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Phase behavior of Basil extractions and their favorable attributes

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Dedication

I dedicate my dissertation work to my beloved homeland; Palestine. To my beloved Al-Quds capital and university.

Also I would like to dedicate it to my supervisor Dr. Ibrahim Kayali and co-supervisor Dr. Mutaz Akkawi who provided me with a continuous support whenever I was in need.

A special feeling of gratitude to my loving husband, who stood by me when things look bleak and supported me throughout the process.

My Mom whom whatever I said, I will not be able to thank her. she has never left my side and are very special, who was my first fan to go in this way and always encouraging me to complete my education.

My sister Aya, who was behind these beautiful invitations. whos words of encouragement and push for tenacity ring in my ears.

To the sweethearts of my heart and the sweetness of my eyes, my beautiful little girls those who bearing my absence.

At last but not least to my dearest friends and to all people in my life who love me, remember me and touch my heart.

Declaration

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

Hadba Mousa Issa Tarayrah

Signed: ..... 21/12/2012 .....

Date: 21-12 -2021.

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Hadba Tarayrah

Abstract:

Nowadays there is a great focus on developing medicines, and searching for plant sources in order to reduce side effects. Moreover, research into new techniques to increase the effectiveness of the active substance obtained. Natural products extraction has played a key role in the discovery of leads for the development of new drugs. Basil plant was selected for our study, which has more than 200 chemical compounds.

In this study, basil leaves and seeds were extracted through the use of the Soxhlet extraction apparatus, This extraction method that we developed and modified in our laboratory was able to extract almost all active compounds from this plant..

The in vitro susceptibility for basil extracts was determined against antibacterial activity by agar disc diffusion method, minimum inhibitory concentration and minimum bactericidal concentration for *Eschericha coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter sp*, and *Pseudomonas aeruginosa* in the presence of positive control (Gentamicin (10 μg/disc) and Penicillin 10 unit).

The findings demonstrated that ethanol extract seeds and leaves exhibit antibacterial action against the majority of bacterial strains. The highest zone of inhibition for seeds extract against *Enterobacter sp*. was 10 mm at 100 mg/ml concentration, whereas the highest zone of inhibition for leaves extract against *Enterobacter sp*. was 25 mm at 100 mg/ml concentration as diffusion (spread). A strain of *Staphylococcus.aureus* has an MIC of 62.5 μg/ml, represents the highest efficacy of the extracts against bacteria strains.

The extracts of leaves and seeds were evaluated for their antioxidant activity (AA) which was determined by using a modified method of the assay of ferric reducing/antioxidant power (FRAP), and the total phenolic content (TPC) by the Folin-Ciocalteu assay. All the analysis was made with the use of a UV-Visible spectrophotometer. Leaves and seeds showed strong antioxidant ability and high phenolic content. The extraction of a seeds part of the plant has 74 ±1.4 mg/g antioxidant activity, comparing this value with those obtained by leaves part 40.4 ±0.8 mg/g. while the total phenolic content (TPC) for Basil seeds (58.2 ±0.9 mg/g) while that of Basil leaves (51 ± 2.4 mg/g).

Moreover, analysis of their flavonoid content (TFC) by using HPLC chromatography at 254 nm. flavonoid compounds were detected in 6.60 minutes for Basil seeds while detected in 6.60 minutes for Basil leaves. Furthermore, total flavonoids content (TFC) was measured by using the aluminium chloride spectrophotometric method. The total flavonoids content (TFC) in leaves (9.0 ± 1.5 mg/g) while for seeds (34.2 ± 3.6 mg/g).

The potential in vitro antimalarial activity of different extracts of the Palestinian Basil herb (*Ocimum*) was done in different solvents (ethanol 99.5%, ethanol 35%, ethyl acetate, petroleum ether and water) using a semi-quantitative assay method it was found that β-hematin in either one

of these solvents is not prevented. This result shown for the first time in this study , rules out one mechanism proposed to explain the plant's antimalarial activity that is inhibition of beta-hematin formation.

This study aims to formulate phase behavior using leaves and seeds extracts from Basil, tween 20 as a surfactant and co-surfactant also was used with absolute ethanol. This investigation is the first report on the formation and production of pseudo ternary phase behaviour for Palestinian Basil (leaves and seeds) extracts. In this research, it was found that Palestinian basil has the capability to the formation of microemulsion in vitro systems.

Microemulsion technology was used to produce a medical cream. The formula is moisturizing, smoothing, good smell, absorb by skin (don't leave film), not irritation pH= 5-5.5, good color, easily spread (not too viscous, apply easily and quickly), and anti-aging, antierythmic, and depigmenting effects when applied topically.

Based on these findings, it is concluded that Basil constitutes a natural source of potent antioxidants, antibacterial, anti-aging, antierythmic, and depigmenting effects. that may prevent many diseases and could be potentially used in cosmetics, and pharmaceutical products.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{وَالْحَبُّ ذُو الْعَصْفِ وَالرَّيْحَانُ} سورة الرحمن آية 12

{فَأَمَّا إِنْ كَانَ مِنَ الْمُقَرَّبِينَ * فَرَوْحٌ وَرَيْحَانٌ وَجَنَّةُ نَعِيمٍ } سورة الواقعة آية 89

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List of Abbreviations, Symbols and Terminology:

Abbreviation	Full word
SE	Soxhlet extraction
RP-HPLC	Reversed phase high-performance liquid chromatography analysis
AA	Antioxidant Activity
RA	Rosmarinic acid
RDA	Recommended Dietary Allowance
PDGF	Platelet-derived growth factor
DW	Dry Weight
TPC	Total Phenolic Content.
TFC	Total Flavonoid Content.
FRAP	Ferric Reducing Antioxidant Power.
GAE	Gallic Acid Equivalent
FDA	Food and drug administration
AOAC	Association of Official Agricultural Chemists
WHO	World Health Organization
MHA	Muller Hinton agar
DMSO	Dimethylsulfoxide
EtOH	Ethanol
min	Minute
MIC	Minimum Inhibitory Concentration
MBC	Minimum bactericidal Concentration

Chapter one :
Introduction

1.1 Basil :

1.1.1-Introduction

Natural products are an important and primary source for drug development. Natural remedies were constructed and evolved over thousands of years in the daily lives of ancient peoples and in the course of their battle against, prevention, and treatment of human ailments, and they have had a positive impact on human civilization. Natural goods have served as the foundation for the development of new drugs. however a high percentage of the studies developed so far fall within the field of analytical chemistry.(Castro et al.,2000)

Basil is a plant in the *Lamiaceae* family that belongs to the *Ocimum basilicum* L. family. This plant is a fragrant herb that develops to be 20–80 cm tall. The stem is glabrous and woody at the base, and the leaves are large, green in color, broadly epitical, 2.5–5 cm 1–2.5 cm in size. Flowers are tiny (3 mm), red, pink, or white, and are arranged in a spikea at the end of the plant. Additionally, the genus *Ocimum* comprises 50 to 150 species of herbs and shrubs. It is regarded the original native of basil due to the excellent climatic conditions in India and China, although it is now widely cultivated commercially in many tropical and temperate nations in Asia, Africa, Central America, and South America. (Ghasemzadeh, et al., 2016; Tran, et al., 2018;Venancio et al.,2011;Javanmardi, et al., 2002;Barbalho, et al., 2012).

Basil is a culinary as well as a decorative herb. Basil leaf, whether dried or fresh, is a common spice and cuisine component. It's a popular herb, prized for its rich, spicy, gently peppery flavor with a hint of mint and clove, and it's been utilized in a variety of food goods and confectionary flavors. (Javanmardi,et al., 2002; Ghasemzadeh, et al., 2016; Tran, et al., 2018).

Natural products' combinatorial chemistry provides more drug-like features to molecules in terms of functional groups, chirality, and structural complexity, which explains why nearly half of FDA-approved chemical drugs for the treatment of human diseases are derived from or inspired by natural products. This necessitates the development of efficient and selective technologies for extracting and isolating bioactive natural compounds. (Bascón et al., 2020; Zhang et al.,2018).

1.1.2- Phytochemical constituents of Basil:

Since 1930, several studies have looked into the phytochemical constituents of sweet basil essential oil, and more than 200 chemical compounds have been identified, revealing a huge diversity in the constituents of its oil from various parts of the world, including monoterpenes, limonene, myrcene, terpinolene, flavonoids (quercetin, kaempferol, rutin), phenolic acids (p-coumaric acid, caffeic acid, caftaric acid), vitamins ,and steroids .(Ghasemzadeh, et al., 2016;Marwat, et al., 2011).

1.1.3- Extract Techniques of Basil :

Extraction is frequently employed as a first step to separate desired natural products from raw materials. Solvent extraction, distillation, pressing, and sublimation are all extraction procedures that follow the extraction principle. Solvent extraction is the most widely used method. It is generally employed as a sample preparation technique in which target compounds are transferred from one phase, the sample or sample-containing phase, to a liquid phase where further processing and/or analysis occurs. Over there many properties may have effects on extraction like solvent efficiency, the particle size of the raw materials (fine, coarse ,etc), the solvent-solid ratio, the temperature and duration of extraction .(Bascón et al., 2020; Zhang, et al., 2018).

Sample extract of plants has been developed for decades using a wide variety of techniques. One of them is conventional Soxhlet extraction. This technique is currently one of the most frequently used extract techniques in plant. Not only in chemical analysis, but also in many other fields. (Bascón et al., 2020; Zhang, et al., 2018; Luque de, et al., 2000).

In conventional Soxhlet, operation is repeated until complete extraction is achieved. The sample (Basil) is placed in extraction thimble-holder and during operation is gradually filled with condensed fresh solvent or extractant (The term used to refer to a solvent utilized for extraction) from a distillation flask, as shown in Fig. 1. When the liquid reaches the level of surplus, a siphon aspirates the contents of each the thimble holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid. This performance makes Soxhlet a crossbred continuous-discontinuous technique. As much as the solvent acts stepwise, the assembly can be considered as a batch system; however, since the solvent extractant is recirculated through the sample, the system also bears a continuous character. (Bascón et al., 2020)

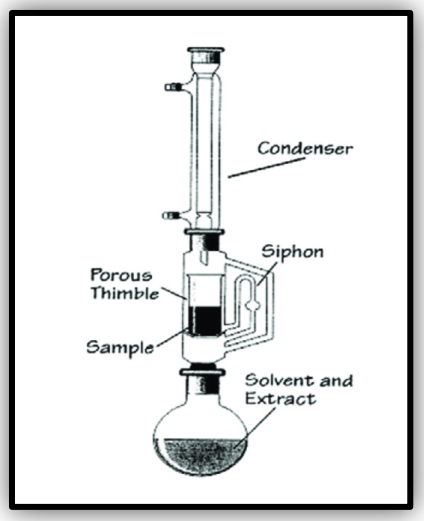


Figure 1.1 Soxhlet extraction apparatus

1.2 Phase Behavior of Basil extract (Emulsification)

Pseudo-ternary phase diagrams are constructed at fixed cosurfactant/surfactant weight ratios. Ternary phase diagrams are constructed to define the region of microemulsion that determines the appropriate concentration ranges of three components (oil, water and surfactant) Which leads to formation of microemulsion , which shall be preweighed into glass vials and titrated with water and stirred well at room temperature. (Mishra, et al., 2014)

Microemulsion, which was first introduced by Hoar and Schulman in 1943, is one of the practical dosage forms. A microemulsion is a transparent dispersion system made up of an aqueous phase, an oil, a surfactant, and a co-surfactant. It forms a single liquid solution, an optically isotropic liquid with droplet diameters typically ranging from 10 to 100 nm and thermodynamically stable systems, and that in a microemulsion, a specific boundary surfactant is located between the oil and aqueous phases, giving the microemulsion a distinct microstructure. (Triastuti, et al., 2019; LEE., 2011).

