**Deanship of Graduate Studies**
**Al-Quds University**

# *"Urtica dioica* leaf extract phase behavior and biochemical composition"

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# "*Urtica dioica* leaf extract phase behavior and biochemical composition"

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**Thesis Approval**

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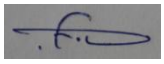
**2-Prof. Fuad Al Rimawi Co-Supervisor, Signature:** 

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**Jerusalem-Palestine**

**2023\1444**

**Declaration**

I certify that this thesis submitted for the degree of master is my own research, expect where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Signed: 

Afaf Abuhilal

20/05/2023

## Dedication

Dear Family,

I dedicate this thesis to you as a token of my gratitude for the unwavering support and encouragement you have given me throughout my Master's degree program. Your love, guidance, and sacrifices have been invaluable in helping me achieve this milestone in my academic journey.

To my mother and father: Your unwavering support and guidance have been the foundation of my success. Your love and encouragement have instilled in me a deep sense of determination and passion for learning. I am forever grateful for your sacrifices and unwavering faith in my abilities.

To my husband and kids: Your love and understanding have been a constant source of motivation and inspiration. Your encouragement and support have given me the strength to overcome the challenges of balancing family life and academic pursuits. I am deeply grateful for your unwavering support and love.

To my brothers and sisters: Your support and encouragement have been a constant source of inspiration and motivation. Your unwavering faith in my abilities has given me the courage to pursue my dreams and reach for the stars. I am grateful for your love and support, and I am proud to share this achievement with you.

To my beloved country Palestine: Your resilience and determination have inspired me to never give up on my dreams. Your rich history and culture have given me a sense of purpose and identity. It is an honor to represent you and to contribute to your growth and development.

I am grateful for the sacrifices you have made and the support you have given me, which have made this accomplishment possible. I could not have done it without you, and I am forever indebted to your love and guidance. I hope that this thesis serves as a testament to your unwavering support and encouragement, and as an inspiration to future generations.

With love and gratitude,

Afaf

## Abstract

*Urtica dioica*, also known as stinging nettle, is a perennial herbaceous plant with a long history of use for its medicinal and nutritional properties. The biochemical composition and phase behavior of *Urtica dioica* leaves extract were investigated in this thesis. The leaves were found to be rich in bioactive compounds such as Polyphenols, Flavonoid*s*, and carotenoids, which possess antioxidant, anti-inflammatory, and anti-cancer properties. The phase behavior of the *Urtica dioica* leaves extract was studied by constructing a Pseudo Ternary phase diagram to determine the regions of microemulsion. The *Urtica dioica* leaf extract dissolved in ethanol concentration of 1mg/ml was used as the oil phase and Tween 80 and Span 20 were used as surfactants. The total phenol content of the *Urtica dioica* leaf extract was analyzed to be 344 mg Gallic acid equivalents (GAE) per gram of extract, indicating the presence of a significant amount of phenol compounds. The Flavonoid content was determined to be 129.33 mg Catechin equivalents (CE) per gram of extract using a colorimetric assay. The DPPH radical scavenging activity of *Urtica dioica*leaf extract was found to be 81.64%. The *Urtica dioica*leaf extracted with methanol was examined using an HPLC device and the result was that it contained a group of compounds: Gallic acid, and Rutin. The extract showed no antibacterial effect against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The phase behavior of the *Urtica dioica*leaf extract determined the formula of Microemulsion, which can be useful for making dosage forms and cosmetic formulations. Further research is needed to fully understand the potential applications of *Urtica dioica* leaves in the pharmaceutical and food industries, given their rich bioactive compound composition.

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## Abbreviations:

- UD: *URTICA DIOICA*
- *UDL*: *Urtica dioica*leaf
- GAE : Gallic Acid Equivalents
- STD : Standard
- GA : Gallic acid
- TFC : Total Flavonoid*s* Content
- TPC: Total Phenolic Content
- DPPH : 1,1-diphenyl 2-picrylhydrazyl (DPPH• ) radical
- GAE: Gallic acid equivalent
- HPLC: High Performance Liquid chromatography
- *S.aureus :Staphylococcus aureus*
- *E.coli: Escherichia coli*

# Chapter One:

# Introduction

## 1.1 Background

As new infections continue to emerge worldwide, the need for novel resources to combat them becomes increasingly crucial. Plants have been a rich and readily available resource for medicinal purposes since ancient times. Among these medicinal plants, stinging nettle stands out. Found in Palestine, it has been utilized in traditional medicine and kitchens for generations.

Urtica dioica, commonly known as stinging nettle, is a perennial herb belonging to the *Urticaceae* family. It is widely distributed in temperate regions of Europe, Asia, and North America, and is characterized by stinging hairs that cover its leaves and stem. The plant grows up to one meter in height and has opposite ovate-lanceolate leaves with toothed margins and an acute tip. The axillary inflorescence consists of many small, green, and unisexual flowers that give rise to achenes as fruit. (Henning et al. 2017)

*Urtica dioica* has a long history of traditional use as a medicinal plant. It has been employed for the treatment of a variety of ailments such as skin diseases, respiratory disorders, joint pain, anemia, and circulatory problems. The use of stinging nettle as a food source is also well documented, as it has been consumed in various forms such as nettle stews and teas. In addition, its leaves have been used in cosmetic preparations for skin and hair care. (Roschek et al. 2009)

Recent studies have shed light on the chemical composition of *Urtica dioica* and its potential health benefits. The plant contains a range of bioactive compounds including Flavonoid*s*, Phenolic acids, lignans, and polysaccharides, among others. These compounds have been found to possess various pharmacological properties such as anti-inflammatory, antioxidant, and antimicrobial activities. Studies have also suggested that *Urtica dioica* may have potential therapeutic effects on conditions such as rheumatoid arthritis, osteoarthritis, and benign prostatic hyperplasia.(Sánchez et al. 2012)

-Chemical Components of Stinging Nettle *Trichomes* and their Effects on the Skin

One of the characteristics of *Urtica dioica,* the stinging sensation when it comes into contact with the skin. This sensation is caused by the release of various chemicals from the plant's *Trichomes*. Understanding the chemical components and their effects on the skin is essential for comprehending the mechanism behind this unique characteristic.

Chemical Components:
The primary chemicals secreted by *Urtica dioica Trichomes* upon contact with the skin include histamine, acetylcholine, serotonin, and formic acid.

Histamine:
Histamine is an important compound involved in immune responses and inflammatory reactions. When released onto the skin, histamine triggers itching, redness, and swelling. These reactions are part of the body's defense mechanism against potential threats.

Acetylcholine:
Acetylcholine acts as a neurotransmitter, facilitating communication between nerve cells. In the context of stinging nettle, it contributes to the pain and irritation experienced upon contact with the plant. The interaction between acetylcholine and specific receptors in the skin amplifies the stinging sensation.

Serotonin:
Serotonin, another neurotransmitter, plays a role in regulating various physiological functions, including mood and pain perception. In stinging nettle, serotonin released by the *Trichomes* enhances the stinging sensation, adding to the discomfort experienced by individuals in contact with the plant.

Formic Acid:
Stinging nettle *Trichomes*may also contain formic acid. This compound, commonly found in ant venom, contributes to the burning sensation and irritation felt upon contact with the plant. Formic acid acts as an additional factor intensifying the skin's reaction.

Effects on the Skin:

The combination of these chemical components leads to temporary discomfort and skin reactions upon contact with stinging nettle. The release of histamine, acetylcholine, serotonin, and formic acid triggers itching, redness, swelling, and a burning sensation. These effects are part of the body's defense mechanism against potential threats, as the stinging sensation acts as a deterrent against further contact.

Stinging nettle *Trichomes* secrete a combination of chemicals that induce a unique stinging sensation upon contact with the skin. The release of histamine, acetylcholine, serotonin, and formic acid triggers various reactions, causing discomfort and temporary skin irritations. Understanding the chemical components and their effects on the skin contributes to our knowledge of the plant's defense mechanisms and the unique characteristics associated with stinging nettle.

## 1.2 Nutritional Facts:

According to Joshi et al. (2014), *U. dioica* L. contains a variety of vitamins and minerals, making it a valuable nutritional resource. (Guerrero et al. (2003) reported that nettle is rich in vitamin C and provitamin A. Additionally, Said et al. (2015) found that protein makes up about 30% of the plant's dry mass, with minerals accounting for approximately 20%. The plant contains various essential amino acids for humans and is a good source of minerals, including zinc, iron, cobalt, potassium, nickel, and molybdenum. The nutritional composition of *U. dioica* is summarized in Table 1 and Table 2.

Table 1 : Nutritional value of 89g of *Urtica dioica:*

**Nutrition Facts**

| | |
|---|---|
| **Portion Size** | **89 g** |
| **Amount Per Portion** | |
| **Calories** | **37** |
| | **% Daily Value** * |
| **Total Fat** 0.1g | **0 %** |
| **Sodium** 3.6mg | **0 %** |
| **Total Carbohydrate** 6.7g | **2 %** |
| Dietary Fiber 6.1g | **22 %** |
| Sugar 0.2g | |
| **Protein** 2.4g | **5 %** |
| Calcium 428mg | 33 % |
| Iron 1.5mg | 8 % |
| Potassium 297mg | 6 % |

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contribute to a daily diet. 2000 calories a day is used for general nutrition advice.

https://www.nutritionvalue.org/ 1cup =89g

**Table: 2: Bioactive compounds**

| Parts compounds | Bioactive compounds |
|---|---|
| **Leaves and Root** | Vitamins ( Vitamins A, C,K and B vitamins), Minerals ( Calcium, iron, magnesium, phosphorus, potassium and sodium), fats ( Linoleic acid, linolenic acid, palmitic acid, stearic acid and oleic acid), amino acids (all of the essential amino acids), polyphenols ( kaempferol, quercetin, caffeic acid, coumarins, and other Flavonoid*s*, pigments ( Beta- carotene, lutein, luteoxanthin, and other carortenoids) |
| **Seeds** | Vitamins ( Vitamin A,B,C,E and K) minerals ( iron, silicone, calcium, magnesium, manganese, phosphorus, potassium), beta-carotene, folic acid, essential fatty acids. |

(Raman and Said 2015).

This suggest that a cup of tea of *Urtica dioica*contain multiplied amounts of Vitamins and Minerals the body needs every day.

### 1.3 Phytochemical constituents of *U. dioica* Extract

Phytochemicals are non-nutrient plant metabolites that are found in a variety of vegetables, fruits, grains, and other dietary substances. These compounds are produced by plants through various metabolic processes and are responsible for their color, flavor, and aroma. Phytochemicals have gained attention due to their potential to prevent the onset of chronic diseases such as cancer, diabetes, and cardiovascular disease.

They have been found to possess various biological activities such as antioxidant, anti-inflammatory, and anti-carcinogenic properties. There are more than 100 different types of phytochemicals that have been identified, including alkaloids, Flavonoid*s*, Phenolic acids, terpenoids, lignans, fatty acids and alcohols. Their chemical composition varies depending on the species of plant, the environmental conditions, and the plant part. (Cianciosi et al. 2018)

An example of each compound, their molecular formulas, and their sources are listed extensively in Tables 3 to 11

**A-Lignins**

Lignin is a complex organic polymer that is an important structural component of the cell walls of plants and some algae. (Ralph 2010) It is the second most abundant natural polymer in the world after cellulose. Lignin is a three-dimensional network of Phenolic compounds that provides strength, rigidity, and waterproofing to the cell walls. ( Ralph 2010)

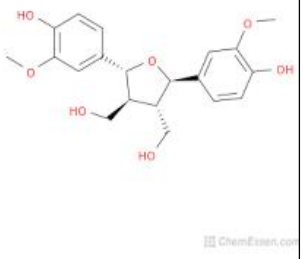
The exact composition and structure
of lignin vary depending on the plant species, tissue type, developmental stage, and environmental conditions. Generally, lignin is highly resistant to degradation and plays an important role in the protection and preservation of plant tissues. (Ibrahim et al.2018) In addition to its structural function, lignin has been found to play a role in plant defense against pests and pathogens, and it can also be used as a source of bioenergy and materials.

