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Formulation and Applications of Borage (Borago Officinalis) seeds oil and leaves extracts and Microemulsion

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Formulation and Applications of Borage (Borago Officinalis) seeds oil and leaves extracts and Microemulsion

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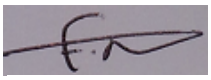
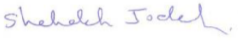
2-	Internal Examiner:	Dr. Fuad Rimawi:	Signature:	
3-	External Examiner:	Prof. Shehdeh Jodeh:	Signature:	

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Dedication:

I would like to dedicate this work to my heroine mother (Mrs Asma Barbarawi) , to my beloved father (Mr. Nour) who have always believed in me , who gave me all they have, all they know, all they can to continue studying and working efficiently to become a person they always wanted me to be.

To my lovely husband, Mohammad. I could never have done this without your support and encouragement.

To my pure love my daughter "Salma "a sweet girl who puts all the sweet colors in my neutral life.

To my precious sisters and brothers, Mohammad, Aya, Danya, Hamza and Tarteel I'm really grateful to all of you . you have been my inspiration and my soul mates, thanks for always having my back.

To my fabulous cousin Diana, thanks for being always around when needed.

To my husband's family for their warm wishes smiles and love.

To all my dear friends, everyone who supported me and helped me, even for a small part of this trip.

Marwa

June, 2020

Declaration:

I certify that this thesis is submitted for the degree of Master graduation in applied industrial technology is my own research, except where otherwise acknowledges, and that this thesis (or any part of the same) has not been submitted for the higher degree to any other university or institute.

Name : Marwa Nour Abd AlKarim Garajah

Signed:

Date: June 4, 2020

Acknowledgment:

{If you are grateful, I will surely give you more and more} Surat Ibrahim, aya7.

All thanks firstly to Allah almighty, Lord of power and Givenness.

My thanks and gratitude to my supervisor **Dr. Ibrahim Kyali** for his special advice, guidance, great efforts to complete this work, l would like to thank **Dr. Wadie Sultan** for providing me the necessary opportunities for completion of my project, special thanks for **Mrs. Reem Yaghmour** for her assistance in the antimicrobial tests.

All heartfelt thanks for my family, my friends and my colleagues, and honest thanks to all who supported me without expecting during my study.

Abstract

The herbal medicine utilizing as one element of complementary and alternative medicine is increasing worldwide, this study aims to prepare a topical borage oil microemulsion formulation to investigate its efficacy and tolerability in the treatment of patients with Atopic Dermatitis. Borage specifically selected in this study due to its abundance in the Palestine Mountains, reachable, the major botanical source of gamma-linolenic acid. The seeds were cultivated upon their ripening season in April of 2016 from the Halhul Mountains.

Soxhlet method was used to extract borage seeds and leaves oil by using ethanol 95%. A ternary phase diagram was constructed by determining appropriate nonionic surfactant to assess the ability for microemulsion formulation and durability of each system. Tween 80 was more suitable to solubilize each of borage seeds and leaves extracts compared with Tween 20 due to its prominent hydrophobic properties.

The antibacterial activity was evaluated in borage seeds and leaves extracts using a well diffusion technique against Streptococcus aureus, Escherichia coli, Candida albicans. The seeds extracts showed an inhibition zone against S. aureus with 12mm higher than inhibition zone that leaves extracts exhibited against gram-positive bacteria (S. aureus) which reach 7.5mm, but no significant effects for both extracts were reported against E. coli and C. Albicans. In addition the antibacterial activity for microemulsions formulation was measured against each of S. aureus, E. coli, and C. Albicans. As expected, they showed minor positive

influence against S. aureus when compared to Penicillin G. which is used as a positive control, in contrast no activity was reported against E. coli and C. albicans.

