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The effects of thermal treatment of extract from Moringa Oleifera and variation on phytochemical distribution

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بسم الله الرحمن الرحيم

كلمة شكر :

احمد الله حمدا كثيرا كما ينبغي لجلال وجهه وعظيم سلطانه.

اتقدم بالشكر الجزيل الى الدكتور لعويني صلاح الدين وزوجته اللذان لم يبخلا علينا بتوجيهاتهما ونصائحهما القيمة طليلة اشرافه على هاذا العمل.

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General introduction

Plants are a major group among the living organisms, which support and help the life line of the mankind as well as other living organisms, they have always sustained human civilization through the biologically active compounds they contain in them. They form the basis of all food webs, most plants are autotrophic, creating their own food using water, carbon dioxide, and light through a process called photosynthesis [1], Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies, although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2 600 BC, and to avoid the side effects of some medicinal plants and to improve theirs effects have been created some methods like extraction.

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. [2] this method make us get many importance compounds like Phenolic compounds and it has been known as plant pigments for over a century and belong to a vast group widely distributed in all foods of plant origin. Phenolic compounds are constituents of fruits, vegetables, nut and plant-derived beverages – tea, Chemically, phenolic compounds have one or more hydroxyl groups attached to a benzene ring. Edible plants contribute to the human diet more than 8 000 different phenolic compounds that can be categorized as flavonoids and non-flavonoid compounds [3], humans have consumed flavonoids and other dietary phenolics since the arrival of human life on earth [4], as any compounds this one can be affected by many factors like pH and Temperature, The thermal stability of polyphenols is crucial and correlated with both the extraction and characterization methods, and the recommendations on their fields of use [5], depending to many investigation has demonstrated that some naturally occurring polyphenolic compounds which are part of our diet are damaged when exposed to high pH. The chemical structure of the phenolic compounds appears to have a profound effect on their susceptibility to such destruction as measured by their absorption spectra [6].

In this study, the effects of thermal treatment of extract from Moringa Oleifera and variation on phytochemical distribution (phenolic compounds, flavonoids and flavanols) were investigated. The HPLC profile of each extract and the effects of thermal treatment on quantity of phenolic compounds were studied.

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I. General and presentation of Moringa oleifera

I.1. Introduction

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, that is native to the sub-Himalayan tracts of India, southeast Asia, Arabia
South America and the Pacific and Caribbean Islands, this rapidly-growing tree know as many names known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree.
And this tree was utilized by the Romans, Greeks and Egyptians but now it's wildly cultivated and became naturalized in many locations in the tropic it's have low quality timber also it's perennial softwood tree but since long time ago, a centuries ago was used for traditional medicinal and industrial uses.

I.2. Description of plant:

Moringa oleifera is a deciduous tree occasionally growing up to 15 m in height but is usually less than 10 m tall it has a large underground rootstock and normally a single main trunk with a wide, open and typically umbrella-shaped crown, lose her leaves from December to January and its florescence between January to March and the fruits ripen from Avril to June. Every part in this plant is important the leaves, the flowers, the seeds (the fruits) and peel of seeds.

Figure 1: Habit of *Moringa oleifera* (photo: Chris Gardiner)

I.2.1. Shoot system (up ground part):

I.2.1.1. The leaves:

The shape of leaves is tri-pinnate her color be from green to dark green, with vegetative shoots contain 8-10 of Pairs of leaves reversed the direction.

Figure 2: Leaves and flowers of Moringa oleifera (photo: Sheldon Navie)

I.2.1.2. The flower:

It has a good smell her long arrange between 0.7 to 1cm, they are born pedicle 12-21mm long and the flower buds are avoiding in shape, the flowers are white to cream.

Figure 3: Flower clusters of Moringa Oleifera (photo: Sheldon Navie)

Figure 4: Close-up of Moringa oleifera flowers (photo: Sheldon Navie)

I.2.1.3. The fruits:

It has a shape of tri-horn with long from 20-60cm, contain 3 part every part from it contain arranged 12-35 seed with avoid shape reproduce with it.

Figure 5: Immature fruit of Moringa Oleifera (photo: Chris Gardiner)

Figure 6: The seeds of Moringa Oleifera

I.2.2. Root system (underground system):

The roots: having tubular structure and can be reached till 1.30m depth also it's have resistance to drought.

Figure 7: Root of Moringa Oleifera

I.3. Scientific Classification of *Moringa oleifera*:

Moringa oleifera is one of the 14 species of the Moringaceae family.

Table 1: Scientific Classification of Moringa [1]

Classification	Specific name
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Moringaceae
Genus	Moringa
Species	Oleifera

I.4. Common plant names:

Table 2: table show the common names of Moringa oleifera in different language [2]

Language	Common names
English	Moringa, horseradish tree, drumstick tree, sujuna, ben tree, ben oil tree
French	Ben ailé, ben oléifère, benzolive, arbre radis du cheval
Spanish	Ben, árbol del ben, paraiso, morango, Moringa
Portuguese	acácia branca, marungo, muringa, moringuiero; cedro (Brazil)
Arabic	الرواق, الحليم,شقر الرواق.
Swahili	mzunze, mlonge, mjungu moto, mboga chungu, shingo
German	Behenbaum, Behenussbaum, flügelsaniger Bennussbaum, Pferderettichbaum

Italian	Sàndalo ceruleo Fon: kpatima, yovokpatin,kpano,yovotin
Gun	èkwè kpatin, kpajima
Yoruba & Nago	èwè igbale, èwè ile, èwè oyibo, agun oyibo, ayun manyieninu, ayèrè oyibo
Fulani	gawara, konamarade, rini maka, habiwal hausa
Hausa	zogall, zogalla-gandi, bagaruwar maka, bagaruwar masar, shipka hali, shuka halinka, barambo, koraukin zaila, rimin turawa
Ibo	Ikwe oyibo
Senegal	nebeday
Philippines	malunggay or malungai (Tagalog)
India	sujuna, sajina, lopa, horseradish or drumstick tree
Haiti	benzolive (Haitian Creole)

I.5. *Moringa oleifera* species:

Table 3: show the pieces of *Moringa oleifera* and her origin place [2]

Species	Origin
Moringa oleifera	India
M. drouhardii	Madagascar
M. cocanensis	India
M. arborea	North Eastern Kenya
M. hildebrandtii	Madagascar
M. oleifera	India
M. borziana	Kenya and Somalia

M. ovalifolia	Namibia and extreme southwestern Angola
M. peregrina	Horn of Africa, Red sea, Arabia
M. longituba	Kenya, Ethiopia, Somalia
M. stenopetala	Kenya, Ethiopia
M. pygmaea	Northern Somalia
M. rivae	Kenya, Ethiopia
M. ruspoliana	Kenya

I.6. Geographical distribution:

It was utilized by the old Roman and Greeks also Egyptian but now it's cultivated in India and South Southeast Asian (particularly Filipino) populations also part from Africa and can be found in some places with tropical and subtropical regions.

I.6.1. Study area:

El Oued Province is bordered to the northeast by Tebessa Province, to the north by Khenchela Province, to the northwest by Biskra Province, to the south and southwest by Ouargla Province, to the southeast by Tunisia's Tataouine Governorate, and to the east by Tunisia's Tozeur and Kebili Governorates [3].

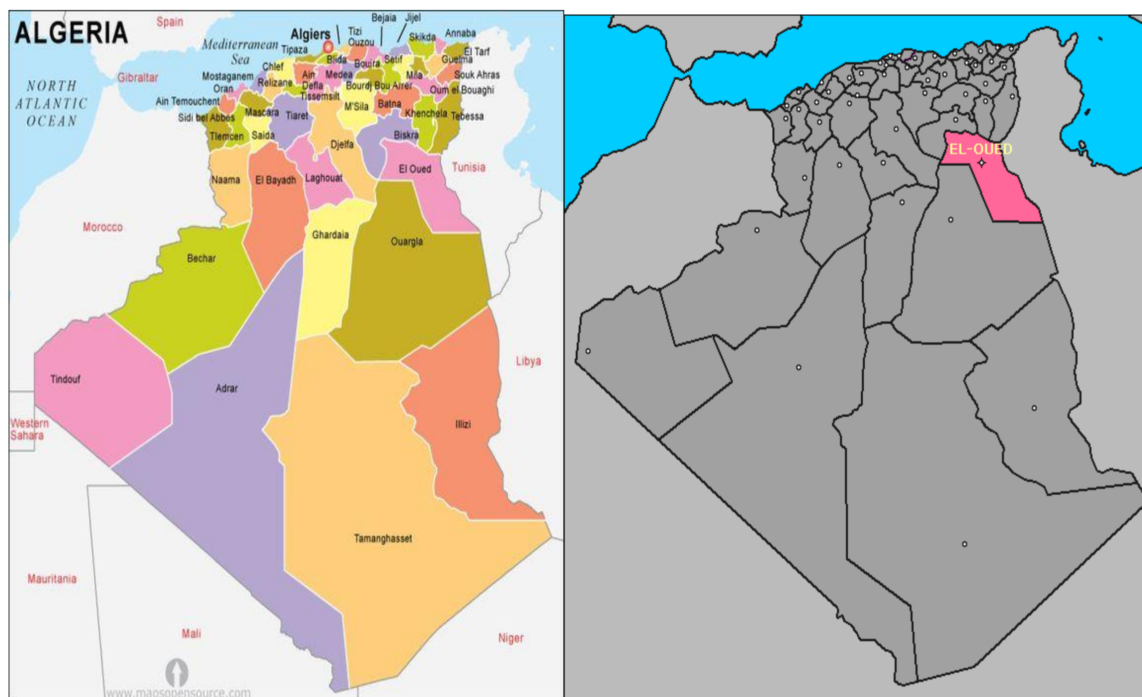

Figure 8: Map show the location of EL OUED [4]

I.7. Chemical Composition and nutritional value of *Moringa oleifera*:

Since the chemical content of plants are affected by the type of soil or climatical condition they grow [5], it would be expected that there will be variation in the chemical composition reported by different researchers also, the method adopted for analysis and the state of the leaves.

whether fresh or dried could contribute to variation in values obtained.

Analysis of *Moringa oleifera* pods, fresh (raw) leaves and dried leaf powder have shown them to contain the following per 100 grams of edible portion.