The capacity to boost the solubilization, dissolving rate, and bioavailability of poorly soluble medicines, as well as superior thermodynamic stability and ease of manufacture, are all advantages of microemulsion. The ability of microemulsion to increase cutancous delivery of hydrophilic and lipophilic medicines has been demonstrated in numerous investigations. (Triastuti, et al., 2019)

Basil is commonly used to treat a variety of ailments. Antioxidant, antibacterial, antimalarial, and hypercholesterolemia characteristics have been documented. Despite multiple articles demonstrating the plant's health advantages, little work has been done to generate microemulsion formulations incorporating Basil extracts. (Triastuti, et al., 2019; Pansang, et al., 2010)

1.3 Medical effect of Basil

1.3.1-Introduction

The therapeutic characteristics, nutritional value, and pharmacological activity of medicinal plants have been widely recorded, including antioxidant, antithrombotic, anti-inflammatory, antiartherogenic, and cardioprotective benefits. Basil is a particularly beneficial herb since the entire plant has been used in traditional medicine around the world in the form of household remedies for a variety of human problems since antiquity. The leaves, stems, seeds, and flowering portions have traditionally been used to treat stomach cramps, gastroenteritis, fever, poor digestion, nausea, headaches, insomnia, and depression as antispasmodic, aromatic, carminative, and digestive remedies. The plant's anti-inflammatory, anti-oxidant, and antibacterial qualities have been used in traditional medicine. (Tran, et al., 2018; Venancio et al., 2011; Baskaran, et al., 2015; Ghasemzadeh, et al., 2016).

Basil can also be used to help with digestion, respiratory circulation, cold symptoms, and digestion problems. As a result, sweet basil leaves are used to make healthful food products and

are commonly used in traditional medicine to relieve stress, prevent gout, and for other health advantages. They've also been used to treat acne, insect stings, snake bites, and skin diseases on the outside. (Tran et al., 2018; Venancio et al., 2011;Ghasemzadeh, et al., 2016).

1.3.2 Anti-aging, antierythmic and depigmenting effects of basil extracts:

Skin diseases are one of the common diseases in different parts of the world. Which includes skin aging, erythmic and hyperpigmentation , wound healing ,ets.

Ultraviolet (UV) irradiation is the major risk factor that leads to skin aging induced when constant exposure to it. Skin aging is a complex process, UV generates reactive oxygen species leading collagen deficiency and eventually skin wrinkling. Irregular pigmentation, increased wrinkles, loss of elasticity, dryness and roughness are features of skin aging (Rasul A., Akhtar N,2011). Hyperpigmentation is the most common pigment disorder and spots, which usually appear because of increase in melanin synthesis or uneven distribution of melanin (Rasul,et al.,2011). Burn trauma and wounds are still a big problem in developing countries, Serious complications often occur and involve high costs of treatment.(Salmah,et al.,2005).

The stratum corneum's (SC) low permeability to medicines often limits dermal and transdermal medication delivery, preventing pharmaceuticals from passing the skin at therapeutic rates. As a result, the administration of medications through the skin has received a lot of attention in the pharmaceutical industry. As a result, the use of compounds that function as skin permeability enhancers has the potential to improve drug delivery. The SC's barrier qualities can also be lowered. Transdermal research has shown that developing new enhancers and investigating the mechanics of permeation enhancement is a very important and busy subject. (Fang,et al.,2004).

Natural products are important sources for biologically active drugs and many plants are used in the modern phytocosmetics (Saleh et al., 2009).furthermore, An important aspect of the use of traditional medicinal remedies and plants to reduce the financial burden. and the same time several plants and herbs have been used experimentally to treat skin disorders. Natural ingredients are attracting a lot of attention as drug permeation enhancers. Fatty acid extracts and essential oils are examples of these enhancers. Medicinal plants having active chemical components that have antioxidant qualities are effective in preventing a variety of degenerative disorders. (Lukmanul et al., 2008;Fang,et al.,2004; Rasul,et al.,2011; Salmah,et al.,2005).

Basil (*Ocimum basilicum*) is an annual plant, with surprising medicinal properties in dermatology. it is rich in essential oils that have been the subject of numerous chemical studies and which contains several antioxidant compounds. Essential oils extracted from 0.basilicum show to be the least toxic extract among different *Ocimum* species. Additionally, no evidence to date has indicated if a 3% *Ocimum* product would cause any skin reaction. (Rasul A., Akhtar N,2011; Fang,et al.,2004; Pansang,et al.,2010).

1.3.3 Antibacterial effect:

The consumption of contaminated foods caused the illness and has a broad economic and public health impact worldwide. Many pathogenic microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Salmonella sp.,* and *Pseudomonas aeruginosa* have been reported as the causal agents of foodborne diseases .and another disease caused by *Klebsiella pneumonia* and *Enterobacter*. Antimicrobial agents are used to inhibit bacteria in food through a variety of different chemical and synthetic compounds. Interestingly, plants are well recognized since the prehistoric times, widely used by all civilizations throughout the millennia as the antimicrobial, antiseptic, and other therapeutic applications. As a result, there has been an upsurge in demand for natural food preservatives. Chemical food preservatives have been discovered as having potential harm. (Hossain et al.,2010; Sienkiewicz et al.,2013).

Plant essential oils are explored as a promising alternative for current used antimicrobials, this activity is attributed to their ability to synthesize aromatic substances, the majority of them are phenols or oxygen-substituted derivatives. Monoterpenes, sesquiterpenes, and their oxygenated derivatives make up essential oils, which are a complex collection of molecules (alcohols, aldehydes, esters, ethers, oxides, phenols, and ketones). Many species of *Ocimum* (including *O.basilicum*) essential oils have antimicrobial activity against multidrugresistant clinical strains of many pathogenic microorganisms and have a broad spectrum of in vitro antimicrobial activities. They have also been used as flavoring agents or preservatives in food, beverage, and confectionery products for a long time. (Hossain et al.,2010; Sienkiewicz et al.,2013; Sakkas, et al., 2017).

The *Lamiaceae* family includes the *Ocimum* L. (basil) genera. This popular herb is used as a fresh and dried food spice, and basil plants are highly respected for their therapeutic potentials in traditional medicine. Essential oils of *Ocimum basilicum* Antibacterial, antifungal, and antioxidant activities are present. (Hossain et al.,2010; Sienkiewicz et al.,2013).

1.3.4 Antioxidant effect:

The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest. *Ocimum basilicum* also possesses significant antioxidant qualities, which have a variety of therapeutic effects on the body, such as acting as a cancer preventative and reducing the occurrence of diseases with the highest death rates worldwide. (Juliani, et al., 2002; Patil, et al.,2011).

Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or drinks and the prevention of diseases. These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others. Natural antioxidants can be found as single chemical entities or as components in

complicated combinations, which has substantial medicinal implications. As a result, the phenolic profile and antioxidant activity of the extract derived from the common herb basil have been examined. (Juliani et al., 2002;Patil, et al.,2011; Damien et al.,2010).

Basils (*Ocimum basilicum*), a popular dietary ingredient from the *Lamiaceae* (*Labiatae*) family, include a variety of essential oils rich in phenolic compounds as well as a variety of other natural products, including polyphenols like flavonoids and anthocyanins. Rosmarinic acid is responsible for basil's antioxidant effects. One of the most prevalent esters of caffeic acid found in Ocimum is rosmarinic acid (a-O-caffeoyl-3-4-dihydroxyphenyl-lactic acid). (Juliani et al., 2002; Patil et al.,2011; Dorman et al.,2010).

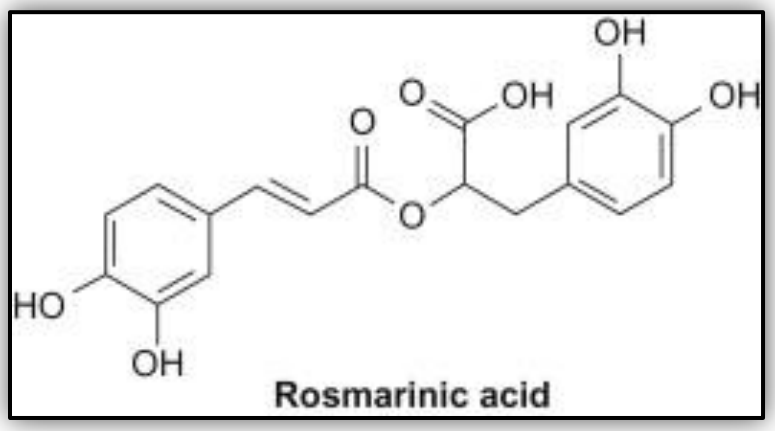


Figure 1.2 : Rosmarinic acid

Antioxidants are the first line of defense against free radical damage and are essential for preserving optimal health and luxury. Free radicals cause cell damage and are thought to play a significant part in the aging process and disease progression. Pollution, cigarette smoke, narcotics, disease, stress, and even exercise all increase free radical exposure, all of which can lead to oxidative stress. As a result, with greater exposure to free radicals, the requirement for antioxidants becomes even more crucial. As a result, many experts consider that the Recommended Dietary Allowance (RDA) for certain antioxidants may be insufficient, and that the demand in some circumstances may be several times the RDA. (Patil et al.,2011)

1.3.5 Antimalarial effect:

Malaria is still one of the most common infectious diseases in the world, killing over half a million people each year, according to WHO figures. In 2006, 247 million people were infected with malaria, putting 3.3 billion people at risk. In 2016, an estimated 216 million cases of malaria were reported worldwide, with 445,000 deaths, the majority of whom were children under the age of five. The majority of malaria cases and deaths were reported in Africa, which accounted for 91% of all malaria deaths.(Akkawi et al.,2018; Mabubu et al.,2015; Inbaneson et al.,2012)

Malaria is caused by parasites such as Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, and Plasmodium vivax. P. falciparum is a Plasmodium protozoan that is transmitted to humans through the bite of an infected female Anopheles mosquito, which then introduces the parasite into the bloodstream of the human host. (Akkawi et al.,2018; Mabubu et al.,2015; Inbaneson et al.,2012)

The parasites developed resistance to the most commonly used anti-malarial medications, while the vectors developed pesticide resistance. Antifolates and artemisinin have brought attention to the critical need for new antimalarial medications to combat this deadly disease. In the treatment of malaria and other diseases, plant extracts are still commonly employed. As a result, many of today's antimalarial medications have their origins in nature; *Ocimum basilicum* is a significant essential-oil producing plant, and its oil has a wide range of chemical structures. (Grayer et al., 1996; Akkawi et al.,2018; Mabubu et al.,2015; Inbaneson et al.,2012)

Secondary metabolites come in a wide range of chemical compositions in nature. Due to their health-promoting antibacterial and antioxidant potentials, naturally occurring secondary metabolites of phenolic phytochemicals from various basil kinds have gotten increased attention. Basil oil contains a wide range of ingredients. It is impacted not just by chemical patterns, but also by factors like harvest day timing. In addition, the phenolic type and composition are largely determined by the basil variety employed and the extraction process used. This could account for some of the discrepancies in the science and reporting on basil's medical value from culture to culture. (Berger, 1985; Martin et al., 2003;Akkawi et al.,2018; Mabubu et al.,2015; Inbaneson et al.,2012)

1.4 Objectives:

The main objective of this study is the preparation and production of pseudo ternary phase behaviour for Palestinian Basil (leaves and seeds) extracts. Furthermore, the formation of microemulsion in vitro systems.

Additionally, to Procedure one of the mechanism proposed to explain the plant's antimalarial activity that is inhibition of beta-hematin formation.

There are other objectives needed to be studied and achieved:

- To check the Antibacterial activity against five clinical pathogens.
- Antioxidant activity (AA) by using a modified method of the assay of ferric reducing/antioxidant power (FRAP).
- Total phenolic content (TPC) by the Folin-Ciocalteu assay.
- HPLC chromatography at 254 nm.
- Total flavonoids content (TFC) by using the aluminium chloride spectrophotometric method.
- Microemulsion technology to produce a medical cream.

Chapter Two:
Literature Review.

Jamal Javanmardi, et al. (2002) evaluated the 23 basil (*Ocimum basilicum* L) accessions from Iran areas, looking at quantitative and qualitative features as well as phenolic acid chemical variation. Using high-performance liquid chromatography, the quantity of phenolic acids was evaluated, and there were significant differences between accessions. Rosmarinic acid is the most abundant phenolic acid in flower and leaf tissues, according to chemical analysis.