The antioxidant activity of borage seeds and leaves extract was investigated by Ferric iron- reducing antioxidant power (FRAP) method, the antioxidant activity was further indicated by the quiet good ability to reduce the FRAP reagents for both extracts with the indication of higher seeds extract activity. This variation is explained by the higher seeds extract content of polyphenol, tocopherol and Vitamin C than leaves extract the content.

In results, we can recommend administrating the borage seeds and leaves extracts within the treatment of patients with Atopic Dermatitis, at least in mild cases who are seeking an alternative treatment, in addition to the potential of using it as an effective ingredient within antibacterial and antioxidant applications.

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Abbreviations, Symbols and Terminology:

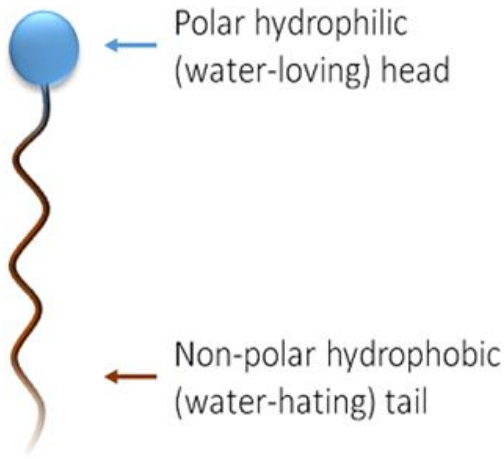
HLB	Hidrophile Lipophile Balance
o/w	oil in water
w/o	water in oil
GLA	gamma-linolenic acid
ALA	α-Linolenic acid
SDA	stearidonic acid
AD	Atopic dermatitis
FRAP	Ferric Ion Reducing Antioxidant Power Assay
PG	Propylene Glycol
Wt.%	weight percent

Chapter One:

Introduction

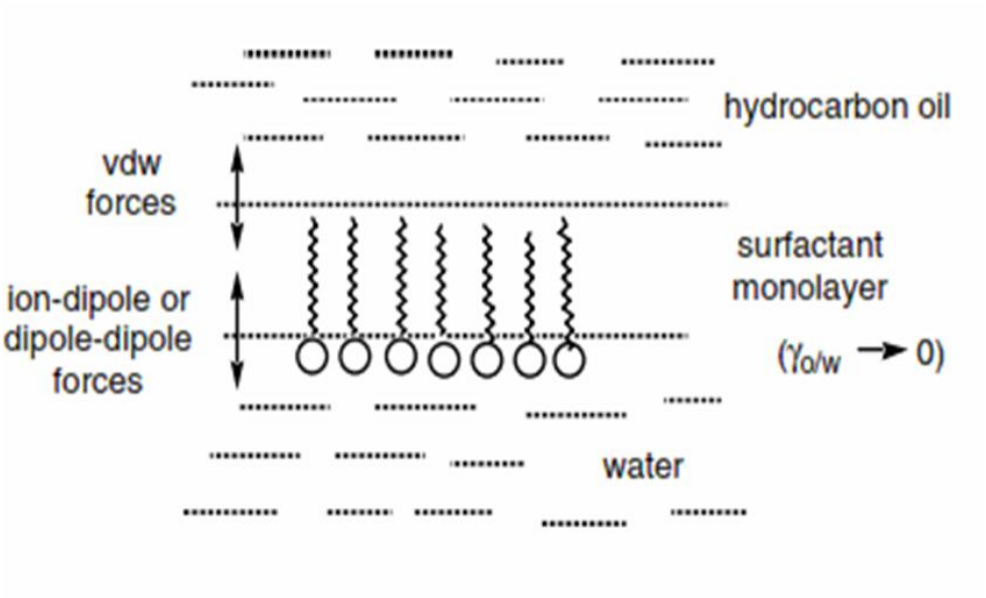
1.1. Surfactant

The word (surfactant) is a combination of the three words " surface active agents". They are organic compounds with a distinctive structures consisting of two parts, one of which is the lyophilic (solvent-loving) group, the other one is lyophobic (grease-loving) group, in the molecule. When the water or an aqueous solution is used at a solvent in which the surfactant is to be used. it is named as hydrophilic" water- loving" and in as hydrophobic" grease loving" are used. Also, a surfactant contains at least one non-polar group and one polar (or ionic) group, as shown in **Figure(1.1)**[1,2].



