FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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COMPARISON OF SOME BIOACTIVE COMPOUNDS OF FOUR MEDICINAL PLANTS

Awaz Faruq ABDULLAH

Master's Thesis

DEPARTMENT OF CHEMISTRY

Division of Biochemistry

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THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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This thesis was approved by the Administrative Board of the Graduate School on

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Signature

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Director of the Graduate School

DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Comparison of Some Bioactive Compounds of Four Medicinal Plants ” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

27 January 2022

Awaz Faruq ABDULLAH

PREFACE

This study titled "Comparison of Some Bioactive Compounds of Four Medicinal Plants " was presented as a Master Thesis in the Department of Chemistry, Institute of Science of Fırat University.

First I would like to thank Allah for the virtues of his blessing for implanting the soul of endurance and faith in me for completing this study, giving me strength and health, and facilitating the ways for me to accomplish my study.

I would like to express my deep gratitude and sincere thanks to my supervisor *Prof. Dr. Mehmet Yaman*, for his encouragement, guidance, professional suggestion, and much useful advice during this study.

I would like to express my sincere gratitude to my beloved family for their supports during my study until this thesis ended.

I appreciate the role of Firat University and the Faculty of Natural and Applied Science, Chemistry Department for giving me this great chance to study and get a certificate that will never be forgotten. Hope you all the best and delight.

I'd want to express my heartfelt thanks to my husband, Fakhir Hassan, who is always by my side and has invested greatly in being kind and patient in taking care of some of my obligations while I'm studying, as well as for his constant encouragement and enormous sacrifice. I'd like to express my gratitude to my parents for their unwavering moral and financial support in helping me achieve my life goals.

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ABSTRACT

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In this study, determination of the antioxidant activity, total phenolic content (TPC) and Total flavonoid content (TFC) as well as some bioactive compounds in four medicinal plants is aimed. The plants*: Urtica dioica, Peganum harmala, Citrullus colocynthis and Syzygium aromaticum clove* were subject to test. The extraction technique used in this study included maceration extraction by employing methanol as a solvent. The obtained values are between 0.2-574 mg/g for TPC, 0.4-8.4 mg/g for TFC, 11.3-484 mg/g for FRAP, and 123-1979 mg/g MCC. Further, HPLC was utilized to evaluate the extrcats obtained. THe bioactive compoubds were determined by using HPLC – DAD device.

Keywords: Herbs, antioxidants, HPLC/DAD method, Lycopenes, Beta-carotene, polyphenols

ÖZET

Dört Tıbbi Bitkideki Bazı Biyoaktif Bileşiklerin Karşılaştırılması

Awaz Faruq ABDULLAH

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Bu çalışmada, dört tıbbi bitkide antioksidan aktivite, toplam fenolik içerik (TPC) ve Toplam flavonoid içeriği (TFC) ile bazı biyoaktif bileşiklerin belirlenmesi amaçlanmıştır.

Bitkiler: Urtica dioica, Peganum harmala, Citrullus colocynthis ve Syzygium aromaticum karanfil teste tabi tutulmuştur. Bu çalışmada kullanılan ekstraksiyon tekniği, çözücü olarak metanol kullanarak maserasyon ekstraksiyonunu içermiştir. Elde edilen değerler TPC için 0,2-574 mg/g, TFC için 0,4 8,4 mg/g, FRAP için 11.3-484 mg/g ve 123-1979 mg/g MCC arasındadır. Ayrıca, elde edilen ekstreleri değerlendirmek için HPLC kullanıldı.

Biyoaktif bileşikler HPLC – DAD cihazı kullanılarak belirlendi.

Anahtar Kelimeler: Otlar, Antioksidanlar, HPLC/DAD yöntemi, Likopenler, Beta-karoten, Polifenoller

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SYMBOLS AND ABBREVIATIONS

Symbols

Fe : Iron
I_2 : Iodine crystals
B : Bath length
C : Concentration (Concentration providing)
IC_{50} : 50%inhibition
Ɛ : extinction coefficient

Abbreviations

Ac : Absorbance of sample
Alco.ext. : Alcoholic extract
AO : Antioxidan
AA : Antioxidant activity
D.W : Distilled water
EDTA : Ethylenediaminetetraacetic acid
FRAP : Ferric reducing antioxidant power
F. ext. : Flavonoid extract
DPPH : Free radical-scavenging activity (diphenylpicryl hydrazine)
GPX : Glutathione peroxidase
HPLC : High-performance liquid chromatography
IR : Infrared
i.d : Internal diameter
O.D : Optical density
MCC : Metal Chelating Capacity
PTLC : Preparative thin-layer chromatography
ROS : Reactive oxygen species
RP : Reducing power
R_f : Retention factor
R_t : Retention time
SMs : Secondary metabolites
SD : Standard deviation
SOD : Superoxide dismutase
TLC : Thin -layer chromatography
TFC : Total flavonoid content
TPC : Total phenol content
TEAC : Trolox equivalent antioxidant capacity
UV : Ultra-violet
V : Volume
HPLC-DAD: High Perpormance Liquid Chromatography with diode array detection

1. INTRODUCTION

Plants provide a range of resources that help people meet their basic requirements for food, clothes, and shelter.

1.1. Historical background

Medicinal and aromatic plants are among the most economically important plants, and they have long been used as therapeutic medicines. Secondary metabolites, which are natural products of metabolism, were first characterized around the turn of the nineteenth century, and the art has since become a part of molecular biology.

Hundreds of plants were analyzed for bioactive metabolites, yielding a large number (35000) of bioactive metabolites. There were roughly 120 pharmacological formulations produced from traditional medicine in the late 19th century, and this number is growing as customers demonstrate a preference for naturally derived pharmaceuticals and bacteria continue developing antibiotic resistance [1]. People are searching for non-drug solutions to symptoms that including arthritis, allergies, insomnia, headaches, anxiety, and depression.

Herbal medicine is one of the most significant aspects of traditional medicine globally. To promote safe herbal medicine use and to assess their potential as a source of novel medications. It is critical to conduct more extensive research on therapeutic plants with a traditional reputation [2]. Plant natural products with higher levels of antibacterial chemicals could allow a new source of antimicrobial compounds. with potentially novel molecular pathways [3]. Unlike synthetic medications, plant-based antimicrobials have few side effects and offer a huge therapeutic promise for treating a variety of infectious disorders [4].

Plants, fungi, bacteria, insects, and animals have all created natural compounds that have been identified as bio-active medicines. Natural products or their products are widely known in the pharmaceutical sector for their extensive structural variety as well as their large range of pharmacological activity, accounting for around one-third of the world's top-selling drugs. Traditional, empirical, and molecular approaches to treating infections have yielded new medicines. This is an age-old practice that was perhaps the only method available in the past.

Conventional medicine is utilized by more than 80% of the world's inhabitants for basic health care, following the World Health Organization (WHO). Herbal medicine has a long history in Asia as a result of human interactions with the environment. Traditional medicine plants offer a variety of chemicals that can be utilized to treat both chronic and infectious ailments [5]. Synthetic medications are resistant to many infectious microorganisms, necessitating the development of alternate treatment.

In the last two decades, there has been a surge in research into natural materials in the hopes of discovering new and better pharmaceuticals [4]. Medicinal plants are also utilized in the bakery, confectionery, alcoholic beverages, food, soft drinks, and pharmaceutical industries for flavorings and scents [6]. Many chemicals with complex molecular structures are synthesized by plants as part of their secondary metabolism, and some of them have been linked to antibacterial characteristics observed in plants as well as their derivatives, these substances include alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes, and phenolic compounds are examples of these chemicals. The antibacterial activity of medicine has been studied using diffusion and dilution methods. Plants also produce a variety of substances that can be utilized to defend against various diseases. Phenols or their oxygen-substituted derivatives make up a large portion of these compounds. They are considered to be secondary metabolites. Plants are collected either at random or in response to leads provided in geographical localities where the plants are found, by local healers [7].

1.2. Phytochemistry and secondary metabolites

Plant chemistry, often described Phytochemistry, has developed in recent years as a different science that sits midway among natural product organic chemistry and plant biology, and is closely connected to both [8] . Plant species are estimated to comprise between 250,000 and 500,000. Plants make a huge number of different chemicals that help them adapt to their surroundings. These classes of compounds have been referred to as secondary metabolites, secondary products, natural products, phytochemicals, and antinational factors in general [8].

Plants produce both primary and secondary metabolites, which are organic substances that are synthesized by them. Many molecules, including phytosterol, acyl lipids, and nucleotides, are primary metabolites with important functions in photosynthesis, respiration, growth, and development. Secondary metabolites, on the other hand, are not included in the primary biochemical activities of cell growth and reproduction. which are not essential for the viability of a cell organism [9]. They don't generate energy, but they are critical for carbon fixation and reduction via photosynthesis, glycolysis, fermentation, and the tricarboxylic acid cycle. Secondary metabolites in plants appear to play an important function in protecting them from herbivores and microbial disease.

It's employed as a pollinator and seed-dispersing animal attractant, an allopathic agent, and a UV protectant [8]. Figure 1.1 shows the synthesis of many kinds of secondary metabolites from primary metabolites. Acetyl coenzyme A and mevalonic acid are important components in the production of terpenoids. The shikimic acid or malonic acid pathways are used to make phenolic compounds. Biosynthesis of nitrogen-containing secondary metabolites like alkaloids is primarily based on amino acids [10].

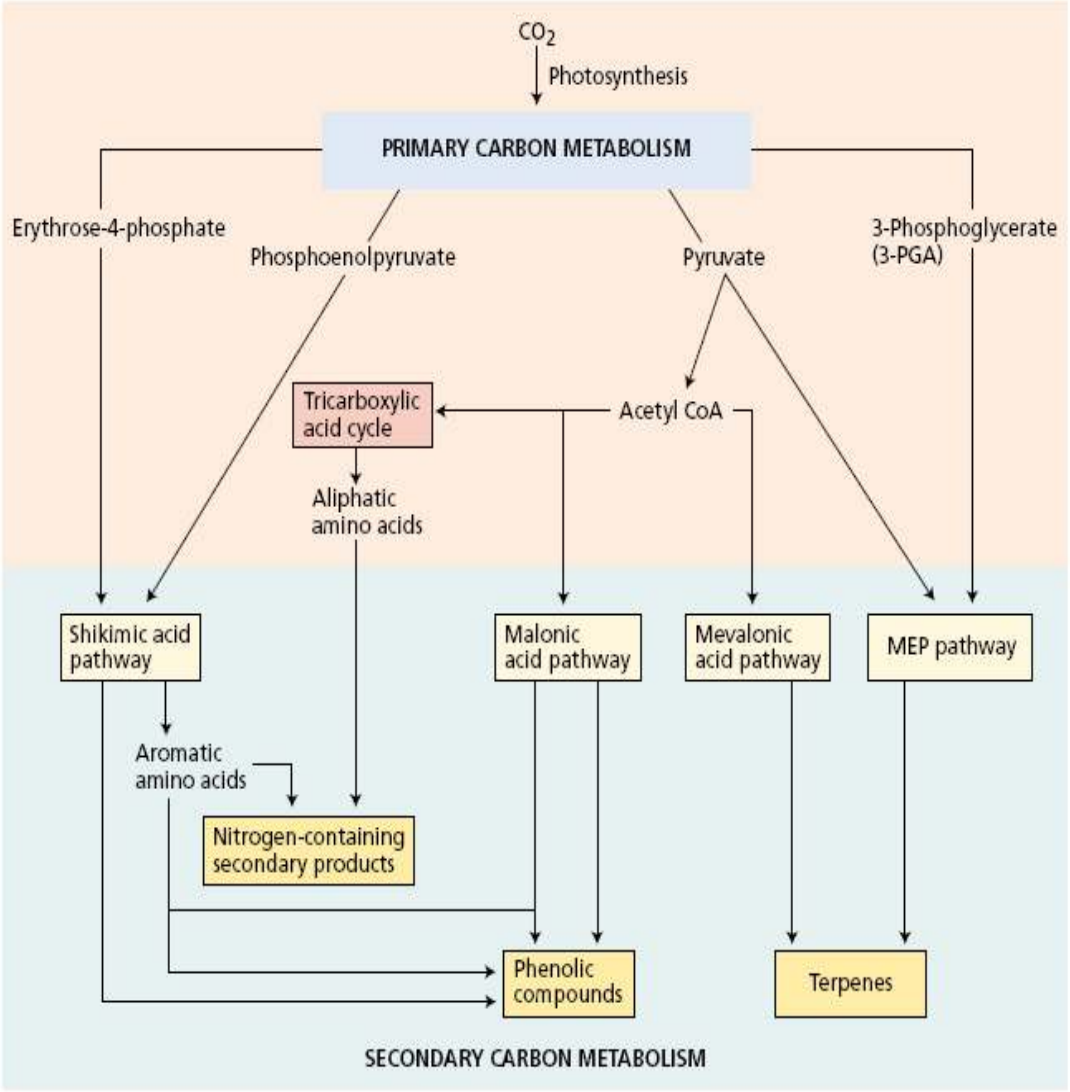


Figure 1.1. Pathway happenings participated for the metabolites biosynthesis plant cells [10]

Plant SMs canvass divided into three main bunches built on the biochemical sources of secondary metabolites:-

1. Polyphenolic compounds.
2. Terpenoids.
3. Nitrogen-containing compounds (alkaloids) [8].

1.3. Polyphenolic compounds

Polyphenolic substances have at a minimum one aromatic ring with one or further hydroxyl groups connected to them [11]. The amount and arrangement of carbon atoms in phenolic compounds. It's possible to split it into two clusters. (non-flavonoids and flavonoid phenolic compounds) [8, 12].