Thien Hien Tran, et al.(2018) used Basil plant as a common source for linalool and estragole. to extract essential oil from Vietnamese basil, microwave-assisted hydro-distillation (MAHD) power of 430 (W) was used with Response Surface Methodology (RSM). Furthermore, gas chromatography-mass spectrometry (GC-MS) was utilized to analyze the chemical composition of the obtained oil. Resulting of GC-MS analysis revealed that essential components of Vietnamese Basil were Estragole (87.869 %), a-Bergamotene (2.922%), T-Cardinal (2.770%), and Linalool (1.347%).

In order to improve the medicinal quality of sweet basil leaves (*Ocimum basilicum*) and phytochemical contents, **Ali Ghasemzadeh, et al. (2016)** tested ultraviolet (UV)-B irradiation at different intensities at the post-harvest stage. Total flavonoid content (TFC) and total phenolic content (TPC) were measured and identified using ultra-HPLC and spectrophotometric methods (TPC). The influence of chalcone synthase (CHS) on flavonoid metabolism was investigated using a CHS test enzyme. As a cipher. Basil extracts were tested for antiproliferative and antioxidant activities against a breast cancer cell line (MCF-7) using MTT and (DPPH) assays, respectively.

Spectrophotometric method, ultra-high performance liquid chromatography analysis, and (UV)-B at different intensities revealed the presence of the highest TFC and TPC for 8 h irradiation. furthermore, the highest CHS activity was determined value of 3.6 W/m² of UV-B. UV-B treated leaves have been reported to possess the highest antiproliferative effect and DPPH activity with a half-maximal inhibitory concentration value. That ultraviolet (UV)-B irradiation with post-harvest irradiation can be considered a promising technique to develop the pharmaceutical properties and healthy-nutritional of sweet basil leaves according to the results were shown when that techniques and methods were applied .

2.1 -Phase Behavior of Basil extract (Emulsification)

According to **Triastuti, et al., (2019)**, they created a microemulsion to boost the plant's economic worth and provide society with a more cheap dosage form. Microemulsions are made up of a mixture of oil, water, and a surfactant (s). Three formulae were produced employing a variety of surfactants (Formula 1 = 10: 1, Formula 2 = 11: 1, and Formula 3 = 12: 1), as well as cosurfactant and P. major extract (0.3 percent). For all formulations, the results revealed homogeneity and stability, as well as meeting the particle size requirements for microemulsions. After topical administration of microemulsions for 4 weeks at different temperatures (4 ° C, 25 °

C, and 40 ° C), ear edema and pro-inflammatory cells in the tissue were reduced, and its effectiveness was comparable to hydrocortisone 1 percent ($p > 0.05$).

Pansang, et al., 2010 investigated Thai *0. basilicum* microemulsion on human subjects using various approaches. The creation of a microemulsion using *Ocimum basilicum* (sweet basil) essential oil, which has been shown to have antibacterial action against Propionibacterium acnes. Thus, utilizing a closed patch epicutaneous approach under semi-occlusion conditions, microemulsion was tested for skin irritation reactions to 3 percent basil oils. (18%) The findings of this investigation demonstrate that a 3% *Ocimum* microemulsion is safe and well-tolerated on male human skin after all items are applied to the upper back of each subject for I h. For all formulations examined, TEWL values were constant from baseline, and erythema indices were lower than baseline.

2.2 Medical effect of Basil

The medical properties were studied by **Ghasemzadeh, et al., (2016)** when used ultraviolet-B irradiation to improvement in flavonoids and phenolic acid production and the pharmaceutical quality of *Ocimum basilicum*. Total flavonoid content (TFC), total phenolic content (TPC), antiproliferative activity, and Antioxidant activity of basil extracts were determined by Spectrophotometric method, ultra-high performance liquid chromatography analysis, DPPH assay, and MTT assays, respectively.

Baskaran, et al., (2015) studied the medical properties of Basil extract, which have been reported to have phytochemical components. The result revealed the Basil possesses antihypercholesterolemic effects. By using reversed-phase high-performance liquid chromatography and Gas chromatography with tandem mass spectrometry analysis, Basil extract has been shown the presence of eicosyl ester, luteolin, 1-heptatriacotanol, naringin, oleic acid, α-tocopherol, apigenin, ascorbic acid, and phenol 2,6- bis(1,1-dimethyl ethyl). The highest inhibition effect of about 74% appeared in B.alba among the 25 medicinal plant extracts. (9%)

2.2.1 Anti-aging , antierythmic and depigmenting effects of basil extracts:

Rasul A. and Akhtar N,(2011) investigated the cream containing Basil leaves and flowers extract effects on antiaging potential, also studied the Formulation containing 3% of the concentrated basil extract by utilizing non-invasive methods. The creams were stored at different temperatures to predict their stabilities. The results demonstrated significant effects on skin moisture and TEWL after applied w/o emulsion of basil formulation. Significant effects were observed decline for Volume, skin roughness, and scaliness, smoothness, and skin wrinkles parameter but increase in the texture parameter of Energy values.

Rasul, et al., (2011) used a topical cream to study the benefits of a newly designed w/o emulsion containing 3 percent basil (*Ocimum basilicum*) extract against skin erythema and skin melanin in healthy human volunteers for 12 weeks. The findings were assessed every two weeks and revealed a reduction in skin pigmentation and erythema without causing discomfort. The formulation had no negative side effects and was safe to use.

Salmah, et al.,(2005) prepared *O. basilicum* L. alcoholic leave extract in combination with honey and solcoseryl – jelly to studied the synergistic effects of this combination on cutaneous wound healing as a topical application in the neck area of male Sprague Dawley rate. Strongly beneficial effects of basil extract appeared through the experiment for the acceleration of the wound healing process and the rates of wounds sterility. The wound showed clean and remain sterile over the experiment.

Fang, et al., (2004) want to enhance medication absorption through the skin. Sweet basil (Ocimum basilicum, OB) essential oils were tested for skin permeation enhancers and irritancy utilizing a variety of in vitro and in vivo methodologies. To employ in this investigation, different carbon counts of Terpenes (mono-, sesqui-, di-, and tri-) were specified in both the higher-polarity fraction (OB-2) and lower-polarity fraction (OB-1). The permeation enhancement of OB-1 was found to be larger than that of OB-2. The permeation enhancement of OB-1 was found to be larger than that of OB-2. Both OB-1 and OB-2, on the other hand, successfully accelerated transdermal medication delivery.

Also, **Pansang, et al.,(2010)** studied skin irritation reactions of a 3% *O.basilicum* microemulsion on human subjects. The cumulative irritancy study was performed on Thirty healthy human subjects for a period of 3-day, by used a closed patch epicutaneous technique under semi-occlusion conditions. The findings of this investigation demonstrate that a 3% *Ocimum* microemulsion is safe and well-tolerated on male human skin after all items were applied to the upper back of each subject for 1 hour. For all formulations examined, TEWL values were constant from baseline, and erythema indices were lower than baseline.

2.2.2 Antibacterial effect:

Hossain et al., (2010) investigated the antibacterial activity of basil against an increasing spectrum of foodborne pathogenic microorganisms. The GC-MS analysis of essential oils and methanol leaves and steam of sweet basil *Ocimum basilicum* L. (*Lamiaceae*) extracted by hydrodistillation. The entire leaf and stem oils are made up of 57 components, which account for 94.9 and 96.1 percent of the total. *O. basilicum* showed promising antibacterial activity against a variety of bacteria, with zones of inhibition ranging from 11.2-21.1 mm and MIC values ranging from 62.5 to 500 μg/ml. These findings suggest that *O.basilicum* could be used as an antibacterial agent in the food and pharmaceutical industries.

Sienkiewicz et al., (2013) conducted the same tests on multidrug-resistant bacterial strains and achieved the same results. Clinical strains acquired from patients with infections of the respiratory tract, abdominal cavity, urinary tract, skin, and hospital equipment were used to demonstrate the actions of basil (*Ocimum basilicum* L.) essential oils. The findings suggest that essential oils have potential against bacteria with various resistance mechanisms, as well as extended-spectrum b-lactamase positive bacteria. not just in treating resistant strains, but also in preventing their spread.

2.2.3 Antioxidant effect:

Juliani, et al., (2002) studied the antioxidant activity of ethanolic basil extract and essential oils. In vitro, the essential oils were tested utilizing two screens ABTS and FRAP, in the FRAP screen the antioxidant activity was related to ascorbic acid while in the ABTS screen the activity was related to Trolox. The results were showed the antioxidant activity in basils is largely due to There was a strong relationship between the total phenolic content and the antioxidant activity.

Patil, et al., (2011) investigated the antioxidant properties of ethanol, chc13, and cc4 extracts of *Ocimum basilicum* using various techniques. DPPH radical scavenging activity was tested on two basil extracts at different concentrations (10-50ig/ml). According to the findings, the ethanol extract of *Ocimum basilicum* has more antioxidant activity than a conventional antioxidant.

Dorman et al.,(2010) studied antioxidant-related activity. *Ocimum basilicum* L. leaf material was extracted by maceration with different solvents, and then examined their iron(III) reductive and free radical scavenging activities were determined in a battery of in vitro assays. Leaves of *Ocimum basilicum* displayed the highest activity as an antioxidant activity for all samples.

2.2.4 Antimalarial effect:

Using RPMI 1640 conditions, **Mabubu et al. (2015)** investigated the in vitro inhibitory effect of crude ethanol extracts of wild basil leaves on the growth of P falciparum at different dosages. Harborne (1989) technique was used to isolate sesquiterpene lactones contained in ethanol extracts of basil, which were then separated by preparative TLC, yielding 11 different

compounds. The potential antimalarial activity of these isolated compounds was then assessed in vitro by looking at their inhibition of dihydrofolate reductase. Two compounds were identified to be inhibitory to DHFR, with specific activity of 17.2 and 8.7 micromole/min/mg protein, respectively, among the eleven compounds eluted from TLC plates and examined.

Chapter Three :
Materials and Methods

3.1 Chemical material:

Materials were purchased from Sigma Aldrich: Absolute Ethanol (EtOH), Ethanol (35%), Methanol, TCA(trichloro acetic acid), PBS(phosphate buffer saline), Tween 20, Tween 80, Span 20, Ethyl acetate, $ALCL_3$, $[K_3Fe(CN)_6]$ Potassium ferricyanide, $NaNO_2$, $FeSo_4.6H_2O$(ferrous iron standard), Flavonoid standards (quercetin) (HPLC). DMSO (Dimethylsulfoxide) purity 99.5%.

3.2- Equipment and Apparatus:

The following equipment was used at Chemistry, Pharmacy, Biology and Environmental Sciences Laboratory lab at Al-Quds university: Analytical Balance from Sartorius CP, Mechanical grinder, Benchtop B4- centrifuge from Jouan., Refrigerator, Soxhlet apparatus, Rotary evaporator from IKA WEREK RV06-ML, Freeze Drying machine (Lyophilizer) from Labconco, Electronic Balance, spectrophotometer (UV2550, Shimadzu, Kyoto, Japan), Desiccators, Stabili therm incubator from Thermo, Hot plate, Water bath from Jouan, Wire brush, Graduated cylinder, Evaporating dish, Test tubes and Test tubes rack, Micro pipets, Spatula, Thermometer, Funnel, Beaker.

3.3-Methods:

3.3.1- Collection and identification of plant materials:

The plant of Basil was collected in summer 2020 from the various region of a cultivated field in Hebron, Palestine. It was identified by Dr. Khalid Sawalha (Associate Professor of Plant Biotechnology) from the Biology Department, Sciences Faculty (Al-Quds University). Where confirmed and identified plant Basil is: *Ocimum basilicum* L. *Lamiaceae = Labiatae*. It is more likely to be this plant with a binomial name. While seeds basil was bought from a local market in Ramallah, Hebron & Jerusalem.