Figure(1.1): general structure of surfactant molecule **.**

Adsorption of a surfactant at the water/oil interface produces a surface with significant very low interfacial energy, the formation of this type of low interfacial energy surface is the basis of the stability of most oil and water emulsions and all microemulsions as shown in **Figure(1.2)**[2].



Figure(1.2) Illustration of the effect of an adsorbed surfactant layer on the interfacial energy between oil and water[2].

The general chemical classification of the surfactant is based on the nature of the hydrophilic part (head), with subgroups based on the nature of the hydrophobic part (tail)[3].

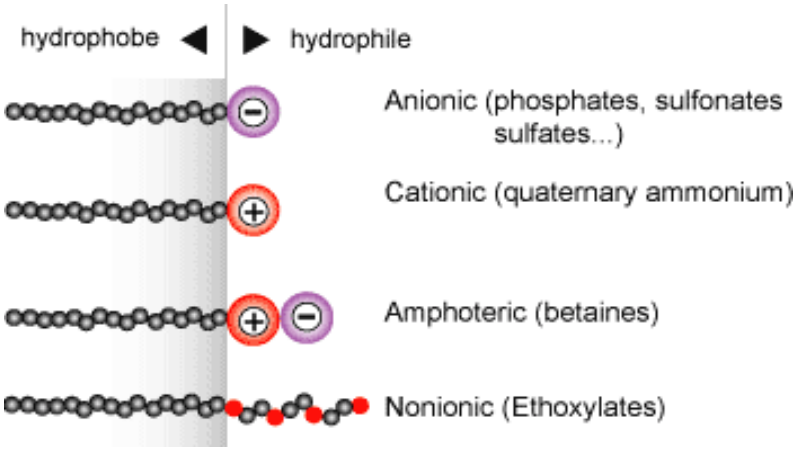
There are four groups of surfactant, these classifications are based upon the composition of the polarity of the head part: anionic, cationic zwitterionicand nonionic as shown in **Figure(1.3**)[1,4].

-anionic surfactant: has a negative charge group in its head

-cationic surfactant: has positive charge group in its head

- zwitterionic surfactant: has an amphoteric group

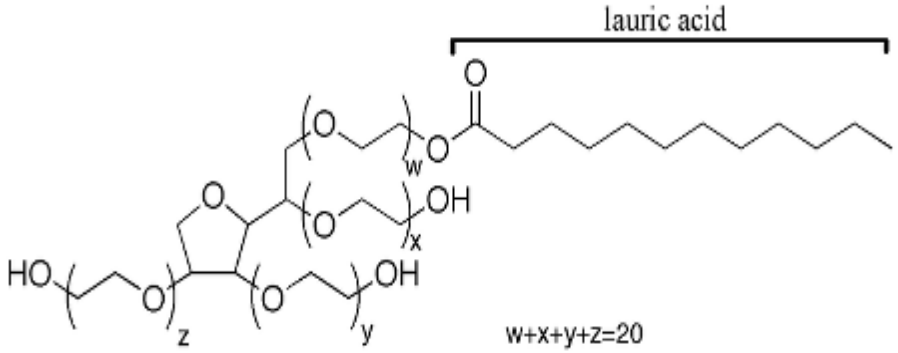
-non-ionic surfactant: has no charged group in its head



Figure(1.3): Schematic illustration of the various types of surfactants[48].