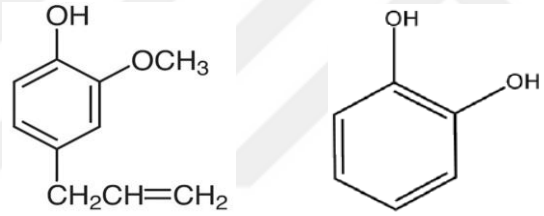
Non – flavonoids are the C_6–C_1 phenolic acids, most particularly gallic acid, that is a precursor of hydrolyzable tannins, the C_6–C_3 hydroxycinnamates, also the polyphenolic C_6–C_2–C_6 are stilbenes, A most paramount non-flavonoids with nutritional significance [8].

1.4. Major groups of secondary metabolites in medicinal plants

Secondary metabolites are substances that plant output to assist them to compete in their climate. These minuscule chemicals have an assortment of impacts on both the plant and other living things.

1.4.1. Simple phenols and phenolic acids

The simplest phenols (Fig.1.2), are one of the main and diverse classes of plants that have their structures contain a minimum of one aromatic ring and one or many hydroxyl groups C_6H_5OH [13, 14].

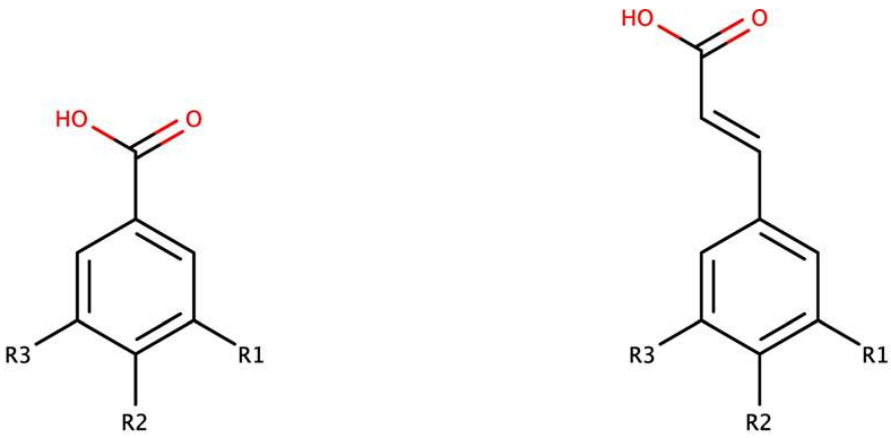


eugenol **catechol**

Figure 1.2. Phenols – the simplest of the phenols.

It may be split into two categories: soluble chemicals such as flavonoids, phenylpropanoids, and quinones, which exist in the plant cell's vacuole, and insoluble compounds such as lignins, hydroxyl cinnamic acid, and condensed tannins, which are found inside of the cell wall [15]. Secondary metabolites classified as phenolic compounds are an important group. Phenolic acid and flavonoids are among them, with the latter being a particularly varied class. Phenolic acids are found in fruits, vegetables, and other plant products that are exhausted and taken in a regular diet daily. It's common knowledge that consuming a diet rich in vegetables and fruits will help protect you from dangerous diseases like cancer. Higher plants produce thousands of distinct phenolic content, also with the amount of completely stated compounds increasing all the time. Glycosides and esters amides of hydroxycinnamic acids, glycosylated flavonoids, notably proanthocyanidins and flavonols, are found in vascular plant leaves. Polymers with phenolic properties include lignin, pollen sporopollenin, and suberin. Many soluble phenolic compounds, such as chlorogenic acid, are widely dispersed; however, because the distribution of many other structures is restricted to specific species or groups, they are used as taxonomic biomarkers [15].

The hydroxycinnamic acids, which are extensively dispersed in the plant world, are a significant family of phenolic chemicals. Caffeic acid is the much more prevalent hydroxycinnamic acid, that is usually found in foods as chlorogenic acid. ester with quinic acid (5-caffeoylquinic acid). In vitro, chlorogenic acid and caffeic acid are the most prevalent antioxidants [16]. Plant polyphenols are aromatic hydroxylated substances that are sources of antioxidants and are willing to take responsibility for several of the taste, flavours, and colors found in a variety of foods that make up a large part of with us nutrition, as well as being also one of the most effective and medicinally able biologically active subjects. The redox characteristics of phenolic compounds are primarily responsible for their antioxidant action. Because they can absorb and neutralize free radicals, as well as quench singlet and triplet oxygen and break down peroxides [8]. They can help block oxidative-related illnesses like atherosclerosis, cardiovascular and neurological diseases, and cancer [17]. Phenolic acids are a type of secondary metabolite that belongs to a broad family of phenolic chemicals found throughout the plant kingdom. They are significant food components that contribute to the flavor, color, and nutritional characteristics of the meal. Phenolic acids are divided into two groups based on their chemical structure: Derivatives of benzoic acid and cinnamic acid Fig.1.3. Plants manufacture hydroxycinnamic acids (HCAs) from phenylalanine via cinnamic acid or directly from tyrosine via tyrosine ammonia-lyase, generating p-coumaric acid, which can then be transformed into caffeic, ferulic, and sinapic acids. [18, 19].



1, R1 = R2 = R3 = H, Benzoic acid
2, R1 = R2 = R3 = OH, Gallic acid
3, R1 = R2 = OH, R2 = H, Protocatechuic acid
4, R1 = R3 = H, R2 = OH, *p*–Hydroxybenzoic acid
5, R1 = OCH_3, R2 = OH, R3 = H, Vanillic acid

6, R1 = R2 = R3 = H, Cinnamic acid
7, R1 = R2 = OH, R3 = H, Caffeic acid
8, R1 = R3 = H, R2 = OH, *p*–Coumaric acid
9, R1 = OCH_3, R2 = OH, R3 = H, Ferulic acid
10, R1 = R3 = OCH_3, R2 = OH, Sinapic acid

Figure 1.3. Structures of benzoic acid (1), common hydroxybenzoic acids (2–5), and common hydroxycinnamic acids (6–10)

1.4.2. Quinones

Quinones are aromatic rings with two ketone replacements that are widely distributed in nature and famously reactive. These vibrant chemicals produce browning in cut or damaged fruits and vegetables and are a component of the human skin's melanin synthesis process [20]. They are present in henna, which provides it with its colored capabilities. Vitamin K is a naphthoquinone with a complicated structure. Its ability to oxidize in bodily tissues may explain its antihemorrhagic properties [21]. For that reason, quinone antimicrobial actions have a wide spectrum of potential. In the microbial cell, surface-exposed adhesions, cell wall polypeptides, and membrane-bound enzymes are all potential targets. Quinones can indeed inhibit it difficult for bacteria gaining access to substrates. Quinones (Fig. 1.4), like other plant-derived antimicrobials, need to be thoroughly researched for any potential side effects [22].

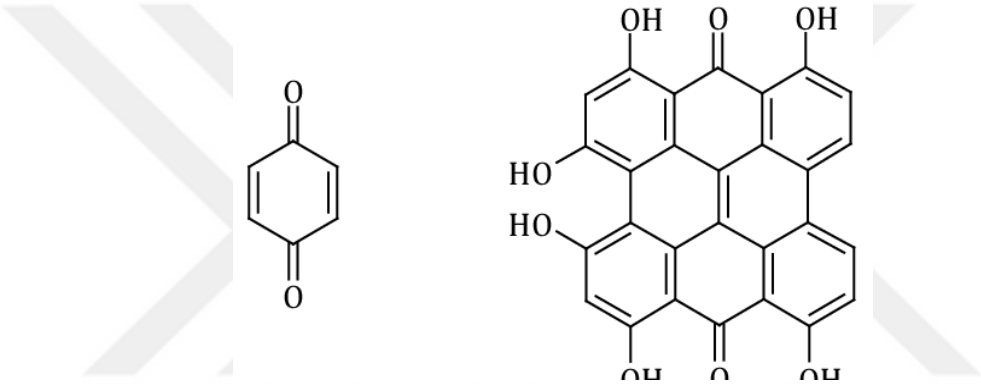


Figure 1.4. Structures of quinone and hypericin

1.4.3. Flavones, flavonoids, and flavonols

Flavones are monocarboxylic phenolic compounds with only one carbonyl group (as adverse to the two carbonyls in quinones). A flavonol is made by adding a 3-hydroxyl group to a compound. Flavanones are prominent by the lack of 2:3 C=C double bonds and the presence of a chiral center at C_2 Figure 1.5. In the enormous amount of natural sources flavanones, the C-ring is connected to the B-ring at C_2 in the -configuration. Hesperetin-7-O-rutinoside (hesperidin) is the most prevalent flavanone glycoside, which is present in citrus peel. Flavonoids are hydroxylated phenolic compounds that are connected to an aromatic ring by a C_6-C_3 unit. Plants are designed to produce these compounds in responding to microbial contagion. It's no surprise that they've been proven to be an effective antibacterial agent against a wide range of microbes in vitro. Their ability to bind to proteins and other macromolecules and soluble proteins, as well as to attach to bacterial cell walls, as quinones do, is most likely the reason for their effect. Microbial membranes may be disrupted by more lipophilic flavonoids. Flavonoids are phenolic chemicals derived from a variety of vascular plants; there are over 8150 flavonoids known. Flavonoids are plant substances that can be present inside cells or on the exterior of different plants parts, so they have a wide range of functions [23].

In plants, they act as antioxidants, antimicrobials, photoreceptors, visual attractors, dietary removers, and sunlight security checks [24]. In many studies, flavonoids have been shown to have antioxidant, genotoxic, antitumoural, antiretroviral, antibacterial, cardioprotective, hepatoprotective, neuroprotective, antimalarial, antileishmanial, antitrypanosomal, and antemedial effects [25].

1.5. Classification of flavonoids

Flavonoids are divided into two subclasses (anthoxanthins and anthocyanidins) number of hydroxyl groups, aromatic ring conjugation, nature, degree of oxidation and unsaturation of the C-ring, number, and position of glycoside and methoxy substituents attached to the benzene rings, and number and arrangement of glycoside and methoxy methyl groups connected to the aromatic rings, as seen in Figure 1.5 [8, 26].

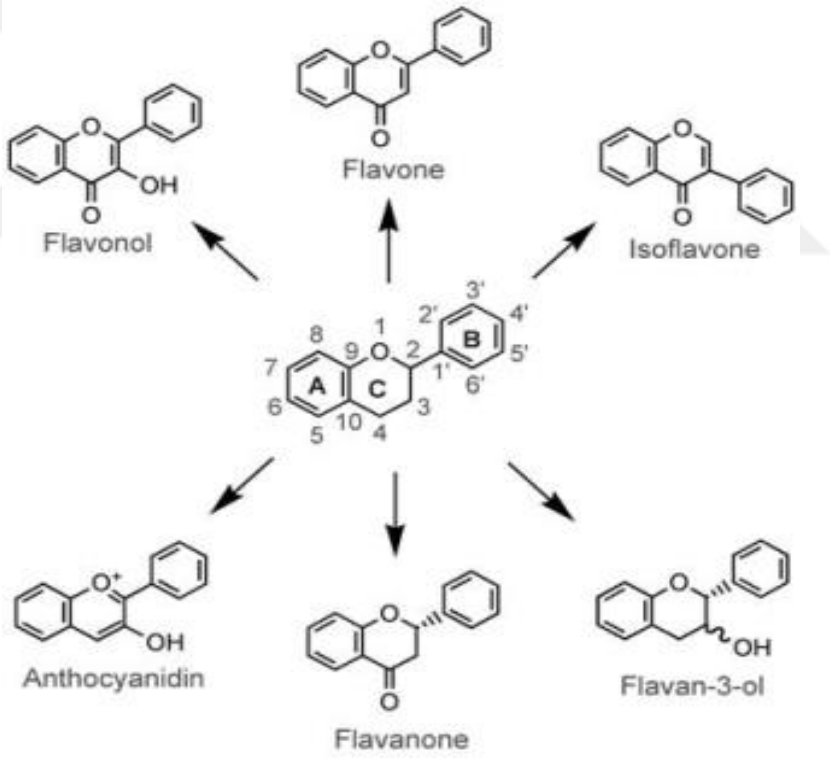


Figure 1.5. Generic structures of major flavonoids [8]

Flavonoids' ability to operate as antioxidants has earned them a prominent position in human health and medicine. Diets high in flavonoids, fruits, and vegetables are preventive against several ailments, including cardiovascular disease and certain types of cancer. Flavonoids' ability to transfer free radical electrons, chelate metal catalysts, is thought to be responsible for their protective actions in biological systems, antioxidant enzymes must be activated, reduce the amount

of alpha-tocopherol radicals, and oxidases are inhibited. The flavones and catechins have appeared to be almost operative in shielding the body from reactive oxygen sort. Flavonoids with biological action are known as bio flavonoids. They are capable of capturing superoxide, hydroxyl, and lipid radicals [27]. Flavonoids have been used medicinally for a long time, primarily to support healthy capillary and blood vessel function. They're advertised as anti-inflammatory and antispasmodic. [28]. Furthermore, flavonoid analogs and their metal complexes have important applications in agriculture, industry, and medicinal chemistry [29]. Subclass of flavonoids or (The best-known flavonols are as follows):

1.5.1. Quercetin

Quercetin (3,3',4',5,7-pentahydroxyflavone,) is a flavonol that cannot be manufactured by the human body and gets its name from the Latin word "Quercetum," which refers to Oak Forest " [30]. Its molar mass is 338.27 g/mol and its chemical formula is $C_{15}H_{10}O_7$. Quercetin, a flavonoid glycoside aglycone derived from plants, has been used as a nutritive health supplement and may help with a variety of diseases. Anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammatory activity, anti-diabetic, gastroprotective advantages, antihypertensive, antitumor, and anti-infective activities are only a few of the beneficial effects [30].