Table 4: The result of analyses 100g from Moringa oleifera [6]

	Pods	Leaves	Leaf Powder
Moisture (%)	86.9	75.0	7.5
Calories	26.0	92.0	205.0
Protein (g)	2.5	6.7	27.1
Fat (g)	0.1	1.7	2.3
Carbohydrate (g)	3.7	13.4	32.2
Fiber (g)	4.8	0.9	19.2
Minerals (g)	2.0	2.3	-

Ca (mg)	30.0	440.0	2,003.0
Mg (mg)	24.0	24.0	368.0
P (mg)	110.0	70.0	204.0
K (mg)	259.0	259.0	1,324.0
Cu (mg)	3.1	1.1	0.6
Fe (mg)	5.3	7.0	22.2
S (mg)	137.0	137.0	870.0
Oxalic acid (mg)	10.0	101.0	0.0
Vitamin A - B carotene (mg)	0.1	6.8	16.3
Vitamin B -choline (mg)	423.0	423.0	-
Vitamin B1 -thiamin (mg)	0.05	0.21	2.6
Vitamin B2 -riboflavin (mg)	0.07	0.05	20.5
Vitamin B3 -nicotinic acid (mg)	0.2	0.8	8.20
Vitamin C -ascorbic acid (mg)	120.0	220.0	17.3
Vitamin E -tocopherol acetate (mg)	-	-	113.0
Arginine (g/16g N)	3.6	6.0	0.0
Histidine (g/16g N)	1.1	2.1	0.0
Lysine (g/16g N)	1.5	4.3	0.0
Tryptophan (g/16g N)	0.8	1.9	0.0
Phenylanaline (g/16g N)	4.3	6.4	0.0
Methionine (g/16g N)	1.4	2.0	0.0
Threonine (g/16g N)	3.9	4.9	0.0
Leucine (g/16g N)	6.5	9.3	0.0
Isoleucine (g/16g N)	4.4	6.3	0.0
Valine (g/16g N)	5.4	7.1	0.0

I.8. Traditional medicinal uses of *Moringa oleifera*:

The medicinal uses or the benefits of *Moringa oleifera* cannot be exhausted, because all the part of this tree the flowers, the leaves, the seeds also the roots have been utilized within traditional medical.

Table 5: Some common medicinal uses of different parts of Moringa oleifera [7]

Plant part	Medicinal Uses
Root	Antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation,
Leave	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling
Stem bark	Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti-tubercular activity
Gum	Used for dental caries, and is astringent and rubefacient; Gum, mixed with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism
Flower	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolaemic rabbits and increased the excretion of faecal cholesterol
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of Moringa pods

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II. Phenolic compounds

II .1. Introduction

There are many processes that can change the structure of the material and its physical or chemical nature such as heat treatment and it’s an operation or combination of operations involving heating at a specific rate, soaking at a temperature for a period of time and cooling at some specified rate.

The aim is to obtain a desired microstructure to achieve certain predetermined properties which can be physical, mechanical, magnetic or electrical. [1]

And here we will see their effects on the Phenolic compounds or phenolics substances which are a chemical compositions possess an aromatic ring with at least one hydroxyl group and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer, they are the most widely distributed secondary metabolites, and universally present in the plant kingdom [2]. More than 8,000 different phenolics have been reported [3] in the flavonoid family alone and the list continues expanding [4] although the term polyphenol is often used as a synonym of phenolic compound, it should be restricted to molecules bearing at least two phenolic rings [5] polyphenols were first defined by Bate-Smith and Swain (1962) as water soluble

Also this one have molecular weights between 500 and 3,000 (Da).

II .2. Main classes:

we can classify the Phenolic compounds into many types and ways because they are constituted in a large number of heterogeneous structures that range from simple molecules to highly polymerized compounds. If we take a look to their carbon chain, phenolic compounds can be divided into 16 major classes [6].

The main classes of phenolic compounds regarding to their carbon chain can be seen in Figure 2. On the other hand, as to their distribution in nature, phenolic compounds can be divided into three classes:

II.2.1. Shortly distributed as simple phenols, pyrocatechol, hydroquinone, resorcinol, Aldehydes derived from benzoic acids that are components of essential oils, such as vanillin.

II.2.2. Widely distributed: divided in flavonoids and their derivatives, coumarins and phenolic acids, such as benzoic and cinnamic acid and their derivatives.

II.2.3. Polymers: which is tannin and lignin [7].

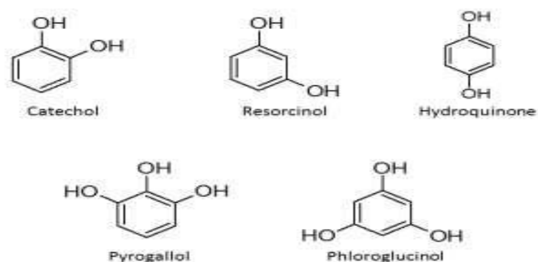

Figure 1: Simple phenols

Class	Basic skeleton	Basic structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naphthoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans and neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

Figure 2: Main classes of phenolic compounds regarding to their carbon chain

II .2.4. Flavonoids:

we can divide flavonoid into 13 [8] classes according to presence of a C2-C3 double bond heterocycling pyrone ring and the degree of hydroxylation the most important being represented by the flavonols, flavones, isoflavones, anthocyanidins o anthocyanins and flavanones [3].

Within these classes there are many structural variations according to the degree of hydrogenation and hydroxylation of the three ring systems of these compounds.

Flavonoids also occur as sulfated and methylated derivatives, conjugated with monosaccharides and disaccharides and forming complexes with oligosaccharides, lipids, amines, carboxylic acids and organic acids, being known approximately 8000 compounds [9]. Some classes of flavonoids are colorless (eg: flavonones) and some other colored (eg: anthocyanins).

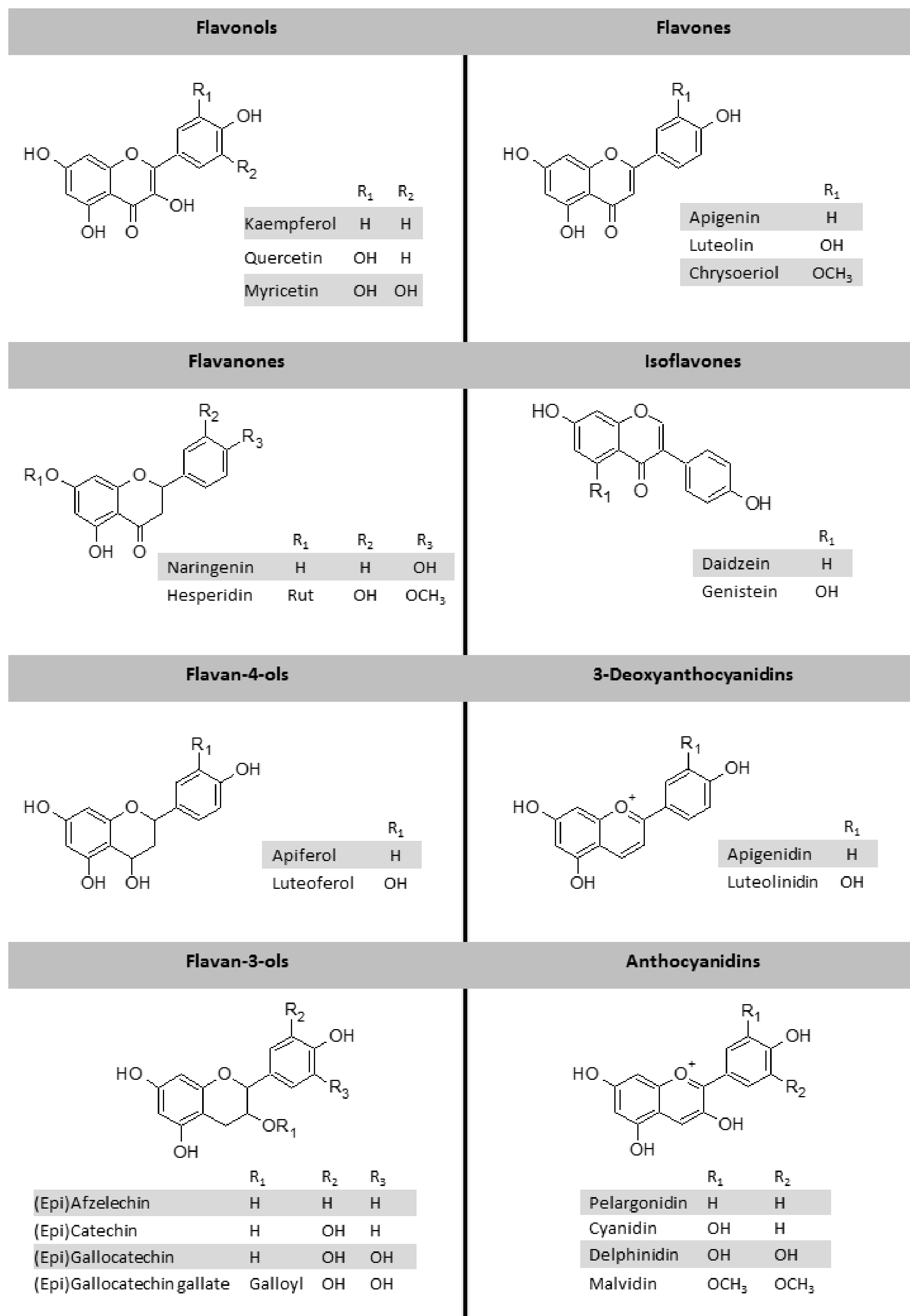

Figure 3: Flavonoids (main classes and examples).

II .2.5. Tannins:

Tannins are a group of water-soluble polyphenols with high molecular weights (500 to 3000 Da), and can be classified into two major groups: hydrolysable tannins and non-hydrolysable or condensed tannins [10] and some of other hand there is a third group of tannins, phlorotannins which are only found in brown seaweeds [11].

The hydrolysable tannins have a center of glucose or a polyhydric alcohol partially or completely esterified with gallic acid or hexahydroxydiphenic acid.

The condensed tannins are polymers of catechin and/or leucoanthocyanidin, not readily hydrolyzed by acid treatment, and constitute the main phenolic fraction responsible for the characteristics of astringency of the vegetables. Although the term condensed tannins is still widely used, the chemically more descriptive term "proanthocyanidins" has gained more acceptance. These substances are polymeric flavonoids that form the anthocyanidins pigments[12].