The Non-ionic surfactant is a surfactant that doesn't ionize in aqueous solution, because their hydrophilic group is a non-dissociable type, such as alcohol,phenol, ether, ester, or amine. Nonionic surfactants are commonly used to formulate microemulsion due to their Good skin tolerance, low irritation potential and toxicity, environmental friendly compatible, and commercial inexpensive surfactant In addition, less sensitive to water hardness and they foam less strongly [2,4 ,5].

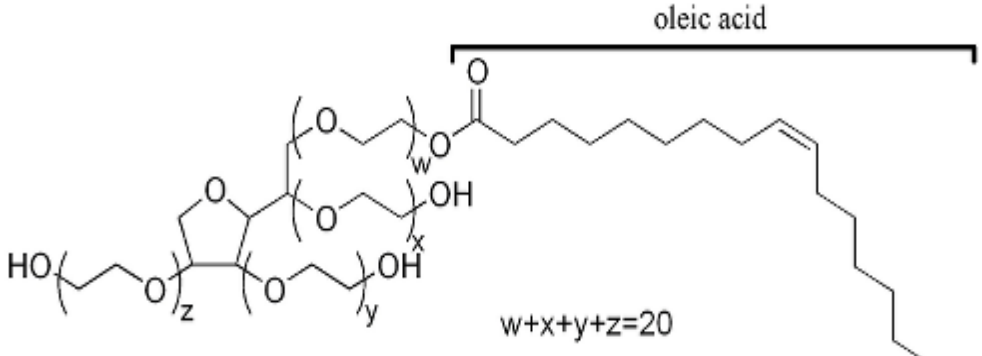
Tween 80 and Tween 20 are the non-ionic surfactants utilized in this study, they are common non-ionic surfactants, emulsifiers, wetting agents and solubilizers that are used in a variety of industrial applications, food products, medications, and cosmetics. Tween 20 is a clear, yellow to yellow-green viscous liquid derived from polyethoxylated sorbitan and lauric acid it has an HLB value16.7 showed in **Figure(1.4)** Tween 80 is a viscose, yellow to amber liquid, derived from polyethoxylated sorbitan and oleic it has an HLB value15.0 showed in acid **Figure(1.5)**[6,7,8].



Tween 20

$C_{58}H_{114}O_{26}$

Figure(1.4): Chemical structure of Tween 20 (Polyoxyethelene (20) sorbitan monolaurate



Tween 80

$C_{64}H_{124}O_{26}$

Figure(1.5): Chemical structure of Tween 80 (Polyoxyethelene (20) sorbitan monooleate)

1.1.1.HLB

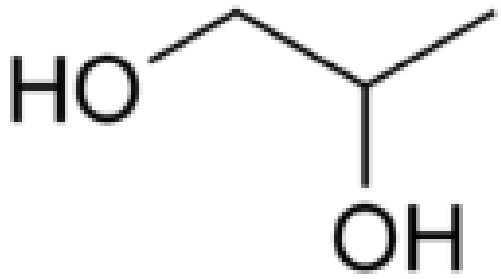
In the bulk aqueous phase, surfactants form masses, such as micelles, where the hydrophobic tails form the core and the hydrophilic heads are immersed in the surrounding liquid. Other types of structures can also be formed, such as spherical micelles or lipid bilayers. The shape of the molecules depends on the balance in size between the hydrophilic head and a hydrophobic tail. A measure of this is the HLB, Hydrophilic-lipophilic balance; which is a system created as a tool to make it easier to selection of suitable surfactant for a given application [1,2,9].

-Higher HLB surfactant (>10): hydrophilic (water loving) _ form O/W (oil-in-water) emulsions.

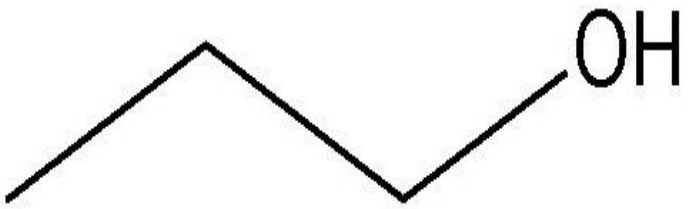
- Low HLB surfactant (1-10): lipophilic _ form (water-in-oil) emulsions.