Quercetin (Fig. 1.6) can also protect against free radicals produced by the surroundings, such as those created by cigarettes. Cigarette tar contains free radicals, which have been found to injure erythrocyte membranes. Quercetin and its conjugate metabolites were also discovered to protect erythrocytes from smoking-induced cellular damage [31].

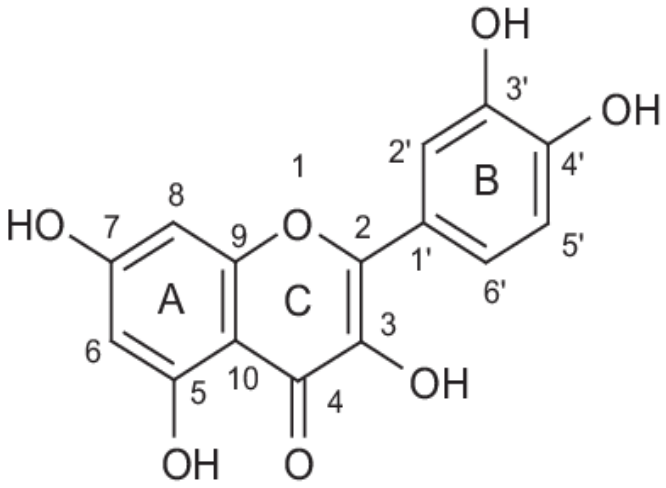


Figure 1.6. Chemical structure of Quercetin

Because of its widespread presence in herbs and food, quercetin, a kind of flavonoid known as flavonols, has garnered a lot of attention. Quercetin, like other natural flavonols, has been proven to have antiviral, anti-inflammatory, and antibacterial actions related to its antioxidant capabilities.

Benzopyran, an aromatic ring, and phenyl substituents compose constitute the bonded ring structure of quercetin Figure1.6 [32].

The antioxidant activity of quercetin is measured using it as a reference material [33]. Plants store quercetin as glycosides such as glucosides and rutinosides [34]. Quercetin is a natural antioxidant that plants by inhibits the enzyme xanthine oxidase, chelating iron, and directly clearance hydroxyl, peroxy, and superoxide radicals to reduce lipid peroxidation. Flavonols, such as quercetin, also aid in the preservation of the anti-oxidative advocate system by increasing the absorption of vitamin C [35].

1.5.2. Isoquercitrin

Isoquercitrin is a flavonoid that is abundantly found in plants. The structural difference between them as rutin derivatives is merely α-l-Rhamnosidases. Isoquercetin is one of the naturally happening glucosides of quercetin. Its molar mass is 464.38 g/mol and its chemical formula is $C_{21}H_{20}O_{12}$. Andhirsutrin is another name for quercetin-3-O-glucoside, quercetin-3-O-ß-D-glucoside, and quercetin-3-O-ß-D-glucoside. Isoquercetin is also known as isoquercitrin, a quercetin-3-monoglucoside that is essentially identical to isoquercetin. Although the two compounds are technically distinct (isoquercetin contains a pyranose ring, whilst isoquercitrin has a furanose ring), they are functionally identical [36].

Isoquercetrin is an important quercetin derivative since it is the substrate for another significant quercetin derivative, enzymatically modified isoquercetrin (EMIQ), as well as having a variety of biological actions [37]. The bioavailability of isoquercetin (quercetin-3-glucoside) was shown to be equivalent to quercetin-4-glucoside Researchers frequently use the terms to the two "glucoside" interchangeably. The current study also showed that isoquercitrin, which has a higher pharmacological activity than rutin, has a significant role in human body aging and diseases due to its special ability to act as an antioxidant.

In addition, isoquercitrin outperforms rutin in protecting injured cells by scavenging free radicals generated by H_20_2 and anoxia/hypoglycemia in cell culture liquid, as well as inhibiting free radical-induced cerebral injury in mice with cerebral ischemia and hepatic injury in mice with CCl_4 exposure [38, 39]. It also regulates blood glucose and cholesterol levels, as well as improving pancreatic islet function. [40].

1.5.3. Rutin

Rutin is one of the bioactive flavonoid molecules found in high concentrations in plants. Rutin has a wide spectrum of physiological functions, according to several studies [41]. Rutin is a lemon flavonoid glycoside establish in oats, also known as rutoside, quercetin-3-rutinoside, and derives primarily. Rutin (quercetin-3-O-rutinoside) is made up of aglycone and rutinose molecules of quercetin. Although rutin, isoquercitrin (isoquercetin), and quercetin have structural similarities, as shown in Figure 1.7, they are structurally different [42]. But it does have the molecular formula $C_{27}H_{30}O_{16}$ as well as a molar mass of 610.53 g/mol, it has very different physical, chemical, and biological properties [43].

Rutin is an important food component for human health because it positively affects capillary fragility and permeability [44]. Rutin, quercetin, and isoquercetin are flavonols found in many medicinal plants, including apple and onion, and are thought to contribute to the therapeutic properties of many botanical medications.

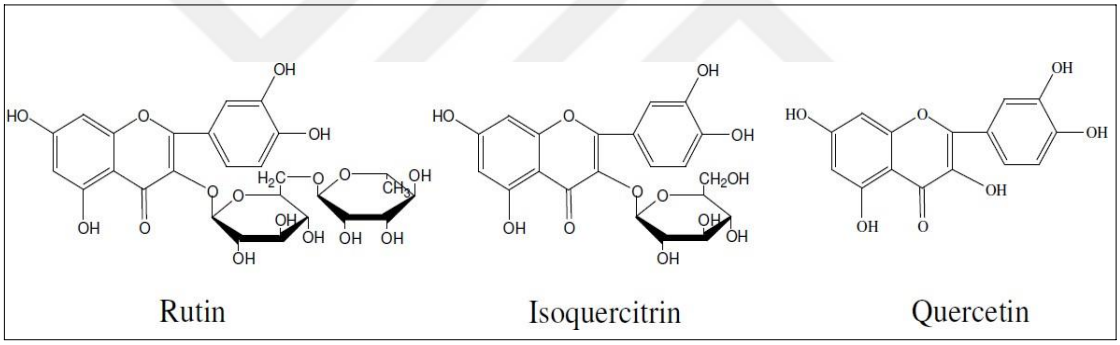


Figure 1.7. Chemical structures of rutin, isoquercitrin and quercetin [42]

1.5.4. Tannins

Tannin is a broad phrase for a kind of polymeric phenolic compound that can suntan skin or precipitate gelatin from solution, a property known as astringency. These proteins have a molecular masses variety from 500 to 3.000 [45]. They can be present in the bark, lumber, leaflets, fruits, and stems of the plant, among other places. Tannins are separated into two categories: hydrolyzable and concentrated tannins. The more compressed tannins there are the better. (also called proanthocyanidins) Flavonoid monomers combine to generate flavonoid polymers. Gallic acid is used to make hydrolyzable tannins, which are usually in the mode of numerous esters with D-glucose. Tannins are composed of flavan compounds that were condensed after being transported to plant fibrous tissues. It can also be synthesized through the polymerization of quinone units. Tannins are water-soluble oligomers with a molecular weight greater than 500 and the capacity to attach or precipitate proteins that seem to be soluble in water. The process of tanning animal skin

to make leather is referred to as tanning [8]. Tanniniferous plants are abundant in nature, and even though much attention has been paid to their study in recent years, the term "tannin" remains a difficult one to define precisely. Indeed, whereas similar phenolic substances like simple phenolics, neolignans, and flavonoids are classed and characterized based on their chemical structure, tannins are a heterogeneous set of molecules that are related primarily by their capacity to bind with proteins Figure 1.8 [46, 47].

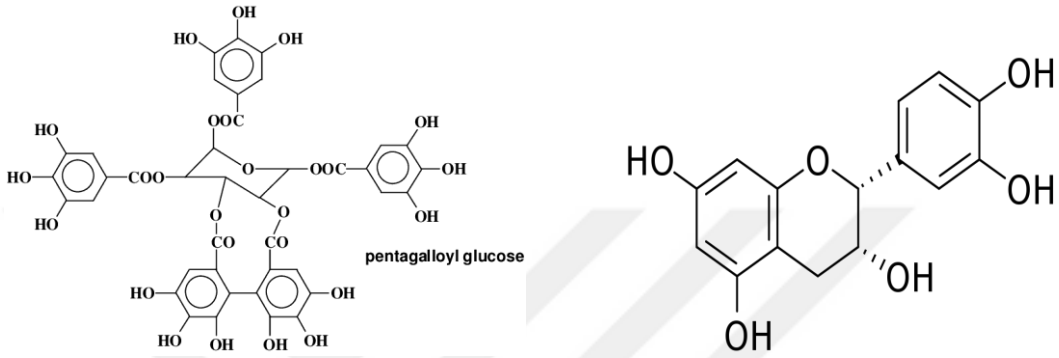


Figure 1.8. Pentagalloylglucose procyanidine B-2 (hydrolysable tannin) (condensed tannin) Tannins

1.5.5. Alkaloids

Alkaloids (Fig. 1.9) are organic heterocycle nitrogen constituents found in plant secondary metabolism, the majority of which are poisonous; they contain at least one heterocycle nitrogen atom.

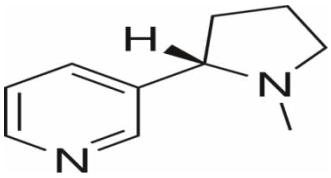


Figure 1.9. Alkaloids structure

The principal secondary metabolites with medicinal potential are alkaloids. By scavenging free radicals or binding with a catalyst, alkaloids can help to prevent a variety of degenerative diseases.

According to much research, alkaloids from many plants offer a broad array of pharmacological applications. Plant-based natural products have been utilized by humans for thousands of years as medications, nutrients, antioxidants, scents, tastes, pesticides, dyes, and pheromones to improve health. Alkaloids are among the most main classes of secondary

metabolites, with a diverse variety of biosynthetic routes and a structure that includes over 20,000 distinct chemicals establish in roughly 20% of all vascular plants discovered [48].

1.5.6. *β*-Carboline Alkaloids

β-Carboline alkaloids are a diverse group of natural and manufactured indole alkaloids with varying degrees of aromaticity, many of which can be obtained from plants in plants, sea animals, insects, and mammals. These compounds have recently gotten a lot of attention because of their intriguing biological and pharmacological features, such as their ability to interact with benzodiazepine receptors. The *β*--Carboline alkaloids, which can be contained in traditional medicines, cuisines, alcohol, and tobacco smoking, have a wide range of biological effects [49]. Numerous simple and complex-carboline alkaloids with saturated or unsaturated tricyclic ring systems have been isolated and identified as major bioactive components of numerous terrestrial plants over the last two decades. Reports on the isolation and characterization of simple-carboline alkaloids, including such nor Harman and Harman, up to 2003 were compiled. They've also been discovered in heat-treated protein-rich foods. Furthermore, in mammals as well as fishes, the intracellular formation of some harmane derivative products has been described. These chemicals (Fig. 1.10) are increasing toxicological importance due to their increased biological processes and harmful consequences on people's health, such as Parkinson's disease, alcohol addiction, and malignancy.

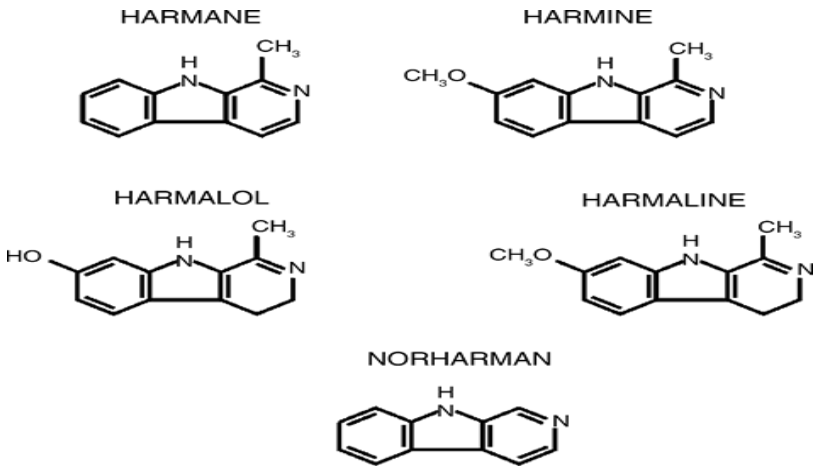


Figure 1.10. Several þ-carboline alkaloids of the harmane group are naturally present in plants and may occur in many plant-derived foodstuffs and beverages [50]

1.5.7. Terpenoids

Terpenes also referred to as terpenoids, are a broad group of plant-derived chemicals that can be divided into mono, di, tri, tetra, and sesquiterpenes dependent on the number of isoprene units.

Tea, cannabis, thyme, citrus fruits, and Spanish sage are common sources of plant terpenes, are these the fundamental constituents of plant-derived volatile oil. Terpenes have a variety of functions in plants, involving signaling pigments, solvents, taste, and medicinal applications [51].

Terpenes have anticancer, antibacterial, antiviral, antifungal, analgesic, antihyperglycemic, antiparasitic, and anti-inflammatory effects. Antioxidants are also used to increase skin permeability and avoid inflammatory illnesses. Terpene is a chemical compound that is used in medicine to make a variety of medications. Phytochemical constituents, anti-oxidants, protein modification reagents, and other terpenoid chemicals are produced by all plants in large amounts. Terpenoids are taxonomically restricted and have been connected to the attraction of organisms [52].

Terpenes, generally known as isoprenoids are arranged in the following head-to-tail arrangement. Squalene (an unsaturated hydrocarbon found in the human and sharks), as well as the side chains of Vitamins A, E, and K, are all terpenes. Terpenes are aromatic compounds that are used in cosmetics, insect repellents, pollination, perfume creation, and have a variety of therapeutic properties [53].