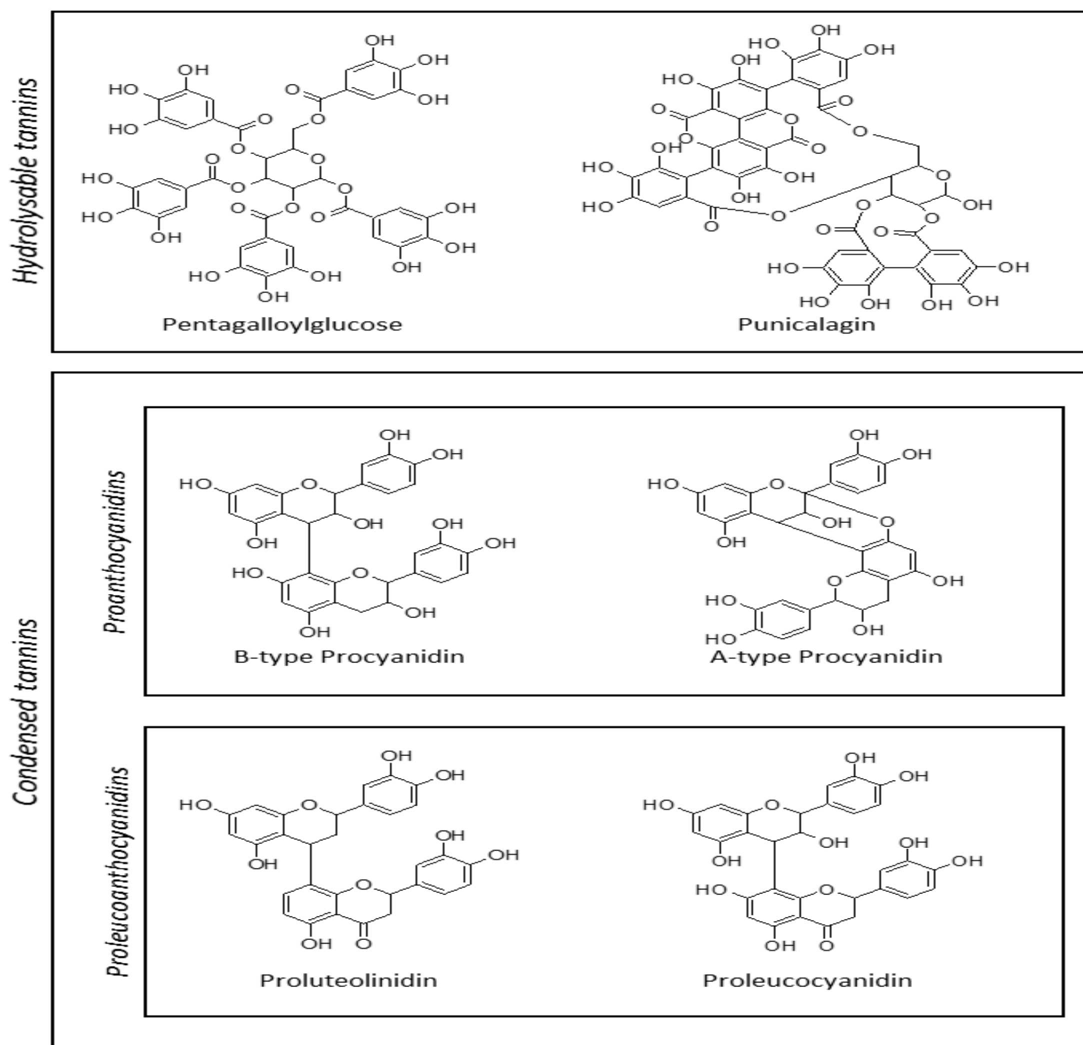

Figure 4: Tannins (main classes and examples).

II .2.6. Coumarins:

Coumarins are lactones obtained by cyclization of hydroxycinnamic acids, belonging to the phenolic compounds group with the basic skeleton of C6-C3. Coumarins are present in plants in free and glycosylated forms.

In general, coumarins are characterized by high chemical diversity, mainly differing in the degree of oxygenation of their benzopyrone moiety.

The major coumarins include simple hydroxylcoumarins (e.g. esculetin and scopoletin), [13]

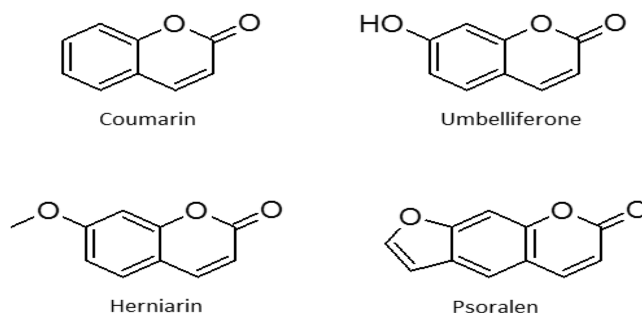

Figure 5: Coumarins (examples).

II .2.7. Anthraquinones :

Also known as hydroxyanthracenic compounds, anthraquinones are a group of colorful dyes common in plants, being very unstable and existing not only in several oxidation states but also bonded to sugars, mainly glucose and rhamnose. The most abundant anthraquinones are chrysophanol, emodins, aloins, rheins and senidins [14].

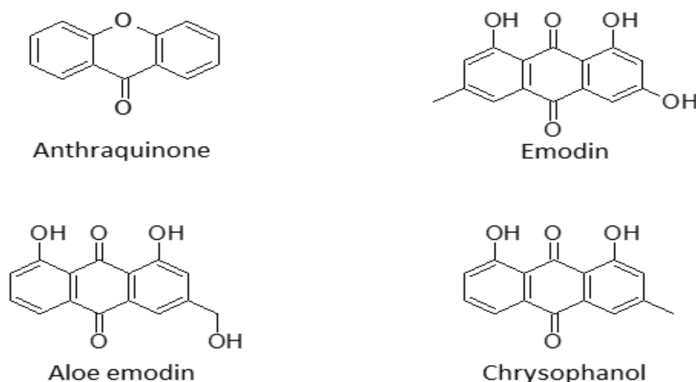

Figure 6: Anthraquinones (examples).

II .2.8. Phenolic acids:

Phenolic acids can be divided into two groups: benzoic acids and cinnamic acids and derivatives thereof. The benzoic acids have seven carbon atoms (C6-C1) and are the simplest phenolic acids found in nature. Cinnamic acids have nine carbon atoms (C6-C3). These substances are characterized by having a benzenic ring, a carboxylic group, and one or more hydroxyl and/or methoxyl groups in the molecule [15].

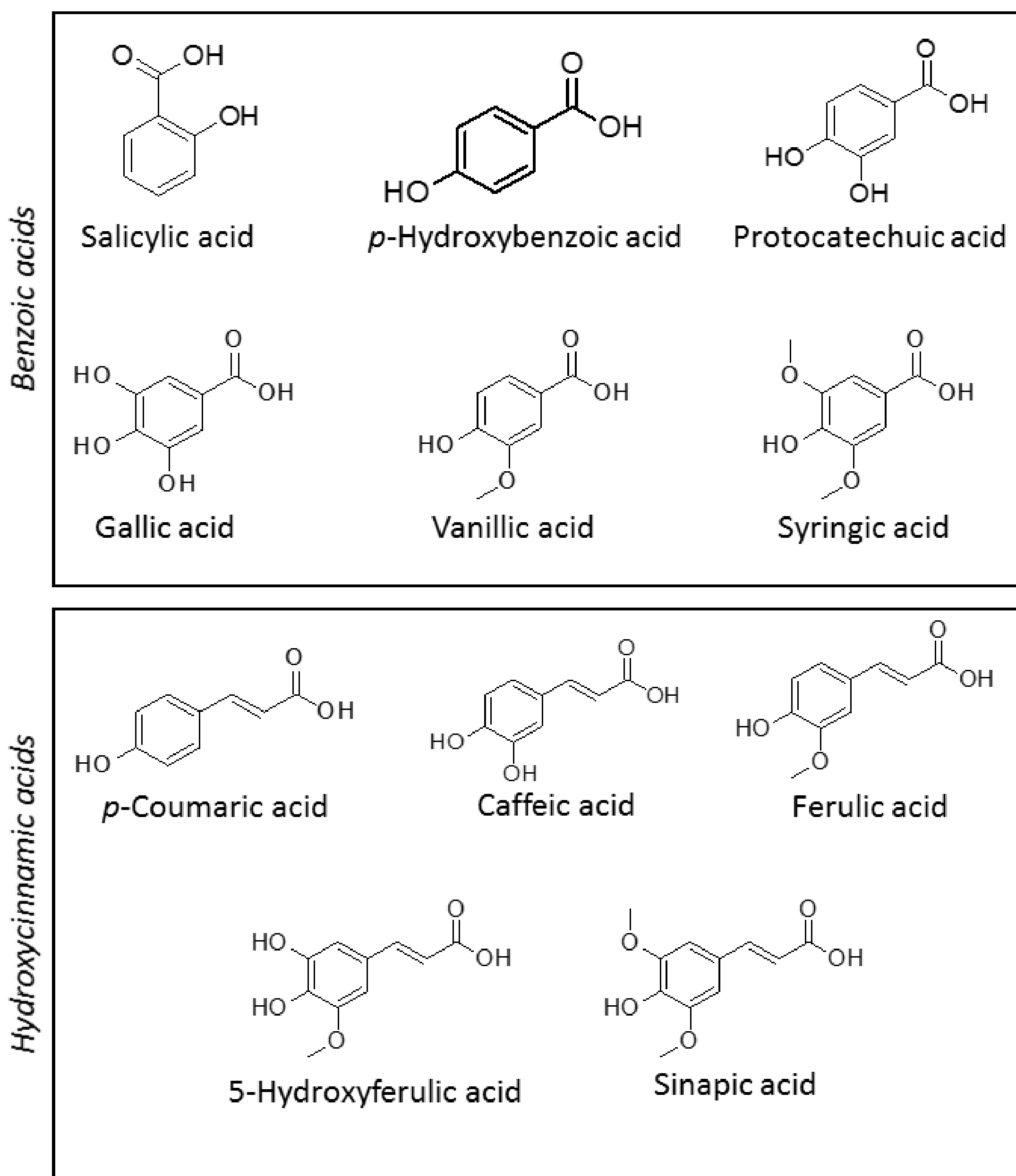

Figure 7: Phenolic acids (examples).

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III.1. Materials and methods:

III.1.1. Reactive chemicals and equipment:

All the chemicals used are of analytical quality. Chemical reagents and solvents were provided by Sigma Aldrich (France), Alpha Asear (France).

The different devices used for us analyzes are as follows:

- Rotavapor from B.U.C.H.I company model R-210 equipped by an upper cooler (Figure 1).
- A spectrophotometer Spectrum Instrumens SP-UV 500DP characterized by a high resolution and an error of less than 0.01 nm. This device linked with a microcomputer to facilitate the processing of results (Figure 2).
- A high performance liquid chromatograph from Shimadzu, HPLC (RP - HPLC) composed of a DGU-20 A3 degasser, two LC-20 AT pumps coupled to a UV-Visible SPD-20 A detector and equipped with a 20 µl injection loop and a column CTO-20 AC (RP C18 size 4.6 mm × 150 mm, with a particle size of 5 µm) and (Figure 3).
- Incubator from binder company characterized by high-temperature accuracy (Figure 4).
- A Spectrometer IR from Thermos fisher company model Nicolet iS5 FTIR (Figure 5).

