequently is the leaves. In the laboratory, the results of the phytochemical analysis showed that the plant contains groups of effective compounds, including: flavonoids, coumarins, alkaloids, tannins. Considerable amount of these active substances in all the studied parts, and the results showed that the leaf part and acetate extract contain the largest amount of phenolics, and when using the high-quality liquid chromatography (HPLC) technique, the following elements were identified: gallic acid, ascorbic acid, vanillin and chronological acid in all Species.

The antioxidant efficacy was evaluated through two techniques: spectroscopy (DPPH, FRAP; CAT) and the electrochemical technique for the three studied parts with different polar extracts. The results confirmed to us that the *Solanum nigrum* L. possesses antioxidant efficacy and this explains the usefulness of its use in folk medicine according to the survey and in the last chapter of this work. The corrosion inhibitory activity of X52 carbon steel was studied in an acidic medium (H_2SO_4,0.5M) of butanol extracts of the three parts by an electrochemical method (Tefal curves, electrochemical impedance) and the results showed that it has good corrosion inhibitory activity.

Key words: extracts, *Solanum nigrum* L., phenol, HPLC, antioxidant, corrosion.

Résumé

Solanum nigrum L. est une plante médicinale originaire d'Asie. Elle est connue depuis l'Antiquité car elle présente des avantages dans la médecine traditionnelle. Pour apprécier l'importance de cette plante qui pousse au hasard dans notre région aride d'El Oued nous avons étudié différents extraits polaires de trois parties de cette plante (feuilles, tige et fruits).

Une étude d'investigation a été menée sur *Solanum nigrum* L., et nous avons constaté que la majeure partie de la population de la région utilise cette plante à des fins thérapeutiques et que la partie la plus utilisée est les feuilles. En laboratoire, les résultats de l'analyse phytochimique ont montré que la plante contient des groupes de composés efficaces, notamment: les flavonoïdes, les coumarines, les alcaloïdes, les tanins. Une quantité considérable de ces substances actives dans toutes les parties étudiées, et les résultats ont montré que la partie de feuille et l'extrait d'acétate contiennent la plus grande quantité de phénoliques. Lors de l'utilisation de la technique de chromatographie liquide de haute qualité (HPLC), les éléments suivants ont été identifiés: acide gallique, acide ascorbique, vanilline et acide chronologique dans toutes les espèces.

L'efficacité antioxydante a été évaluée à travers deux techniques: la spectroscopie (DPPH, FRAP; CAT) et la technique électrochimique pour les trois pièces étudiées avec des extraits polaires différents. Les résultats nous ont confirmé que le *Solanum nigrum* L. possède une efficacité antioxydante et cela explique l'utilité de son utilisation en médecine traditionnelle selon l'enquête et dans le dernier chapitre de ce travail. L'activité inhibitrice de corrosion de l'acier au carbone X52 a été étudiée en milieu acide (H_2SO_4,0,5M) d'extraits de butanol des trois parties par une méthode électrochimique (courbes Tefal, impédance électrochimique) et les résultats ont montré qu'il a une bonne activité inhibitrice de corrosion.

Mots clés: extraits, *Solanum nigrum* L., phénol, HPLC, antioxydant, corrosion.

الملخص

عنب الذئب هي نبتة طبية اصلها من اسيا . معروفة منذ القدم ان لها فوائد في الطب الشعبي التقليدي ,لتثمين اهمية هذه النبتة التي تنمو عشوائيا في منطقتنا الصحراوية (الوادي) ,قمنا بدراسة مستخلصات مختلفة القطبية لثلاث اجزاء من هذه النبتة (اوراق, ساق, فواكه)

اجريت دراسة استقصائية حول نبتة عنب الذئب ووجدنا ان اغلب سكان المنطقة يستعملونها لغرض علاجي وان الجزء المستخدم بكثرة هو الاوراق, مخبريا اظهرت نتائج التحليل الفيتوكيميائي احتواء النبتة على مجموعات مركبات فعالة منها: الفلافونيدات ,الكومارينات, القلويدات,التانينات كما تم تقدير محتوى الفينول والفلافونيدات والفلافانول للنبتة واظهرت النتائج وجود كمية معتبرة من هذه المواد الفعالة في جميع الاجزاء المدروسة, كمااظهرت النتائج ان جزء الورقة ومستخلص الاسيتات يحوي الكمية الاكبر من المواد الفينولة. و عند استعمال تقنية الكروماتوعرافيا السائلة ذات الجودة العالية (HPLC) تم التعرف على العناصر التالية: حمض الغاليك, حمض االسكوربيك, الفانيلين و حمض الكرونولوجيك في كافة الانواع.

تم تقييم الفعالية المضادة لالكسدة بتقنيتين: الطيفية (DPPH ,FRAP;CAT) و التقنية الكهروكيميائية لثلاث الاجزاء المدروسة بمستخلصات مختلفة القطبية وأكدت لنا النتائج أن نبتة عنب الذئب تمتلك فعالية مضادة للأكسدة وهذا ما يفسر فائدة استعمالها في الطب الشعبي حسب الدراسة الاستقصائيةو في الفصل الاخير من هذا العمل تمت دراسة الفاعلية التثبيطية للتآكل الفولاذ الكربوني X52 في وسط حمضي (H_2SO_4,0.5M) لمستخلصات البوتانول للاجزاء الثلاثة بطريقة الكتروكيميائية (منحنيات تفال,الممانعة اللالكتروكيميائية) وقد اظهرت النتائج ان لها فعالية تثبيطية جيدة للتآكل

الكلمات المفتاحية: مستخلصات ,عنب الذئب, الفينول, HPLC, مضادة للأكسدة ,التاكل.