1.6. Antioxidants

Halliwell and Gutteridge (1999) defined an antioxidant as "any chemical that significantly slows or prevents oxidation of an organic substrate when present at low concentrations compared to those of that substrate." Many antioxidants protect the organism from oxidative harm when free radicals are produced in vivo. As the first line of defense, precautionary antioxidants like peroxidases and strong adsorption proteins reduce the formation of reactive oxygen radicals or free radicals. As a second line of defense, radical-scavenging antioxidants like multivitamins, ascorbic acid, and α-tocopherol scavenge radicals to prevent chain propagation and oxidation process activation. Due to a reaction between two radicals, these can apply to the ending chain's conclusion. Maintenance and autosomal recessive enzymes function as the last line of defense, repairing and synthesizing membranes. Among these are lipases, proteases, DNA mending enzymes, and transferases. Figure 1.11, shows the two components of the antioxidant defense mechanism in most cells: antioxidant enzymes (endogenous antioxidants) and small-molecule antioxidants (exogenous antioxidants) [54].

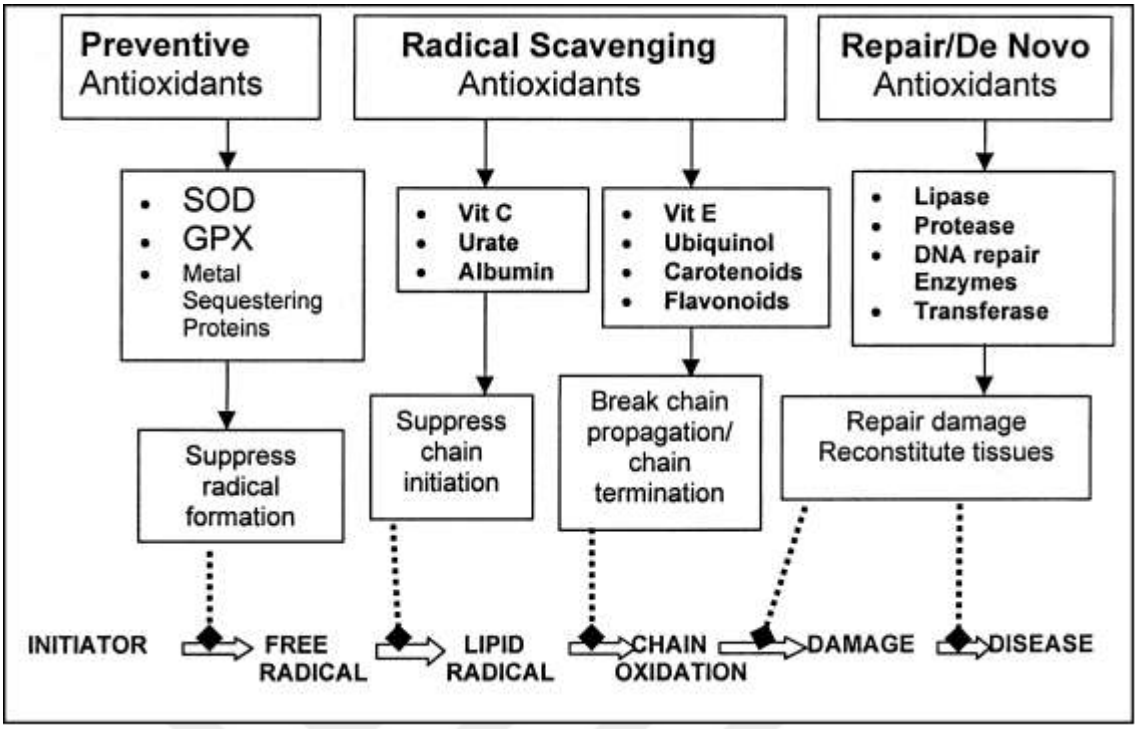


Figure 1.11. Antioxidant groups and actions [55]

1.6.1. Endogenous antioxidants

There is a big array of internal and external antioxidants that serve a variety of activities within each region of defense. H_2O_2 is changed to O_2 and H_2O by catalase, while the superoxide radical is converted to H_2O_2 and O_2 by superoxide dismutase (SOD) [56]. Some antioxidant enzymes have many versions, such as glutathione peroxidase, which has a cell wall, cytoplasmic, and blood manifestations, and SOD, which has a protective layer, cytosolic, and extracellular forms. The quantities and positions of these antioxidants should be carefully monitored for cells to survive. SOD, glutathione peroxidase (GPX), and catalase (CAT) are antioxidant enzymes that work within cells to eliminate the majority of oxidizing chemicals and sources of free radicals previously they react with metal ions and make extra reacting free radicals. Per oxidative chain events begun by free radicals that overcame antioxidant status are prevented by chain-breaking aqueous or lipid-soluble antioxidants [55].

1.6.2. Exogenous antioxidants

Diet plays an important part in the formation of antioxidant defenses by providing essential dietary antioxidants such as ascorbic acid, tocopherol, and β-Carotene. Flavanoids and fundamental minerals, among many other antioxidant plant phenols, form key antioxidant enzymes. The mineral that is found in SOD and selenium are found in glutathione peroxidase [55, 56].

Diet has an impact on the substrates that are oxidized; it is a significant factor of an oxidation reaction. A better illustration is the oxidation of lipids. Polyunsaturated fatty acids with two or many double bonds become much sensitive to free radical damage as the number of double bonds increases. [57]. Antioxidants present at the site of a radical assault interrupt the cycle of oxidation by being selectively oxidized by the attacking radical, inhibiting oxidation of the fatty acid nearby [55]. Given good conditions, the body might be in a stable state with free radicals created and quenched by the antioxidant enzyme. Oxidative stress arises when an endogenous and exogenous antioxidant system breaks down to balance off the free radicals produced in human cells. Lipids, proteins, and DNA can all be damaged by oxidative stress [58]. Antioxidants may reduce tissue oxidative damage in two ways: by improving natural cell defenses and/or by directly scavenging free radical species that are required for other molecules to perform specific activities [59].

1.7. Carotenoids

Plant carotenoids accumulate in the chloroplast of several organs and have important roles, especially in chlorophylls, where they aid energy transduction in light-harvesting complexes and play a role in photo defense mechanisms. Lycopene and β-carotene are mostly present in blossoms, where they aid in pollination and seed dispersal. Carotenoids are beneficial to human health. α-carotene is the most important predecessor of retinol or axerophthol, and β-cryptoxanthin has some pro-Vitamin A activity, although not as much as β-carotene. Plants and microorganisms, but not animals, produce carotenoids, a family of colored chemicals. They support the photosynthetic apparatus in plants and protect them from photo damage. As common chemical properties, Along the connected chain of the double bond, carotenoids have a polyisoprenoid configuration having approximately symmetrical around in the central double bond [60]. More than 600 carotenoids have been discovered in nature so far. A normal human diet should consist, however, only around forty are present. from the gore tissue of humans, around 20 of these 40 carotenoids have been found. β-carotene, α-carotene, lycopene, lutein, and cryptoxanthin account for around ninety percent of the carotenoids inside the food system and body. Carotenoids' antioxidant properties are the freelance intervention of pro-vitamin A act while are linked to their ability to bind singlet oxygen via conjugated double bond systems, with carotenoids with more than nine double bonds providing the most protection. Lycopene has better antioxidant effectiveness than β-carotene as a result of this. Lycopene andβ-carotene, on the other hand, can also have a pro-oxidant effect whenever there is a high oxygen partial pressure or a high concentration of carotenoids [61].

Carotenoids, which have antioxidant or provitamin A activity, seem to be well biologically useful natural compounds. The preparative technique, the kind of extraction solvent, and the method of their determination are all important factors in the detection and quantification of plant bioactive chemicals. The most common techniques for identifying and measuring natural

compounds are spectrophotometry and HPLC. The carotenoids were extracted in two organic combinations using the selection method, and the processing technique was utilized to improve extraction efficiency. [62] Figure 1.12. Demonstrates the structures of β-carotene and lycopene.

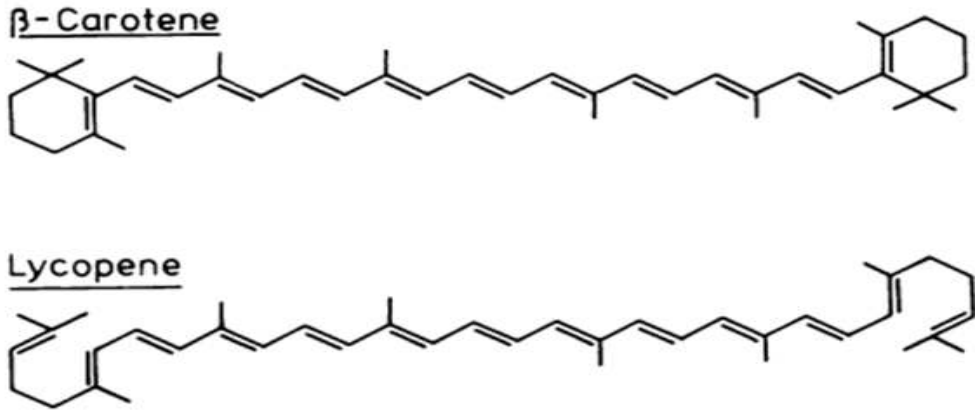


Figure 1.12 Structures of β-Carotene and Lycopene

2. LITERATURE REVIEW

Plant-produced bioactive chemicals have pharmacological or toxicological impacts on humans and animals. Although nutrients (e.g. vitamins and minerals) can have pharmacological or toxicological effects when consumed in large doses, nutrients in plants are not usually considered bioactive plant compounds.

2.1. Bioactive compounds in plants

Bioactive molecules are gaining popularity in a variety of fields, Geomedicine, plant physiology, industrial medicine, agricultural chemicals, aesthetics, foodservice establishments, nanomaterials, and other fields are only a few examples.

2.1.1. Definition of bioactive compounds in plants

"Bioactive" is a combination of 2 dialogues: bio- and active. The term "bio-" is derived from the Greek "bios" which meaning "living." But(–active), which is come from the Latin phrase "activist," means "dynamic," "energetic," or "vigorous." [63, 64], or it entails an action [63]. This activity encompasses all events that result in the manifestation of a living thing, a functioning, or a mechanism. [63]. The keyword "bioactive" is a synonym for "biologically active" in a purely academic understanding [63]. A bioactive chemical, basic sense, is a substance that has biological activity [63]. In medical terminology, a biological substance is described as a component that has an influence [63], a reaction's cause [63], or elicits a reply in [63] tissue that is alive If a compound (or substance) has a direct influence on a live organism, it is said to have biological activity. Depending on the drug, amount, and bioavailability, these effects might be beneficial or negative [63].

. Secondary metabolites are the primary sources of bioactive chemicals in plants. As a result, the following is a definition of plant bioactive compounds: a plant that is secondary [65].

Plants create a vast range of chemically varied substances called bioactive compounds (also known as defense bioactive compounds) in response to insect herbivores and microbial diseases. Plant-derived bioactive chemicals are widely employed in the cosmetic, food, and pharmaceutical industries [66].

2.1.2. Bioactive compounds from medicinal plants.

Medicinal plants have been at the core of many societies' healthcare systems. More than 60% of the world's population, including 80% of those in underdeveloped nations, rely on plants for their medicine [67]. Medicinal plants are now being taken into account as part of the global

biodiversity strategy. They are known as the "sleeping giant" and will continue to be a key source of medications since they are produced cheaply and provide a steady stream of novel goods that appear to be in short supply [67]. Botanical medicine, often known as herbal medicine, is the use of plant seeds, roots, berries, leaves, flowers, or bark for medical purposes. It has been used by people all over the world since prehistoric times to treat, control, and manage a range of conditions. Infectious diseases have become a primary cause of death worldwide, thus their research has become a global concernInfectious diseases have become a primary cause of death worldwide, thus their research has become a global concern [68]. The advent of multidrug resistance in microorganisms is putting many existing antibiotics' clinical efficacy in jeopardy. Throughout history, herbal remedies have been used to treat a variety of infectious disorders. Plant extracts, whether as standardized natural products or as pure chemicals, have provided limitless opportunities for novel medications due to their unrivaled chemical variety. For re-emerging and new infectious illnesses, there is an urgent and ongoing need for new antimicrobial agents with unique modes of action and different chemical structures [69].

2.2. Analysis of medicinal plants by HPLC

For the isolation of numerous natural compounds, the HPLC analytical technique is applied. HPLC is a chromatographic method used to detect, quantitate, and cleanse the mixture of the specific compound in phytochemical and analytical chemistry [70]. his methodology is presently gaining preferred as the advisable method for biometric investigations for herbal plant quality control, according to various analytical techniques [71]**.** Natural products are frequently extracted after a biological experiment is used to thoroughly describe the characteristics of a relatively crude extract. HPLC's resolving capacity is appropriate for the quick quantitative and sample preparation level production of manifold compound mixtures. Multiple authors address how to characterize and quantify secondary metabolites in plant extracts using high-performance liquid chromatography (HPLC) [72-75]. The separation of flavonoids has been aided by a variety of chromatographic and electrophoretic methods [76]. These approaches have been used to evaluate flavonoids in a variety of matrices, including edible plant parts, commercial products, and biological fluids in nature [77-80]. Due to the polarity, non-volatility, and efficient ionization of these samples, HPLC-ESI-MS/MS stands for high-performance liquid chromatography connected to a process called ionizing simultaneous mass spectrometry. shown tremendous promise due to its high reliability, unequaled reproducibility, and high sample throughput [81].

2.3. General description of plants and their pharmacological properties

Conventional medicine has been possessed from the commencement of time, and it will continue to be well-delivered and utilized by the general public. From the beginning of time, plants have been employed as a source of medicine.