Figure 1: Rotavapor of extract recovery

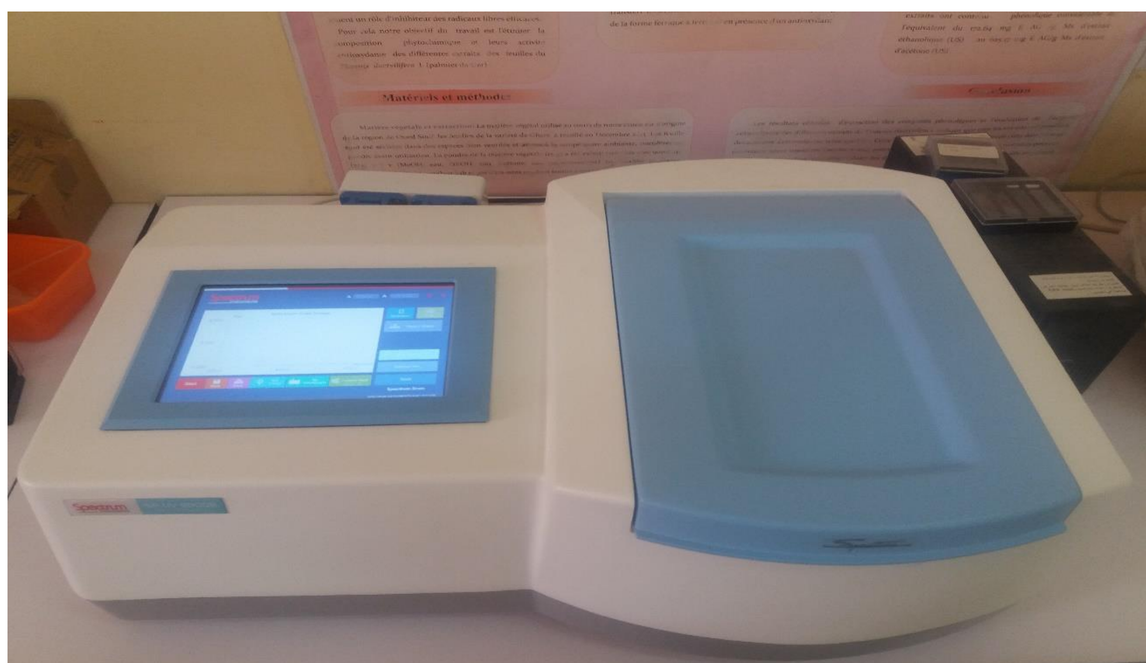

Figure 2: Spectrophotometer Spectrum Instrumens model SP-UV 500DP

Figure 3: High-performance liquid chromatogram (HPLC)

Figure 4: Incubator

Figure 5: Spectrophotometer IR

III.1.2. Plant materials:

The plant materials used in our study are from the origins of the region of Oued Souf the leaves and vegetative shoots (from a tree was planted in university Hamma lakhdar in Oued Souf) during the month of February.

III.2. Methodology:

III.2.1. Samples preparation:

Samples preparation is the first importance for any reliable analysis, many sample preparation methods have been developed to determine phenolic compounds.

Sample preparation procedures for the analysis of phenolic compounds vary greatly depending on the nature of the compounds to be analyzed, these must take into account numerous parameters among which: the polarity, the acidity of the molecules, the number of hydroxyl groups and aromatic nuclei. The most commonly described assay methods include several steps of sample preparation, each of them aims to increase the sensitivity and selectivity of the assay. Unfortunately, the various steps can sometimes introduce interference that creates artifacts, which is not without consequences on the reproducibility of the assay and requires an average of several tests.

It is therefore important to monitor the entire preparation and evaluate the influence of these effects on the analysis of the results. In general, the solid samples are subjected to grinding and sieving, often

preceded by a step of drying in the open air of the plant material to be analyzed the liquid samples are centrifuged and filtered, we do many step to get the final test for all the uses devises, we begin with:

III.2.1.1. Drying:

The plant material was washed with water to remove soil, dried in a circulating-air oven (37 ± 2 °C), like what's shown in the picture below for 3 days and check its weight on last day every 2 hours to make sure it's completely has been dried.

Figure 6: Drying the leaves and Vegetative shoots

Figure 7: Drying the leaves and vegetative shoots on open air

After its completely drying its weight was 105g for the leaves and for the vegetative shoots was 31.15g, then we grinding it until its be a powder each one separated from the other.

III.2.1.2. Maceration:

On this step, we take the powder of leaves and vegetative shoots and put them in beakers contain ethanol, for the leaves we use 632ml and for the vegetative shoots 186.9ml (the weight×6 to find the size of ethanol added)

Leave everyone from these beakers on hot plate magnetic stirrer for 24 hours and at 40C

Figure 8: Begin Maceration process for the vegetative shoots

And after 24 hours we stop this process to begin the next one.

III.2.1.3. Filtration:

On this step, we take the liquid of each one, and filter it with filter paper and re-process it for 3times to make sure it's been filter completely.

III.2.1.4. Evaporation:

To separate the extract from the liquid we use the Rotavapor and deny all the liquid from it.

Then we division the extract of the leaves and vegetative shoots to 5 parts and gets

A: for leaves extract

B: for vegetative shoots extract

Table 1: Division of extract to many samples

Samples	**weights**					**Total weights**
A	A0=2.07g	A1=2.05g	A2=2.08g	A3=2.08g	A4=2.06g	10.4g
B	B0=0.28g	B1=0.27g	B2=0.27g	B3=0.29g	B4=0.27	1.5g

III.2.1.4. Heat treatment:

The samples A and B (A for the extract of leaves and B for extract of vegetative shoots) set for varying temperatures for 5min in Incubator, A1and B1 set on 80 °C, A2 B2 set on 100 °C, A3 B3 set on 120 °C, A4 B4 set on 140 °C and let A0 and B0 without heating to be the reference of our compare.

Table 2: table relate every simple and there temperature heat on it

A	A0	A1	A2	A3	A4
B	B0	B1	B2	B3	B4
	Reference	80°C	100°C	120°C	140°C

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Figure 8: Begin Maceration process for the vegetative shoots 30

IV.1. Determination of total polyphenolic compounds:

IV.1.1. Results and yield discussions of compound extractions phenolic:

Extraction yields of phenolic compounds for the leaves and the vegetative shoots

% yield = (the mass of extract / initial mass) X 100

For the leaves: 9.90%

For the vegetative shoots: 4.76%

IV.1.2. Infrared spectroscopy:

IR spectroscopy is the spectroscopic technique which uses the Infrared light and studies its interaction with the molecules. The spectra generated can give a lot of information about the molecule, especially about the functional groups associated.

as we can see from sample A0 which is the sample without heat treatment and we will use as a reference for comparison with others simples.