Figure 3.1 *Ocimum basilicum*

3.3.2-Plant Sample:

Basil leaves were separated from stems and then washing with tap water flow and make sure to dry the basil really well. Cleaned basil leaves were air-dried at room temperature in the shade for about two days (make sure the leaves not too many on top of each other and completely dry them to prevents grow mold), grinded through the mechanical grinder and converted into a coarse powder, and then kept in the refrigerator until use. While The *basilicum* seeds were cleaned manually to remove husks and foreign matter, then uniformly grinded using a mechanical grinder to make a fine powder.

3.3.3-Basil extraction:

3.3.3.1-Preparation of Basil leaves ethanolic extract:

To identify the chemical components contained in basil, an ethanolic extract was generated using a soxhlet apparatus (type FA-46) extraction method utilizing 15gm of dry powder (After grinding the dry leaves) in 99.5 percent ethanol for 2 hours. The plant's powder to solvent ratio was 1:9 (wt/vol) during extraction using the soxhlet apparatus method in 99.5 percent ethanol. The extract was then filtered using MN615.110 mm filter paper after extraction.

Figure 3.2. Soxhlet apparatus (model FA-46)

After filtration, the crude ethanolic extract Basil was concentrated using a rotary evaporator under reduced pressure and Lyophilization (Freeze Drying) at -40C° and pressure 0.095 mbar until a consistent weight was reached. Lyophilization is the process of isolating a solid substance

from an aqueous solution by freezing the solution and evaporating the ice under vacuum utilizing a simple physics mechanism known as sublimation.
On a dry weight basis, the dried powder obtained was 1.2gm (8%), which was determined using the following equation:

$$\text{Percentage yield} = \frac{\text{Wt of dried extracts} \times 100}{\text{Wt of powder taken}}$$

The dry extract was stored in an opaque bottle and placed in a refrigerator until analysis by HPLC, also future tests and for further pharmacological studies. (Shah et al., 2017)
the same method was used to prepare the leaves and seeds extracts by utilized several solvents (ethanol 35%, ethyl acetate and petroleum ether) to carry out for antimalarial assay.

3.3.3.2-Preparation of Basil seeds ethanolic extracts:

Basil ground seeds extraction carried out by using the Soxhlet apparatus as described in the AOAC (1990) with ethanolic (99.5%) as a solvent. the cycles of ethanol were run till complete defatting was obtained. Then, the crude solvent extracts of Basil seeds were dried at room temperature and stored in an opaque bottle, and placed in a refrigerator until analysis by HPLC, also future tests and for further pharmacological studies. (Gajendiran,2016; Rabie,2013)

3.3.3.3-Preparation of Basil leaves and seeds water extracts:

2-grams of the Basil was soaking in 150 ml of distilled hot water at 90°C for 20 minutes, then cooled at room temperature, and filtered through MN 615 number 110mm filter paper in a filter funnel. Followed by using a rotary evaporator to gentle remove excess solvent under reduced pressure at 60-70°C, concentrated extracts were obtained. The residue was freeze-dried and stored in the opaque bottle until analysis by HPLC, also future tests and for further pharmacological studies. (Akkawi et al.,2018)

3.3.3.4-Solubility study of crude extract

The solubility of basil extract was tested using distilled water. By three folding the quantities of basil extract, the solubility of the extract was measured at R.T (25℃). Increasing the concentration gradually until the extract was no longer soluble. By gently heating the extract in a water bath and shaking it, the maximum solubility in water was reached. (Shah et al., 2017)

Table 3.1: Solubility test for ethanolic extract of Basil leaves in different solvents.

Extract: Solvent (w/v)	Solubility	
	Distilled water	Ethanol
1mg/ml	Freely soluble	Freely soluble
3mg/ml	Freely soluble	Freely soluble
6mg/ml	Freely soluble	Freely soluble
9mg/ml	Freely soluble	Freely soluble
12mg/ml	soluble	Freely soluble
15mg/ml	soluble	Freely soluble
18mg/ml	soluble	Freely soluble
21mg/ml	insoluble	Freely soluble

Table 3.2: Solubility test for ethanolic extract of Basil seeds in DMSO solvents.

Extract: Solvent (w/v)	Solubility
	DMSO
Img/ml	Freely soluble
3mg/ml	Freely soluble
6mg/ml	Freely soluble
9mg/ml	Freely soluble
12mg/ml	Freely soluble
15mg/ml	Freely soluble
18mg/ml	Freely soluble
21mg/ml	Freely soluble

* DMSO; Dimethyl sulfoxide.

3.3.4 Phytochemical screening:

3.3.4.1 Phytochemical screening for Basil leaves extract:

For phenolic content, flavonoids, saponins, tannins, alkaloids, triterpenes, and steroids, the phytochemical constituents of Basil leaves extract were qualitatively examined. Basil leaves extract, both freeze-dried and desiccated, was used in the phytochemical studies. (Baskaran,2015)

►Test for flavonoids: Basil extract (0.5 mg) was boiled in ethyl acetate (10 ml) for 3 minutes over a steam bath. Then, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of 10% ammonia solution. The formation of yellow color indicates the presence of flavonoids. (Baskaran,2015)

► Test for saponins: 0.5 g basil extract was combined with 5 ml distilled water and violently stirred. The presence of saponins is determined by the production of foam after 15 minutes. (Baskaran,2015)

► Test for tannins: Basil extract (0.5 g) was cooked in 10 ml water in a test tube and filtered with the help of a water bath. A few drops of a 1% ferric chloride solution were then added. The presence of hydrolysable tannins is indicated by blue-black color development, whereas the presence of condensed tannins is indicated by brownish-green precipitate. (Baskaran,2015)

3.3.4.2 Phytochemical screening for Basil seeds extract:

The phytochemical qualitative reactions of alkaloids, flavonoids, saponins, tannins, and phenolic acids were screened using basil seeds extract. As an indicator, the color intensity of the precipitate that was generated was used. Basil seeds extract, both freeze-dried and desiccated, was used in the phytochemical testing.

►Test for alkaloids (Wagner's test): A small amount of extract was placed in the test tube, and 35 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) were added. The presence of alkaloids in the extract was revealed by the production of a reddish-brown precipitate or coloring. (Gajendiran,2016)

► Test for saponins (Foam test): A total of 0.1 g of extract was combined with 5 ml of distilled water and brought to a boil. 2 drops of olive oil were applied to 1ml of filtrate after filtration. The emulsion and froth development were noticed after shaking the mixture. The 1 ml filtrate was diluted to 4 ml by adding distilled water. The mixture was briskly agitated, and a steady foam was noticed. (Gajendiran,2016)

►Test for flavonoids: For 1 minute, 0.5 g of sample and 10 ml of ethyl acetate were heated together. The filtrate (4 ml) was agitated with a 1 percent ammonium chloride solution after filtration (1 ml). The presence of flavonoids was revealed by the production of a yellow tint in the presence of ammonium solution. (Gajendiran,2016)

►Test for phenols (Ferric chloride test): 1 ml basil extract was combined with 1 ml distilled water and heated on a hot plate. 2 ml ferric chloride solution was then added. The existence of phenols was established by the production of a green or blue tint. (Gajendiran,2016)

►Test for tannins: A water bath was used to boil about 5 g of sample in 20 ml of water in a test tube. The mixture was then filtered. A few drops of ferric chloride were added to this. The presence of tannins was indicated by the emergence of brownish-green or bluish-black colour. (Gajendiran,2016)

3.3.4.3 Total phenolic content (Folin–Ciocalteu assay)

The total phenolic content was determined using the Folin-Ciocalteu assay and a spectrophotometric technique. Crude Basil (leaves/seeds) extracts (0.20 mg) were dissolved in ethanol (99.5%) (20 ml), and 500 µl of the solution was diluted in ethanol (99.5%). (10 ml). In 1 ml of sample, Folin-Ciocalteu reagent (diluted 10-fold; 5 ml, w/v) was added, followed by sodium carbonate 7.5 percent (5 ml, w/v). the reagents were mixed well, and the mixture was incubated in total darkness for 30 minutes at R.T to complete the reaction. subsequently, by utilizing a spectrophotometer (UV2550, Shimadzu, Kyoto, Japan) the absorbance of the solution was read at 269 nm. The calibration curve was created using different amounts of gallic acid ranging from 20 to 70 ppm. The results are given in milligrams of gallic acid equivalents. (mcg GAE per gram of sample) The measurements were carried out in triplicate, with the average of three replicates.

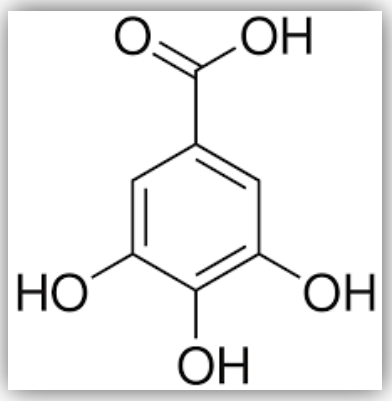


Figure 3.3: Structure of the phenolic compound was used as standard (Gallic acid)

3.3.4.4 Total Flavonoid Content (TFC)

Using the aluminum chloride spectrophotometric technique, total flavonoids in crude Basil extracts were determined. Crude Basil (leaves/seeds) extracts (0.20 mg) were dissolved in ethanol (99.5%) (20 ml), and 500 µl of the solution was diluted in ethanol (99.5%). (10 ml). Extracts (1 ml) were mixed with methanol (3 ml) and $AlCl_3$ solution (0.2ml,1:10, w/v), furthermore potassium acetate (0.2ml,0.98:10,w/v) was added and distilled water (5.6ml). The reagents were mixed well, and the mixture was incubated in total darkness for 30 minutes at R.T to complete the reaction. Subsequently, by utilizing a spectrophotometer (UV2550, Shimadzu, Kyoto, Japan) the absorbance of the solution was read at 420 nm. The calibration curve was created using different concentrations of quercetin standard ranging from 10 to 75 ppm. The results were represented as quercetin equivalents in milligrams. (mg QE per gram of sample) The measurements were carried out in triplicate, with the average of three replicates.

3.4 RP-HPLC analysis of flavonoids

The high performance liquid chromatography (HPLC) technique is frequently used to assess the flavonoids concentration in natural extracts, for both separation, quantification, and identification of these chemicals. The crude raw extracts of the two types of plant parts were identified using HPLC, which is an effective instrument. By dissolving 10 mg of crude extract in 10 ml of corresponding solvent 99 percent ethanol, samples of crude extracts (leaves/seeds) were generated at a concentration of 1 mg/ml.

HPLC with UV detector method was used for quercetin analysis using C18 column (15 cm with 4 micro meter particle size) at 254 nm wavelength and a mobile phase of water: methanol (50:50 v/v) at a flow rate of 1.0 ml per minutes. standard of 0.01 g per 10ml was used.

3.5 phase behavior

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

The phase behavior of the systems consisting of Basil seeds ethanolic extract, water phase [Basil leaves ethanolic extract with ethanol 99.5% as co-surfactant (1:9 (wt./vol))] and Tween 20 may be represented by a phase tetrahedron, with the apexes representing the pure components.

1g of a combination containing basil oil and tween 20 in various weight ratios - as given in table (3.3) - was made in glass test tubes with screw caps and swirled by the vortex at room temperature (25°C).

Table: 3.3: weight ratios of oil phase and surfactant.

Tube No.	Oil phase(g)	Surfactant(g)
1	0.1	0.9
2	0.2	0.8
3	0.225	0.775
4	0.25	0.75
5	0.3	0.7
6	0.4	0.6
7	0.5	0.5
8	0.6	0.4
9	0.7	0.3
10	0.8	0.2
11	0.9	0.1
12	1	0

Titrating these samples with water phase, which was added until the end limit was reached -as stated in table (3.4)- All of the aqueous phase additions on a vortex mixer were followed by vigorous stirring. The interval for equilibration between each addition was normal, and if necessary, a centrifuge was used for 5 minutes.

Table 3.4-a : The amount of water added each time in 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

Table 3.4-b : The amount of water added each time in titration.