2.3.1. *Urtica dioica*

Urtica dioica (stinging nettle), a common perennial weed found in temperate and tropical wasteland zones around the world, belongs to the *Urticaceae* family [82]. The *Urticaceae* family includes the herbaceous perennial flowering plant common nettle (*Urtica dioica*). Leaves of stinging nettle, in the shape of a grass infusion, are used in traditional Balkan herbal medicine to cure diarrhea, vaginal discharge, and internal/external bleeding [83].

Lecithin, carotenoids, flavonoids, sterols, tannins, histamine, serotonin, choline, and other chemical substances are found in this plant. It has a variety of pharmacological effects and applications, which include adjunctive treatment for prostate cancer [84], When the liver is subjected to ischemia, that has hepatoprotective benefits or tetrachloride of carbon, antidiabetic, the effect of tissue regeneration, In addition to other effects, hypocholesterolemic, anti-HIV, antibacterial, antifungal, anticancer, anthelmintic, and antiulcer [83].

The release of a combination of formic acid, histamine, acetylcholine, serotonin, and leukotrienes B4 and C4 from the trichomes of the plant's leaves and stem causes contact dermatitis when they are touched [85]. Various phenolics were discovered in U.dioica leaves by HPLC, including chlorogenic acid ($C_{16}H_{18}O_9$), rutin, quercetin 3-O-glucoside, kaempferol 3-O-rutinoside, and isorhamnetin 3-O-rutinoside [86]. (Urticaceae) grows in the temperate zone of the earth naturally. Protein, flavonoids, phenolic acids, terpenoids, alkaloids, fatty acids, vitamins, and minerals are all found in this plant [87]. Chlorophyll ($C_{55}H_{72}MgN_4O_5$), ascorbic acid($C_6H_8O_6$), phylloquinone, panthotene acid ($C_9H_{17}NO_5$), carotenoids, B group vitamins (thiamin (vitamin B1) riboflavin (vitamin B2), tannins(complex polyphenolic substances), essential oil, proteins, and minerals are all found in the stinging nettle leaf (Iron, Copper, Manganese, and Nickel) [88]. Acetylcholine and histamine($C_5H_9N_3$)are found in the hairs of stinging nettles [83], Flavonoids are found in the stem and root. When the skin comes into contact with the hairs and bristles on the leaves and stems of nettle, it can cause a painful sting. *Urtica dioica* has been recognized as a therapeutic plant in many nations around the world in recent years due to its significant impacts on human health world. The leaves of *U. dioica* are used to make an herbal tea that is utilized to treat some illnesses, counting high blood pressure while benign prostatic hyperplasia. The leaves involve Caffeic acid and chlorogenic acid [89].

2.3.2. *Citrullus Colocynthis*

Citrullus colocynthis belongs to the *Cucurbitaceae* family. These are mainly trailing perennial herbs. Wild on the sands of the North West, Punjab, Sind, and Central and Southern India, as well as the Coromandel Coast. Arabia, West Asia, Tropical Africa, and the Mediterranean region are also home to this species [90]. The Cucurbitaceae group provides one of the most biologically diverse clusters of restorative plants in the plant kingdom. Drought-tolerant plants, intolerant of moist, frost-sensitive soils, and waterlogging represent the bulk of the plants in this group [91]. *Colocynth,* bitter apple, and bitter cucumber are all names for *Citrullus colocynthis.* It's a desert viny plant that can be found in the Mediterranean and Asian regions. It has the appearance of a conventional melon plant, but it bears tiny, hard fruits with a bitter taste. The pharmacological effects of phytochemical substances have been studied using traditional screening methods. *Citrullus colocynthis* has been utilized in folk medicine and as a source of energy for millennia. Bio - power source and oil-seeds, for example. The leaves have a diuresis effect and are employed to cure jaundice and asthma. The root is used to treat breast inflammation, amenorrhea, rheumatism, and joint aches, as well as ophthalmia and uterine difficulties, externally [92].

The *C. colocynthis* contains a few fluid active compounds. The herb *colocynthis* was investigated. Saponins, sugars, tannins, glycosides, alkaloids, flavonoids, and essential oils are among the various types. Any portion of the plant, such as the leaves, roots, flowers, stems, fruits, and seeds, can be used to extract plant-based distinctive elements. Various secondary plant metabolites, such as flavonoids and cucurbitacins, have now been identified in *C. colocynthis* [93]. This plant has formerly been relied on to fight tumors, skin disorders, ulceration, influenza, pneumonia, bladder incontinence, expansion of the spleen, TB glands of the neck, dyspepsia, constipation, anemia, and throat, disorders have all been treated with this herb in the previous[94].

2.3.3. *Peganum harmala*

Peganum species can be found in African, the Arab World, Asia, Latin America, Mexico, and the southern United States, (family *Nitrariacea*)**.**

Coughs, rheumatism, Allergies, high blood pressure, hyperglycemia, and other maladies have all been treated using *Peganum harmala* seeds and the whole plant in Turkey, Iran, and China for centuries. From the seeds, leaves, flowers, stems, and roots of *P. harmala*, different chemical components including alkaloids, steroids, flavonoids, anthraquinones, amino acids, and polysaccharides have been isolated [95]. The alkaloids, predominantly β-carbolines such harmine, harmaline, harmalol, harmol, and tetrahydroharmine, have always been discovered to be the major chemicals accountable to *P. harmala's*. antibacterial, antidepressant, antinociceptive, analgesic, anticancer, and vasorelaxant actions.Plants that have medicinal properties are thought to have

significant exporters of novel chemical compounds with medicinal promise. The discovery of therapeutic agents, as well as new sources of commercial resources such as oil and gums, can be aided by understanding the chemical composition of plants. Alkaloids, tannins, flavonoids, and phenolic chemicals are the most important bioactive elements of these plants [96].

P. harmala, like many other medicinal plants, is considered to have allelopathic characteristics, which may help it dominate its ecosystems by becoming widespread in the southern United States [97]. Allelopathy is thought to impact species division and plenty within plant state, as well as add to the success of many exotic plants' invasions. Allelopathy is the term used to describe any indirect or directly adverse or positive impact that one plant has on another through the manufacture of chemical substances into the environment. Allelopathy is considered to influence species distribution and abundance within plant communities, as well as contribute to the invasion success of many exotic plants. Allelopathy refers to any direct or indirect harmful or beneficial effect by one plant on another through the production of chemical compounds that are released into the surrounding environment [98].

2.3.4. Sy*zygium Aromaticum Clove*

Cloves seem to be the parched, unopened floral buds of the Myrtaceae family's evergreen tree *Syzygium aromaticum*(Gaertn) Linn. They're employed more as a flavour enhancer, a spice for livening chewing tobacco, and as a component of betel chew. Aromatic, stimulating, and carminative, they're utilized to treat dyspepsia and stomach irritation. The major ingredient in *clove* is eugenol, which is harnessed as an antiseptic, anti-microbial, and analgesic in conventional healers. It is now employed as a flavoring additive in medicinal and food goods, as well as beverages. Eugenol's medicinal properties are well-known. It has recently been investigated for several interesting biological features. It is said to take part in phytochemical reactions and to have insecticidal, antioxidant, and anti-inflammatory properties [99].

Eugenol, a methoxyphenol constituent of clove (*Syzygium aromaticum, Myrtaceae)*, provides anti-inflammatory, painkiller, anesthetic, anti-pyretic, hypertensive, anti anaphylactic, antidepressant, anticonvulsant, antidiabetes, antimicrobial activity, and antitumor are all observed. Eugenol is extracted from a variety of plants, including *Syzygium aromaticum and Ocimum sanctum,* and has a variety of uses and benefits [100].

Syzygium aromaticum (Clove) is regarded as one of the most valuable spices in the world and has long been used as a food preservative and for a variety of medical uses. *Syzygium aromaticum* is a medium-sized (8-12m) tree that originated in Indonesia and was quickly spread to many other nations across the world. *Clove* is known for its high levels of phenolic chemicals such gallic acid, eugenol, and eugenol acetate. The major bioactive ingredient in clove is eugenol, which

is found in amounts ranging from 9 381.70 mg per 100 g of fresh substance vegetation to 14 650.00 mg per 100 g of fresh plant material [101].

Clove is a prominent exporter of phytochemicals like flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propane in the plant kingdom [101]. Among the phenolic acids, gallic acid has the highest content (783.50 mg/100 g fresh mass). Some gallic acid derivatives, like hydrolyzable tannins, are present in larger concentrations (2 375.8 mg/100 g) [102]. Other phenolic acids provided in *cloves* consist of caffeic, ferulic, elagic, and salicylic acids. *Cloves* include lower levels of flavonoids such kaempferol, quercetin, and their byproducts (glycosylated). *Clove* is an essential therapeutic plant because of the extensive spectrum of pharmacological properties it has accumulated over centuries of traditional use and documented in the literature. *Clove (buds)* exhibited the uppermost antioxidant activity and polyphenol concentration of any of the spices studied [103].

2.4. Aims of study

The utilization of complementary medicines has lately risen, enticing the interest of numerous researchers throughout the world. The present study involves the collection, extraction phytochemical evaluation. Each plant sample was randomly collected and shed at room temperature. The principal target of the research is to identify several bioactive compounds in four plants. *Urtica dioica*, *Peganum harmala*, *Citrullus colocynthis,* and *Syzygium aromaticum clove*. In addition, Determination of the (TPC), (TFC), (FRAP), and (MCC) are among the objectives of this study. Furthermore, the antioxidant activity of the produced plant extracts was investigated by comparing various methods, including β –carotene & lycopene content. This study was utilized to discovery the most active antioxidant extracts with the highest phenolic content, which were then submitted to a thorough chromatographic and spectroscopic estimate to determine the chemicals accountable for their high antioxidant activity.

3. MATERIALS AND METHODS

Our research was conducted in the analytical chemistry lab at Firat University in Turkey, where we used a variety of chemicals, glassware, and laboratory instruments. To achieve a reasonable and acceptable outcome.

 1.Scientific name: *Urtica dioica* Kingdom: Plantae - Plants Division: Magnoliophyta - Flowering plants Class: Magnoliopsida – Dicotyledons Order: Urticales Family: *Urticaceae* - Nettle family Genus: *Urtica L.* Species: *Urtica dioica L.* [104].	 2. Scientific name: *Citrullus Colocynthis.* Kingdom: *Plantae* Division: *Magnoliophyta* Class: *Magnoliopsida* Order: *Cucurbitales* Family: *Cucurbitaceae* Genus: *Citrullus* Species: *C. colocynthis* [105]
 **3.**Scientific name: *Pegnum harmala* Kingdom: Plantae Division: Magnoliophyta Class: Magnoliopsida Order: Sapindales Family: Nitrariaceae Genus**:** Peganum Species: *harmala* [106]	 4.Scientific name: Syzygium aromaticum clove Kingdom: Plantae Division: Magnoliphyta, Spermatophyta Class: Magnoliopsida Order: Myrtales Family: Myrtaceae Genus: Syzgium Species: aromaticum L [107]

Figure 3.1. Taxonomy of plants

3.1. Collection of plants material

In October 2020, in northern Iraq, Urtica dioica has been collected, dried in shade for two weeks, and finally ground in the electrical grinder to obtain fin powder. Parts and fruits of *citrullus colocynthis* were collected in early September 2020 during the flowering and fruiting stage in north Iraq. The mature seed (Fig. 3.1) was separated manually from the pulp of the plant and the seeds were separated manually from the pulp of the plant and then the pulp was dried in shadow and grind in a grinder into a coarse powder and prepared for analysis. The seeds of *Peganum harmala* were collected from Soran Iraq. Forestall the humidness impact and so hold on at room temperature till additional use. Dried *Syzygium aromaticum clove* samples were bought from the local markets of

Soran \ Iraq. Grinding was done for clove bud with the grinder in the laboratory of Researches. The samples were kept in closed containers after being chopped into small pieces (1mm).

3.2. Scientific classification of plants (taxonomy)

The science of discovering, recognizing, describing, categorizing, and naming plants is known as plant taxonomy. It's one of the most crucial features of taxonomy (the science that finds, describes, classifies, and names living things) Figure 3.1 taxonomy of plants.

3.3. Chemicals

The chemicals that were utilized in this study and their provisioner were abbreviated in Table 3.1.

3.4. Apparatus and equipment's (Instruments)

1. Sensitive Balance.
2. Grinding Machine.
3. Hot plate magnetic stirrer, Stuart.
4. Rotary Evaporator, Buchi Rota vapor-Re.((Buchi, Rotavapor R-100)
5. Micro wave.
6. Oven.
7. Incubator.
8. Shaker water bath, Lab Tech.
9. Water bath, Grant.
10. Vortex, Bibby.
11. Micropipettes
12. Disposable tips.
13. Centrifuge.
14. UV-Visible Spectrophotometer, UV Spectroscopy measurements were performed with UV-VIS (Isolab, Germany).
15. Spectrophotometer.
16. Coupled with a heating bath (B-100)
17. A vacuum bath (V-100) was used to evaporate solvent for sample preparations.
18. A microwave oven (Mars Express) was used for the extraction.
19. HPLC instruments.