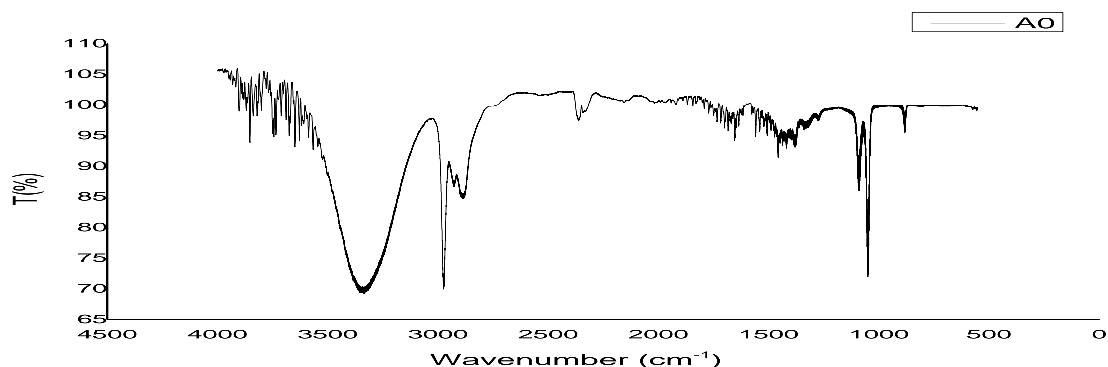

Figure 1: IR analysis for sample A0

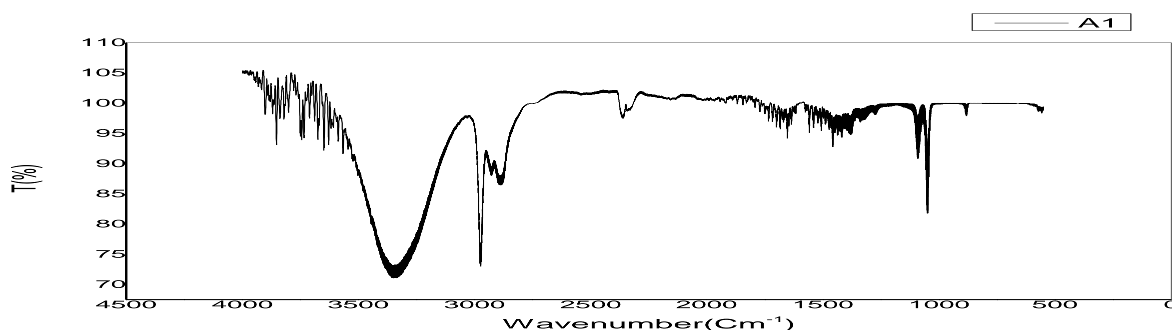

Figure 2: IR analysis for sample A1

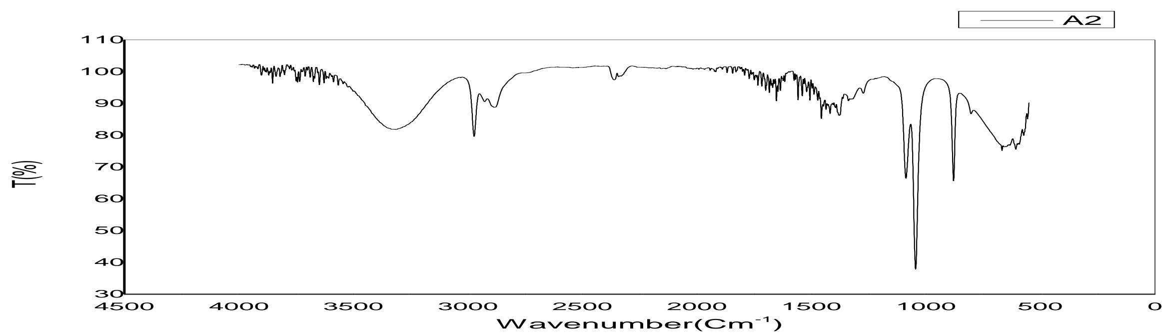

Figure 3: IR analysis for sample A2

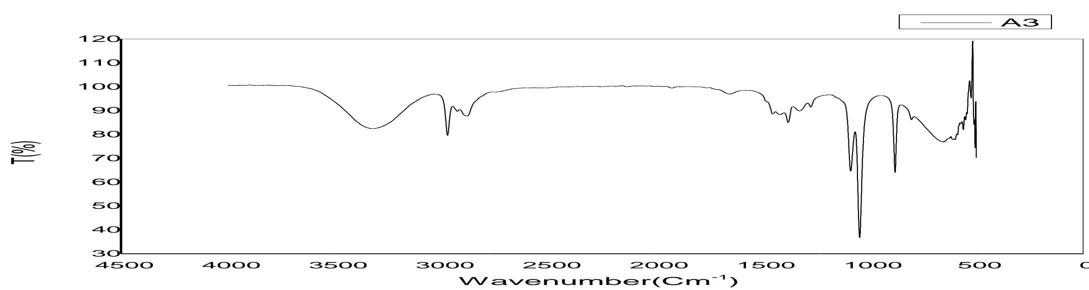

Figure 4: IR analysis for sample A3

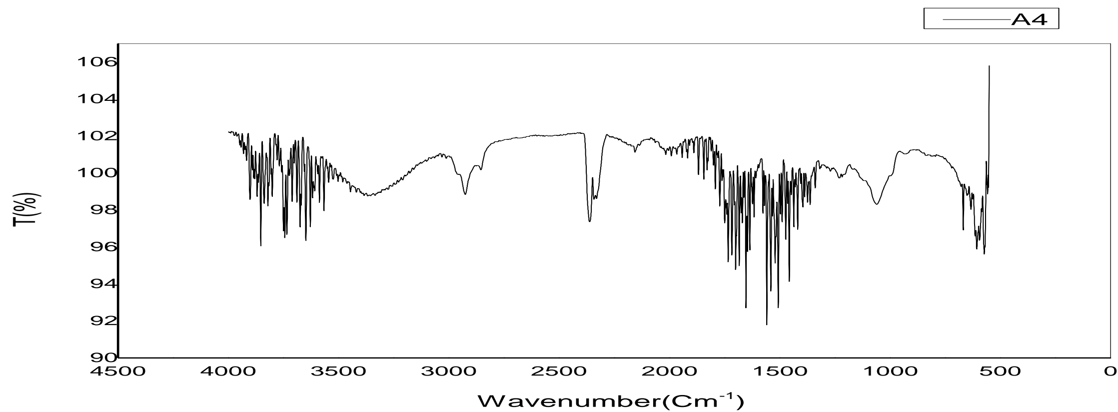

Figure 5: IR analysis for sample A4

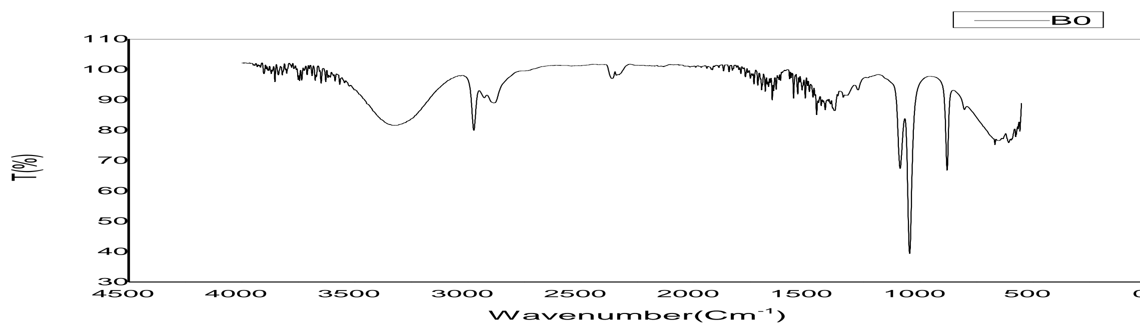

Figure 6: IR analysis for sample B0

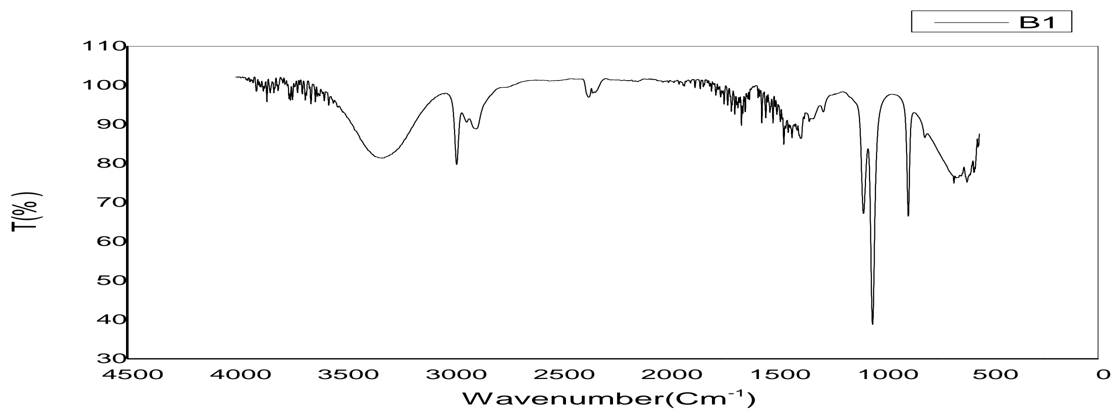

Figure 7: IR analysis for sample B1

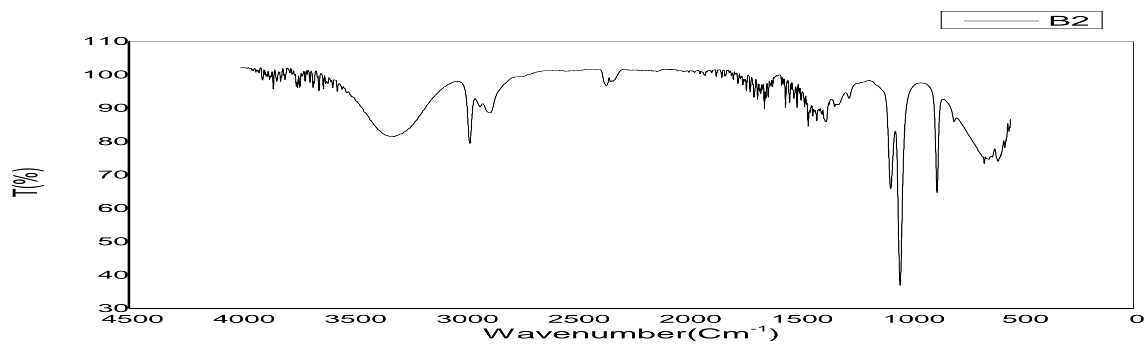

Figure 8: IR analysis for sample B2

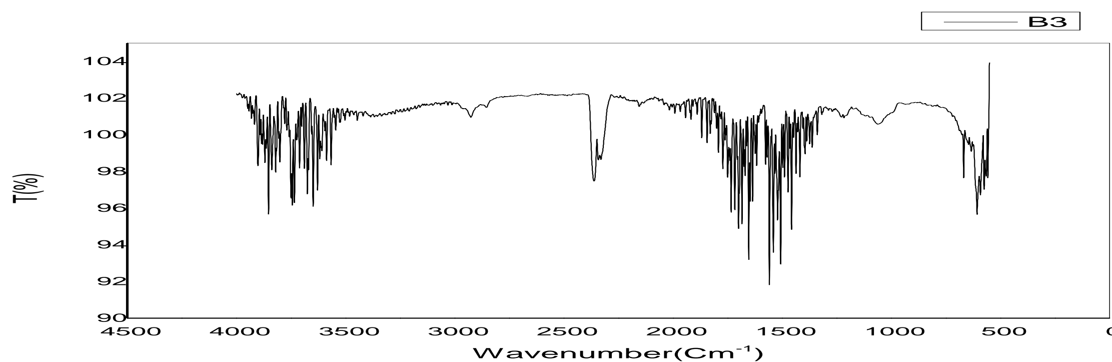

Figure 9: IR analysis for sample B3

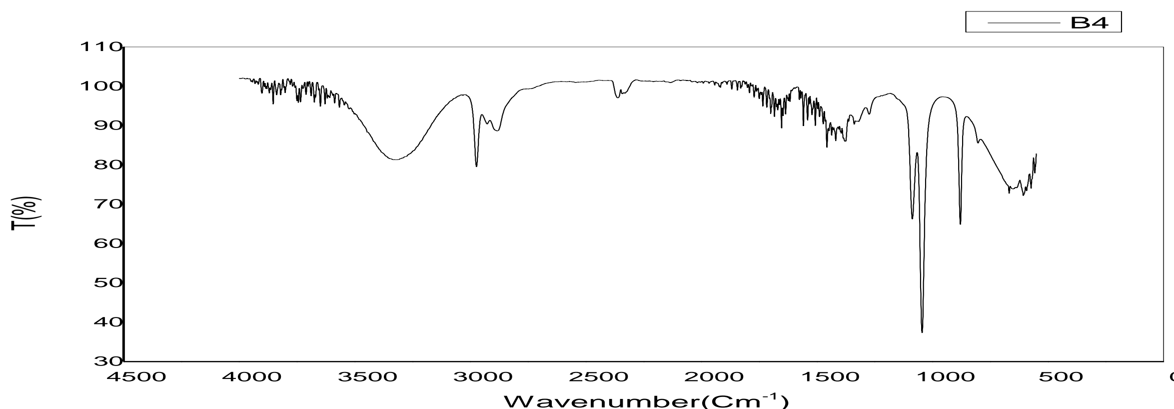

Figure 10: IR analysis for sample B4

Sample A0:

At 900 wavenumber have little stretch of C(3links) C-H, at 1041 wavenumber stretching and it's for C-O Alcohol, at 1090 we have stretched and it's for C-O Alcohol, at 2885 wavenumber stretching for C-H alkane, at 2926 wavenumber stretching for C-H alkane and at 3334 wavenumber streaking for O-H Alcohol.

Sample A1:

In this simple still have stretched at 1000 wavenumber of Alcohol C-O, stretching at 2900,2973 wavenumber of Alkane C-H stretch and stretching at 3290 wavenumber of alcohol O-H.

Sample A2: at 679 wavenumber stretching for C-H Alkyl alkene, at 1045 and 1087 wavenumber stretching for C-O Alcohol, at 3000 wavenumber stretching for C-H Aromatic, at 3200 wavenumber stretching for O-H hydroxyl.

Sample A3:

At 890 wavenumber stretching for C-H of Alkene, at 1070 wavenumber stretching for
C-O of alcoholat 1100 wavenumber stretching for C-O of alcohol, at 2900 wavenumber stretching C-H of Alkane and at 3350 wavenumber stretching C-H of Aromatic.