*Urtica* species have been found to contain over 23 lignin compounds, and an example can be found in table 3.( Ibrahim et al. 2018)

Although lignin is mainly known for its structural role in plants, there is increasing interest in its potential medicinal benefits. Some studies have suggested that lignin and lignin-derived compounds may have anti-inflammatory, antioxidant, antimicrobial, and antitumor properties. However, more research is needed to confirm these potential benefits and to determine the mechanisms of action.

Table 3:lignin compound found in *Urtica dioica*( Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Neoolivil | C20H24O7 | *Urtica dioica* |  |

**B.Sterols**

Sterols are a class of lipids that have a characteristic chemical structure consisting of four fused rings, including three cyclohexane rings and one cyclopentane ring. They are found in high concentrations in cell membranes and are essential components of many biological systems, including hormones and vitamins. (Vance)In the genus Urtica, 11 different steroidal alcohols have been reported so far (Ibrahim et al. 2018), an example of a sterol found in *UDL*extract can be found in Table 4.( Ibrahimet al. 2018)

Sterols have been found to have a number of potential medicinal benefits. Here are a few examples:

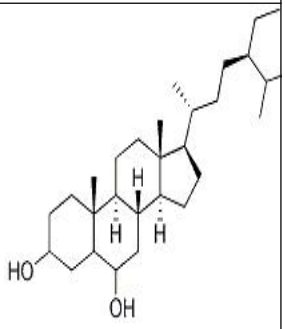
1. Cholesterol-lowering: Plant sterols, such as sitosterol, stigmasterol, and campesterol, have been shown to reduce cholesterol absorption in the gut, leading to lower levels of LDL ("bad") cholesterol in the blood. This has potential implications for reducing the risk of cardiovascular disease. (law 2000)

2. Anti-inflammatory: Some studies have suggested that plant sterols may have anti-inflammatory effects, potentially reducing the risk of chronic diseases such as arthritis and diabetes. (Salari and Moghaddam 2016)

3. Immune system support: Animal studies have shown that sterols may enhance immune function by stimulating the production of certain immune cells, such as T cells and natural killer cells. (Bouic 1999)

4. Anti-cancer: Some studies have suggested that plant sterols may have anti-cancer properties, potentially inhibiting the growth and spread of certain types of cancer cells. (Awad 2000)

Table 4: Sterols found in *Urtica dioica*( Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| Stigmas-tane-3,6-diol; (3b,a6a,24R)-form | C29H52O2 | |  |

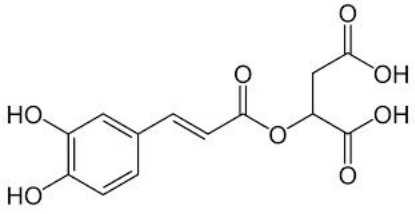
## C.Phenolic Acids

Phenolic acids are a class of organic compounds that are characterized by a phenol ring with one or more carboxylic acid groups attached to it. (Sánchez 2012) They are widely distributed in the plant kingdom *Urtica dioica*and are important components of many plant-based foods and beverages, including fruits, vegetables, grains, and coffee.(Clifford 2000)
There are several types of Phenolic acids, including hydroxybenzoic acids (such as gallic acid and salicylic acid) and hydroxycinnamic acids (such as caffeic acid, ferulic acid, and p-coumaric acid). These compounds are known for their antioxidant properties and are thought to have a range of potential health benefits, including anti-inflammatory, antimicrobial, and anti-cancer effects.(Kaur 2002)

Phenolic acids are also important contributors to the taste and aroma of many foods and beverages. For example, caffeic acid and chlorogenic acid are responsible for the bitter taste of coffee, while ferulic acid is a major contributor to the flavor of vanilla. Overall, Phenolic acids are an important class of compounds with a range of potential health benefits and sensory properties. Various species of *Urtica* have

been found to contain 10 different Phenolic acids, as reported by Ibrahim et al. 2018 .An example of a Phenolic acid in *UDL* extract can be found in Table 5.

Table 5: Phenolic Acids found in *Urtica dioica*( Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| Caffeoylmalic acid | $C_{13}H_{12}O$ | *Urtica dioica* |  |

**D. Fatty Acids**

Fatty acids are a type of organic molecule that are important building blocks of fats, oils, and other lipids. They are composed of a long chain of carbon atoms with a carboxyl group (-COOH) at one end and a methyl group (-CH3) at the other end.

Fatty acids can be classified into three main categories: saturated, monounsaturated, and polyunsaturated. Saturated fatty acids have no double bonds between carbon atoms in their chain and are typically solid at room temperature. Monounsaturated fatty acids have one double bond and are typically liquid at room temperature, while polyunsaturated fatty acids have two or more double bonds and are also liquid at room temperature.An example of a fatty acid can be found in *UDL* extract table 6.

Fatty acids are one of the many bioactive compounds found in *Urtica dioica*leaf extract, and they may contribute to some of its medicinal benefits. Here are some of the potential contributions of fatty acids in the medicinal benefits of Urtica dioica:

Anti-inflammatory effects: Fatty acids, such as alpha-linolenic acid and linoleic acid, have been shown to have anti-inflammatory effects, which may contribute to the anti-inflammatory properties of *Urtica dioica*leaf extract.(Roscheket al. 2009)

Prostate health: Fatty acids, such as palmitic acid and stearic acid, have been shown to have beneficial effects on prostate health. These fatty acids may contribute to the ability of *Urtica dioica*leaf extract to reduce the symptoms of an enlarged prostate.(Lopatkin et al. 1995)

Antioxidant properties: Fatty acids, particularly omega-3 and omega-6 fatty acids, have antioxidant properties that can help protect the body against damage from free radicals. These fatty acids may contribute to the antioxidant properties of *Urtica dioica*leaf extract.(Negi et al. 2005)

Blood sugar control: Some fatty acids, such as linoleic acid, have been shown to help regulate blood sugar levels in people with diabetes. This may contribute to the ability of *Urtica dioica*leaf extract to regulate blood sugar.(Eidi 2009 et al )

It's important to note that the contribution of fatty acids to the medicinal benefits of *Urtica dioica*leaf extract may vary depending on the specific fatty acids present in the extract, as well as the concentration and dose of the extract used. More research is needed to fully understand the mechanisms behind the medicinal benefits of *Urtica dioica*leaf extract.

Table 6: Fatty Acids found in *Urtica dioica*( Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| Alpha-Linolenic Acid | $C_{18}H_{30}O_2$ | *Urtica dioica* |  |

**E.Alkaloids**

Alkaloids are a class of nitrogen-containing organic compounds that are commonly found in plants. Many alkaloids have medicinal properties and are used in traditional medicine and modern pharmacology.(Fattorussom & Taglialatela-Scafati 2008)

*Urtica dioica*leaf extract contains several alkaloids, including histamine, serotonin, acetylcholine, and scopoletin. These alkaloids are believed to contribute to the medicinal benefits of the plant.(Roschek et al. 2009 )

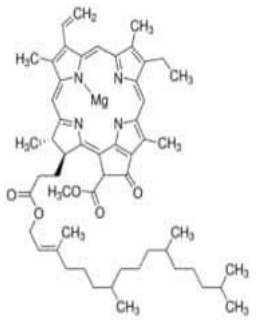
For example, histamine and serotonin are known to have anti-inflammatory effects, which may help explain Urtica dioica's traditional use in treating conditions such as arthritis and allergies. (Stark &Hartmann 2013 ) .Acetylcholine is a neurotransmitter that plays a role in cognitive function and may contribute to the plant's purported benefits for memory and brain function. (Haam &Yakel 2017)

Scopoletin has been shown to have antioxidant and anti-inflammatory effects and may contribute to the plant's traditional use in treating skin conditions.

However, it's important to note that the mechanisms behind the medicinal benefits of *Urtica dioica*leaf extract are complex and not fully understood. Further research is needed to fully elucidate the role of alkaloids and other bioactive compounds in the plant's therapeutic effects..

An example of an alkaloids in *UDL* extract can be found in table 7.

Table 7. Alkaloids found in *Urtica dioica*( Ibrahim et al.2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| Chlorophyll a | $C_{55}H_{72}MgN_4O5$ | *Urtica dioica* |  |

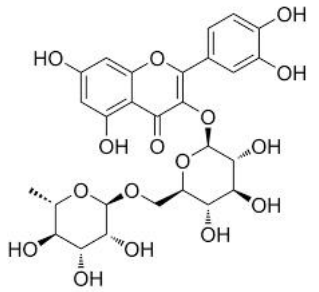
**F.** Flavonoid*s*

Flavonoid*s* are a group of natural compounds found in plants that have been associated with a variety of health benefits, including antioxidant, anti-inflammatory, and anticancer activities (Manach et al. 2004). They are commonly found in fruits, vegetables, and tea, , and are responsible for the colors of these foods.
To date, 17 Flavonoid*s* have been identified from various plants in the genus Urtica, as reported by Ibrahim et al 2018. Please refer to Table 8 for an example.

There is evidence to suggest that Flavonoid*s* in *Urtica dioica*leaf extract may have a number of health benefits. For example, one study found that *Urtica dioica*leaf extract reduced inflammation and oxidative stress in rats with arthritis, possibly due to the presence of Flavonoid*s* (Ibrahim et al. 2018). Another study found that *Urtica dioica*leaf extract had a protective effect on the liver in rats, which was also attributed to its Flavonoid content (Çölet al. 2016).

Table 8: Flavonoid*s* found in *Urtica dioica*( Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Rutin | $C_{27}H_{30}O_{16}$ | *Urtica dioica* |  |

**G.Terpenoids**

Terpenoids are a group of natural products that are similar to terpenes. They are composed of repeated units of five-membered carbon isoprenyl units, arranged in various ways. Terpenoids are also known as isoprenoids, named after their precursor. The carbon skeleton arrangement and functional groups vary across different compounds, with many of them containing multiple rings. This class of natural products is the largest and can be found in almost all living organisms.

Terpenoids account for approximately 60% of all available natural compounds. As per Ibrahim (2018) et al., 34 different compounds belonging to this class have been identified in the genus Urtica.. An example is given in table 9

There are several terpenoids present in *Urtica dioica*leaf extract that may contribute to its medicinal benefits. Here are some potential correlations:

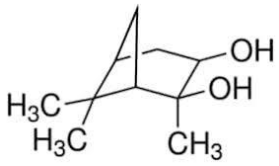
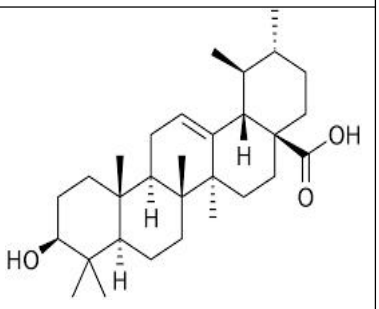
Anti-inflammatory effects: Terpenoids, such as beta-sitosterol, have been shown to have anti-inflammatory effects, which may contribute to the ability of *Urtica dioica*leaf extract to reduce inflammation in the body (Javadiet al. 2017).

Antioxidant properties: Terpenoids, such as carotenoids, have antioxidant properties that can help protect the body against damage from free radicals. These terpenoids may contribute to the antioxidant properties of *Urtica dioica*leaf extract (Gülçin et al. 2011).

Allergy relief: Terpenoids, such as quercetin, have been shown to have antihistamine properties and may help relieve symptoms of allergies, such as runny

nose and itchy eyes. *Urtica dioica*leaf extract has been found to contain significant amounts of quercetin (Roschek et al. 2009).