1.1.2.Co_surfactant

Are usually alcohols or amines with ranging from C1 to C10, its function represented in the formation and stabilization of micelles / microemulsions. Co_surfactant is often used to increase the oil- solubilizing capacity of microemulsion surfactant system [10]. in our study we used propylene glycol and propanol as a co surfactant with molecular formula ("CH_3CHCH_2OH" "$CH_3CH_2CH_2OH$ ") respectively they shown in **Figure(1.6)a** and **Figure(1.6)b**.



Figure(1.6)a: structural formula of propylene glycol



Figure(1.6)b: structural formula of propanol

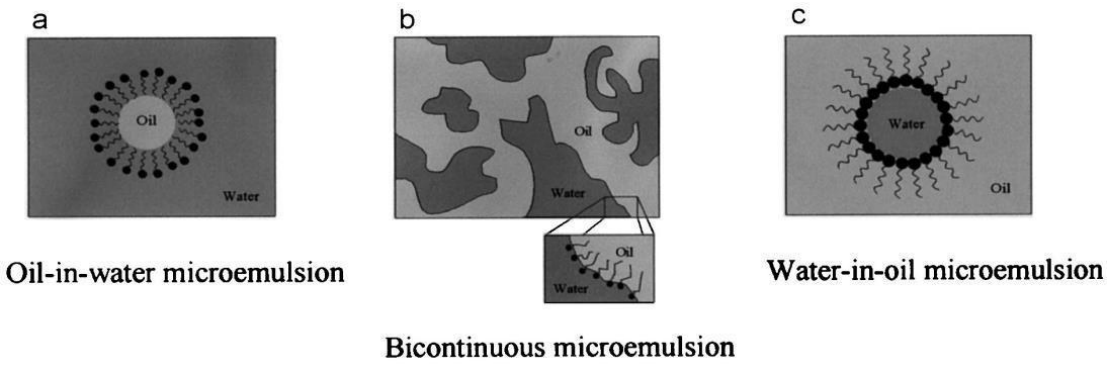
1.1.3.Microemulsions

They are isotropic, transparency particles by microscope, thermodynamically stable system compound of hydrophilic, hydrophobic and an amphiphilic component , in conjunction with surfactant, sometimes co-surfactant as another component[11,12].

In addition, microemulsion can be categorized according the ratios between the components as shown in the following classifications:

1. Oil in- water (O/W) microemulsions: the oil particles are dispersed in the continuous aqueous phase.
2. Water in-oil (W/O) microemulsions: the water particles are dispersed in the continuous oil phase.
3. Bicontinuous microemulsions: the micro ranges of oil and water are inter dispersed inside the system.

The three types of microemulsions, the interface is stabilized by using surfactants and/or co-surfactants as shown in **Figure(1.8)** [13].



Figure(1.8) Emulsion micro structures[13]:

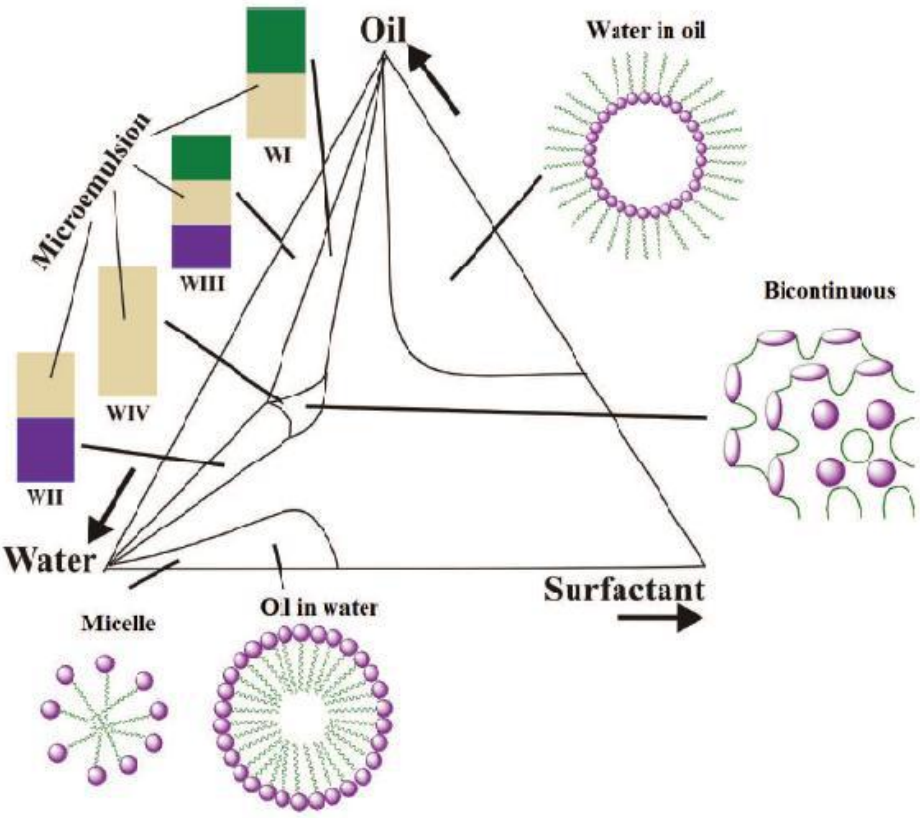
(a) Oil-in-water, **(b)** Bi-continuous, and **(c)** Water-in-oil microemulsion

Microemulsions and Nano emulsions have a larger surface area per unit volume than do macroemulsions because of their sizes, whereas in microemulsions the droplet size of the dispersed phase is less than 100 nm. On the other hand, both macroemulsions and nano emulsions have a thermodynamically instability in contrast the microemulsions. All kinds vary in shapes between spherical to cylindrical and lamellar [14].

The phase diagram represents the relevance between the phase behavior of mixture and its composition microemulsion is basically characterized by analyzing the phase behavior of the system comprised of surfactant, co-surfactant, oil, and water in which it is possible to represent through pseudo-ternary diagram[15]. According to Winsor classification, four types of equilibrium[16,17] as shown below:

1. Winsor I: oil in-water(o/w) microemulsion are formed, and the surfactant_ rich water phase coexists with the oil phase where the surfactant is only exist as Monomers

2. Winsor II: water in- oil (w/o) microemulsion, are formed and the surfactant _ rich oil phase coexists with surfactant _ poar aqueous phase.
3. Winsor III: (middle phase) a three phase system where a Bicontinuous middle _ phase microemulsion (rich in surfactant) coexists with both excess water and oil phases.
4. Winsor IV: a single phase (is tropic) miceller solution (microemulsion), that from upon addition of a sufficient quantity of amphiphile. And this classification is illustrated in **Figure(1.9)**.



Figure(1.9): Schematic Ternary phase diagram of various types of microemulsion systems as classified by Winsor[47].

There has been a revolution in the last two decades in exploitation of microemulsion in a variety of chemical and industrial process, include use it in

enhanced oil recovery, Detergency, as coatings and textile finishing, cosmetics, food, biotechnology, analytical application, microemulsion gel technique, liquid membranes and in pharmaceutical. In talking about the microemulsion application we consider microemulsion as promising tools as delivery systems, allowing both types of drug release, Controlled as well as sustained, during various routes of administration. In addition, microemulsion have different unique distinctive features as a delivery system, main features of being less toxic, facilitating enhanced absorption of drugs, and regulating the drug release rates[17,18].