Samples A4: from1365 to 1436 wavenumber many of stretching -C-H of Alkane, at 1520 wavenumber stretching C-C of aromatic and at 2850 wavenumber stretching C-H of Alkane.

Sample B0: at 1045 wavenumber stretching C-O of alcohol, at 2990 wavenumber stretching C-H of Alkane and at 3300 wavenumber stretching C-H of the alkene.

Sample B1: at 1045 wavenumber stretching C-O of alcohol, at 1350 wavenumber bending of C-H of alkane, at 2990 wavenumber stretching C-H of Alkane and at 3300 wavenumber stretching C-H of the alkene.

Sample B2: at 1045 wavenumber stretching C-O of alcohol, at 1350 wavenumber bending of C-H of alkane, at 2990 wavenumber stretching C-H of Alkane and at 3300 wavenumber stretching C-H of alkene

Sample B3: at 1350 to 1480 wavenumber bending for C-H alkane, at 1400-1600 wavenumber stretching C=C of Aromatic and at 3500-3700 wavenumber stretching O-H of Alcohol

Sample B4: at 1045 wavenumber stretching C-O of Alcohol, at 2990 wavenumber stretching C-H of Alkane and at 3300 wavenumber stretching C-H of Aromatic

IV.2. Determination of total phenolic compounds:

IV.2.1. By Folin-Ciocalteu method:

The reagent used is «Folin-Ciocalteu»; it's a mixture of complexes of phosphotungsten ($H_3PW_{12}O_{40}$) and phosphomolybdenum ($H_3PMo_{12}O_{40}$) acids of yellow color.

Steps: first step prepare Folin diluted 10 times and that by add 18ml from Distilled water to 2ml of Folin go to next step which is prepare the Na_2CO_3 diluted 10 times 1ml from Na_2CO_3 add to it distilled water until its be with size of 10 ml to the last step which is preparation the simples on utilizing 1ml from our extract for each sample add to it 1ml from diluted Folin and 0.2ml from Na_2CO_3 to the final step which is, leave it in dark for 40mn.

Table 1: Folin-Ciocalteu method

Preparation of Folin	2ml +18ml (distilled water)
Preparation of Na_2CO_3	1ml+10ml (distilled water)
The sample extract A,B	An extract with 0.1 concentration
Preparation the sample extract A,B	1ml extract +1ml Folin + 0.2 Na_2CO_3

The read on Uv spectrophotometer device show:

$C_{extract}$= 0.1mg/ml   ƛ= 700   and leave it in darker place for40 min

Table 2: The result on Uv visible for samples A

The sample A	A0	A1	A2	A3	A4
Absorbance	0.424	0.795	0.781	0,784	0,810

Table 3: The result on Uv visible for samples B

The sample B	B0	B1	B2	B3	B4
Absorbance	0.940	0.626	0.687	0.686	0.635

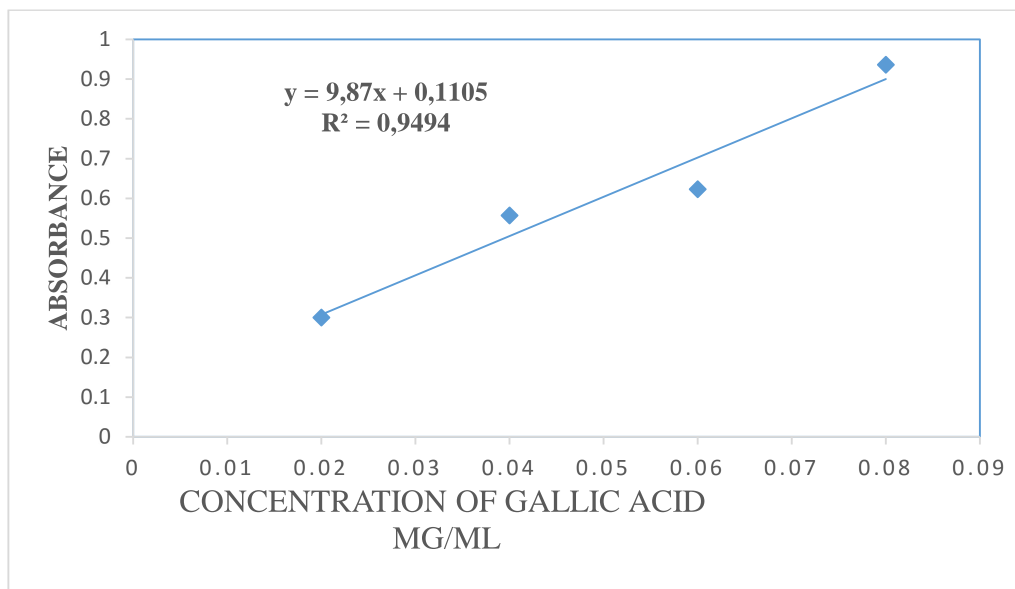

Figure 11: Calibration curve for the determination of polyphenols for A and B

IV.2.2. Flavonoid:

The total flavonoid content (mg/mL) was determined using aluminum chloride ($AlCl_3$) method1g $AlCl_3$ +**62** ml ethanol= $AlCl_3$ (of density 2%)

The assay mixture consisting 0.5ml of $AlCl_3$ (2%) +0.5 of plant extract(C=0.5mg/ml) and stay in dark place for hour.

$C_{axtract}$=0.5mg/ml ƛ= 420 leave it in dark for =60 min

Table 4: The result on Uv visible for samples A

The sample A	A0	A1	A2	A3	A4
Absorbance	0,442	0,588	0,956	0,221	1,077

Table 5: The result on Uv visible for samples B

The samples B	B0	B1	B2	B3	B4
Absorbance	1,20	0,914	1.236	0,568	0,966

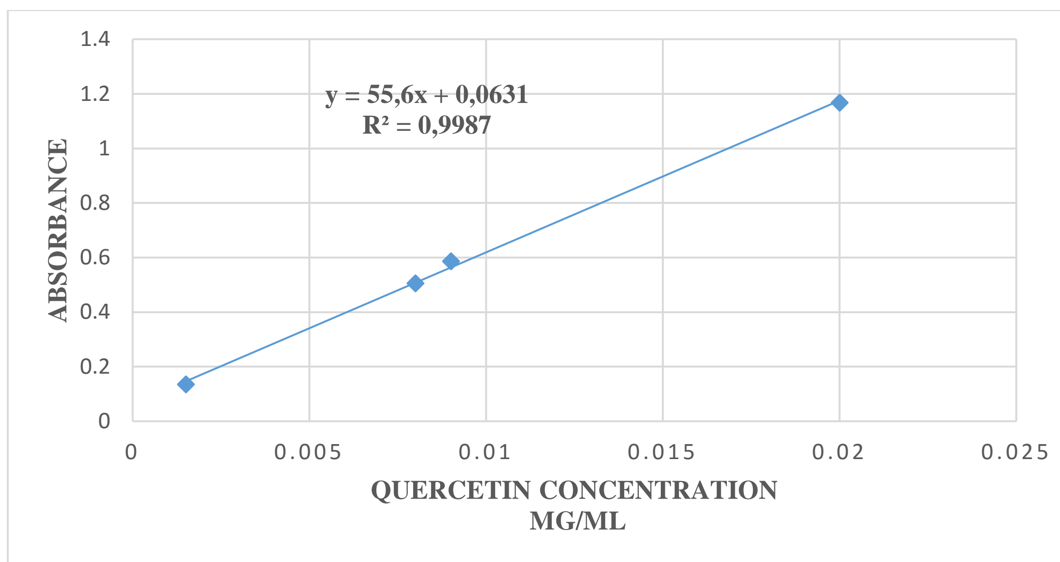

Figure 12: Calibration curve for the determination of flavonoids for A and B

IV.2.3 The total flavanols:

The total flavanols content (mg/mL) was determined using acetate sodium and aluminum chloride. Acetate sodium concentration of 50g/l.

The assay mixture consisting of 1ml of extract and 1ml of $AlCl_3$ and1.5ml from acetate sodium and leave the final mixture in the dark place for 150min.

The samplesA and B $C_{extract}$= 1mg/ml / ƛ= 440 /and leave in dark for =150 min

Table 6: The result on Uv visible for samples A

The samples A	A0	A1	A2	A3	A4
Absorbance	0.678	0,345	0,566	0,990	0,857

Table 7: The result on Uv visible for samples B

The sample B	B0	B1	B2	B3	B4
Absorbance	1,061	0,615	0,839	0,512	0,453

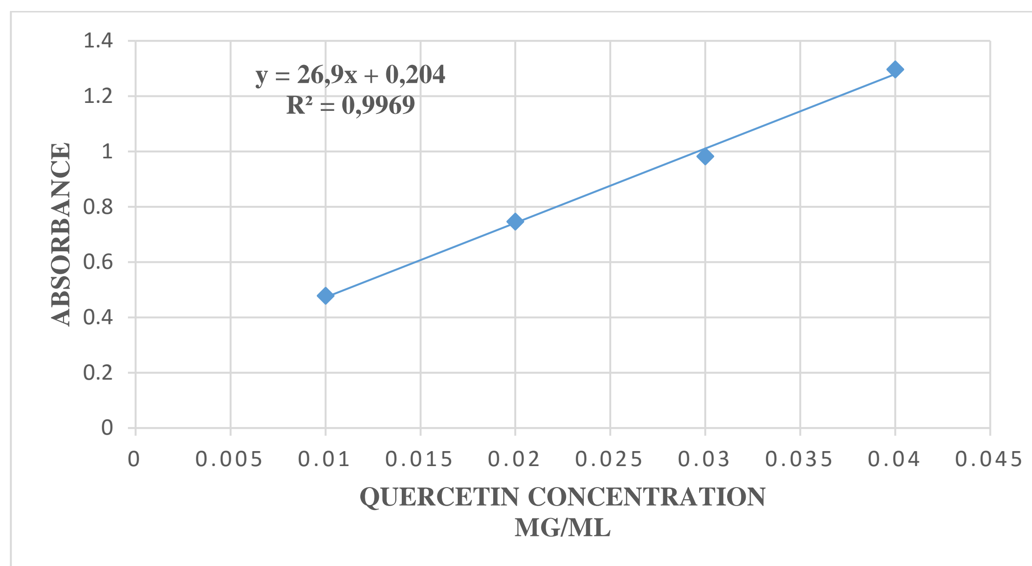

Figure 13: Calibration curve for flavanols determination for A and B

IV.2.4. HPLC

High-performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations including HPLC, operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.[1]

Using this method to analysis our samples and get this graph:

For samples **A0, A1**, **A2**, **A3**, and **A**4 which is the samples was taken from the leaves with and without heat treatment (**A0** without heat treatment and the other with heat treatment).