Table:9 Terpenoids found in *Urtica dioica*(Ibrahim et al. 2018)

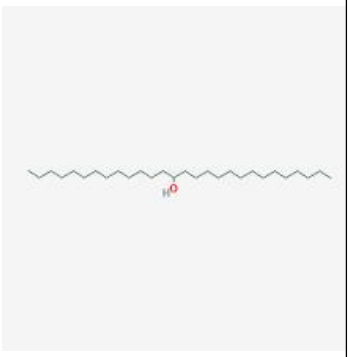
| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| 9,10-Pinanediol | $C_{10}H_{18}O_2$ | Urtica dioica |  |
| Ursolic acid | $C_{30}H_{48}O_3$ | Urtica dioica |  |

**H. Alcohols**

Alcohols are organic compounds that contain one or more hydroxyl (-OH) groups attached to a saturated carbon. The term "alcohol" was originally used for ethyl alcohol (a primary alcohol), which is a major component of wine and other alcoholic beverages. The suffix "-ol" is used by the IUPAC system to indicate the presence of a hydroxyl group in the compound's highest priority functional group. For example, the names cholesterol and paracetamol indicate that these compounds are alcohols. However, some compounds, such as fructose and mannose, which also contain -OH groups,

do not use the "-ol" suffix. Four alcohol compounds have been reported from various plants of the genus Urtica to date.

Table 10 Alcohols: found in *Urtica dioica*(Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| 14-Octacosanol | C28H58O | *Urtica dioica* |  |

**I.Benzopyranoids:**

Benzopyranoids are a class of organic compounds that contain a benzene ring fused to a pyran ring. They are commonly found in plants and have been found to have medicinal properties.

There are several benzopyranoids present in *Urtica dioica*leaf extract that may contribute to its medicinal benefits. Here are some potential correlations:

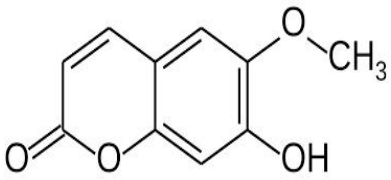
Anti-inflammatory effects: Benzopyranoids, such as quercetin, have been shown to have anti-inflammatory effects, which may contribute to the ability of *Urtica dioica*leaf extract to reduce inflammation in the body (Lesjak2018)

Antioxidant properties: Benzopyranoids, such as kaempferol, have antioxidant properties that can help protect the body against damage from free radicals. These benzopyranoids may contribute to the antioxidant properties of *Urtica dioica*leaf extract (Gülçin& Topal 2011).

Anti-cancer effects: Benzopyranoids, such as scopoletin, have been shown to have anti-cancer effects, which may contribute to the ability of *Urtica dioica*leaf extract to inhibit the growth of cancer cells.

Currently, six compounds belonging to this class have been identified from various Urtica plant species (Ibrahim et al.2018).an example can be found in Table 11

Table 11: Benzoyranoids found in *Urtica dioica*(Ibrahim et al. 2018)

| Name | Molecular formula | Species | Chemical structure |
|---|---|---|---|
| Scopoletin | $C_{10}H_8O$ 4 | Urtica dioica |  |

## 1.4 Pharmacological activities of U. dioica leaf extract

### 1.4.1 Anti-diabetic effects

The prevalence of diabetes mellitus, a chronic metabolic disorder, has been increasing globally. Diabetes is characterized by hyperglycemia, which results from the body's inability to produce or use insulin effectively. Several synthetic anti-diabetic drugs are available, but they have limitations such as adverse effects and high cost. Therefore, there is a need to develop safe and effective alternative therapies. *Urtica dioica*is one of the traditional medicinal plants that have been reported to possess anti-diabetic effects.

Several studies have investigated the anti-diabetic effect of *Urtica dioica*leaf extract. For example (Eidi et al. 2009) conducted a randomized controlled trial on 50 patients with type 2 diabetes mellitus. The patients were randomly assigned to receive either *Urtica dioica*leaf extract (500 mg/day) or placebo for three months.

The results showed that the extract significantly reduced fasting blood glucose, glycosylated hemoglobin, and insulin resistance compared to the placebo group. The study suggested that *Urtica dioica*leaf extract may be a safe and effective adjuvant therapy for type 2 diabetes mellitus.

The antidiabetic effect of *Urtica dioica*leaf extract may be attributed to its various bioactive compounds, including polyphenols, Flavonoid*s*, and triterpenoids. These compounds have been reported to possess antioxidant, anti-inflammatory, and hypoglycemic properties. For instance, one study reported that *Urtica dioica*leaf extract exhibited potent antioxidant activity and protected pancreatic beta-cells from oxidative stress-induced damage (Kamalinejad et al. 2006). Another study suggested that *Urtica dioica*leaf extract may reduce insulin resistance by modulating adipokine levels and improving lipid metabolism (Saeed et al. 2015). Furthermore, *Urtica dioica*leaf extract has been shown to stimulate insulin secretion from pancreatic beta-cells and enhance glucose uptake in peripheral tissues (Sulaiman et al. 2016).

### 1.4.2 Anti- inflammatory effects:

One of the most well-studied pharmacological effects of *Urtica dioica*leaf extract is its anti-inflammatory activity. Inflammation is a key driver of many chronic diseases, including cardiovascular disease, diabetes, and cancer. *Urtica dioica*leaf extract has been shown to exhibit anti-inflammatory activity through various mechanisms.

The anti-inflammatory properties of *Urtica dioica*leaf extract are attributed to the presence of several bioactive compounds, including Flavonoid*s*, Phenolic acids, and lignans. These compounds have been shown to inhibit the production of inflammatory mediators such as prostaglandins and leukotrienes (Riehemann et al. 1999). In addition, *Urtica dioica*leaf extract has been shown to inhibit the activation of nuclear factor-kappaB (NF-kappaB), a key transcription factor involved in the regulation of inflammatory responses (Safieh Garabedian et al. 2000).

One study evaluated the anti-inflammatory activity of *Urtica dioica*leaf extract in a rat model of paw edema induced by carrageenan. The results showed that *Urtica dioica*leaf extract significantly reduced the paw edema and inhibited the

production of inflammatory mediators, such as prostaglandins and leukotrienes (Roschek et al 2009). Another study investigated the anti-inflammatory activity of *Urtica dioica*leaf extract in a mouse model of acute lung injury induced by lipopolysaccharide. The results showed that *Urtica dioica*leaf extract significantly reduced lung inflammation and inhibited the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) (Zhang et al. 2017).

In addition to its anti-inflammatory properties, *Urtica dioica*leaf extract has been shown to exhibit antioxidant activity, which may contribute to its anti-inflammatory effects. The plant contains high levels of Phenolic compounds, which have been shown to scavenge free radicals and reduce oxidative stress in vitro and in vivo (Kamalinejad et al. 2006). Oxidative stress is a key driver of inflammation, and reducing oxidative stress may help to reduce inflammation.

Overall, *Urtica dioica*leaf extract is a promising natural anti-inflammatory agent with potential therapeutic applications for the treatment of inflammatory diseases. Its anti-inflammatory activity is attributed to the presence of several bioactive compounds, including Flavonoid*s*, Phenolic acids, and lignin's, which inhibit the production of inflammatory mediators and the activation of NF-kappaB. Further studies are needed to elucidate the mechanisms underlying its anti-inflammatory activity and to determine its safety and efficacy in humans.

### 1.4.3Anti-hypertensive effects:

One of the potential therapeutic benefits of *Urtica dioica*leaf extract is its antihypertensive effect. Hypertension, or high blood pressure, is a major risk factor for cardiovascular disease, stroke, and other health problems. *Urtica dioica*leaf extract has been shown to reduce blood pressure through various mechanisms.

The antihypertensive effect of *Urtica dioica*leaf extract is attributed to several bioactive compounds, including Phenolic acids, Flavonoid*s*, and lignans. These compounds have been shown to exert vasorelaxant effects and to improve endothelial function, which can lead to a reduction in blood pressure (Kianbakht &Mozaffari 2013). In addition, *Urtica dioica*leaf extract has been shown to inhibit the activity of the renin-angiotensin-aldosterone system (RAAS), a key regulator of blood pressure (Milojkovic-Opsenica et al. 2015).

One study evaluated the antihypertensive effect of *Urtica dioica*leaf extract in a rat model of hypertension induced by a high-salt diet. The results showed that *Urtica dioica*leaf extract significantly reduced blood pressure and improved endothelial function (Vieira et al. 2017). Another study investigated the antihypertensive effect of *Urtica dioica*leaf extract in a clinical trial involving hypertensive patients. The results showed that *Urtica dioica*leaf extract significantly reduced systolic and diastolic blood pressure after 8 weeks of treatment (Kianbakht &Mozaffari 2013).

### 1.4.4 Diuretic effects

*Urtica dioica*has been traditionally used for its diuretic effects.Several studies have investigated the diuretic effects of *Urtica dioica*leaf extract. A study published in the Journal of Ethnopharmacology in 2005 found that *Urtica dioica*leaf extract increased urine volume and sodium excretion in healthy volunteers (Obertreis et al). Another study published in the journal Phytotherapy Research in 2010 demonstrated that *Urtica dioica*leaf extract increased urine volume and decreased sodium and potassium excretion in rats (Tahri et al. 2010).

The diuretic effects of *Urtica dioica*leaf extract may be due to its high potassium content. A study published in the Journal of Medicinal Food in 2017 found that *Urtica dioica*leaf extract had a high potassium content, which may contribute to its diuretic effects by increasing the excretion of sodium and water (Najafzadeh et al. 2017).

The diuretic effects of *Urtica dioica*leaf extract may also have clinical relevance. A randomized controlled trial published in the Journal of Herbal Medicine in 2019 investigated the effects of *Urtica dioica*leaf extract on blood pressure and kidney function in patients with hypertension. The study found that supplementation with *Urtica dioica*leaf extract significantly decreased blood pressure and improved kidney function compared to the placebo group (Mozaffari et al. 2013).

In conclusion, *Urtica dioica*leaf extract has demonstrated diuretic effects in both humans and animals, which may be due to its high potassium content. The evidence supporting its use as a diuretic is promising, although further research is needed to fully understand its mechanisms of action and clinical applications.

### 1.4.5 Anticancer effects

In recent years, studies have investigated the potential anti-cancer effects of *Urtica dioica*leaf extract.

Several studies have shown that *Urtica dioica*leaf extract exhibits anti-cancer activity in various types of cancer cells. A study published in the journal BMC Complementary and Alternative Medicine in 2011 found that *Urtica dioica*leaf extract inhibited the growth of breast cancer cells and induced cell death (Namazi et al. 2011). Another study published in the journal PLoS One in 2016 demonstrated that *Urtica dioica*leaf extract had anti-tumor effects in colon cancer cells (Tanget al. 2016).

The anti-cancer effects of *Urtica dioica*leaf extract may be due to its ability to inhibit cancer cell proliferation and induce apoptosis, or programmed cell death. A

study published in the journal Nutrients in 2018 found that *Urtica dioica*leaf extract induced apoptosis in human prostate cancer cells by activating certain enzymes involved in programmed cell death (Jafari 2018 et al). Another study published in the journal BioMed Research International in 2019 demonstrated that *Urtica dioica*leaf extract inhibited the growth of ovarian cancer cells by blocking the cell cycle and inducing apoptosis (Akrami et al. 2019).

In addition to its direct anti-cancer effects, *Urtica dioica*leaf extract may also have potential as an adjuvant therapy in cancer treatment. A study published in the journal PLoS One in 2013 found that *Urtica dioica*leaf extract enhanced the anti-tumor effects of the chemotherapy drug cisplatin in lung cancer cells (Tanget al. 2016).

While the evidence supporting the anti-cancer effects of *Urtica dioica*leaf extract is promising, further research is needed to fully understand its mechanisms of action and potential clinical applications.