Table 3.1. Chemicals

No.	Chemicals	Chemical structure	Company
1.	Methanol	CH_3OH	Carlo Erba (Sabadell, France)
2.	Hexane	C_6H_{14}	Carlo Erba (Sabadell, Spain)
3.	Acetic acid	CH_3COOH	Merck (Germany)
4.	Butanol	C_4H_9OH	Sigma-Aldrich (Germany
5.	Ethyl acetate	$CH_3COOC_2H_5$	Sigma-Aldrich (Germany
6.	Formic acid	HCO_2H	Sigma-Aldrich (Germany
7.	Carbon tetrachloride	CCl_4	Sigma-Aldrich (Germany
8.	Ammonia	NH_3	Carlo Erba (Sabadell, Spain)
9.	Chloroform	$CHCl_3$	Sigma-Aldrich (Germany
10.	Ethanol	C_2H_5OH	Carlo Erba (Sabadell, Spain)
11.	Quercetin	$C_{15}H_{10}O_7$	Sigma-Aldrich (Germany)
12.	Aluminium chloride	$AlCl_3$	Merck (Germany)
13.	Sodium acetate trihydrate	$CH_3COONa.\ 3H_2O$	Merck (Germany)
14.	Potassium ferricyanide	$K_3Fe(CN)_6$	Merck (Germany)
15.	Dipotassium hydrogen phosphate	K_2HPO_4	Merck (Germany)
16.	Phosphoric acid	H_3PO_4	Carlo Erba (Sabadell, Spain)
17.	Sodium dihydrogen phosphate	NaH_2PO_4	Carlo Erba (Sabadell, Spain)
18.	Potassium hydroxide	KOH	Merck (Germany)
19.	Sodium hydroxide	NaOH	Merck (Germany)
20.	Trichloroacetic acid	$CCl_3\ CH_3COOH$	Merck (Germany)
21.	Ferric chloride trihydrate	$FeCl_3.3H_2O$	Merck (Germany)
22.	Phenanthroline monohydrate	$C_{12}H_8N_2$	Merck (Germany)
23.	Ferric chloride	$FeCl_3$	Merck (Germany)
24.	Ferrous sulfate heptahydrate	$FeSO_4 \cdot 7H_2O$	Merck (Germany)
25.	Folin-ciocalteu's phenol reagent	……..	Merck (Germany)
26.	Sodium carbonate	Na_2CO_3	Merck (Germany)
27.	Gallic acid	$C_6H_2(OH)_3CO_2H$	Iso Lab (Eschau, Germany)
28.	Iodine crystals	I_2	

3.5. Preparation of plants extracts

Extraction is an essential step in the process of identifying bioactive components in medicinal plants.

3.5.1. Conventional solid/liquid extraction under stirring (Maceration)

In a grinder, the dried sample plants will be powdered individually. 10 grams of dried ground from each *Urtica dioica*, *Citrullus Colocynthis*, *Pegnum harmala*, and *Syzygium aromaticum clove*. The powdered sample plants were weighed and placed in a glass balloon with 75 ml methanol. A magnetic stirrer was used to continually stir the balloon at room temperature for 24 hours. Three times this procedure was repeated. Finally, all of the extracts were mixed and evaporated at 40 °C under 150 mbar using a rotary evaporator. The extract was defatted with hexane after the evaporation. The extract was then dried entirely in a vacuum oven after being air-dried first (Nuve 180). For further analysis, the dried extracts will be packed in tubes and kept in the refrigerator at 4–6 [108]. As shown in Figure 3.2.

Figure 3.2. Alcoholic extracts of plants at room temperature

3.5.2. Yield calculation

The ratio between the weight of the extracts obtained and the beginning weight of plants employed is the extraction yield [109]. The following formula is being used to estimate the yield as a percentage:

$$Y\,(\%) = 100\ m/m_0$$

Y: Yield in %.

m: masse of the extract.

m_0: initial mass of the plant.

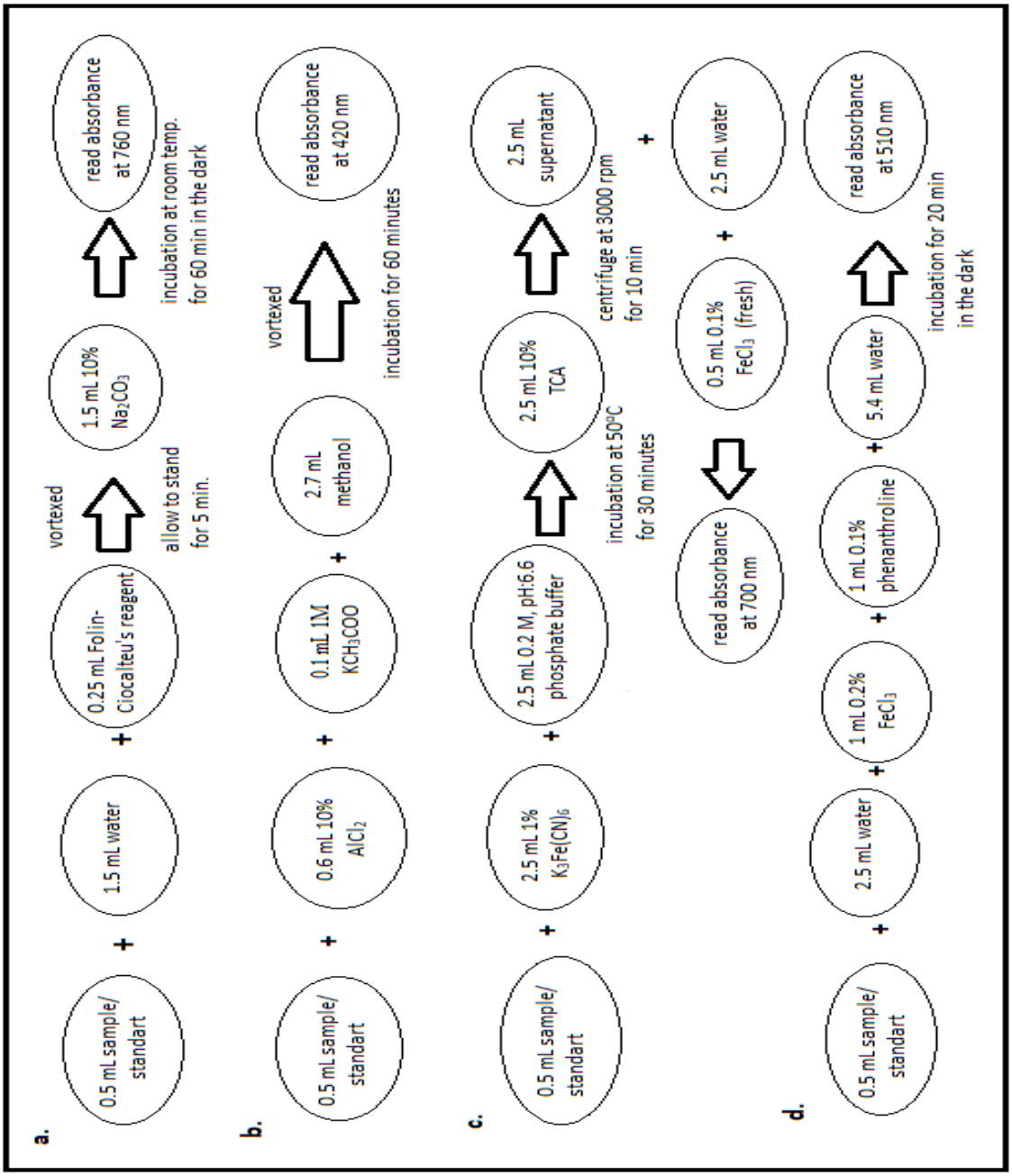


Figure 3.3. The experimental procedures of (a. TPC), (b. TFC),(c. FRAP), and(d. metal-chelating capacity assays) [108].

3.6. Phytochemical study

Samples preparations were made by dissolving 0.05 gm dried extracts in 5.0 mL methanol and agitating for 30 minutes at room temperature in the bath. The antioxidant content of the samples was evaluated using the solutions produced. As illustrated in Figure 3.3.

3.6.1. Total Phenolic Content estimation (TPC)

Total phenolic content in plant extracts was determined using the Folin-Ciocalteu colorimetric technique based on the oxidation-reduction reaction [110]. Figure 3.3a shows the total phenolic content assay technique. Gallic acid stock solutions have been obtained by dissolving (0.005g gallic acid in 5.0 ml methanol) in a bath at room temperature for 30 minutes continuously stirring. Gallic acid solutions in methanol at various concentrations. 0.5 ml of sample extracts/standard was added with 1.5 ml of distilled water to prepare the solution and 0.25 mL of Folin-Ciocalteau reagent (1:1 Folin: methanol) was also added. After vortexing, the tubes for 5 minutes, they were allowed to stand. Immediately, 1.5 ml of Na_2CO_3 (10%) was added to the mixture. Then the mixture was incubated for 1 hour at room temperature in the dark. A UV-Vis Spectrophotometer was used to measure the absorbance of all samples at 760 nm. The construction of the calibration curve (2.5, 5, 10, 20,50, 100, and 150 ppm) was obtained with gallic acid. The results are expressed in mg GAE/gdw (milligrams of gallic acid equivalents per gram of dry weight).

3.6.2. Total flavonoid content estimation (TFC)

Using the aluminum chloride colorimetric technique, the total flavonoid content of the sample was intended. [111-114]. The standard calibration curve for total flavonoid determination has been created using quercetin. The stock quercetin solution is prepared by dissolving (0.005gm quercetin in 5.0 ml methanol) in a bath for 30 minutes at room temperature, stirring continuously, subsequently, by serial dilutions in methanol were used to prepare the standard quercetin solution. With the use of quercetin, a calibration curve (1, 5,10,20,50,100 ppm) was constructed. A mixture of 0.5 ml diluted standard quercetin solutions or extracts, 0.6 ml of 10% aluminum chloride, 0.1 ml (1M Sodium acetate trihydrate $CH_3COONa \cdot 3H_2O$), also 2.7 ml methanol was added and thoroughly mixed. After mixing, the solution was incubated at room temperature for 60 minutes Figure 3.3b. At 420 nm, the absorbances were measured. The results are represented as mg QE/gdw.

3.6.3. Ferric reducing antioxidant power (FRAP) assay

Relied on their antioxidant principle, The methanolic extract's reducing power (FRAP) was determined to produce a colored complex with potassium ferricyanide [115].

In test tubes containing 0.5 ml sample or standard solutions (quercetin) were added 2.5 ml 1% potassium ferricyanide ($K_3Fe(CN)_{6)}$ and 2.5 ml (0.2 M phosphate buffer) (pH: 6.6), after the incubation for 30-minute at 50°C. Ferricyanide $[Fe(CN)_6]^{3-}$ can be converted to ferrocyanide $[Fe(CN)_6]^{4-}$ by reducing it.

Add-on 2.5 ml of 10% (w/v) trichloroacetic acid ($C_2HCl_3O_2$) and centrifuging for 10 minutes at 3000 rpm, the reaction was halted by addition. Finally, 2.5 ml of the upper part was mixed with 2.5 ml of D.W and 0.5 ml of newly prepared (0.1%of $FeCl_3$). Using a standard solution of (0.005gm quercetine in 5ml methanol), the calibration curve was created by using different quercetin concentrations between (15, 30, 50, 100, 150, 200, 300, 400, and 500 ppm). The absorbances were measured at 700 nm. The results are given in (mg) of quercetin equivalents per (g) of dry weight (mg QE/gdw) Figure 3.3c.

3.6.4. Metal Chelating Capacity (MCC) assay

A modified phenanthroline method were added in test tubes, 0.5 mL sample, or standard solutions (iron II sulfate). Then, 2.5 ml distiller water (D.W), 1 ml FeCl3, and 1 ml phenanthroline have been added to the admixture, which was then blended with 5.4 ml D.W. After vortexing thoroughly, the tubes were incubated for 20 min in the dark Figure 3.3d. The score is expressed as iron II sulfate equivalents on the dry plant basis (mg Fe2+/gdw). The calibration curve was obtained by utilizing (0.02gm FeSO4.7H2O in 20ml distilled water) different Fe2+ concentrations between (10, 20, 50, 100, 150, and 200 ppm). UV-V is a spectrophotometer that was used to measure the absorbance at 510 nm. The results are expressed in mg Fe^{2+} E/gdw (milligrams of Fe^{2+} ion equivalents per gram of dry weight) [108].

3.7. β –carotene & lycopene content

The desiccated extract was filtered through Whatman No.4 filter paper after being vigorously agitated for 1 minute with a 10ml acetone-hexane combination (4:6). The absorbance was measured at 453nm, 505nm, and 663nm. The contents of β-carotene and lycopene were calculated using the equations below[116].

$$\text{Lycopene (mg/100mg)} = -0.0458\ A_{663} + 0.372 A_{505} - 0.0806\ A_{453}$$

$$\beta\text{-Carotene(mg/100mg)} = 0.216\ A_{663} - 0.304\ A_{505} + 0.452 A_{453}$$

3.8. Thin Layer Chromatography (TLC) was employed for separation and identification

Thin-layer chromatography is a quick control process that includes a thin, homogenous layer of dried silica as the stationary phase, coarsely powdered and put on a suitable support, with a thickness of 0.25cm (aluminum paper or glass). By capillarity, the mobile phase or eluent spreads across the plate's surface [117]. The most efficient solvent systems are seen in Table 3.2.

Table 3.2. Solvent systems used as a mobile phase in TLC

Methanolic extracts	Migration solvents	Ratio
Urtica dioica	Butanol-Acetic acid-Distilled water	4V:1V: 5V
Citrullus Colocynthis	Ethyl acetate -Formic acid- Acetic acid-Distilled water	9V:1V:1V:2.3V
Pegnum harmala	CCl_4- Methanol -NH_3	10V:5V:0.08V
Syzygium aromaticum (clove)	Chloroform- Ethanol	4V:1V

3.9. Phenolic Compound Analysis by HPLC-DAD

Analysis of Phenolic Compounds: The qualitative and quantitative determination of polyphenols was carried out using a Shimadzu HPLC, equipped with Shimadzu DGU-20A5 model vacuum degasser and Shimadzu 20 ADXR solvent pump. Separations were performed using a Clipeus C18 5 µm reversed-phase column (250 mm × 4.6 mm). Detection was performed with a Shimadzu SPD-M20A photodiode array detector. To prepare analytically pure polyphenol standards, 1000 mg/L stock solutions were prepared by dissolving 0.010 g polyphenol into deionized pure water and completed with 10 mL methanol and water (1:1 v/v). By using these stock solutions standard solutions of each polyphenol were prepared daily. The determination of phenolic compounds was carried out by the HPLC-DAD system. In the determination by applying gradient elution, Solvent A: 4.5% acetic acid solution and Solvent B: acetonitrile was used as mobile phase. The elution conditions are given in Table 3.3.