Guide for Chromatogram:

Black for **A0**, pink for **A1**, blue for **A2**, red for **A3** and green for **A4**.

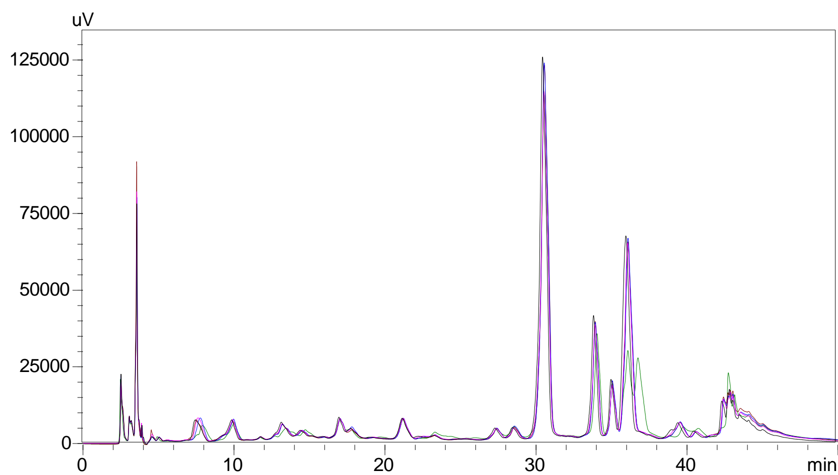

Figure 14: HPLC analysis of samples **A**

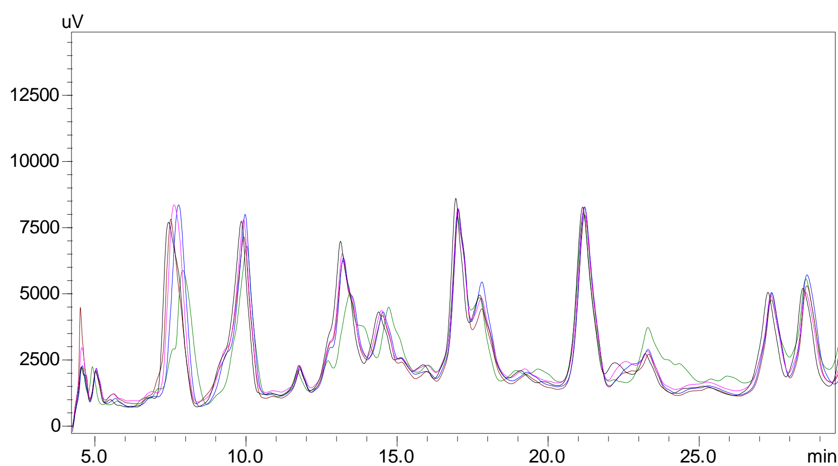

Figure 15: HPLC analysis for samples **A** with a zoom of 130%

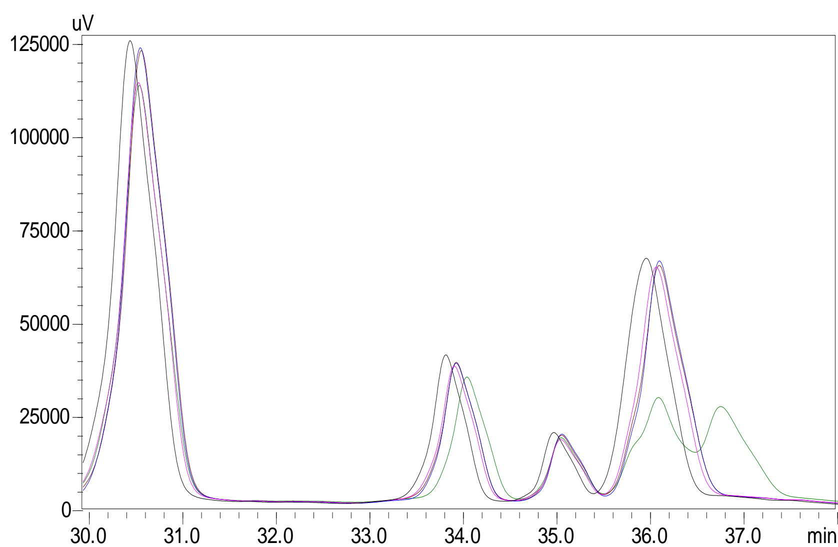

Figure 16: HPLC analysis for samples **A** with a zoom of 170%

For more clarification on each sample alone.

For A0 (the extract of leaves without heat treatment)

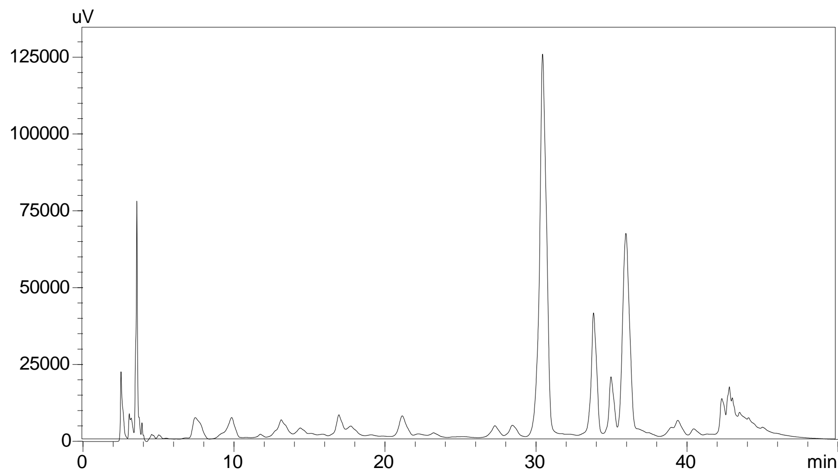

Figure 17: HPLC analysis for sample **A0**

From the Chromatogram

For **A1**

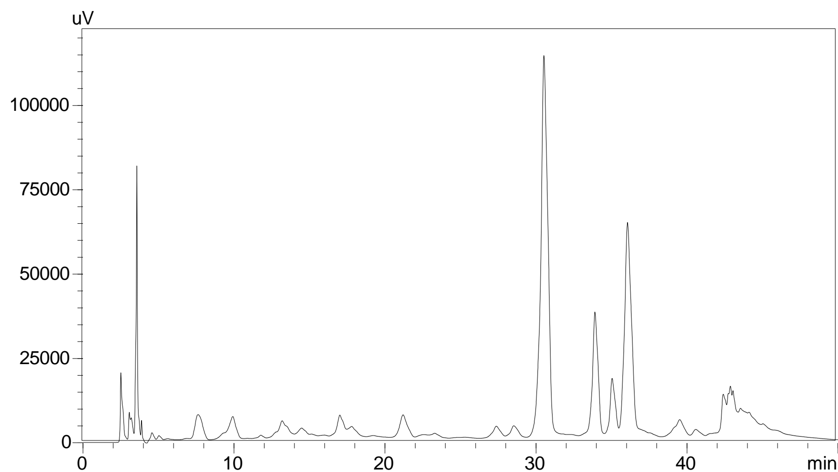

Figure 18: HPLC analysis for sample **A1**

For **A2**

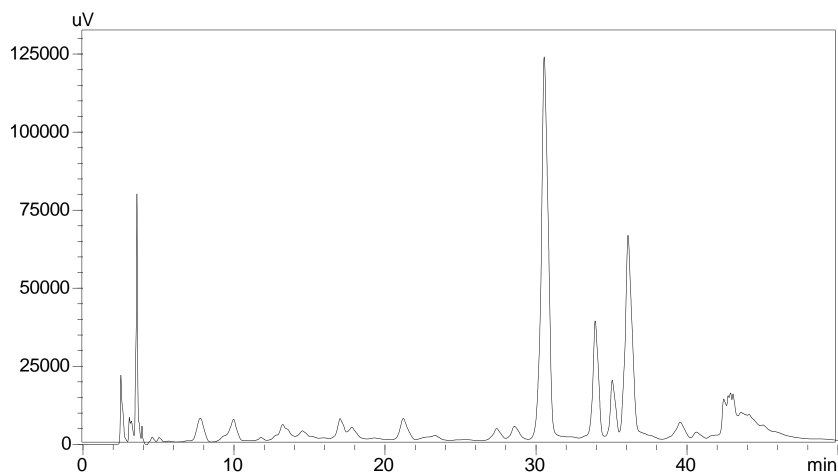

Figure 19: HPLC analysis for sample **A2**

For **A3**

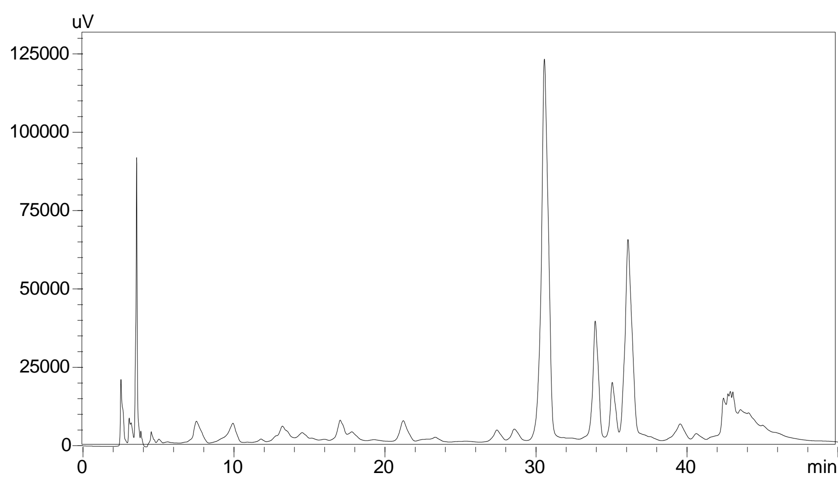

Figure 20: HPLC analysis for sample **A3**

For **A4**

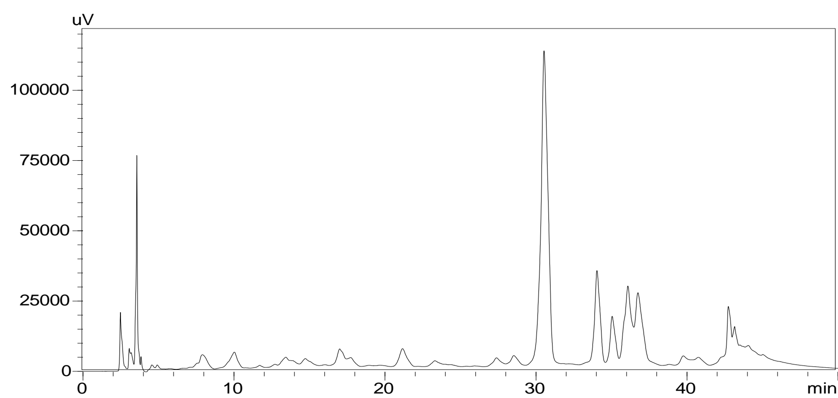

Figure 21: HPLC analysis for sample **A4**

IV.3. Results and Discussion:

The content of phenolic compounds in plant materials can be determined by several separation methods high-performance liquid chromatography (HPLC), Uv visible, Folin flavanols and flavonoids, as a set of individual substances or by a specific chemical reaction as a group of chemically similar reactive compounds

IV.3.1. Phytochemical composition:

By doing many methods to collect the quantity of polyphenols, we reach many results.