### 1.4.6Prostatic hyperplasia:

Benign prostatic hyperplasia (BPH) is a common condition in men, characterized by the non-cancerous enlargement of the prostate gland. It affects a large proportion of aging men and can lead to bothersome urinary symptoms such as difficulty in urination, frequent urination, and incomplete bladder emptying.

The currently available pharmacological treatments for BPH have been associated with various side effects, leading to an increasing interest in alternative and complementary therapies.

*Urtica dioica*has been studied for its potential role in the treatment of BPH due to its anti-inflammatory, diuretic, and antioxidant properties. This article will review the current evidence regarding the efficacy and safety of *Urtica dioica*in the treatment of BPH.

One of the mechanisms of action of *Urtica dioica*in the treatment of prostatic hyperplasia is its ability to inhibit the interaction of prostatic growth factors. In a study by Hirano et al. (1994), it was demonstrated that *Urtica dioica*extracts could

inhibit the binding of epidermal growth factor (EGF) to its receptor in the prostate gland, leading to a reduction in the growth of prostatic cells.

Another mechanism of action is the modulation of sex hormone-binding globulin (SHBG), a protein that plays a key role in the development of prostatic hyperplasia. Ganßer and Spiteller (1995) investigated the effects of *Urtica dioica*extracts on SHBG binding capability and found that the presence of secoisolariciresinol and a mixture of isomeric acids led to a lower binding capability of SHBG.

Clinical studies have also shown the efficacy of *Urtica dioica*in the treatment of prostatic hyperplasia. In a randomized, double-blind, placebo-controlled trial by Safarinejad 2007, 620 patients were treated with either *Urtica dioica*root extract or placebo for six months. The patients who received *Urtica dioica*extract showed significant improvements in the International Prostate Symptom Score (IPSS), maximum urinary flow rate (Qmax), and postvoid residual urine volume (PVR), compared to the placebo group.

Similarly, in a study by Lopatkin et al 1995 , patients with prostatic hyperplasia who were treated with *Urtica dioica*root extract for six months showed significant improvements in symptoms, compared to a control group. The *Urtica dioica*group also showed a reduction in prostate size and an increase in urinary flow rate.

In conclusion, *Urtica dioica*has shown promise as a natural treatment for prostatic hyperplasia. The plant has demonstrated multiple mechanisms of action, including the inhibition of prostatic growth factors and modulation of SHBG binding capability. Clinical studies have also shown significant improvements in symptoms and urinary function in patients treated with *Urtica dioica*root extract. Further research is needed to fully understand the potential of this plant in the treatment of prostatic hyperplasia.

### 1.4.7Therapeutic Window of Urtica DioicaExtract.

The therapeutic use of *Urtica dioica*extract also requires careful consideration of its safety profile and dosage range. The therapeutic window of *Urtica dioica*extract refers to the range of doses at which the extract can provide therapeutic benefits without causing significant adverse effects. Understanding the therapeutic window of *Urtica dioica*extract is crucial for its safe and effective use in the management of various health conditions. This can be achieved through rigorous preclinical and clinical studies aimed at determining the optimal dosage range, administration route, and duration of treatment for different health conditions.

The therapeutic window of *Urtica dioica*dosage form has not been well established. There is limited clinical evidence on the optimal dosage of *Urtica dioica*for different therapeutic applications. However, some studies have used doses ranging from 300 to 1200 mg/day of *Urtica dioica*extract in divided doses. It is important to note that the optimal dosage of *Urtica dioica*may vary depending on various factors such as the patient's age, weight, health status, and the severity of the condition being treated. Therefore, it is recommended to consult a healthcare professional before using *Urtica dioica*or any other herbal supplement for therapeutic purposes.

The clinical trial published in the Journal of Ethnopharmacology in 2007 concluded that the administration of *Urtica dioica*root extract at a dose of 300 mg/day of *Urtica dioica*was used in a study to examine patients with benign prostatic hyperplasia for six months, the symptoms were improved in 50 patients .No significant adverse effects were reported during the study. (Safarinejad 2007)

A dose of 500mg/ day of *Urtica dioica*was used in a study in which 20 patients with type 2 diabetes were given *Urtica dioica*leaf extract. After three months of treatment, the patients experienced a significant decrease in fasting blood glucose levels and hemoglobin A1c levels. (Kianbakht & Khalighi 2005)

There is limited scientific evidence on the toxic levels of Urtica dioica. However, some studies suggest that high doses of *Urtica dioica*may cause adverse effects. High dose of *Urtica dioica*extract (2,000 mg/kg) caused liver damage in rats. Karakus et al.A high dose of *Urtica dioica*extract (500 mg/kg) caused kidney

damage in rats.However, these studies used very high doses of *Urtica dioica*extract, which are unlikely to be consumed by humans in normal circumstances. Overall, *Urtica dioica*is considered safe when consumed in normal amounts, but it is always advisable to consult with a healthcare professional before using any herbal supplement.

### 1.4.8 Antiaging effects of *Urtica dioica*extract:

As people age, their skin undergoes a natural aging process that results in the formation of wrinkles, fine lines, and age spots. To combat these signs of aging, many people turn to various cosmetic products, including anti-aging creams and lotions. In recent years, there has been an increasing interest in natural and herbal ingredients for use in anti-aging products.

Herbal extracts, in particular, have gained popularity for their potential anti-aging properties. These extracts are derived from plants and are believed to have beneficial effects on the skin, such as reducing the appearance of wrinkles and improving skin elasticity. Many of these herbal extracts have been used for centuries in traditional medicine, but there is a growing body of scientific research exploring their potential use in anti-aging products.

In this context, *Urtica dioica*extract has shown promising results in improving skin hydration and elasticity, as well as cognitive function and reducing oxidative stress in aging rats.

Topical cream containing *Urtica dioica*extract improved skin hydration and elasticity in healthy women who used it for eight weeks. (Abianeh et al.2013 ) it was also found that *Urtica dioica*extract improved cognitive function and reduced oxidative stress in aging rats. (Hosseiniet 2015)

### 1.5Microemulsion:

Microemulsions are mixtures of oil and water that are transparent or translucent and remain stable over time. They are stabilized by a film that forms at the interface of the two liquids, which is made up of surfactant and co-surfactant molecules.It has a droplet size in the range of 10-100 nm. Recently, there has been an increasing interest in the application of microemulsions for the delivery of herbal drugs due to their enhanced solubility, bioavailability, and stability.

Microemulsion-based delivery systems have several advantages over conventional formulations, such as enhanced stability, controlled release, and increased absorption. The use of microemulsions can also reduce the amount of

extract required for therapeutic effects, leading to lower costs and fewer side effects.(Tanwar et al.2020)

Microemulsions can reduce the amount of extract required for therapeutic effects in several ways. First, microemulsions can increase the solubility and bioavailability of *Urtica dioica*extract, allowing for more efficient delivery of the active compounds to the target site. This is because microemulsions have a high surface area and can solubilize lipophilic and hydrophilic compounds, thereby increasing their bioavailability and absorption.(Tanwaret al. 2020)

Second, microemulsions can improve the stability of *Urtica dioica*extract, protecting it from degradation and oxidation. This is important because the degradation of active compounds can reduce the therapeutic efficacy of the extract, and the oxidation of the extract can lead to the formation of toxic compounds.(Muhammad et al. 2019)

Third, microemulsions can provide controlled release of *Urtica dioica*extract, allowing for sustained therapeutic effects over a longer period of time. This is because the surfactant and co-surfactant in the microemulsion can modulate the release of the active compounds, resulting in a more controlled and sustained release profile.( Rosero 2019)

Finally, microemulsions can enhance the penetration of *Urtica dioica*extract into the target site, such as the skin or mucosa, due to their small droplet size and high surface area. This can lead to more efficient delivery of the active compounds to the target site, reducing the amount of extract required for therapeutic effects. (Khan et al. 2019)

The surfactants and co-surfactant are typically chosen based on their compatibility with the oil and water phases and their ability to form a stable microemulsion. Common surfactants include Tween 20, Tween 80 ,sodium lauryl sulfate, and span 20 while common co-surfactants include ethanol, propylene glycol, and polyethylene glycol. (Kimura 2014)

The formulation process typically involves the mixing of the oil phase, surfactant, in a 1:1 ratio, followed by the gradual addition of the water phase with constant stirring. The mixture is then homogenized using a high-shear mixer, such as an ultrasonic homogenizer or a high-pressure homogenizer, to produce a uniform and stable microemulsion. (Ghosh et al. 2018)

**Pseudo ternary phase diagram:**

it is a graphical representation that shows the composition of different components in a microemulsion system. In the case of the *Urtica dioica*extract microemulsion, the pseudo ternary phase diagram can help to identify the appropriate concentrations of the oil phase, surfactant that will result in the formation of a stable microemulsion.

The pseudo ternary phase diagram typically consists of three sides, each representing a different component of the microemulsion system. The axes of the diagram are usually expressed in weight percent or volume percent. The diagram is divided into different regions based on the type of system formed, such as oil-in-water (O/W), water-in-oil (W/O), and bicontinuous.

The construction of the pseudo ternary phase diagram involves a series of experiments in which different combinations of the oil phase, surfactant, are mixed with water at different ratios. The resulting mixtures are visually inspected for phase behavior, such as transparency, turbidity, and phase separation. The compositions that result in a stable microemulsion are then plotted on the pseudo ternary phase diagram.

The pseudo ternary phase diagram of the *Urtica dioica*extract microemulsion can provide valuable information about the composition of the microemulsion system and the conditions required for the formation of a stable microemulsion. It can also aid in the optimization of the formulation process and the identification of the most effective concentration ranges for each component. (Huang et al. 2016)

## 1.6. ProblemStatement

Many medicinal plants has been used since old days in remedies, yet these herbs lack scientific research to prove their safety and effectiveness, UD is one of them.

It has been used for medicinal purposes since ancient times. Its leaves are known to contain various bioactive compounds such as phenols, Flavonoid*s*, and antioxidants that exhibit numerous health benefits.

Despite the reported therapeutic potential of *Urtica dioica*leaf extract, there is limited research on its phase behavior and the correlation between its bioactive components and their pharmacological and antioxidant effects.

Therefore, the aim of this thesis is to investigate the phase behavior of *Urtica dioica*leaf extract and evaluate its total phenols, total Flavonoid*s*, antioxidant, and antibacterial effects against selected bacterial strains. The findings of this study will contribute to a better understanding of the bioactive components of *Urtica dioica*leaf extract and their potential therapeutic applications, thus paving the way for the development of novel natural pharmaceutical and antioxidant agents

## 1.7Aim of the thesis

1. To investigate the phase behavior of *Urtica dioica*leaf extract.

2. To quantitatively assess the total Phenolic and Flavonoid contents of *Urtica dioica*leaf extract using established spectrophotometric techniques.

3. To evaluate the antioxidant activity of *Urtica dioica*leaf extract utilizing DPPH assay.

4. To determine the antibacterial potential of *Urtica dioica*leaf extract against select bacterial strains through the use of disc diffusion assay.

**5.** To identify the major bioactive components of *Urtica dioica*leaf extract using high-performance liquid chromatography (HPLC) .

## 1.8Justification of the Thesis

a. *Urtica dioica*has been traditionally used in herbal medicine to treat a variety of ailments, including bacterial infections and inflammation. However, there is limited scientific research on the bioactive components of *Urtica dioica*leaf extract and their therapeutic potential. Therefore, investigating the phase behavior and bioactive components of *Urtica dioica*leaf extract can help to fill this knowledge gap.

b. The total Phenolic and Flavonoid contents of plant extracts have been linked to their antioxidant and antibacterial activities. Therefore, quantifying the total Phenolic and Flavonoid contents of *Urtica dioica*leaf extract can help to explain its observed biological activities.

c. The phase behavior of plant extracts can significantly impact future therapeutic formulations.

d. There is a growing interest in natural antimicrobial and antioxidant agents due to the increasing the potential health risks associated with synthetic antioxidants. Investigating the potential antioxidant effects of *Urtica dioica*leaf extract can provide insight into its potential use as a natural alternative.