1.2. Borage

Borage officinalis from Boraginaceae family, as known as borage, bourrache, buglass, borage. Borage is an seasonal, herbal and hairy plant, the height of its stems ranges between 70_100cm. In addition, they are straight, often branched, hollowed and covered by tough fibers, its leaves grow in an alternating pattern with wavy edges and covered with tough fibers while the flowers are mostly blue and rarely occur white or rose colored[19,20,21,23].as shown in the **Figure(1.9)a**. Each flower produces a fruit; With 3_4 brownish nutlet after growing, this fruits are small brownish oval wrinkled nutlet[19,22]. as shown in the **Figure(1.9)b.**

Figure(1.9)a: Borage plant

Figure(1.9)b : brownish nutlet

1.2.1.Cultivation

The appropriate time to cultivate this plant in early spring, it can also be harvested in autumn or late winter. Many researchers have proven that early seeding increases seed performance and quality in comparison with late seeding. There is an obvious relation between seed performance and gamma-linolenic acid (GLA) level which can be related to the cultivation's date, more precisely, the amount of GLA is reduced as temperature reduction during growth period. One of the serious problems during the production of borage seeds is unlimited falling of flower and seeding, and this problem that we also encountered [21,24,25, 26].

Several fatty acids combination in Borage oil, the amount of linolenic acid, ALA, GLA, SDA and erucic acid are a special important chemotaxonominic inside this plant. The oil of Borage seeds is the richest plant source of GLA (Gamma - linolenic acid) in which its amount ranges between 30%_40%. GLA is one of the volatile fatty acid which synthesized just by a few plant species, and mostly this fatty acid found in their seeds. Moreover, GLA has shown a positive role in treating a number of patients whose have a clinical condition caused by GLA deficiency, such as Atopic Dermatitis which indicated in this thesis [27].

Borage oil also contains minor components have another important roles, such as tocopherols, phenolic acid, δ-bornesitol, sterols, pyrrolizidine alkaloids, flavonoids Rosmarinus acid, anthocyanins, saponins, unsaturated terpenoids and sterol. Tocopherols also have an antioxidants effect, and borage species have high

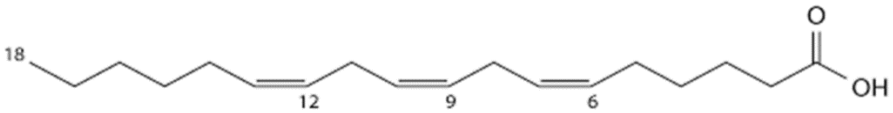
amounts of δ-tocopherols.Phenolic compounds also occur in oil seeds, several studies have proved their antioxidant features[19,28].

1.2.2.Gamma linolenic acid

GLA is an 18 carbon polyunsaturated fatty acid containing three double bonds **Figure(1.12)**, It is an δ_6 desaturated product in the metabolism of LA. The richest plant source of the gamma- linolenic acid (GLA) is the borage (seeds and oil) which contains a high amount of the GLA (30% _ 40%)[19], in which commonly used as nutritional supplement and pharmaceutical prescription to control heart disease, diabetes arthritis, multiple sclerosis, eczema and atopic dermatitis[29,30].

Essential fatty acids abnormalities and defects could contribute to A.D is two ways[19]:

- ❖ through a direct effect on the skin structure and function.
- ❖ By affecting maturation and sensitization of the immune system affecting the skin.



Figure(1.12): Gamma- Linolenic Acid _18:3n-6

1.3. Atopic dermatitis (eczema)

is an inherited chronic dermal disease which usually begins in childhood period; though anyone can get the disease, this disease is one that affects a large percentage of children reach to 5%-10%, and the incidence is increasing[31].A. Dermatitis is an eczematous disease with severe signs includes very itchy, red rashes on the back of the neck and knees and in elbow creases. The main cause of atopic dermatitis is idiopathic. However, many studies shown that the pathogenesis of AD is multifactorial includes an environmental, immunological and genetic factors.The pathophysiology of atopic dermatitis involves skin barrier defects causes an increase in transepidermal water loss (TEWL) beside increased permeability to irritants and allergens[32,33,34].