Table 3.3. The gradient program applied by HPLC

Analysis time (min)	Solvent B (%)	Flow rate (mL/min)	Column temp. (°C)	Injection vol. (µL)	Wavelength (nm)
0.01	0	1	30	20	(271,275,279,283, 306,309,324,326 ,354, 371)
7	5				
12	15				
20	40				
25	100				
30	100				
40	5				

4. RESULTS

For the four medicinal plants, phytochemical experiments were done using or in the presence of different phytochemical analyses of the methanolic extracts, which yielded positive results for a variety of efficacy purposes.

4.1. Percentage yields of extracts

The process of extraction from 10g of plant materials with methanol made it potential to obtain a dry residue of methanolic extracts, and the results indicate that the yield of methanolic extracts of *(Urticadioïca*, *Citrullus Colocynthis*, *Pegnum harmala*, and *Syzygium aromaticum Clove*) to afford (1.1584gm,0.6945gm,1.928gm 3.0192gm). The percentage yield for all prepared extracts from all plants used in this study had been calculated. Table 4.1 and Figure 4.1 exhibit the mentioned results.

Table 4.1. The percentage yield of all extracts

Plants	Plant parts	Type of extracts	% Yield
Urtica dioica	*Leaves & flower*	Methanol	11.584%
Citrullus Colocynthis	*Fruits*	Methanol	6.945%
Pegnum harmala	*seed pod*	Methanol	19.28%
Syzygium aromaticum Clove	*Clove*	Methanol	30.192%

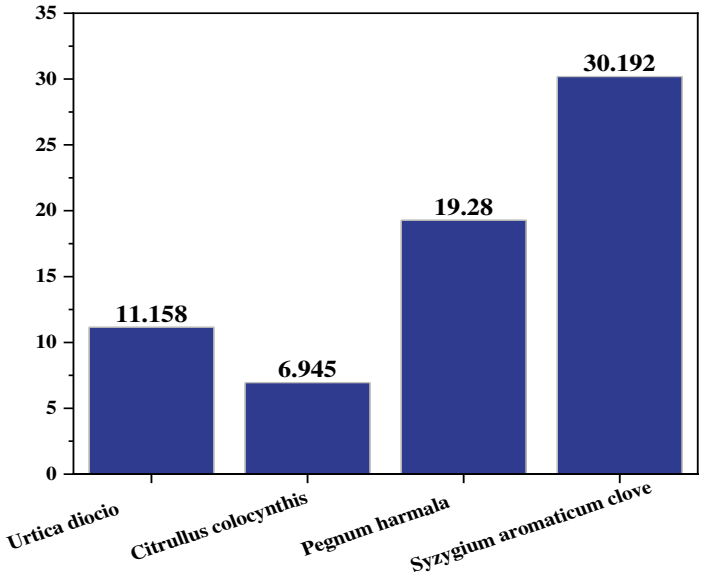


Figure 4.1. The percentage yield of all extracts

4.2. Phytochemicals Investigation

The number of phytochemicals found was counted. Drawing a calibration curve of TPC, TFC, FRAP, and MCC of the examined samples yielded results for Overall. ((Gallic acid, quercetin, and ferrous sulfate heptahydrate) and expressed in(mg GAE/g, mg QE/g, mg QE/g, and mg Fe E(II)/g) dry plant samples of extracts. Phytochemical screening shows the presence of the following chemical groups and the construction of calibration curve and calculation of TPC, TFC, FRAP, and MCC content, in different plant extracts. As shown in Table 4.2, and demonstrate this in Figure 4.2a, 4.2b, 4.2c, and 4.2d. Figure 4.2 shows the TPC, TFC, FRAP, and MCC in different plants extracts.

Table 4.2. Quantitative Phytochemical Analysis of plants extracts[1]

Methanolic extracts	Phytochemicals			
	TPC quantity (mg/g)	TFC quantity (mg/g)	F RAP quantity (mg/g)	MCC quantity (mg/g)
Urtica dioica	38.3 ± 5.2	4.8±0.65	47.6±6.2	422.6±5.5
Citrullus Colocynthis	0.23±0.05	0.43±0.07	11.2±17	123.3±14.7
Pegnum harmala	83.6±9.5	8.4±9.7	48.4±6.5	259.6±28
Syzygiumaromaticumclove	573.6±59.4	1.72± 0.23)	483.6±51.6	1979±255

[1]Values are mean ± standard deviation of triplicates.

(a)

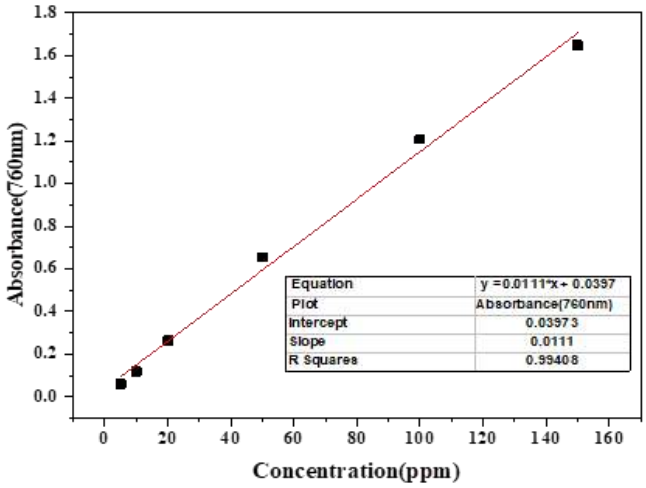
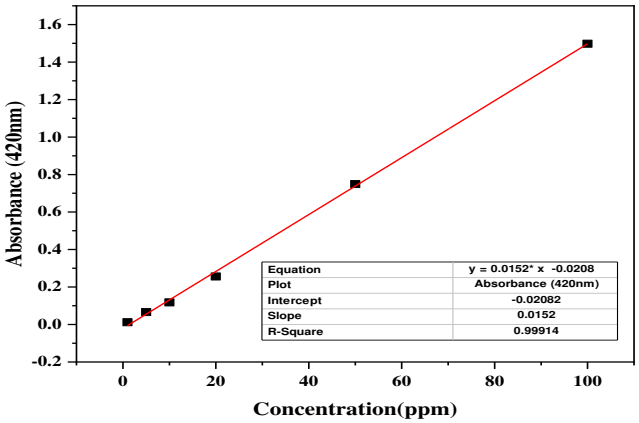
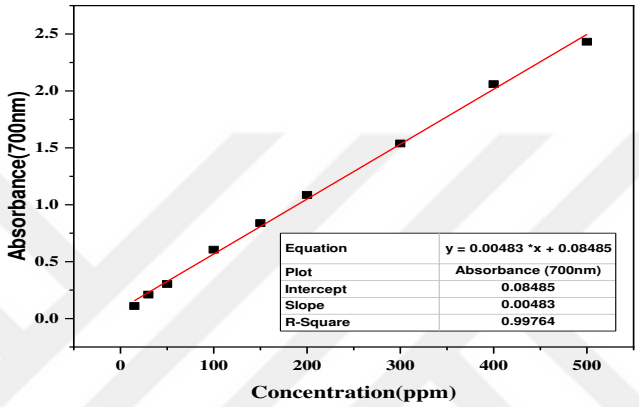


Figure 4.2. (a) Calibration curve for gallic acid, (b) Quercetin calibration curve for TFC (c) Quercetin calibration curve for FRAP, and (d) Calibration curve for Iron (II).

(b)



(c)



(d)

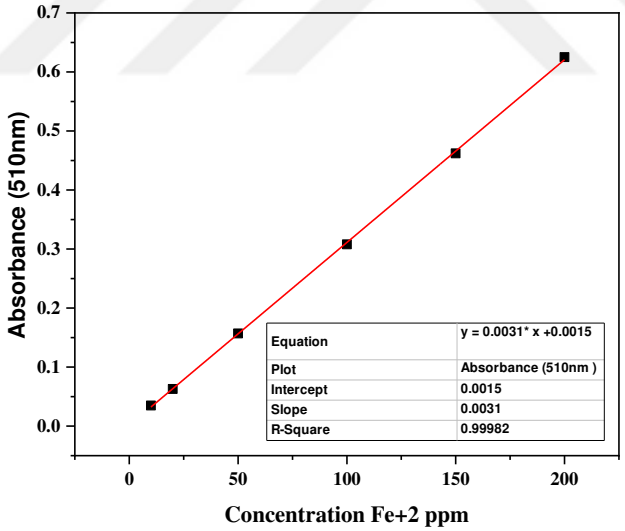


Figure 4.2. (a) Calibration curve for gallic acid, (b) Quercetin calibration curve for TFC (c) Quercetin calibration curve for FRAP, and (d) Calibration curve for Iron (II) (Continue)

4.3. Indicates the Results of Quantitative Estimation of Lycopenes, and β – Carotenoids

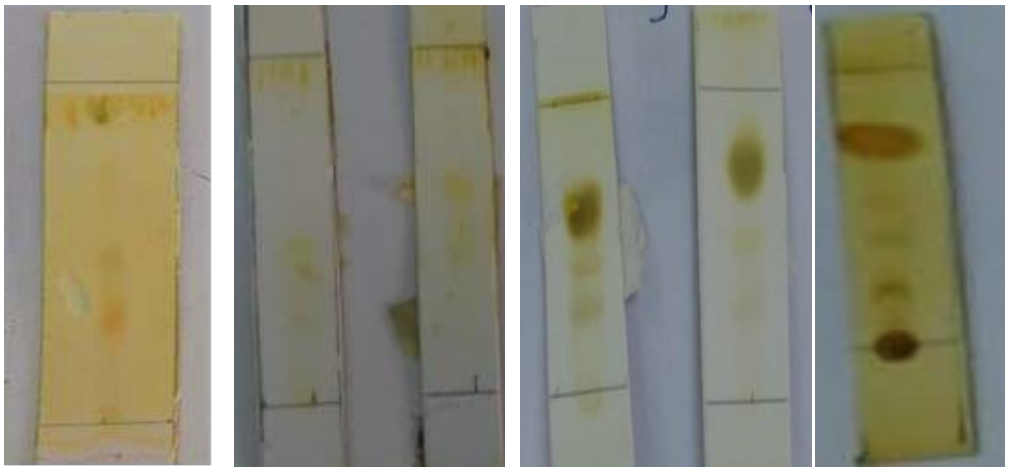
The results show that the quantity of concentration that produces beta-carotene and lycopene in four medicinal plants differs. Higher quantities of β – carotene and lycopene result from the enrichment, making four medicinal plants a rich and good source of carotene.

Table 4.3. Results of quantitative estimation of Lycopenes and β –Carotenoids

Methanolic extracts	Phytochemical Results	
	Lycopenes mg/g	β –Carotenoids mg/g
Urtica dioica	7420±750	10674±1102
Citrullus Colocynthis	681±70	564±58
Pegnum harmala	3314±436	7764±750
Syzygium aromaticum clove	3443±351	4578±461

4.4. Phytochemical analysis of the methanolic extracts

Constituents of medicinal plants' phytochemicals the existence of different bioactive molecules in plants grant therapeutic efficacy. The TLC results of methanolic extracts are shown in Figure 4.3 using the Iodine visualization method. A study on the methanolic extracts of *(Urticadioïca*, *Citrullus Colocynthis*, *Pegnum harmala*, and *Syzygium aromaticum Clove*) are in agreement with our results for some chemical components.



A **B** **C** **D**

Figure 4.3. Separation of the phytochemicals extracted from (A) *Urtica dioica*, (B) *Citrullu Colocynthis*,(C) *Pegnum harmala,* and (D)*Syzygium aromaticum* (clove), TLC containing silica-gel plate using iodine fumes.

4.5. HPLC Analyses of Phenolic Component of the Methanolic Extracts

The total phenolic content and antioxidant activity of plant samples obtained from various locations and cities in north Iraq differed significantly. The findings of a study on methanolic extracts of *(Urtica dioica, Citrullus colocynthis, Pegnum harmala, and Syzygium aromaticum Clove*) are presented in Table 4.4.

Table 4.4. Analysis of phenolic compounds of methanolic extracts by HPLC-DAD (ppm, mg L^{-1} extract)

Methanolic extracts	Phenolic compounds				
	Gallic acid	Catechin	Chlorogenic Acid	p-Coumaric acid	Rutin
Urtica dioica	0,07±0.008	32,0±0.4	16±2	1,7±0.2	67±7
Citrullus Colocynthis	0,23±0.03	8,0±0.9	N.D	0,112±0.023	N.D
Pegnum harmala	N.D	8,2±0.9	N.D	N.D	N.D
Syzygium aromaticumclove	38,1±4.5	N.D	N.D	N.D	N.D

N.D: Not Detected

5. DISCUSSION

The study also looks into the idea of employing four medicinal plants as natural antioxidants in the food industry. The plants were examined for phytochemicals to see whether they might be utilized for therapeutic purposes.