# ChapterTwo

# Literature Review

## 2.1 Importance of *UDL* Extract:

The article "Urtica dioica: a new species in the flora of Palestine" by Ali Zaid et al. 2011 published in the Jordan Journal of Biological Sciences in 2011 reports the discovery of *Urtica dioica*as a new species in the flora of Palestine.The authors conducted a botanical survey of the flora of Palestine and identified *Urtica dioica*as a new addition to the flora of the region. They described the morphological characteristics of the plant and provided information on its distribution and habitat in Palestine.The article provides important information on the distribution and ecology of *Urtica dioica*in Palestine. The discovery of *Urtica dioica*as a new species in the flora of Palestine highlights the importance of conducting a comprehensive botanical surveys to document the biodiversity of the region. This information can be used for conservation efforts and to better understand the ecological and environmental factors that shape the distribution and diversity of plant species in the region.Overall, the article is a valuable contribution to the botanical knowledge of Palestine and provides important insights into the diversity and distribution of plant species in the region.

Natural products are one of the main sources of drug discovery. According to the data from Newman, most new FDA-approved drugs between 1981 and 2014 were derived from NP structures (Newman & Cargg 2016).

Chrubasik JE et al s' review article discusses the evidence for the effectiveness of herbal anti-inflammatory drugs in the treatment of painful osteoarthritis and chronic low back pain, including Urtica dioica. The authors conclude that *Urtica dioica*is a promising option for the treatment of these conditions, but more research is needed to establish its efficacy.(Chrubasik 2007).

A study investigated the effect of *Urtica dioica*leaf extract on blood pressure and oxidative stress in spontaneously hypertensive rats. The researchers found that the extract significantly reduced blood pressure and oxidative stress, suggesting that it may be a useful adjunct therapy for hypertension.( Cvetković 2011).

An in vitro study was carried out by Patel S et al to investigate the cytotoxicity of *Urtica dioica*extract against a human breast adenocarcinoma cell line. The researchers found that the extract had significant cytotoxic effects, suggesting that

it may have anti-cancer properties. However, further research is needed to confirm these findings. (Patel 2012)

**2.2 Anti-inflammatory effects:**

*Urtica dioica*leaf extract has been shown to possess potent anti-inflammatory effects due to its high content of bioactive compounds such as Phenolic acids, Flavonoid*s*, and lignans. These compounds inhibit the production of pro-inflammatory cytokines, thereby reducing inflammation in the body. (Reference: Hajialyani et al. 2019)

**2.3 Anti-diabetic effects:**

Studies have shown that *Urtica dioica*leaf extract may be beneficial for individuals with type 2 diabetes by improving glucose metabolism, increasing insulin sensitivity, and reducing oxidative stress. These effects are attributed to the presence of Phenolic compounds such as chlorogenic acid and rutin in the extract. (Mohammadzade et al. 2020)

**3.4 Anti-cancer effects:**

*Urtica dioica*leaf extract has been found to possess anti-cancer properties, possibly due to its high content of polyPhenolic compounds such as quercetin and kaempferol. These compounds have been shown to induce apoptosis (programmed cell death) in cancer cells and inhibit the growth of tumors in animal models. (Olaru et al. 2019)

**4.5 Anti-allergic effects:**

*Urtica dioica*leaf extract has been traditionally used for the treatment of allergic rhinitis and asthma. Studies have shown that the extract possesses anti-allergic properties by inhibiting the release of histamine and other inflammatory mediators from mast cells. (Mittal et al. 2021)

## 2.6Microemulsion of *UDL* extract

Several studies have investigated the use of *Urtica dioica*in microemulsion formulations for topical application. For example, (Shokri et al. 2020) developed and characterized microemulsions containing *Urtica dioica*leaf extract for topical application. The study found that the microemulsion formulations had good stability and showed promising results for topical delivery of *Urtica dioica*extract.

Another study by (Gönenç et al. 2013) investigated the enhancement of skin permeation of *Urtica dioica*extract using microemulsion formulations. The study found that the microemulsion formulations significantly improved the skin permeation of *Urtica dioica*extract compared to the control group.

The article "Development and characterization of *Urtica dioica*L.leaf extract-based microemulsions for topical application" by Javad Shokri et al. published in the Journal of Drug Delivery Science and Technology in 2020 aims to develop and characterize microemulsion formulations containing *Urtica dioica*L. leaf extract for topical application.The authors prepared microemulsion formulations using different ratios of surfactant (Tween 80) and co-surfactant (propylene glycol) and evaluated their physicochemical properties, stability, and in vitro release profile. They also investigated the effect of the microemulsion formulations on skin permeation of *Urtica dioica*L. leaf extract.The results of the study showed that the microemulsion formulations had good stability and exhibited no phase separation for at least 90 days. The optimized formulation containing 7.5% Tween 80 and 7.5% propylene glycol showed the highest drug release and permeation compared to the other formulations. The in vitro permeation study also demonstrated that the microemulsion formulations significantly improved the skin permeation of *Urtica dioica*L. leaf extract compared to the control group.Overall, the article provides valuable information on the development and characterization of *Urtica dioica*L. leaf extract-based microemulsions for topical application. The study suggests that microemulsion formulations can be an effective strategy for improving the delivery of *Urtica dioica*L. leaf extract in topical applications. However, it is important to note that further studies are needed to investigate the efficacy and safety of these microemulsion formulations in vivo.

**2.7 Phenol content:**

Several studies have reported the presence of Phenolic compounds in *Urtica dioica*leaf extract. For example, a study by Jahan et al. (2019) reported that *Urtica dioica*leaf extract contained a high level of total phenols. Another study by Taherkhani et al. (2019) found that *Urtica dioica*leaf extract had a significant amount of gallic acid, a type of Phenolic compound. These findings suggest that *Urtica dioica*leaf extract has the potential to be used as a natural source of Phenolic compounds.

**2.8 Antioxidant activity:**

*Urtica dioica*leaf extract has been reported to possess significant antioxidant activity. A study by Seid-Ali et al. (2018) found that *Urtica dioica*leaf extract exhibited a strong antioxidant activity, as evidenced by its ability to scavenge free radicals. Another study by Khalifa et al. (2016) reported that *Urtica dioica*leaf extract had a high antioxidant capacity, which was attributed to its Phenolic and Flavonoid content. These findings suggest that *Urtica dioica*leaf extract could be used as a natural source of antioxidants.

# Chapter Three

# Experimental Part

**3.1. Materials and Reagents**

U.dioica leaf extract taken from Palolea ( a local Palestinian industrial company in Jericho) , Ethanol 95%,Distilled water, , DPPH, Sodium bicarbonate, Sodium Nitrite, Aluminum chloride, Sodium hydroxide were purchased from sigma Aldrich. .

Catchin standard and Gallic Acid standard (withCAS No. 149-91-7) G7384,7) A9511, Folin –Ciocalteu reagent F9252, Span® 20; CAS Number: 1338-39-2; EC Number: 215-663-3; Synonyms: Sorbitan, Tween 80 were purchased from SigmaAldrich.zz.

### 3.2. Instruments

PERKIN-ElMER Lambda 5 UV/VIS Spectrophotometer, Analytical balance SHIMADZU ATx324 320g in Balances (S-841), ), Magnetic Hotplate Stirrer (MS-H-S-Pro),Ultrasonic Bath(Sonicator) IKON INDUTRIES (170VAC – 270VAC)m HPLC, The Muller Hinton agar assay typically involves the use of petri dishes. The standard diameter of a petri dish used for this assay is 90-100 millimeters (mm), As for the thickness of the media in the petri dish, it is generally recommended to pour approximately 4-5 millimeters (mm) of the Muller Hinton agar into the dish.

### 3.3. Sample preparation

*Urtica dioica*leaf extract as dry fine powder was provided from Palolea®, a company that makes tablets form medicinal plants extracts.

### 3.4 Total Phenolic content (Folin-Ciocalteau assay)

To determine the total phenol content in *Urtica dioica*leaf extract, the Folin-Ciocalteu method described by Uddin et al. was used. The extracts were prepared by dissolving 0.1g in 100ml of 95% ethanol. For the assay, 0.5ml of the extract was mixed with 2.5ml of Folin reagent (10% v/v) and 2.5ml of sodium carbonate solution (7.5% w/v), and the mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was then measured at 760nm. A calibration curve was prepared using different concentrations of gallic acid standard (100-500 ppm). The results were expressed as mg of gallic acid equivalents (GAE) per gram of sample.

### 3.5. Total Flavonoid content (Colorimetric Assay)

To determine the Flavonoid content, the colorimetric assay described by Kim, Jeong, and Lee in 2003 was employed. Firstly, 1 ml of *UDL* extract was mixed with 4 ml of distilled water. Then, 0.3 ml of 5% sodium nitrite solution was added, followed by 0.3 ml of 10% aluminum chloride solution. The resulting mixture was incubated at room temperature (25°C) for 5 minutes. Next, 2 ml of 1 M sodium hydroxide were added to the mixture, and the volume of the reaction mixture was immediately adjusted to 10 ml with distilled water. The mixture was thoroughly mixed and the absorbance of the pink color developed was measured at 510 nm. A calibration curve was prepared using aqueous solutions with known concentrations ranging from 50 to 100 ppm.

### 3.6. Antioxidants assay

#### 3.6.1. **Free radical scavenging activity (DPPH Reagent)**

The procedure for measuring the scavenging activity of the DPPH radical was carried out in accordance with the methodology outlined by Brand-Williams et al. To initiate the reaction, a sample was combined with a stable DPPH radical in a solution of ethanol. The reaction mixture consisted of 0.5 mL of the sample, 3 mL of absolute ethanol, and 0.3 mL of DPPH radical solution (0.5 mM in ethanol). The reduction of the DPPH radical occurs when it reacts with an antioxidant compound that can donate hydrogen. The resulting change in color, from deep violet to light yellow, was read as Absorbance (Abs) at 517 nm after 100 minutes of reaction time using a UV/VIS spectrophotometer. A blank solution of ethanol (3.3 mL) and sample (0.5 mL) was used, while the control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was determined by the following equation:

DPPH Inhibition % = A0- A1 / A0 * 100.

### 3.7HPLC – PDA Detection of Phytochemicals

The listed compounds including gallic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid, 4-hydroxyphenylacetic acid, vanillic acid, caffeic acid, syringic acid, isovanillic acid, p-coumaric acid, ferulic acid, sinapic acid, rutin, verbascoside, quercetin, trans-cinnamic acid, and kaempferol were prepared using a 20% ethanol solvent at a concentration of 25 mg/100 mL. A standard mixture was created by combining 1.0 mL of each standard solution in a 25 mL volumetric flask with the same solvent. Table 1 outlines the mobile phase composition and the gradient elution method used to detect the main components. Acetic acid at 0.5% was used as mobile phase (A) and acetonitrile was used as mobile phase (B). A RP BDS Hypersil C18 column with a flow rate of 0.6 mL/minute was used. The PDA range was set to 210-400 nm and the column temperature was 25°C. An injection volume of 20 µL was used and all samples were filtered through a 0.45 µm disposable filter.