Addition No	Mass of addition(g)	. Addition percentage (%)
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
37	0.5	85.07
38	0.8	87.5
39	1	88.8
40	1	90
41	1	90.9
42	2	92.3
43	2	93.3
44	3	94.4
45	3	95.23
46	4	96
47	4	96.55
48	5	97.05
49	5	97.43
50	10	97.95
51	10	98.3
52	10	98.55
53	20	98.87
54	20	99.08
55	20	99.2
56	30	99.37
57	40	99.49
58	50	99.59
59	100	99.71
60	100	99.77
61	>500	100

At a temperature of 25°C, the phase diagram was studied. Observing multiple phases with 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 single-phase boundary; then, using Origin Pro 2021 software, construct the phase diagram.

3.6 Preparation of cream :

A cream is a topical preparation that is typically used on the skin. Creams are considered pharmaceutical items because even cosmetic creams are founded on pharmacy principles, and unmedicated creams are widely utilized in a range of skin diseases (dermatoses). Creams are emulsions, or oil-water combinations, that are semi-solid.

Table 3.5 :The detailed cream formula.

Phase	%w/w input	Ingredient
A	72.35	Water
A	0.10	Disodium EDTA
B	5.00	Glycerin
B	0.3	Xanthan Gum
C	1.50	GSC: Glyceryl Stearate Citrate
C	8.00	Cetearyl Alcohol, Ceteareth-20 (Emulgade 1000 NI)
C	2.00	Ceteargl alcohol
C	4.00	Caprylic/Capric Triglycerides (Myritol 318)
C	3.00	Isopropyl myristate (Crodamol IPM)
C	2.00	Shea butter
D	0.80	[(Phenoxyethanol, Ethylhexylglycerin (Euxyl PE9010)
D	0.60	Tocopherols
D	0.10	Basil (Ocimum basilicum)essential oil
D	0.05	Basil (Ocimum basilicum)essential leaves

Water and Disodium EDTA (Phase A) were measured. Made a slurry of phase B(Glycerin and Xanthan Gum), then added to phase A with mixing. phase A/B was Heated to 75°C. phase C was Combined and heated to 75°C. Next to that, phase C [GSC: Glyceryl Stearate Citrate, (Cetearyl Alcohol, Ceteareth-20 (Emulgade 1000 NI)), Ceteargl alcohol, (Caprylic/Capric Triglycerides (Myritol 318)), Isopropyl myristate (Crodamol IPM), Shea butter] were added to phase A/B under high shear stirring and mixing until a smooth, glossy, homogenous emulsion forms. the mixture was allowed to cooling under low shear stirring until less than 40°C.

Phase D [(Phenoxyethanol, Ethylhexylglycerin (Euxyl PE9010)), Tocopherols, (Basil (*Ocimum basilicum*)essential oil and leaves)] was Added and stirred through under low shear until homogenous. The mixture was allowed to cooling less than 25°C. Check/adjust final pH to 5-

5.5. .Finally, the mixture was Covered and given stir the next day before pouring off. The percentage of basil extract in cream 0.15%.

Cream stability tests were carried out at 8°C+0.1°C in the refrigerator, as well as at 25°C+1°C, 40°C+1°C, and 40°C+1°C in an incubator with a relative humidity of 75%. (RH).

3.7 Antibacterial assay:

The antibacterial test was carried out by agar disc diffusion method (Murray et al., 1999). Negative controls were prepared using the same solvents (ethanol 99.5%) employed to dissolve the samples. Standard antibiotic, Gentamicin (10 micrograms/disc) and Penicillin (10 unit) were used as positive control for the tested bacteria. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition around disc against the tested bacteria.

3.7.1 Micro organism : Clinical Isolates of *Eschericha coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter sp and Pseudomonas aeruginosa* were all supplied by the College of Health Professions Department of the University of Al-Quds.

3.7.2 Preparation of medium: Mueller – Hinton Agar was supplied by the Department of Microbiology, Faculty of sciences and technology , Al quds University.

In order to grow bacteria very specific environmental conditions were required, food energy, suitable temperature and humidity. when growing this cells in the laboratory we have to create these conditions using culture media. solid culture media is the Muller Hinton agar in the Petri dishes. After the preparation of bacterial inoculum in broth, Mueller Hinton Agar (MHA) was prepared and sterilized by autoclaving for about 30 min. For the next step the media was poured in sterile petri plates. They were Left for 10-15 minutes to solidify. Plates were turned upside down to prevent moisture from condensing on the surface of the media and then stored in a plastic bag until use.

3.7.3 Culture of bacteria: The bacterial different species were inoculated into nutrient broth and left into an incubator at 37 ^{0}C for 24 hours then a culture is diluted to have a concentration compared to that of MacFarland nephlometer tube no. 0.5 10^{8} cfu/ml) using spectrophotometer at 625 nm, (optical density 0.08 to 0.1).

After solidification of MHA, the inoculum was dispersed uniformly on the surface of MHA by cotton swab. This is also called carpet culture.

3.7.4 Antimicrobial assay: 5 types of bacteria were studied for antibacterial activity by disc diffusion method . the disc (5mm diameter) was sterilized by autoclaving for about 30 minutes.

The prepared bacterial species were seeded onto prepared plates of Muller Hinton agar.

Reference antibiotic disc on the surface of MHA as positive control. Then sterile discs were impregnated with (50 microliter) of the plant extracts (with different concentrations) , negative controls (the solvent) with 50µl in each disc. Then the plates had been incubated at 37ºC for 24 hours .

3.7.5 Measuring the minimum inhibitory concentration (MIC): The MIC is the lowest antimicrobial concentration that inhibits bacterial growth or causes no observable growth. The MIC for all of the microorganisms examined was determined using the serial broth dilution technique.

For each microbe, a set of four tubes was made, 2 ml of nutrient broth was put in all tubes, then 2 ml of the aqueous extract of Basil (seeds, leaves) (with a concentration of 100 mg/ml) was added to the first tube via micropipette and thoroughly mixed. Then, 2 ml of the first tube's solution were transferred to the second tube, well mixed. Two milliliters of a solution in the second tube was then transferred to the third tube, and subsequently to the fourth tube. After the dilution, 200 microliters of bacterial suspension standardized to an optical density (0.08-0.1) relating to McFarland Scale (10^8 CFU/ml) was added to all four tubes. The extract concentration in the first tube was 1000 mg/ml, and it was diluted three times. Nutrient Broth and bacterial culture were used to make positive control tubes. Nutrient Broth with a solvent was used to make the negative control tubes. For the bacteria, the tubes were incubated at 37°C for 24 hours. The clear tubes (showing inhibitory activity) were seen after incubation for each microbe, and the MIC was determined by taking the least clear tube from each set. This test was done three times for confirmation, and all processes were completed in sterile settings, using a hood and autoclave to sterilize instruments. (Jaradat and Abu-hadid 2014).

3.7.6 Minimum bactericidal concentration (MBC) Which is the minimum concentration that is required to kill the bacteria, had been tested after the results of the MIC.

For the bacteria with controls, the tubes of the MIC for whole samples (seeds and leaves basil) that indicated no growth (clear) of microorganisms were sub-cultured into nutrient agar plates and incubated at 37oC for 24 hours. The MBC is the concentration of extract that did not exhibit any colony growth. (Jaradat and Abu-hadid 2014).

3.8 Antioxidant assay :

The antioxidant activity of Basil (leaves and seeds) extracts was determined using a modified method of the assay of ferric reducing/antioxidant power (FRAP) of Oyaizu M. (1986). Freshly 0.5 ml of dissolved sample was mixed with 0.75 ml phosphate buffer (0.2M, pH 6.6) as well as 0.75 ml of a 1% (w/v) potassium hexacyanoferrate solution. After that, 0.75 ml 10% (w/v) aq

was added to the reaction mixture and incubated at 50 °C. The solution of trichloroacetic acid (TCA) was added, and the mixture was centrifuged for 10 minutes (2000 rpm). Finally, a 0.75 ml aliquot from centrifuged material was combined with 0.75 ml H_2O and 0.25 ml 0.1 percent (w/v) aq. Solution of $FeCl_3.6H_2O$. Absorbance at 700 nm was read with reference to a reagent blank containing distilled water which was also incubated at 50 °C. Aqueous solution of known Fe(11) concentrations in the range of (2-5 mM) ($FeSO_4.6H_2O$) were used for calibration.

3.9 Antimalarial assay:

A semi-quantitative in vitro assay method was used to study the potential antimalarial activities of different parts of Palestinian basil plant (leaves and seeds) using different solvent extracts .

The protocol for assessing bio-mineralization hemin, according to (Akkawi et al., 2018), is to incubate a test combination in a typical non-sterile flat bottom 96-well plate at 37 °C for 18-24 hours. The following ingredients were added in the following order: 50 L (0.5 mg/mL hemin chloride) freshly dissolved in dimethylsulphoxide (DMSO). 100 liters of 0.5 M sodium acetate buffer (pH 4.4), followed by 50 liters of antimalarial drug solution or solvent. Meanwhile, the mixture was incubated at 37°C for 18-24 hours in a bottom plate.

The plate was then centrifuged for 10 minutes at 4000 rpm. The reaction's pH was measured after the supernatant was removed, and the final pH was between (5 - 5.2). To remove free hemin chloride, the remaining pellets were suspended in 200 L of DMSO. The dish was then centrifuged once more, and the supernatant was discarded. The β-hematin precipitate was dissolved in 150 L of 0.1 M NaOH to produce alkaline hematin for direct spectroscopic measurement using an ELISA reader at wavelength 405 nm.

Basil leaves dried extracts obtained with different solvent (absolute ethanol, ethanol 35%, ethyl acetate and water) were dissolved in pure water, while Basil seeds extracts with different solvent (absolute ethanol, ethanol 35%, ethyl acetate, water and petroleumether) were dissolved in DMSO. Ultra-pure water and DMSO were used as negative controls and Chloroquine diphosphate salt as positive controls.

Chapter Four:
Results and Discussion

4.1- Phytochemical screening:

4.1.1- Phytochemical screening of basil ethanolic extracts (leaves & seeds):

Phytochemical screening of basil ethanolic extracts revealed the presence of medically active constituents such as Flavonoids, Saponins, and Tannins:

Table 4.1:Qualitative analysis of Phytochemical constitutes.

	Phytochemical tested	***Test performed***	***Test Result***	
			Leaves extract	Seeds extract
1	Flavonoids	Ethyl acetate test	+	+
2	Saponins	Frothing test	+	+
3	Tannins	ferric chloride test	+	+

4.1.2-Total Phenolic Contents (TPC):

Table 4.2 shows the TPC of basil plant extracts for two-part leaves and seeds. As can be seen from the table, the TPC of Basil extract varies depending on whatever portion of the plant is extracted. The highest TPC was found for the plant material when extracted the seeds with 99.5% ethanol, followed by plant material extracted leaves with 99.5% ethanol. The results showed that the Basil seeds plant studied in this study had more phenolic compounds (58.20.9 mg/g) than the Basil leaves plant (51.24 mg/g). Because plant phenolics have multifunctional properties, including the ability to act as singlet oxygen quenchers and scavenge free radicals, the presence of significant amounts of these compounds in Palestinian Basil (seeds and leaves) promotes the latter as an important source of antioxidants, which, if consumed properly, may reduce the risk of degenerative diseases and provide a health-promoting benefit. Comparing the TPC of Palestinian Basil to Basil from other places is fascinating.

Table 4.2: Total phenolic content (TPC as mg Gallic acid/g DW), total flavonoids contents (TFC as mg Quercetin /g DW), Antioxidant activity FRAP(mg/g DW).

	TPC (mg/g)	**TFC (mg/g)**	**Antioxidant activity FRAP(mg/g)**
Basil leaves ethanolic extract (99.5 %)	51.0 ± 2.4	9.0 ± 1.5	40.4 ± 0.8
Basil seeds ethanolic extract (99.5 %)	58.2 ± 0.9	34.2 ± 3.6	74 ± 1.4

4.1.3-Total Flavonoid Content (TFC)

Table 4.2 shows the results of the aluminum chloride test for assessing flavonoids content. The highest TFC was for the plant material when extracted the seeds with 99.5% ethanol, followed by plant material extracted leaves with 99.5% ethanol. The results showed that the Basil seeds plant studied in this study (34.2 3.6 mg/g) had four times the amount of basil leaves extracted (9.0 1.5 mg/g). When comparing the TFC and TPC content of different parts of the plant, there is a parallelism in the two patterns, with the maximum TPC and TFC concentration found in Basil seeds extracted. Furthermore, the TPC is a higher value than their corresponding TFC.