5.1. Quantitative Analysis for Plants Extracts

The percentage yields of plant extracts are shown in Table 4.1. The alcoholic extract yields a higher percentage than other extracts. This finding could be explained by the use of polar solvents like methanol, which can extract polar components more efficiently than non-polar solvents regardless of plant species. According to a study the yield of the methanolic extract (%) of *Urticadioïca*, *C. colocynthis*, *P. harmala* of seeds, and *Syzygium aromaticum Clov* was found to be 11.584%, 6.945%,19.28%, and 30.192%. w/w on a dry weight basis, respectively Table 4.1. The findings of this study revealed that the methanol extracts of the plants studied are in the order listed above. Because of its appropriate polarity and ability to solubilize and recover optimum amounts of phenolics from plant matrices, methanol is usually recognized as an effective solvent for the extraction of antioxidant compounds, according to previously documented literature. 30.192 percent was the maximum extract yield. The *clove* extract with the highest yield contained the most extractable antioxidant constituents. This could indicate that *clove* has more polar constituents than polar one's constituents. This does not mean that its polar constituents are less biologically active than their semi-polar or non-polar counterparts.

Table 4.2 shows the phytochemical screening of the methanolic extracts, with total phenolic contents, total flavonoid contents, Ferric reducing antioxidant power, and metal chelating capacity content being the most similar as compared to the table. Many phenolic component studies have found that environmental, climatic, or geographic conditions, as well as extraction processes, can have a considerable impact on phenolic component quality and quantity. Phenolic chemicals are a type of antioxidant that serves as good proton donors, resulting in free radical terminators, and contribute to plant antioxidant activity.

The total phenolic contents of the *Urtica dioica, Citrullus Colocynthis, Pegnum harmala, and Syzygium aromaticum clove* extracts as gallic acid the equivalent was found to be more in methanol extracts where the results of TPC of methanolic extracts from dry *Syzygium aromaticum clove* extract showed a high total phenolic content, comparing these results of TPC (574mg GAE/g) depending on the extraction technique used. The results reveal that methanol extracts' antioxidant activity is proportional to the number of phenolics present in the extract. Because *Syzygium aromaticum cloves* having a high phenolic content, they may be a useful source of antioxidants.

Total flavonoid content. Flavonoids are polyphenolic compounds It has a variety of biological effects, including that have anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, antiviral, and anticancer properties. Enzymes like reductase and xanthine oxidase are also inhibited by these compounds. Because of their phenolic hydroxyl groups, they can effectively scavenge reactive oxygen species and are potent antioxidants. Flavonoids were determined using the aluminum chloride colorimetric method. According to our findings in Table 4.2, high flavonoid content in *Pegnum harmala* seeds significantly contributed to antioxidant activity by neutralizing free radicals, which had roughly comparable amounts of total flavonoids content (8.4 mg /g). The flavonoid content *of Peganum harmala* seeds was calculated using a quercetin calibration curve and expressed as quercetin equivalents in mg/g extract.

The FRAP method assesses antioxidants' potential to act as reducing agents. In the FRAP experiment, potassium ferricyanide was utilized as the ferric reagent. Transition metal ions are utilized to improve lipid oxidation by breaking lipid hydroperoxides into more reactive peroxyl and alkoxyl radicals via the Fenton reaction. The ferrous ions form •OH radicals, which are highly reactive and contribute significantly to oxidative stress, as a result of the Fenton reaction.

$$Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+OH^{-}+OH\cdot \quad \text{Fenton reaction}$$

Proteins, carbohydrates, cellular lipids, and nucleic acids are all damaged by the hydroxy radicals that result, resulting in cellular damage. Cu^{+}, $Ti3^{+}$, Cr^{2+}, and Co^{2+}, as well as their complexes in lower oxidation states, similarly react with H_2O_2 as Fe^{2+}, and combinations of these metal ions with H_2O_2 were dubbed "Fenton-like" reagents. Antioxidants, which are potent metal chelators, can quickly deactivate prooxidant metal ions, preventing or slowing lipid oxidation caused by metal ions. Phenolic compounds are metal chelators and scavengers of hydroxyl and other radicals produced by Fenton reactions involving iron. In other words, following creation, polyphenols sacrificially reduce ROS/RNS, such as $^{\bullet}OH$, $O^{2\bullet-}$, $NO^{\bullet}$, or $OONO^{-}$, preventing damaging to biomolecules or the synthesis of more reactive ROS. The ferric reducing power assay works on the idea that compounds with reduction potential combine with potassium ferricyanide (Fe^{3+}) to generate potassium ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride to form a ferric–ferrous complex with a 700 nm absorption maximum. When a reductant, such as antioxidants found in plant extracts, is present, the Fe3+ ferricyanide complex is reduced to the ferrous form, Fe2+. The methanol extracts' transformation of iron (III) to iron (II)-reducing activity in of the methanol extracts. Table 4.2 shows that *Syzygium aromaticum* (*Clove*) extract has the highest reducing power activity (484 mg/g d.w).

The approach was used to calculate the amount of Fe^{+2} chelated by extracts. Ferrozine can form compounds with Fe^{+2} in a quantitative manner. The complex formation is interrupted in the presence of chelating chemicals, resulting in a reduction in the red color of the complex. As a result,

the color reduction can be used to allow estimate the chelating activity of the coexisting chelator. Fe^{+2}, a transition metal ion, can transfer single electrons, allowing it to form and propagate numerous radical reactions, even those that start with relatively non-reactive radicals. Chelating metal ions is the principal technique for avoiding ROS formation, which is related to redox-active metal catalysis. The total results from Table 4.2 indicated that *Syzygium aromaticum* (*Clove*) contains high amounts of TPC, FRAP, and MCC; this observation was also reported by several Scientists, who concluded that *Syzygium aromaticum* (*Clove*) is one of the most important herbal plants seen all over the world due to its high contents of different numerous beneficial constituents.

5.2. Quantitative estimation of Lycopenes and β – Carotenoids

Carotenoids are essential dietary sources of vitamin A, and determining carotenoid concentrations following β-carotene retinol bioconversion into pro-vitamin .A could be another objective of this study. Vitamin A and related substances are composed mainly of carotenoids. β-carotene is among the most well-known carotenoids, and it is a powerful antioxidant as well as a growth agent in the diet. Carotenoids make another important group of nutrients present in *U. dioica.* Higher β-carotene content in Urtica dioica may be associated with their photoprotective role. The obtained data in Table 4.3 have shown the highest content of β-carotene in *Urtica dioica* (10674 $mg.g^{-1}$) in an acetone-hexane mixture. It is interesting to note that the content of lycopene in *Urtica dioica* was significantly lower (7420$mg.g^{-1}$) compared to β-carotene content. The results obtained in this study showed that *Urtica dioica* leaves could be used by humans as healthy food, taking into account the carotenoids they contain ,due to their high amounts of some carotenoids. B-carotene, lycopene, and lutein isomers were the major carotenoid in leaves. Significantly, *Pegnum harmala* (7764 mg/g) had the second-highest value of β – Carotenoids, whereas *Pegnum harmala* (3314 mg/g) had the greatest value of Lycopenes. Carotenoids are important because of their vibrant hues, antioxidant action, and role as vitamin A precursors. These are safe substances that can be utilized in nutraceuticals and food supplements. Carotenoids are vital in the prevention of diseases and the maintenance of good health in humans.

Cloves have the least lycopene and the most B-carotene, according to the results (3443, 4578) . The complex loses its chromophore and characteristic orange hue as β-carotene molecules lose their double bonds due to oxidation, which can be measured spectrophotometrically. While carotenoids, which contribute to color and flavor, play a significant part in keeping good health due to their antioxidant activity and contribution of vitamin A to the diet, they also play an important function in maintaining good health.

Citrullus Colocynthis had a decreased lycopene and ß carotene ratio (681,564) comparable with anther plants. Carotene is an accessory pigment found in plants that serve to power photosynthesis by collecting light wavelengths. Defined lycopene as an acyclic isomer of β-carotene,

one of the carotenoid family's members. It is soluble in lipids and antioxidants, which are produced by a variety of plants, microbes, algae, and fungus, but not by animals or humans. Lycopene is a light-gathering pigment that helps shield plants and microorganisms from the harmful effects of oxygen and light.

5.3. Chromatographic Technique for Methanolic Extracts

The purity of compounds was determined using the TLC technique. It was also tested with a variety of solvents and stationary phases to determine the optimal combinations for column chromatography. The identification of the compounds is based on a comparison of theR_f and colors noticed under the Iodine visualization method spots appearing on TLC with standards noted under the identical experimental circumstances. The plate obtained has an excellent migration and, as a result, a decent separation, allowing for qualitative flavonoid compound analysis. The supplied frontal reports are then computed using the relation following Figure 4.1.

Thin-layer chromatography of the methanolic extract of *Urtica dioïca*. A set of spots was obtained by the solvent system (Butanol, Acetic Acid, Distilled Water) (4: 1: 6) of the methanolic extract. The chromatogram obtained comprises a series of spots, which appear. The solvent mixture of ethyl acetate, acetic acid, formic acid, and water (9: 1:1: 2.3 upper layer v/v) gave the best results of the methanolic extract of *Citrullus colocynthis*. Thin-layer chromatography of *Peganum harmala*. Mobile phase Methanol, tetrachloromethane, ammonia (5: 10: 0.08) Thin-layer chromatography(TLC) of the extract of *Peganum harmala* seeds showed a presence of 3 spots after the revelation by the Iodine visualization method spots appeared on TLC.

The clove extract was analyzed using TLC following the usual technique. TLC silica gel plate with aluminum sheet cover was utilized. The sample was extracted using capillary pipettes to dissolve 0.015gm of sample in 1ml of methanol, and the sample and standard were put to a TLC plate. The sample and standard were separated by 2 cm from the radical and 1 cm from the TLC sheets' sides. The TLC sheets were then prepared using a mix of chloroform and ethanol (4:1). The TLC sheets were checked after 30 minutes using the Iodine visualization method, which revealed spots on the TLC sheets.

5.4. Identification of Phenolic Compounds in four medicinal plants extracts

Plants play a significant role in these natural commodities, which are applied as food, cosmetics, and medicine. Humans utilize these natural compounds because they consist of worthy

bioactive molecules with significant antioxidant, anti-inflammatory, and antibacterial capabilities. Phenolic molecules, which are secondary metabolites, are accountable for a wide range of bio-properties. Table 4.4 displays the results of the analysis by HPLC of methanolic extracts. The phenolic component of all samples in this study was identified using five antioxidant standards.

In compared to other samples and studies, phenolic components of *Urtica dioica* sample were shown to be dramatically higher .The phenolic content of *Urtica dioica* components (leaves) was determined qualitatively and quantitatively utilizing HPLC-DADAccording to the findings. As demonstrated in Table 4.4, Urtica dioica has a higher concentration of (rutin 66,95 ppm, catechinis 32,04 ppm, chlorogenic acid 15,94 ppm, p-Coumaric acid 1,66 ppm, and gallic acid 0,07 ppm) than other plants. The presence of phenolic chemicals (mainly Catechin and Rutin) and their antioxidant activity are linked to the medicinal action of these plant extracts.

The recognition of *Citrullus Colocythis* components phenolic extracts in methanolic extracts by HPLC was based on a comparison of their retention periods with those obtained for the same standard compounds. We conclude that the extracts contain the three benchmark phenolic compounds identified, although their quantities varied from one to the other, either by the area of the peaks or by recorded values such as (Gallic acid 0,23 ppm, Catechin 8,03 ppm, and p-Coumaric acid 0,11 ppm).

P. harmala seeds were analyzed using HPLC DAD. One phenolic compound, was found in the chromatogram, like (Catechin 8,21ppm).

Moreover, the *Syzygium aromaticum clove* contains phenolic acids (Gallic acid 38,06 ppm). The findings demonstrate that the *clove* extract had much more phenolic compounds than the other plants extracts.

6. CONCLUSIONS

U. dioica has a wide range of therapeutic properties, and it is often used to treat a variety of inflammatory illnesses. The highest level of carotenoids of Lycopenes and β –Carotenoids– Carotenoids was found in this plant in traditional medicine. The leaves of *Urtica dioica* are rich in numerous secondary environments, biological qualities, and medicinal virtues, according to the phytochemical screening of the methanolic extract. *Urtica* dioica metabolites involved in the plant's adaptation to its leaves suggest that they could be used as a source of antioxidant phenolic compounds, with methanolic extracts having the greatest antioxidant effects as determined by FRAP assays. We also used a high-performance liquid chromatography (HPLC) system to detect numerous phenolic compounds in extracts, recording the presence of all reference phenolic compounds (05compounds), which include Gallic acid, Catechin, Chlorogenic Acid, p-Coumaric Acid, and Rutin. *Citrullus colocynthis* phytochemical research reveals important information about the chemicals present in this plant. Although several compounds and many pharmacological activities have been discovered, the results of this study show that due to the high amount of TPC, TFC, FRAP, MCC, and carotenoids (Lycopenes and β –Carotenoids) while carotenoids, which contribute flavor and color, play a significant role in maintaining healthy through their antioxidant activity and also because they make a contribution vitamin A to the diet. Since various chemicals and pharmacological activity have been discovered in this plant species, additional research is needed because many of the traditional uses that have been described thus far must be verified through investigation. *Peganum harmala* seeds may be a significant component of this plant, which has a high scavenging capacity for free radicals. A large amount of antioxidant activity in this organ could be linked to the high number of secondary metabolites. These findings revealed that the phenolic compound played a substantial role in the antioxidant capacity of the plant species studied. Many research groups have demonstrated a beneficial relationship between total phenolic content and antioxidant activity. *Peganum harmala* has the highest concentration of Lycopenes and –Carotenoids in this study. Based on the data results present in this study comparable, it is possible to infer that *Syzygium aromaticum* (*Clove*) is a fascinating herb with tremendous potential as a food preservative and a rich source of antioxidant chemicals. Its biological activity has led to the development of medical medicines for humans and animals, confirming that this plant has been used for ages virtually all over the globe. Finally, our findings have paved the way for selecting the most active antioxidant extracts with higher phenolic content and subjecting them to comprehensive chromatographic and spectroscopic analysis to discover the chemicals responsible for their high antioxidant activity. These herbs are recommended for both low-calorie diets and health reasons.

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WORK EXPERIENCE

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