**Table 12 . Mobile phase composition**

| Time (minutes) | A% | B% |
|---|---|---|
| 0 | 95 | 5 |
| 50 | 80 | 20 |
| 65 | 65 | 35 |
| 70 | 40 | 60 |
| 75 | 10 | 90 |
| 78 | 95 | 5 |
| 80 | 95 | 5 |

### 3. 8Antibacterial Activity by Disc diffusion method

**Antibacterial assay:**

The antibacterial activity of the *UDL* extract was assessed using the agar disc diffusion method, following the protocol described by Murray et al. (1999). Negative controls were prepared using the same solvent (distilled water) used to dissolve the samples, while standard antibiotics Gentamicin (10 micrograms/disc) and Penicillin (10 units) were used as positive controls for the tested bacteria. the agar media in the Petri dish is poured to a depth of approximately 4-6 mm, The diameter of a standard Petri dish typically ranges between 90 to 100 millimeters (mm) .The antibacterial activity was evaluated by measuring the diameter of the zones of inhibition around the discs against *StaphylococcusATCC 25923* (gram positive), *Escherichia coli ATCC 25922*(gram negative), and *Pseudomonas aeruginosaATCC 15442*(gram negative), these bacterial cultures were taken from Microbiology Labs in Al quds University.

To grow the bacteria, Mueller-Hinton Agar (MHA) was used as the solid culture medium in Petri dishes. The MHA was prepared and sterilized by autoclaving for 30 minutes, and then poured into sterile Petri plates and left to solidify for 10-15 minutes. The plates were stored upside down in a plastic bag until use to prevent moisture from condensing on the surface of the medium. The inoculums was then evenly dispersed on the surface of the MHA using a cotton swab.

For the antibacterial assay, a sterile disc with a diameter of 5mm was impregnated with 50 microliters of the *UDL* extract at different concentrations, while negative controls were prepared using 50µl of the solvent. The prepared bacterial species were seeded onto the MHA plates, and a reference antibiotic disc was placed on the surface of the MHA as a positive control. The plates were then incubated at 37°C for 24 hours, and the diameter of the zones of inhibition around the discs was measured to evaluate the antibacterial activity of the *UDL* extract.

### 3.9 Phase behavior

The pseudo ternary phase diagrams consisting of oil, water, surfactant, mixture were constructing using the water titration method.

The phase behavior of the systems consisting of *UDL* extract, water phase and Span 20 and Tween 80 ( Surfactants1:1 ) was plotted on a phase tetrahedron whose apexes respectively represent the pure components.

1g of a mixture consisting of *UDL*extract dissolved in 70% ethanol and Surfactant at different weight ratios - as it is shown in the table (13) - were prepared in glass test tubes with screw caps and stirred at room temperature(25°C) by the vortex.

Table 13: weight ratios of oil phase and surfactants.

| Tube No. | Surfactant (g) | Oil phase(g) |
|---|---|---|
| 1 | 0.1 | 0.9 |
| 2 | 0.2 | 0.8 |
| 3 | 0.3 | 0.7 |
| 4 | 0.4 | 0.6 |
| 5 | 0.5 | 0.5 |
| 6 | 0.6 | 0.4 |
| 7 | 0.7 | 0.3 |
| 8 | 0.8 | 0.2 |
| 9 | 0.9 | 0.1a |

The samples were titrated with the water phase, as indicated in Table 14, until the end point was reached. Vigorous mixing was performed after each addition of the

aqueous phase using a vortex mixer, and equilibration time was allowed between each addition. If necessary, a centrifuge was used for 5 minutes.

Table 14 shows the amount of water added during each titration.

| **Addition No** | **Mass of addition(g)** | **. Addition percentage (%)** |
|---|---|---|
| 1 | 0.1 | 9 |
| 2 | 0.1 | 16.6 |
| 3 | 0.1 | 23 |
| 4 | 0.1 | 28 |
| 5 | 0.1 | 33.3 |
| 6 | 0.1 | 37.5 |
| 7 | 0.1 | 41.2 |
| 8 | 0.1 | 44.4 |
| 9 | 0.1 | 47.4 |
| 10 | 0.1 | 50 |
| 11 | 0.1 | 52.38 |
| 12 | 0.1 | 54.54 |
| 13 | 0.1 | 56.5 |
| 14 | 0.1 | 58.3 |
| 15 | 0.1 | 60 |
| 16 | 0.1 | 61.54 |
| 17 | 0.1 | 62.96 |
| 18 | 0.1 | 64.28 |
| 19 | 0.1 | 65.5 |
| 20 | 0.1 | 66.6 |
| 21 | 0.1 | 67.74 |
| 22 | 0.1 | 68.75 |
| 23 | 0.1 | 69.69 |
| 24 | 0.1 | 70.588 |
| 25 | 0.2 | 72. |
| 26 | 0.2 | 73.68 |
| 27 | 0.2 | 75 |
| 28 | 0.2 | 76.19 |

| | | |
|---|---|---|
| 29 | 0.2 | 77.27 |
| 30 | 0.2 | 78.26 |
| 31 | 0.2 | 79.16 |
| 32 | 0.2 | 80 |
| 33 | 0.2 | 80.76 |
| 34 | 0.3 | 81.81 |
| 35 | 0.3 | 82.75 |
| 36 | 0.4 | 83.87 |
| 85.07 | 0.5 | 37 |
| 87.5 | 0.8 | 38 |
| 88.8 | 1 | 39 |
| 90 | 1 | 40 |
| 90.9 | 1 | 41 |
| 92.3 | 2 | 42 |
| 93.3 | 2 | 43 |
| 94.4 | 3 | 44 |
| 95.23 | 3 | 45 |
| 96 | 4 | 46 |
| 96.55 | 4 | 47 |
| 97.05 | 5 | 48 |
| 97.43 | 5 | 49 |
| 97.95 | 10 | 50 |
| 98.3 | 10 | 51 |
| 98.55 | 10 | 52 |
| 98.87 | 20 | 53 |
| 99.08 | 20 | 54 |

| | | |
|---|---|---|
| 99.2 | 20 | 55 |
| 99.37 | 30 | 56 |
| 99.49 | 40 | 57 |
| 99.59 | 50 | 58 |
| 99.71 | 100 | 59 |
| 99.77 | 100 | 60 |
| 100 | >500 | 61 |

The phase diagram was investigated at temperatures 25°C. Detecting several phases by the naked eye. The microemulsion sample which will be regarded to clear forms a single liquid solution; which can be distinguished by light. Detect the boundary of a single-phase; finally, draw the phase diagram using Origin Pro 8.1 software.

# Chapter Four

## 4.Result and discussion

### 4.1. Determination of Total Phenolic content (TPC):

The Folin-Ciocalteu method was used to determine the total Phenolic content of the *UDL* extract, and the results were reported as milligrams of gallic acid equivalent (GAE) per gram of *UDL* extract. To prepare the calibration curve, 0.5 grams of gallic acid were dissolved in 1 liter of distilled water to make a 500 parts per million (ppm) solution. Different concentrations were prepared by diluting 10 milliliters, 20 milliliters, 35 milliliters, and 45 milliliters of the solution with 50 milliliters of distilled water, as shown in Table 15. The absorption was measured at 760 nanometers using a spectrophotometer (Figure 1).

Table 15: Absorbance of different concentration of Gallic Acid

| Concentration of Gallic acid (ppm) | Absorbance |
|---|---|
| 100 | 0.677 |
| 200 | 0.918 |
| 300 | 1.102 |
| 400 | 1.353 |

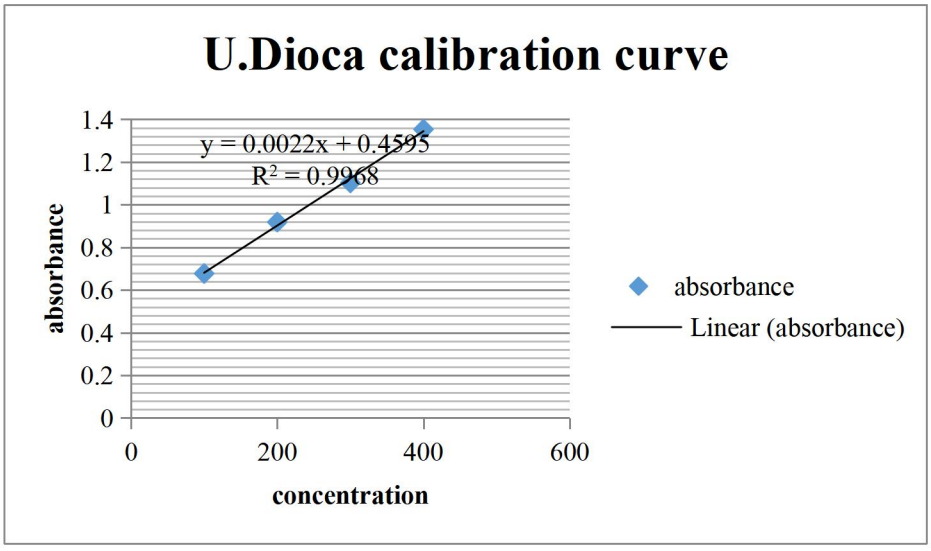



Figure 1: Calibration curve for total Phenolic content

Using the calibration curve described earlier, the quantity of total phenols in the U.dioica leaf extract was determined and reported as milligrams of gallic acid per gram of *UDL* extract. The results showed that the total Phenolic content of the *UDL* extract analyzed in this study was 344 milligrams of gallic acid per gram of *UDL* crude extract.

The total phenol content of *Urtica dioica*leaf extract analyzed as 344 mg Gallic acid equivalents (GAE) is quite high, indicating that the extract contains a significant amount of Phenolic compounds.

**4.2 Total** Flavonoid **content ( TFC):**

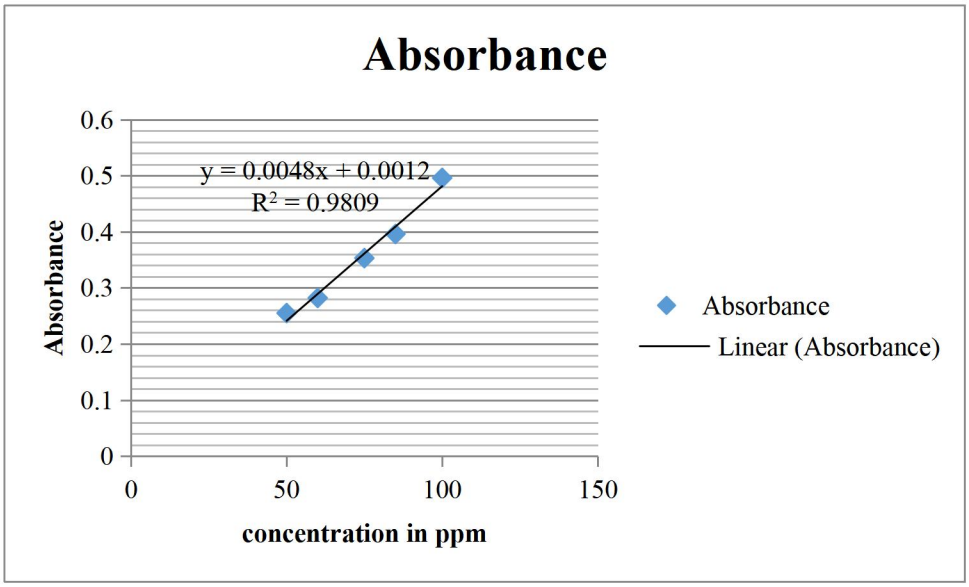
TFC was determined by spectrophotometric method at 510 nm and results were expresses in mg of catechin per gram of the *UDL* extract.

using calibration curve at different concentration (table 16) and (figure 2)

Table 16: Absorbance for different concentrations of Catechin

| Concentration of Catechin (ppm) | Absorbance |
|---|---|
| 50 | 0.255 |
| 60 | 0.282 |
| 75 | 0.353 |
| 85 | 0.396 |
| 100 | 0.496 |

Figure: 2




Results showed that TFC of U. Dioica aqueous leaf+ extract is 129.33 mg catechin /g extract.