4.2 RP-HPLC analysis of flavonoids

The crude extracts were diluted in ethanol at a concentration of 1 mg/ml, injected into an HPLC chromatograph, and their flavonoids were determined. To determine the presence of this chemical in the crude extracts, flavonoid standards were injected and separated concurrently. Individual standard calibration curves were also created at 1000 ppm concentration level.

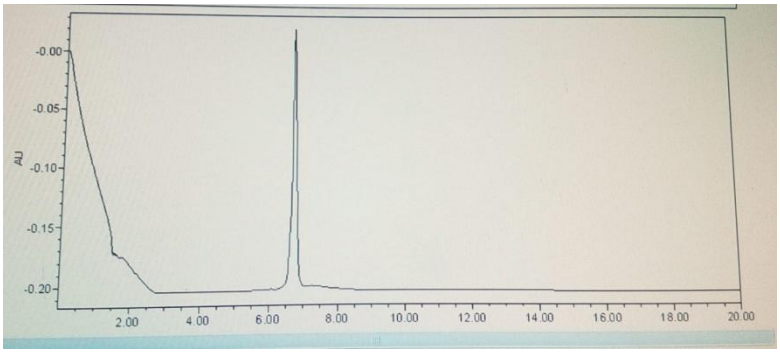


Figure 4.1: Standard (Quercetin)

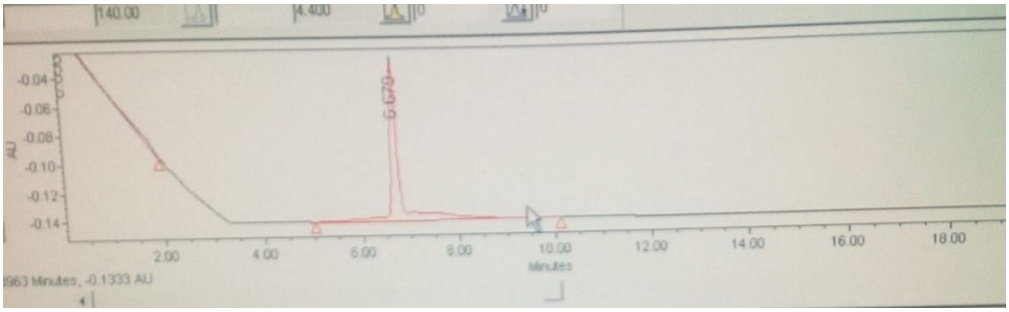


Figure 4.2: Basil seeds extract showed a major peak only.

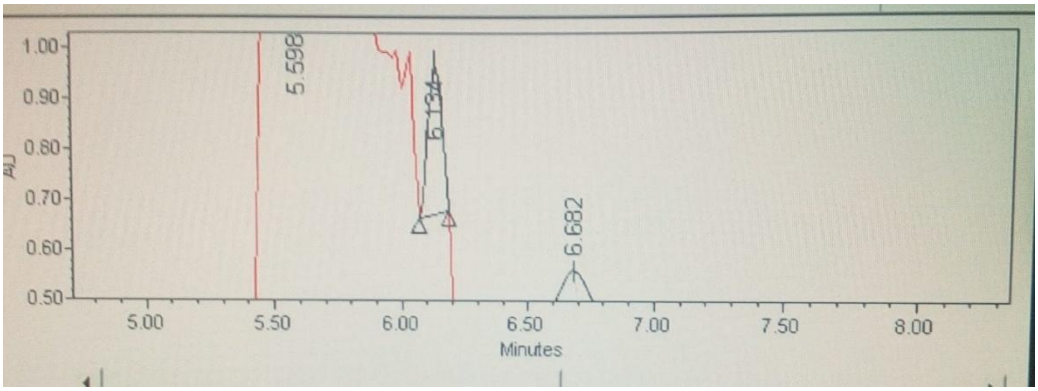


Figure 4.3: Basil leaves extract HPLC analysis.

An overlay chromatogram of the crude extracts at 254 nm is shown in Figure 4.2. In 6.679 minutes, flavonoid chemicals were discovered. This wavelength was chosen since the primary peaks had a highest absorption near it. Flavonoid molecules were discovered in 6.682 minutes at the same wavelength, as shown in Figure 4.3.

4.3 Phase behavior

At 25°C, the addition of water phase to tween 20 / Basil oil with a varied ratio system resulted in a ternary phase diagram (Fig 4.4). Ternary phase behavior of the Basil ethanolic extracts were obtained serial microemulsion reigns under the same formulation conditions. using two different extract part plant which is [Basil leaves ethanolic extract with ethanol 99.5% as co-surfactant (1:9 (wt./vol))] and Basil seeds ethanolic extract at 25^{o}C.

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 before the addition of the first 9% water from the point containing 20% and less ethanol oil extract and 80% and more Tween 20, and extend to up to the water apex (100%). Thus the remainder of the phase diagram represents the turbid region based on visual identification. finally, the phase diagrams were drawn using Origin 2021.

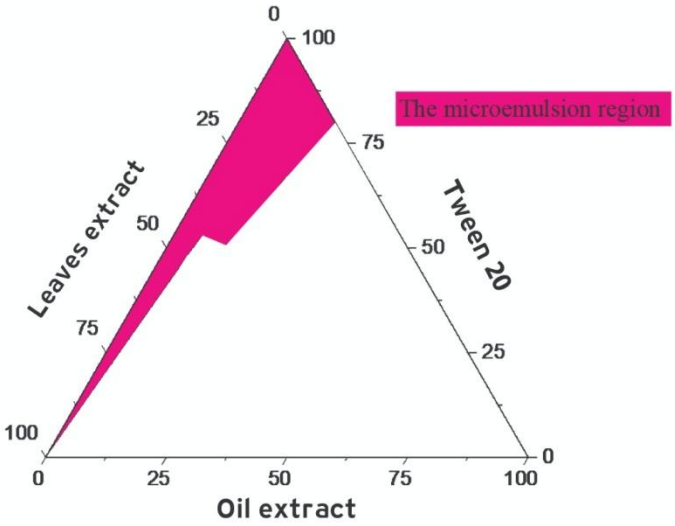


Figure 4.4: Ternary phase behavior of the Basil ethanolic extracts.

4.4 Preparation of cream

The Formula is moisturizing, smoothing, good smell, absorb by skin (don't leave film), not irritation ph= 5-5.5, good color, easily spread (not too viscous, apply easily and quickly), and anti-aging, antierythmic and depigmenting effects when applied topically.

Figure 4.5. cream of Basil.

The samples stored at 8°C and 25°C showed no signs of liquefaction. The sample remained stable in the evaporimeter at 40°C and 40°C+75 percent RH until the 17th day. At various temperatures, the formulation remained stable. This could be owing to basil's antibacterial capabilities, which protect the emulsion against contamination and destruction by microbes.

Calculation : The percentage of basil extract in cream = 0.15/99.8*100% = 0.15 %

Figure 4.6 . Evaporimeter (left) creams in 40°C (middle) creams in 40°C+75% RH (right).

4.5-Antibacterial assay:

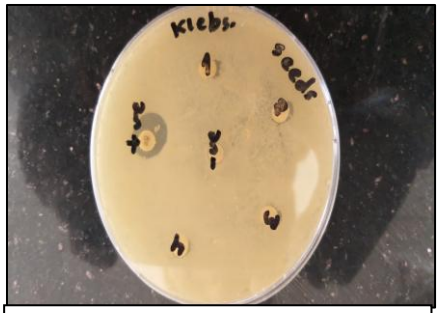
4.5.1.Antibacterial assay:

Plants have been used to treat infections for centuries through herbal medicine and self-medication. Plant use in Palestine, in particular, is a long-standing practice that is based primarily on observation rather than scientific experimentation. Nonetheless, because the effective life span of any antibiotic is limited by bacteria resistance and toxicity, humanity is continually on the lookout for new developing antimicrobial medicines. As a result, preliminary testing of the antibacterial activity of *Ocimum basilicum* essential oil against several types of organisms was carried out in the following study.

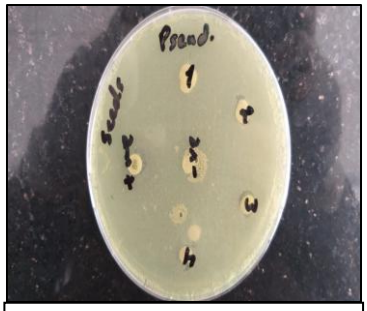
Antibacterial activity of ethanol extract of *Ocimum basilicum* seeds and leaves were checked against five clinical pathogens (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter sp,*and *Pseudomonas aeruginosa***)**. Using the disc diffusion method in the presence of positive controls (Gentamicin (10 µg/disc) and Penicillin (10 unit). The zones of inhibition (Fig.4.7) were measured, and the average results of the zones of inhibition (Table 4.3) were summarized.

Figure 4.7-a: Zone of inhibition of *Ocimum basilicum* (seeds).

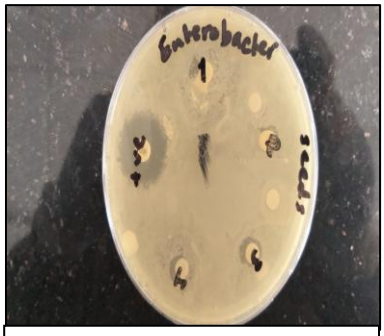
Ocimum basilicum oil antimicrobial activity against *Staphylococcus aureus*



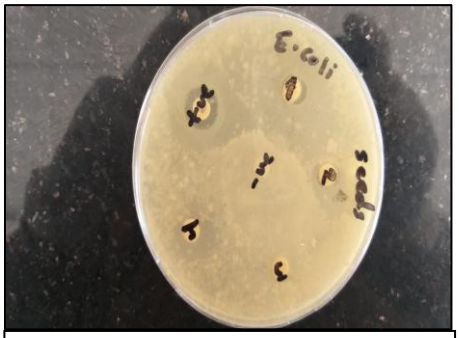
Ocimum basilicum oil antimicrobial activity against *Klebsiella pneumonia*



Ocimum basilicum oil antimicrobial activity against *Pseudomonas aeruginosa*



Ocimum basilicum oil antimicrobial activity against *Enterobacter sp*



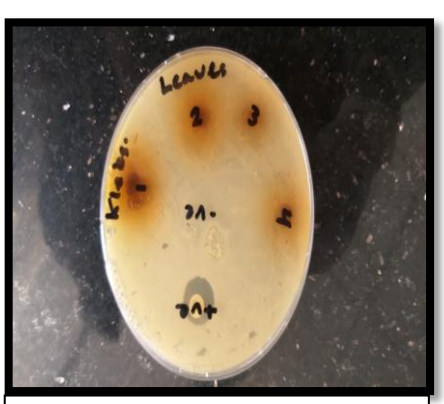
Ocimum basilicum oil antimicrobial activity against *Eschericha coli*

Figure 4.7-b: Zone of inhibition of *Ocimum basilicum* (leaves).