IV.3.1.1. phenolic compounds for the extract of leaves and vegetative shoots, and get this quantity for each sample

Table 8: The quantity of polyphenols in leaves extract with varying temperatures by Folin–Ciocalteu method

The sample A	**A0**	**A1**	**A2**	**A3**	**A4**
The quantity of polyphenols mg/g	317.6	693	679	682	708.7

Table 9: The quantity of polyphenols in vegetative shoots extract with varying temperatures by Folin–Ciocalteu method

The sample B	**B0**	**B1**	**B2**	**B3**	**B4**
The quantity of polyphenols mg/g	840.4	522.2	584	583	531.4

For **A0** and **B0** they are the reference of other samples extract which they are without heat treatment, we find the quantity of polyphenols on vegetative shoots more than leaves in this situation, and for leaves the quantity is the least of all leaves extract, unlike the vegetative shoots which had the most polyphenols quantity.

After applying a heat treatment of 80°C (the samples **A1** and **B1**) the quantity of polyphenols augments for leaves and decrease for the vegetative shoots.

On 100 °C (the samples **A2** and **B2**) the quantity of polyphenols has been decreasing little bit for leaves extract and decrease with high value for vegetative shoots.

On 120°C (the samples **A3** and **B3**) for leaves, the quantity increases little bit and for vegetative shoots decrease with little value. for the last samples the set on 140 °C (**A4** and **B4**), the leaves get the most quantity compared to all leaves extract samples, and the vegetative shoots get the less quantity for all other samples from vegetative shoots.

IV.3.1.2. Flavonoids:

Table 10: The quantity of polyphenols in leaves extract with varying temperatures by flavonoid method

the sample A	A0	A1	A2	A3	A4
The quantity of polyphenols mg/g	136	188	32	5.6	364

Table 11: The quantity of polyphenols in vegetative shoots extract with varying temperatures by flavonoid method

the samples B	B0	B1	B2	B3	B4
The quantity of polyphenols mg/g	408	306	210	181.6	324

The result of this method is volatile for the leaves and vegetative shoots, in natural case which is without heat treatment the quantity of polyphenols on vegetative shoots more than the leaves.

For the leaves extract the quantity of polyphenols increase from A0 to A2 to decrease on A3 and increase again on A4.

For the vegetative shoots, the quantity decrease from B0 to B3 after it increase on B4.

IV.3.1.3. Flavanols:

Table 12: The quantity of polyphenols in leaves extract with varying temperatures by flavanols method

the samples A	A0	A1	A2	A3	A4
Polyphenols quantity	0.0176	0.3374	0.0134	0.0292	0.0242

Table13: The quantity of polyphenols in vegetative shoots extract with varying temperatures by flavanols method

the sample B	B0	B1	B2	B3	B4
Polyphenols quantity	0.0318	0.0152	0.0236	0.0114	0.00956

The result of this method is volatile for the leaves and vegetative shoots, stay the quantity of polyphenols on vegetative shoots more than the leaves.

For samples **A** the quantity of polyphenols increases after applying an 80 °C and decrease when applying 100 °C to back increase with 120 °C and finally decrease again on 14°C.

For samples B the quantity of polyphenols decreases after applying 80 °C and increase on applying 100°C to back again decrease on 120°c and 140°C.

IV.3.2. HPLC analysis:

For HPLC chromatograms: According to Chromatogram (Figure 7 to Figure 11) many changes appear on the structure of chemical compounds also their quantities.

From disappear to appear of chemical compounds on sample **A0** which is the sample without heat treatment and it's the reference for other samples have 0µg/ml from Gallic acid, 0 µg/ml from Chlorogenic Acid, 1631.668088µg/ml from Rutin and 1283.38255

From Quercetin, this is the natural and basic chemical composition for *Moringa Olfeira* leaves extract without any unnatural factors, after exposure to heat many changes on its structure has been seeing.

For **A1** which is the extract of leaves exposure to 80 °C the changes set on 2 chemical compound disappear Rutin and augment Quercetin by 1885.113197µg/ml

For **A2** the 2nd sample set on 100°C by compared with **A0** its new chemical compound has been appearing its Chlorogenic Acid with quantity of 3532.964074µg/ml and augment the quantity of Quercetin compared with **A0** but its decrease according to **A1**.

For **A3** the 3rd sample this one set at 120°C the change on this one is augmented on the quantities of Chlorogenic Acid and Quercetin with keeping lose Rutin.

For **A4** the 4th sample has been heated on 140°C much changes on it augment and decrease and appear, appear new chemical composition which is Gallic Acid and decrease quantity of Chlorogenic Acid according to **A3** and augment the quantity of Quercetin according to all samples **A0, A1, and A3.**

Table 14: The concentration of individual phenolic compounds in extract after heat treatments

Compounds **Quantity (µg/ml)**	**Samples**				
	A0	**A1**	**A2**	**A3**	**A4**
Gallic Acid	0	0	0	0	73.60874893
Chlorogenic Acid	0	0	3532.964074	5361.904762	4708.03908
Rutin	1631.668088	0	0	0	0
Quercetin	1283.38255	3168.495747	2309.981342	2729.288789	3616.260449

IV.3.3. IR spectroscopy:

For the samples A:

The sample A0: have many wavenumber, at 900 wavenumber have little stretch of C(3links) C-H, at 1041and 1090 wavenumber stretching and it's for C-O Alcohol, at 2885 wavenumber stretching for C-H alkane, at 2926 wavenumber stretching for C-H alkane, at 3334 wavenumber streaking for O-H Alcohol.

The sample A1: nothing changes on this sample all functions group are the same.

The sample A2: just appear new function group which is C-Cl Alkyl Halide at 679 wavenumber.

The sample A3: nothing changes, same function group of sample **A2.**

The sample A4: lost most the function group with save the Alkane C-H and appear new one which is C-C Aromatic.

For samples B:

The sample B0: same as sample **A0** has a function group C-O alcohol and C-H Alkane and C-H alkene.

The sample B1: nothing changes on this sample still have the same function groups C-O of Alcohol, C-H Alkane and Alkene.

The sample B2: just appear new function group which is C-H Alkane.

The sample B3: appear new function group its C=C of Aromatic.

The sample B4: lose C-H alkene and get C-H Aromatic.

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General conclusion:

In this study phenolic compounds in *Moringa Olfeira* extract, the thermal stability of extracts was investigated, the quantity of polyphenols in leaves extract increase with applying more heat on it, unlike the vegetative shoots whenever temperature increase the quantity of polyphenols decrease.

For Flavonoids, the effect of heat treatments volatile, increase with more temperature apply on leaves extracts just when it reaches to 120°C, its decrease with any more heat adds.

For the vegetative shoots decreasing with more temperature apply on the samples just when it reaches to 120°C stop decreasing and increase with more temperature adds.

for the Flavanols, the effect of heat on this one volatile with varying temperatures for the leaves extract, increase after applying 80 °C and decrease with a higher temperature which is 100 °C and increases again with applying 120°C to decrease a little bit with 140°C.

For the vegetative shoots, decrease after applying any temperature.

From the other hand, HPLC analysis shows that the Quercetin and Rutin had been founded in leaves extract without heat treatments, after applying some heat treatments the Rutin will disappear and the quantity of Quercetin will augments with more temperature apply unlike from 80°C to 100°C its decrease and back increase again, and get the best quantity in 140°C Chlorogenic Acid we get this one on applying temperature between 100°C and 140°C but the best quantity it was on 120 °C, Gallic Acid was appeared when applying a temperature more than 120°C.

ABSTRACT

This work was aimed to determine phytochemical composition and individual phenolic compound (HPLC) from *Moringa Olfeira* (leaves and vegetative shoots) and investigate their stability with heat treatment, by applying many varying temperatures which are 80 °C, 100 °C, 120°C and 140°C. The result before and after treatment found that the HPLC analysis show varying phytochemical composition. The Rutin acid decrease after applying any heat treatment and for Quercetin acid, the extreme quantity obtained at 140 °C. For Chlorogenic Acid, the high concentration obtained at 120°C for determine the quantity of phenolic compound using the Folin-Ciocalteu method show that before heat treatment the shoots have the extreme quantity, and after heat treatment this quantity was decrease.

For FTIR analysis, the results confirmed that the hydroxyl group of phenolic compounds was decrease with. Further study anti-bactrian and anti-activity was investigated.

Keyword

Moringa Olfeira, phenolic compounds, heat treatments, HPLC analysis, stability, phytochemical composition, Folin-Ciocalteu.

الملخص

تعرضنا في هاذا العمل الي استخلاص المركبات الفينولية من نبات المورينغا أولفيرا حيث كانت الاشكالية الاساسية هي معرفة تاثيرالمعالجة الحرارية على المركبات الفينولية و استقرارها, فقد عرضت العينات الى درجات حرارة متفاوة في القيمة وهي 80,100,120,140 درجة مئوية, وقد اظهرت النتايج باستخدام الة الاستشراب السائلي عالي الأداء قبل وبعد المعالجة الحرارية ان حمض الروتين يختفي بعد تعريض المستخلص لاي من درجات حرارة المذكورة, اما حمض الكروستين يوجد باعلى تركيز له في 140 درجة مئوية اما بالنسبة لحمض الكلوروجنيك وجد اعلى تركيز له عند التعريض لدرجة حرارة تساوي 120 درجة مئوية, ومن جهة اخرى باستخدام طريقة الفولان سيكالتو نجد قبل المعالجة الحرارية ان الاغصان تحتوي قيمة أعلى للمركبات الفنولية من الاوراق وبعد المعالجة الحرارية عند تطبيق درجة حرارة اقل من 140 يتناقص تركيزها. وقد تم التحقق من النتائج باستخدام الة الأشعة تحت الحمراء. مستقبلا يجب التحقق من اسقرار مضادات الاكسدة و مضادات البكتيريا.

الكلمات المفتاحية

المركبات الفينولية, المورينغا أولفيرا, المعالجة الحرارية, استقرار المركبات الفينولية, لاستشراب السائلي عالي الأداء, التركيب النباتي , طريقة الفولان سيكالتو .