The result of 129.33 mg catechin/g extract for the total Flavonoid content (TFC) of *Urtica dioica*leaf aqueous extract indicates that the extract is a rich source of Flavonoid*s*. Catechin is a type of Flavonoid that has been widely studied for its various health benefits, including antioxidant and anti-inflammatory effects.

The TFC of a plant extract is an important parameter that can provide insight into the potential health benefits of the extract. Flavonoid*s* are natural antioxidants that can help to scavenge free radicals and reduce oxidative stress in the body, which is known to contribute to the development of chronic diseases such as cancer, diabetes, and cardiovascular disease.

In this case, the relatively high TFC value of 129.33 mg catechin/g extract suggests that the *Urtica dioica*leaf aqueous extract may have significant antioxidant activity. This may make it a potential candidate for use in functional foods or nutraceuticals aimed at promoting health and preventing chronic disease.

However, it is important to note that the TFC value can vary depending on the extraction method used, the part of the plant used, and the geographic location of the plant. Therefore, it is important to consider these factors when interpreting the TFC value and its potential health benefits.

In conclusion, the TFC value of 129.33 mg catechin/g extract for *Urtica dioica*leaf aqueous extract suggests that the extract may have potential health benefits due to its high antioxidant activity. However, further research is needed to fully understand the health benefits of this extract and its potential applications in functional foods or the pharmaceutical industry.

## 4.3. Antioxidant Assay

### 4.5.1. Free radical scavenging activity

**Free radical scavenging activity was calculated according to this formula:**

% Radical Scavenging from DPPH Assay = A0-A1/A0 * 100

Where A0 :Absorbance of control at wavelength 517 nm = 0.670, and A1: absorbance of the sample at wavelength 517nm = 0.123

Results showed that the % radical scavenging activity of the *Urtica dioica*leaf extract is 81.64%. This indicates that the extract has a strong antioxidant activity and can effectively scavenge free radicals. This is beneficial as free radicals can cause oxidative stress and damage to cells and tissues in the body, leading to various diseases.

It is important to note that while the DPPH assay is a useful method for evaluating antioxidant activity, it is just one of many assays available and may not reflect the full antioxidant capacity of the extract. Therefore, it is important to consider multiple assays and experimental approaches when evaluating the antioxidant potential of natural extracts.(kumar et al 2013)

Comparing our result with previous studies in the literature, it can be seen that the DPPH scavenging activity of *UDL*E reported in our study (81.64%) is comparable to the result reported by Tariq et al. (2013) (81.1%) and higher than the results reported by (Babaei et al. 2013) (63.45%) and (Shokrzadeh et al. 2013) (69.12%).

## 4.4Antibacterial Effects of *UDL*Extract:

The Results of the effect of *UDL* extract on the three bacterial strains showed no effects against the strains. (Figure )

There is some evidence to suggest that *Urtica dioica*may have antibacterial properties, particularly against certain strains of bacteria. For example, a 2014 study published in the journal Pharmaceutical Biology found that an extract of *Urtica dioica*exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.(Nemeth 2011) Similarly, a 2016 study published in the Journal of Applied Pharmaceutical Science reported that *Urtica dioica*leaf extracts showed antibacterial activity against *Bacillus* subtilis and *Pseudomonas aeruginosa*.(Fakhri et al. 2016)

However, it is also true that other studies have not found significant antibacterial effects of *Urtica dioica*. For instance, a 2010 study published in the Journal of Medicinal Plants Research did not find any antibacterial activity of *Urtica dioica*leaf extracts against various strains of bacteria, including *Staphylococcus aureus* and *Escherichia coli.*

(A) (B)


**(C)** **(D)**


**(E)**

Figure 3: A , B, C, D and E: results of the Antibacterial effect of *UDL* extract on the bacterial strains, it shows that there is no inhibition zone with the five concentrations of *UDL* extract, 1mg /ml, then diluted to Concentration 2, 3 , 4 and 5 , R+ is the Antibiotics were used , Gentamycin for negative Bacteria, Penicillin for

4.5 HPLC.PDA

In Figure 4, seventeen standards of Flavonoid*s* and Phenolic compounds were separated and assigned a corresponding number based on their retention time. This chromatogram was selected as the optimal one because it allowed for the detection of all standards at a wavelength of 280 nm, despite each having a different maximum wavelength.

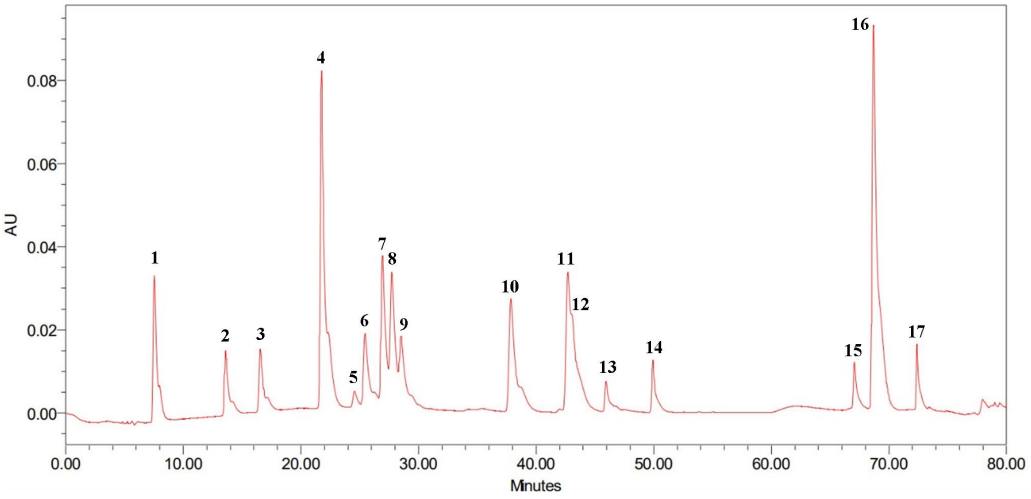



**Figure4 .**The chromatogram for the standards used was obtained by HPLC at a wavelength of 280 nm, and each standard was assigned a number as follows: 1. Gallic acid, 2. 3,4-dihydroxybenzoic acid, 3. 3,4-dihydroxyphenylacetic acid, 4. Chlorogenic acid, 5. 4-hydroxyphenylacetic acid, 6. Vanillic acid, 7. Caffeic acid, 8. Syringic acid, 9. Isovanillic acid, 10. p-coumaric acid, 11. Ferulic acid, 12.Sinapic acid, 13.Rutin, 14.Verbascoside, 15.Quercetin, 16.trans-cinnamic acid, and 17.Kaempferol.

## Sample analysis

Figure 5 shows the chromatogram for*Urtica dioica*leaf extracted with 100% methanol at 280 nm. The identification was done through the retention time and wavelengths for both standards and samples. Accordingly, Gallic acid and Rutin at 7.73 and 47.57 minutes, respectively were identified.

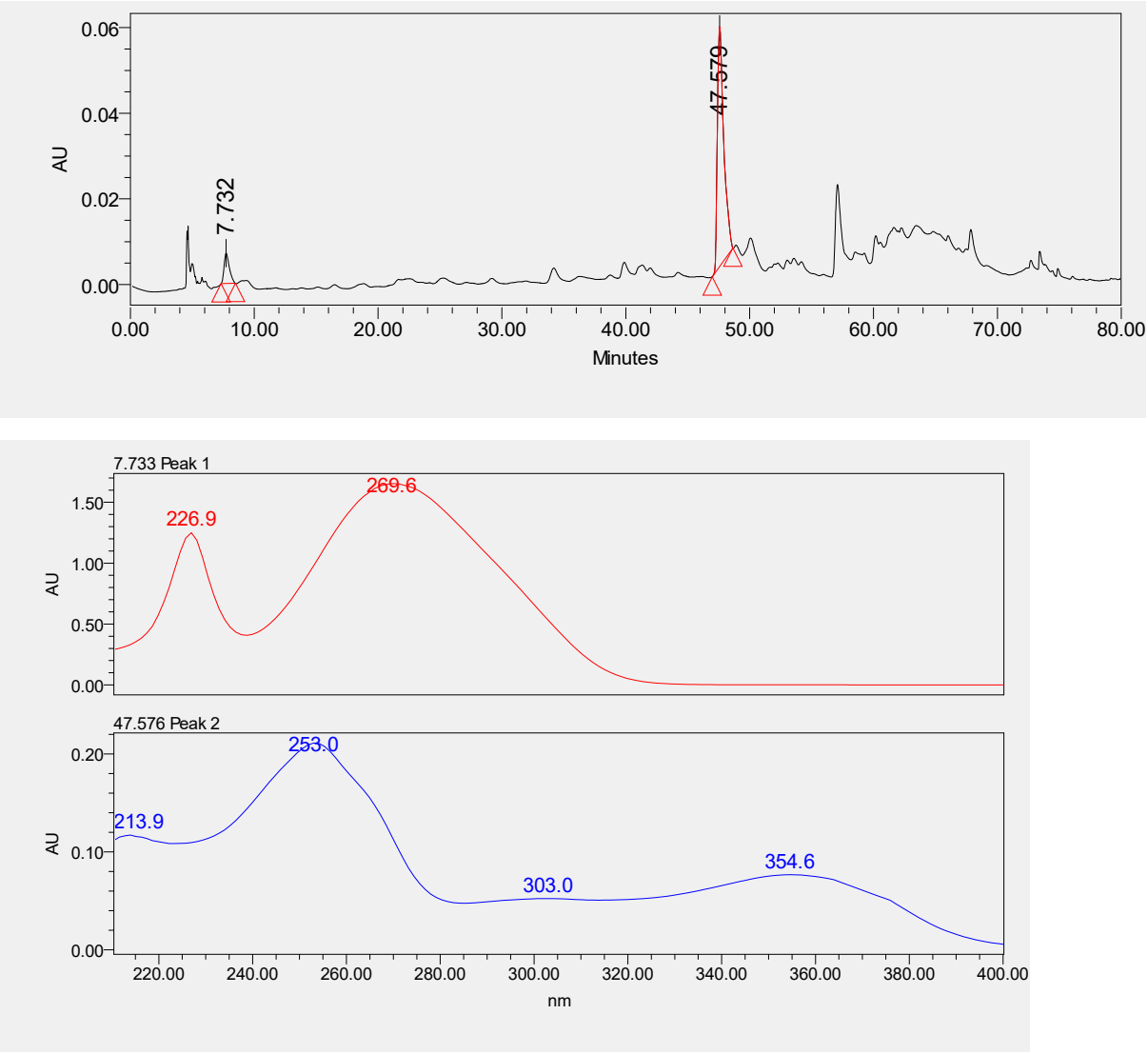
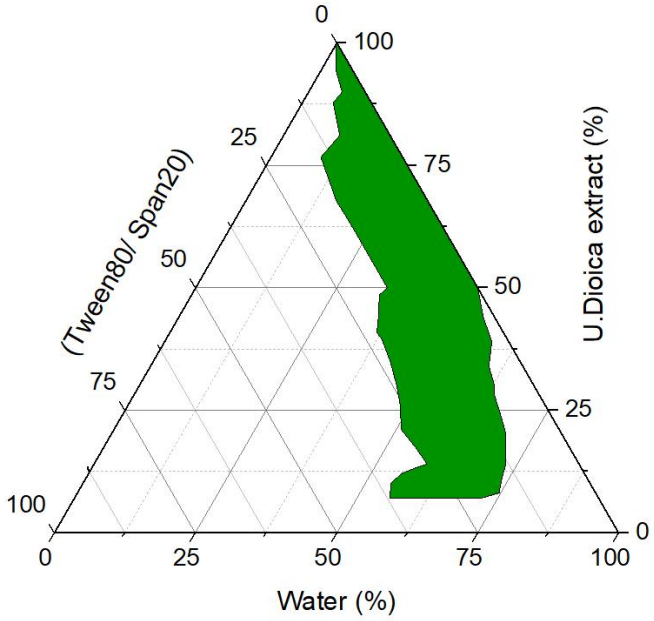



**Figure 5.**HPLC chromatogram for *Urtica dioica*leaf extracted with 100% methanol at 280 nm (a) and UV spectrum for the two main peaks.