Ocimum basilicum (leaves) antimicrobial activity against *Eschericha coli*

Ocimum basilicum (leaves) antimicrobial activity against *Staphylococcus aureus*

Ocimum basilicum (leaves) antimicrobial activity against *Enterobacter sp*



Ocimum basilicum (leaves) antimicrobial activity against *Klebsiella pneumonia*

Ocimum basilicum (leaves) antimicrobial activity against *Pseudomonas aeruginosa*

Comparison between antimicrobial activities (seeds) is illustrated in Fig.4.8-a

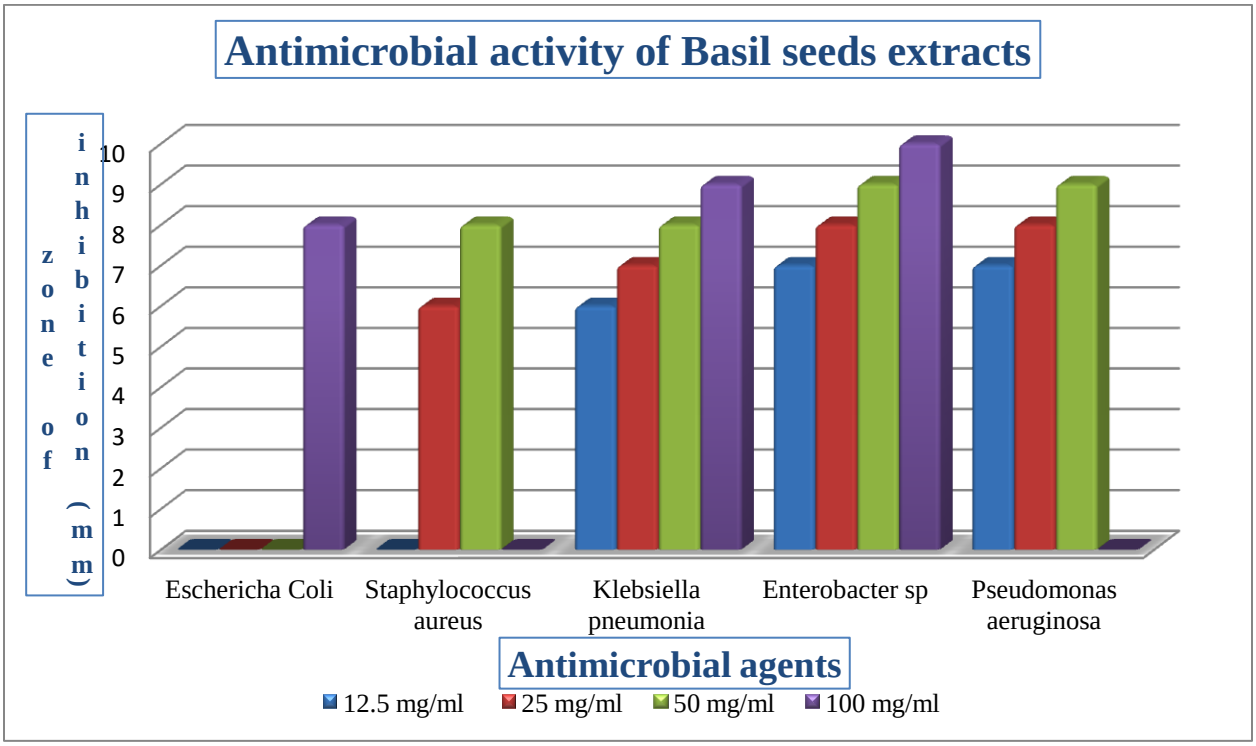


Figure 4.8-a : Antimicrobial activity of Basil seeds extracts.

Comparison between antimicrobial activities (leaves) is illustrated in Fig. 4.8-b.

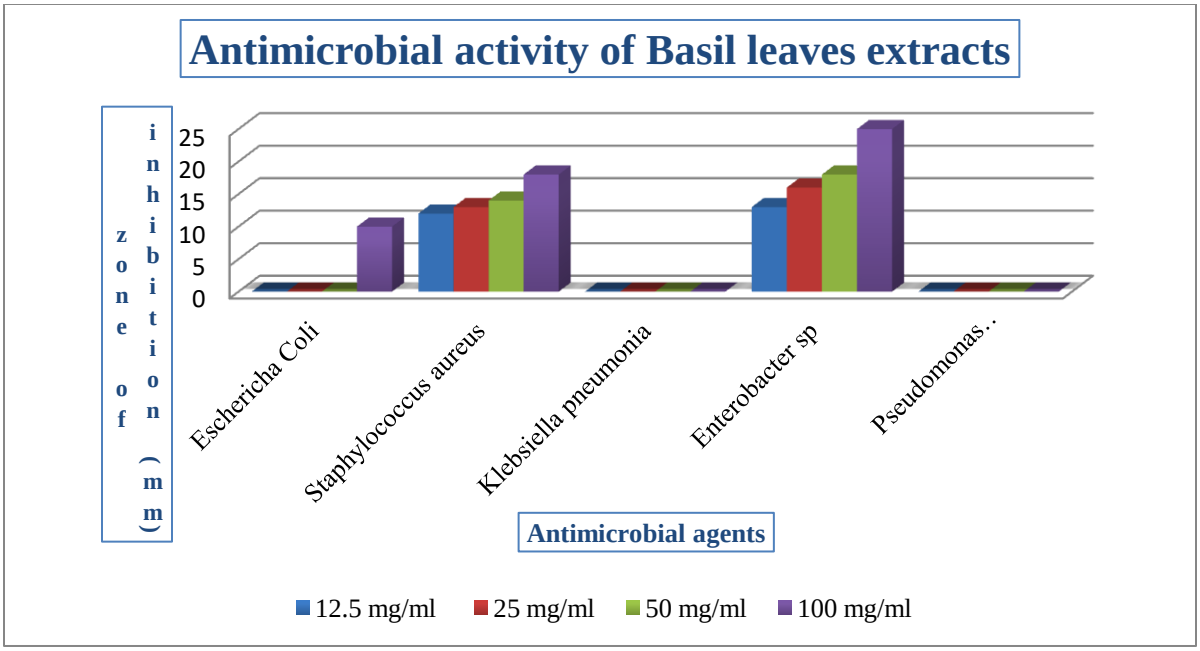


Figure 4.8-b : Antimicrobial activity of Basil leaves extracts.

During antimicrobial testing, seeds and extracts demonstrated a good zone of inhibition against practically all species, according to our findings. While leaves extracts fight bacteria in some organisms but as diffusion (spread) and not exactly an inhibition area. So leaves extracts were demonstrated significantly lower activity. In addition, clinical strains of *Enterobacter sp*. were discovered to be sensitive to the essential oils of basil (*Ocimum basilicum* L.) seeds and leaves at various doses. *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were not effective for basil leaves concentrations ranging from 12.5 µg/ml to 100 µg/ml. The Highest zone of inhibition for seeds extract is 10 mm at 100 mg/ml concentration against *Enterobacter sp*. while Highest zone of inhibition for leaves extract is 25 mm at 100 mg/ml concentration against *Enterobacter sp* as diffusion (spread).

Table 4.3-a. The diameter of zone of inhibition of *Ocimum basilicum* seeds against clinical pathogens.

Clinical pathogens	**Average zone of inhibition (mm)**			
	12.5 mg/ml	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
Eschericha coli	ND	ND	ND	8
Staphylococcus aureus	ND	6	8	ND
Klebsiella pneumonia	6	7	8	9
Enterobacter sp	7	8	9	10
Pseudomonas aeruginosa	7	8	9	ND

*ND: Not detected.

Table 4.3-b. The diameter of zone of inhibition of *Ocimum basilicum* leaves against clinical pathogens.

Clinical pathogens	**Average zone of inhibition (mm)**			
	12.5 mg/ml	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
Eschericha coli	ND	ND	ND	10
Staphylococcus aureus	12	13	14	18
Klebsiella pneumonia	ND	ND	ND	ND
Enterobacter sp	13	16	18	25
Pseudomonas aeruginosa	ND	ND	ND	ND

*ND: Not detected.

4.5.2- MIC and MBC :

The MIC and MBC values were determined in this investigation. Both leaves and seeds were determined, as indicated in Table 4.4. The tubes were tested for turbidity after incubation, which indicates the presence of microorganisms; the organism will grow in the negative control tube, which contains no antimicrobial substance to impede growth.

Because lower amounts of the extract are required to inhibit and kill bacteria, the seed extract was more powerful against *Staphylococcus.aureus* than another (62.5 µg/ml). In *E.coli* and *Klebsiella*, the seed has the same MIC value (500 µg/ml), which is consistent with earlier research. This indicates that their potency is equivalent. because their MIC and MBC results are identical *Pseudomonas* has a MIC value of 12.5 µg/ml of extract (250 µg/ml).

Seeds extract was slightly more potent than the leaves extract, Where MIC value for seeds extracts in *Staphylococcus.aureus* was 62.5 µg/ml while for leaves extracts has 250µg/ml. Because 500 micrograms is the maximum concentration employed in this study, all values above 500 µg/ml require a higher concentration to determine the actual value. The MBC results for each seeds and leaves show slightly higher concentration values than the MIC results.

Minimum concentration of extracts that show zone of inhibition in the disc diffusion method was selected for determination of MIC.

Table 4.4 : MIC, MBC of leaves and seeds extract:

		Minimum inhibitory concentration (MIC) (µg/ml)		Minimum bactericidal concentration (MBC) (µg/ml)	
Microorganism	Min conc. of extracts(mg/ml)	Leaves	Seeds	Leaves	Seeds
E.coli	100	500	500	>500	>500
Staphylococcus aureus	25	250	62.5	500	125
Pseudomonas	12.5	NT	250	NT	500
Klebsiella	12.5	NT	500	NT	>500
Minimum inhibitory concentration and Minimum bactericidal concentration (values in µg/ml). *NT: Not tested					

Comparison between MIC of Basil seeds is illustrated in Fig. **4.9-a**

Comparison between MIC of Basil leaves is illustrated in Fig **4.9-b**

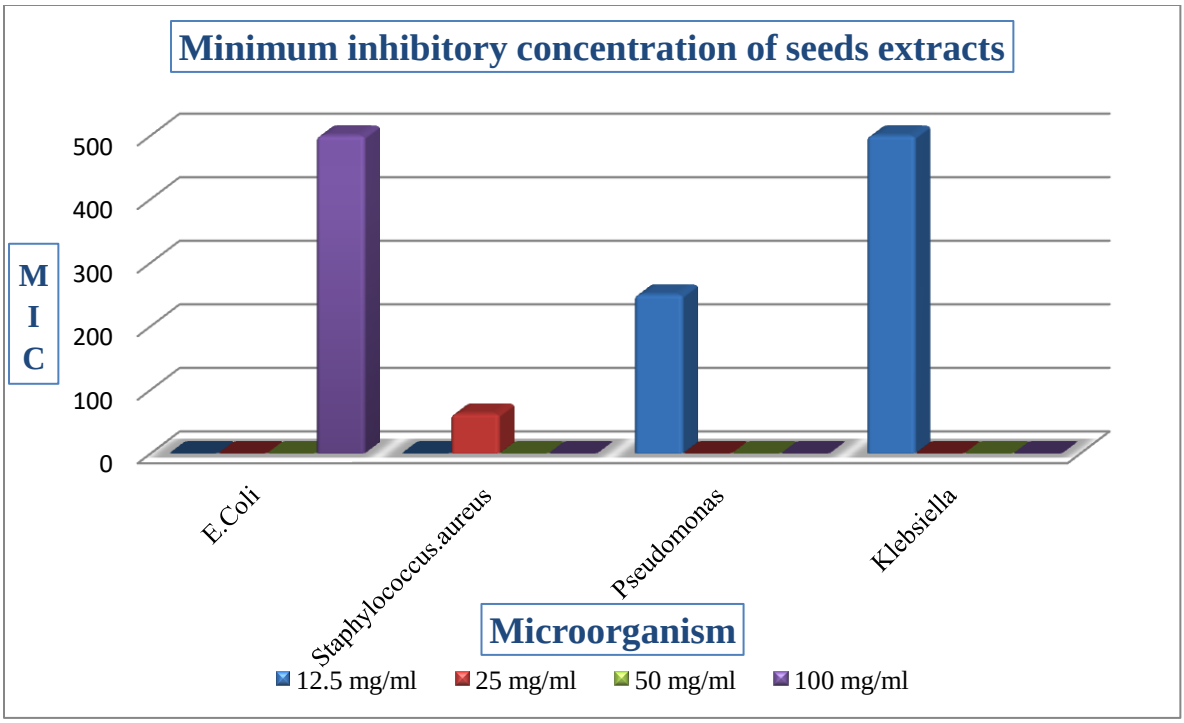


Fig. **4.9-a** : Minimum inhibitory concentration of Basil seeds.

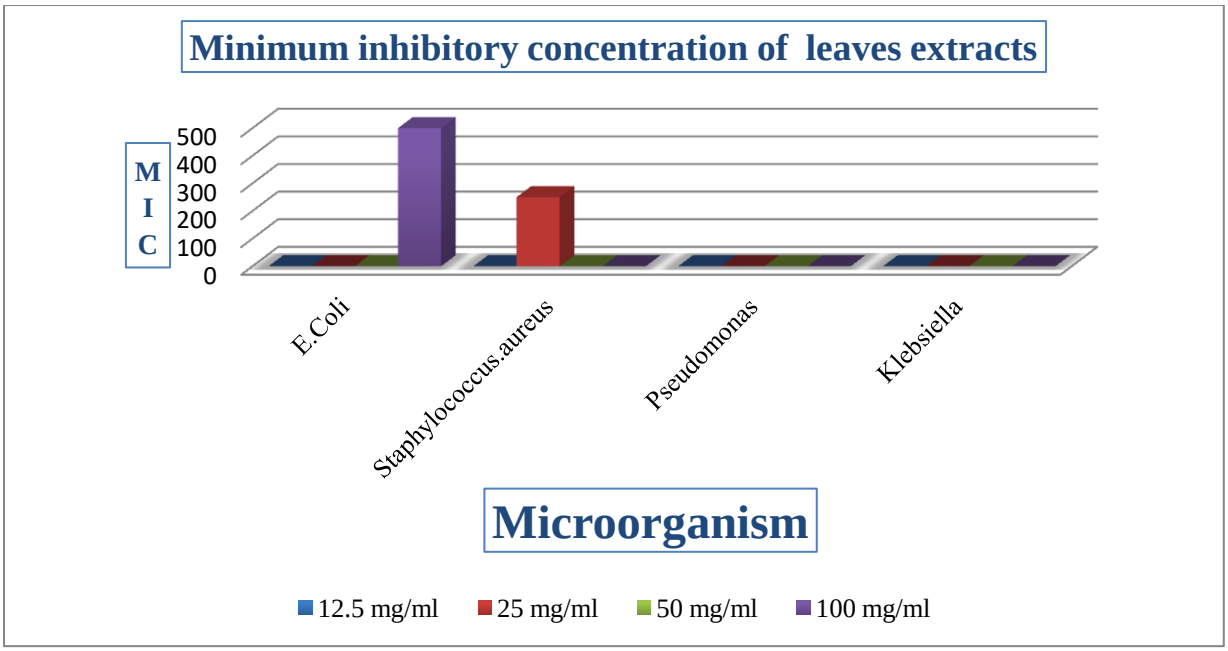


Fig. **4.9-b** : Minimum inhibitory concentration of Basil leaves.

4.6 Antioxidant assay:

The FRAP assay was used to determine antioxidant activity. This assay indicates the electron donating capacity of tested compounds by measuring the ability of the substance to decrease Fe+3 to Fe+2 ions. At 700 nm, the Fe+2 ion is detected spectrophotometrically by determining its colored combination with ($FeSO_4.6H_2O$).

Table 4.2 shows the antioxidant activity of the two-part Palestinian Basil plant studied in this study. As can be seen from the data, the extraction of a plant's seeds contains 741.4 mg/g. When compared to the antioxidant activity of leaves part 40.4 0.8 mg/g, Palestinian seeds portion in this study have a higher antioxidant activity than leaves part.

This result agrees perfectly with the overall phenolics content, which was calculated as milligram gallic acid per gram of Basil plant (DW).

4.7 Antimalarial assay

More than one mechanisms were assumed to explain the potential in vitro antimalarial effect of the alcoholic (ethanolic) extract of *Ocimum* species and this warranted further investigation (Inbaneson et al.,2012).

In the current study, the Basil leaves and seeds extracts, prepared using several solvents (absolute ethanol, ethanol 35%, ethyl acetate, petroleum ether and water) were screened for their potential inhibitory effect on beta-hematin formation using a semi-quantitative in vitro method according to Akkawi et al. The efficiency of these extracts in inhibiting β–hematin formation in vitro is compared to positive (CQ-chloroquine 0.1mg/ml) and negative controls (water) at 405nm. The Palestinian Basil herb (*Ocimum*) extracts as anti-malarial agents were found not to prevent the formation of β-hematin under specific chemical and physiochemical conditions and thus rule out one of these possible mechanisms.

The intraerythrocytic stage of the parasite's life cycle is our target; during this stage, Plasmodium parasites reside inside the host erythrocytes degrading hemoglobin, resulting in the accumulation of free heme; Ferriprotoporphyrin (IX), toxic to the parasite and capable of producing oxygen radicals. The heme is detoxified by being incorporated into an insoluble crystal known as hemozoin, or malaria pigment.

Purified hemozoin, a polymer for in vitro investigations in anti-malarial medication research, is structurally, chemically, and spectroscopically identical to β-hematin, a synthetic polymer produced from Ferriprotoporphyrin- IX.

5. Conclusions and future work

5.1 Conclusions:

The chemical contents of Palestinian basil from various sites in Palestine, as well as some of its pharmacological effects, were investigated.

To our knowledge, this is the first study dealing with the in vitro, work was carried out on the creation of pseudo ternary phase behavior and the production of Microemulsion technology, Basil microemulsion had been successfully produced by combining Basil leaves and seeds extract, and Tween 20 as the surfactants. this technology was used to produce a medical cream to treat many skin diseases.

Plant extract's antioxidant qualities have piqued interest due to their potential usage as natural additions to replace synthetic ones. Sweet basil's antioxidant activity was largely linked to flavonoids, followed by phenolic acids. It was discovered that seeds had a higher concentration of phenolic and flavonoid components in the extracts than leaves.

The HPLC technique was utilized and found to be precise, accurate and reliable in the identification of the flavonoids components of Basil palaestina.

The antibacterial efficacy of basil essential oils against resistant *Escherichia coli* clinical strains, *Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter sp,* and *Pseudomonas aeruginosa* was discovered in these investigations. Furthermore, the tested basil oil (seeds) was more effective than basil oil against all clinical strains (leaves). As a result, essential oil action against bacteria with various resistance mechanisms may be effective not only in treating but also in avoiding the spread of resistant strains.

Finally, under certain chemical and physiochemical conditions, the activity of Palestinian Basil herb (*Ocimum*) as an anti-malarial agent that does not hinder the synthesis of beta β-hematin in vitro.

5.2 Future work:

We can then conduct more studies and use new techniques to reach the best possible results and apply them to treat many diseases such as in treating the Blood Cortisol Level and Blood Serotonin Level in depression treatment, reducing the locomotor activity, and treating migraine headaches, in addition to using it as an anesthetic. We can also produce nanoencapsulation or microencapsulation, and measuring P.S and DSE.

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Phase behavior of Basil extractions and their favorable attributes

سلوك مرحلة المستحلبات الدقيقة لمستخلصات الريحان وصفاتها المفضلة

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ملخص :

في الوقت الحاضر هناك تركيز كبير على تطوير الأدوية والبحث عن مصادر نباتية لتقليل الآثار الجانبية. بالإضافة الى البحث في تقنيات جديدة لزيادة فعالية المادة الفعالة التي يتم الحصول عليها. ان استخلاص المنتجات الطبيعية لعبت دورًا رئيسيًا في تطويرأدوية جديدة. حيث تم اختيار نبات الريحان في هذه الدراسة لإحتوائه على أكثر من 200 مركب كيميائي.

في هذه الدراسة تم استخلاص أوراق الريحان والبذور من خلال استخدام جهاز الاستخلاص (Soxhlet), هذه الطريقة قادرة على استخلاص جميع المركبات النشطة تقريبًا من هذا النبات. وقمنا بتطويرها وتعديلها مخبريا.

تمت دراسة الفعالية المضادة للبكتيريا باستخدام طريقة القرص, تم تحديد اقل تركيز من الماده الفعاله يمكن ان يمنع نمو الكائنات الدقيقه او يقضي عليها كليا (MBC,MIC) لكل من *Eschericha coli* ، و *Staphylococcus aureus* ، و *Klebsiella pneumonia* ، و *Enterobacter* sp ، و *Pseudomonas aeruginosa* في وجود تحكم إيجابي (Gentamicin 10) ميكروغرام / قرص) ووحدة بنسلين 10).

أظهرت الدراسة أن كلا من مستخلصات البذور والأوراق بالإيثانول لها نشاط مضاد للجراثيم على معظم السلالات البكتيرية. فقد كان أعلى تركيز لتثبيط مستخلص البذور هي 10 ملغم/ مل بتركيز 100 ملغم/ مل ضد *Enterobacter sp*. بينما تبلغ أعلى تركيز لتثبيط مستخلص الأوراق 18 ملغم/ مل بتركيز 12.5 ملغم/ مل ضد *Enterobacter sp* و *Staphylococcus aureus* على شكل انتشار.

تم تحليل مستخلص البذور والأوراق باستخدام طرق الفحص القياسية, حيث تم فحص نشاطها المضاد للأكسدة (FRAP) ومحتوى الفينول (TPC) بطريقة فولين Folin-Ciocalteu. وقد تم التحليل باستخدام جهاز UV-Visible spectrophotometer. وقد أظهرت الدراسة ان بذور وأوراق الريحان تحتوي على قدر عالي من مضادات الأكسدة ومحتوى الفينول. حيث بلغ محتوى نسبة مضادات الأكسده في البذور على 1.4± 74 ملغم/غم, بينما في الاوراق 0.8± 40.4 ملغم/غم. أما نسبة المحتوى الفينولي (TPC) في البذور 0.9± 58.2 ملغم/ مل كانت أعلى من محتوى أوراق الريحان ± 51 2.4 ملغم/ مل.

بالإضافة الى ذلك, تم تحليل محتوى الفلافونويد (TFC) باستخدام HPLC عند 254 نانومتر. تم اكتشاف مركبات الفلافونويد في 6.679 دقيقة لبذور الريحان بينما تم اكتشافها في 6.682 دقيقة لأوراق الريحان. أما نسبة محتوى الفلافونويد (TFC) فتم قياسها بطريقة الفحص اللونية Aluminium chloride. حيث كانت بالبذور 3.6 ± 34.2 ملغم/ مل, بينما في الأوراق ± 9.0 1.5 ملغم/ مل.

تم أيضًا فحص فعاليتها المضادة للملاريا وقيامه بمنع تكوين البيتا هيماتين في المختبر تحت ظروف فيزيائية وكيميائية. لم يلاحظ أي نشاط ضد الأنشطة المضادة للملاريا لأجزاء مختلفة من مستخلصات الريحان (الأوراق والبذور) في المذيبات المختلفة.

تهدف هذه الدراسة إلى صياغة سلوك مرحلة المستحلبات الدقيقة باستخدام مستخلصات الريحان (الأوراق والبذور) ومنشط السطوح(Tween 20) كمادة خافضة للتوتر السطحي ومساعد منشط السطوح (co-surfactant) وهو الايثانول. هذه الدراسة هي أول تقرير عن تكوين وإنتاج سلوك المرحلة الثلاثية لمستخلصات الريحان الفلسطيني (الأوراق والبذور). في هذا البحث، وجد أن الريحان الفلسطيني لديه القدرة على تكوين مستحلب المايكرو(microemulsion) في الأنظمة المخبرية.

تم استخدام تقنية Microemulsion لإنتاج كريم طبي مقاوم للشيخوخة والتأثيرات المضادة للطفح الجلدي ومزيل للتصبغات عند تطبيقها موضعيًا. يمتاز بالترطيب والتنعيم والرائحة الطيبة كما انه يمتص بسهولة بواسطة الجلد ولا يحدث تهيجا pH = 5-5.5، كما ان لونه جيدا وينتشر بسهولة (ليس لزجًا ويطبق بسهولة وبسرعة).

بناءً على هذه النتائج، استنتج أن الريحان يشكل مصدرًا طبيعيًا لمضادات الأكسدة القوية، ومضادات الجراثيم، ومضادة للشيخوخة، ومضادة للطفح الجلدي، ومزيلات التصبغ. التي قد تمنع العديد من الأمراض ويمكن استخدامها في مستحضرات التجميل والمنتجات الصيدلانية.