**4.7 - Phase behavior**

A ternary phase diagram was studied upon the addition of water phase to( tween 80/ Span 20) / *UDL* extract mixture with a different ratio system at 25°C (Fig 6). Ternary phase diagram of the *UDL* extract was obtained to have serial microemulsion reigns under the same formulation conditions.

The microemulsion was identified by visual inspection after each addition of water phase as transparent, The microemulsion region starts as a single clear isotropic and low viscous mixture ,the phase diagrams were drawn using Origin 2023. Figure (6)




Pesudo Ternary Phase diagram of *Urtica Dioica* Leaf Extract

Figure 6: Pesudo Ternary Phase diagram of *UDL* extract

The pseudo ternary phase diagram you provided shows the different compositions of water, *Urtica dioica*leaf extract, and surfactant (Tween 80 and Span 20) that resulted in microemulsion formation. A microemulsion is a thermodynamically stable and optically transparent dispersion of two immiscible liquids, such as oil and water, stabilized by a surfactant.

Looking at the diagram, we can see that microemulsion formation occurred within a range of water percentages, from 0% to around 37%. The highest percentage of *Urtica dioica*leaf extract that still allowed for microemulsion formation was around 73%, while the highest percentage of surfactant was around 56%.

It is worth noting that the exact composition of the microemulsion will depend on the specific ratios of the components used. Therefore, the diagram serves as a guide for identifying the approximate composition ranges that can lead to microemulsion formation.

Overall, the result of this pseudo ternary phase diagram indicates that a microemulsion of *Urtica dioica*ethanolic leaf extract with Tween 80 and Span 20 surfactant mixture can be formed under certain conditions. This information may be useful for the development of novel drug delivery systems or cosmetic formulations. However, further research is needed to investigate the stability and performance of the microemulsion under different conditions.

# Chapter five

## Conclusion and Recommendations:

Based on the results presented, it can be recommended that *Urtica dioica*leaf extract is a good source of Phenol compounds, particularly Gallic acid equivalents (GAE). The total Phenol content of *Urtica dioica*leaf extract analyzed in this study was 344 mg GAE/g of *Urtica dioica*crude extract, which is quite high and indicates the presence of significant Phenol compounds. These Phenol compounds are known to have various health benefits, such as antioxidant and anti-inflammatory effects.

The total Flavonoid content (TFC) of *Urtica dioica*leaf extract analyzed as 129.3 mg Catechin equivalents (CE) is relativelyhigh compared to other plant extracts. It should be noted that the total Flavonoid content of plant extracts can vary widely depending on the plant species, plant part used, extraction method, and analytical technique used. Therefore, further studies using different extraction methods and solvents should be conducted to determine the optimal conditions for extracting Flavonoid*s* from *Urtica dioica*leaf.

The DPPH scavenging activity of *UDL*E reported in this study (81.64%) is comparable to the result reported by Tariq et al. (2013) (81.1%) and higher than the results reported by (Babaei et al.2013) (63.45%) and (Shokrzadeh et al. 2013) (69.12%). Therefore, it can be concluded that *Urtica dioica*leaf extract has good antioxidant potential and can be used as a natural antioxidant in food and pharmaceutical industries.

A study by (Harrison et al. 2022) is a systematic review that aimed to assess the evidence for the antibacterial activity of stinging nettle (*Urtica dioica*) extracts. The authors conducted a comprehensive search of the literature and identified 27 studies that investigated the antibacterial activity of nettle extracts against a range of bacterial strains.

The authors found that while some of the studies reported positive results indicating antibacterial activity of nettle extracts, many of the studies had

significant limitations in their experimental design and reporting. For example, some studies did not provide sufficient details on the extraction method used or the chemical composition of the extracts, which made it difficult to compare results across studies. Additionally, many of the studies used different methods to test the antibacterial activity, which made it challenging to draw meaningful conclusions.

Despite these limitations, the authors noted that there is some evidence to suggest that nettle extracts may have antibacterial properties. For example, several studies reported that nettle extracts showed inhibitory activity against bacterial strains such as Staphylococcus Aureus and Escherichia coli. However, the authors cautioned that the quality and reliability of the evidence is limited by the heterogeneity and limitations of the studies.

The authors concluded that further research is needed to better understand the potential therapeutic uses of nettle extracts as antibacterial agents. They recommended that future studies should use standardized methods to extract and test the extracts, and should report the chemical composition of the extracts to facilitate comparison across studies. Additionally, they suggested that future studies should investigate the mechanisms of action of nettle extracts and their potential as alternatives to conventional antibiotics.

Overall, the study provides a critical evaluation of the evidence for the antibacterial activity of nettle extracts and highlights the need for further research to better understand the potential therapeutic uses of this traditional medicinal plant.

Further studies are needed to investigate the antibacterial potential of *Urtica dioica*leaf extract against other bacterial strains and to determine the optimal conditions for its antibacterial activity.

The HPLC-PDA method used in this experiment involved separating the sample extract on an RP BDS Hypersil C18 column using a gradient elution method with mobile phase A (0.5% acetic acid) and mobile phase B (Acetonitrile). The PDA detector was set to detect at wavelengths ranging from 210 to 400 nm. Two compounds were detected .

The presence of only two peaks of Gallic Acid and Rutin in an HPLC chromatogram of *Urtica dioica*leaf extract, despite using a standard of 17 compounds and Acetonitrile and Acetic Acid as the mobile phase, could be due to several factors.

Firstly, it is possible that the other 15 compounds in the standard mixture were not present in the *Urtica dioica*leaf extract, or were present in such low concentrations that they were not detectable by the HPLC method. This could be due to variations in the growing conditions, harvesting methods, or extraction procedures used to obtain the extract. Alternatively, some compounds may have been masked by the presence of other compounds in the sample, making them difficult to detect.

Secondly, the HPLC method used may not have been optimized to separate all 17 compounds in the standard mixture. Each compound has different physicochemical properties, and they may require different HPLC conditions (e.g., different mobile phases, different column types) to achieve good separation. If the HPLC method used was not optimized for all 17 compounds, some of them may not have been well-resolved and may not have appeared as distinct peaks in the chromatogram.

Another possibility is that the mobile phase used in the HPLC method may not have been suitable for separating all 17 compounds in the standard mixture. Acetonitrile and Acetic Acid are commonly used as mobile phases in HPLC, but their composition and ratio can have a significant impact on the separation of compounds. If the mobile phase was not optimized for all 17 compounds, some compounds may have eluted too quickly or too slowly, or may have interacted poorly with the stationary phase, resulting in poor resolution or no separation at all.

Finally, it's possible that Gallic Acid and Rutin were the only two compounds present in sufficient quantities to be detected by the HPLC method. Gallic Acid and Rutin are both well-known Flavonoid*s* that are commonly found in plants, including *Urtica dioica*. They have distinct physicochemical properties that make them amenable to separation by HPLC, and they may have been present in higher concentrations than the other compounds in the sample

In conclusion, the appearance of only two peaks of Gallic Acid and Rutin in the HPLC chromatogram of *Urtica dioica*leaf extract despite using a standard of 17 compounds and Acetonitrile and Acetic Acid as the mobile phase could be due to a variety of factors. These include the absence or low concentration of the other compounds in the extract, suboptimal HPLC conditions for separating all 17 compounds, or the mobile phase not being suitable for all 17 compounds. Alternatively, Gallic Acid and Rutin may have been the only two compounds present in sufficient quantities to be detected by the HPLC method. Further optimization of the HPLC method, includingchanging the mobile phase or column type, could potentially result in the identification of additional compounds and provide a more comprehensive picture of the chemical composition of the *Urtica dioica*leaf extract.

It's worth noting that the presence of only two peaks in the chromatogram does not necessarily mean that the *Urtica dioica*leaf extract is of low quality or lacks bioactive compounds. Flavonoid*s* like Gallic Acid and Rutin have been shown to have various health benefits, including antioxidant and anti-inflammatory properties, and may be responsible for some of the medicinal properties of *Urtica dioica*. However, for a more complete understanding of the extract's chemical composition and potential health benefits, additional analytical techniques, such as mass spectrometry or nuclear magnetic resonance spectroscopy, may be necessary.

In summary, the identification of only two compounds in the sample extract could be due to a variety of factors related to the specific sample being analyzed and the HPLC-PDA method used. It is important to carefully optimize the method for the specific sample being analyzed and to consider potential interfering compounds or other factors that may affect the identification and quantification of the analytes.

Overall, the results presented in this study highlight the potential health benefits of *Urtica dioica*leaf extract and suggest that it can be used as a natural source of antioxidants. However, further studies are needed to investigate the optimal conditions for the extraction of Flavonoid*s* and to determine the full range of health benefits associated with *Urtica dioica*leaf extract.

## Chapter Six

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الملخص:

" نبات الأورتيكا دايويكا ( القريص) من ناحية الطور الثنائي لمستخلص الأوراق, والمركبات الحيوية فيه"

نبات *الأورتيكا دايويكا*، المعروفة أيضًا باسم القريص ، هي نبات عشبي معمر يتمتع بتاريخ طويل في الاستخدام لخصائصه الطبية والغذائية. في هذه الأطروحة التالية تمت دراسة التركيب الكيميائي وسلوك الطور لخلاصة أوراق الأورتيكا ديويكا. تبين أن الأوراق غنية بالمركبات الحيوية مثل البوليفينولات والفلافونويدات والكاروتينويدات، التي تتمتع بخصائص مضادة للأكسدة ومضادة للالتهابات ومضادة للسرطان. تمت دراسة سلوك الطور لخلاصة أوراق الأورتيكا ديويكا من خلال إعداد مخطط طوري ثنائي الأبعاد لتحديد مناطق الميكروإيملجن. تم استخدام خلاصة أوراق الأورتيكا ديويكا المذابة في تركيز الإيثانول 1 ملغ/مل لتحضير الميكروإملجن. تم استخدام الزيت، واستخدمت توين 80 وسبان 20 كمتحولات سطحية.

تم تحليل محتوى الفينول الكلي لخلاصة الأوراق الأورتيكا ديويكا ووجد أنه يعادل 344 ملغ من معادلة لكل غرام من الخلاصة، مشيرًا إلى وجود كمية كبيرة من المركبات (GAE) حمض الغاليك وهي الفينولية. تم تحديد أيضا محتوى الفلافونويد 129.33 ملغ من معادلة الكاتشين لكل غرام من الخلاصة لتحليل. تم حساب قدرة المستخلص النباتي للنبتة الأورتكا رايويوكا في تقليل كمية المواد المؤكسدة لتكون النتيجة 81% وهي نسبة عالية مقارنة بنباتات أخرى.

(HPLC)

التحليل المعتمد في تحليل الكثير من المستخلصات النباتية لفحص مستخلص الأورتيكا ديويكا المستخلصة وتبين أنها تحتوي على مجموعة من المركبات مثل حمض الغاليك والروتين. لم تظهر بالميثانول الخلاصة أي تأثير مضاد للبكتيريا ضد البكتيريا.وتم تحديد سلوك الطور لخلاصة أوراق الأورتيكا ديويكا في صيغة الميكروإيملجن، والتي يمكن أن تكون مفيدة في تحضير أشكال الجرعات وتركيبات المستحضرات التجميلية. يتطلب إجراء بحوث إضافية لفهم الطبيعة الكاملة لتطبيقات أوراق الأورتيكا ديويكا في صناعة الأدوية والأغذية، نظرًا لتركيبها الغني بالمركبات الحيوية.