Several researches have proposed that patients with AD have an association with an abnormality in essential fatty acid metabolism particularly affecting GLA production. Now, local corticosteroids is usually used to remove inflammatory and itchy rashes appears in patient, mostly all medicines given to AD patient have a various side effect. Borage seed oil is also used for chronic skin inflammatory diseases due to prevent apparition of these effect. It has been proposed that atopic dermatitis is associated with an abnormality in essential fatty acids metabolism particularly affecting GLA production[32].

Objectives of research

1. To extract of Borage seeds and leaves by Soxhlet extraction with ethanol 95%.
2. To evaluate the efficacy of borage seeds oil, which contains a high concentration of γ-linolenic acid (GLA) for atopic dermatitis treatment.
3. To investigate the best ternary Phase Diagram for both extracts based on non-ionic surfactant.
4. To evaluate the antioxidant activities of both seeds and leaves extracts.
5. To investigate the antimicrobial activity of both seeds and leaves extracts.
6. To study the effect of using 1-Propanol and propylene glycol as solubilization enhancers.

Chapter two:

Literature Review

2. Literature Review

C.R. LAILI and S. HAMDAN investigated the phase behavior of stable W/O microemulsion regions formed by non-ionic surfactants, the non-ionic surfactants were chosen in this study because of their mild effect and low cost as compared to other types of surfactant. Topical microemulsion depends on surfactants which are used as emulsifying agents and stability oxidative. Tween 80 selected to provide stable microemulsion incorporating with co-surfactant which used in drug delivery system to produce very low interfacial tension on the surface, thereafter spontaneously with a small droplet in diameter of microemulsion, so the non-ionic surfactants more favorite according to the HLB value[15].

N. Grampurohit *et. al.* were chosen The non-ionic surfactants in this study about using of topical microemulsions due to their major role in the drug solubility in oil phase during the preparation of microemulsion. In addition to their good cutaneous tolerance, lower irritation and toxicity. For instance, Tween 80 and Tween 20 which have been selected due to their HLB values (15, 17 respectively), beside the co-surfactant like as propylene glycol [35].

N. Grampurohit *et. al*. investigated the ability of microemulsion as promising delivery system that allows sustained or controlled drug release for precutaneous, peroral, topical, transdermal or any one of other administration routes. While microemulsions are used in many fields, this review focused on topical applications for microemulsions. The topical administration is to conveniently deliver drugs to a localized area of skin, topical microemulsion have the ability to deliver larger amounts of water and topically applied agents through the skin than water alone or other traditional vehicles because they act as a better reservoir for a poorly soluble drug through their capacity for enhanced

solubilization, so they enhanced drug absorption with minimal systemic absorption, reduced side effects and decreased toxicity[35].

P. Kotnik *et. al.* investigated supercritical carbon dioxide extraction of Borage seed oil. Borage seed oil is a major commercial source of ɣ-linolenic acid, the fatty acid has a great potential therapeutic role in the treatment of many diseases. In this experiment the researchers made a comparison between SFE and conventional extraction (soxhlet). In this context, about SFE, the rate of the process is lower at higher temperature, the optimum temperature was 40°C while the highest yield is achieved at 300 bar where 98% of total oil can be obtained after using 35-40 g of CO_2/g of raw material. As results showed the GLA content increases from 11.65% to 16.89% by increasing the pressure from 200 to 300 bar at 40°C. So, it can be supposed that the maximum amount of oil from the seed can be obtained at 40°C and 300 bar. The composition and quality of borage seed oil extracted by SFE is similar to that extracted and obtained with n-hexane using Soxhlet although longer times and higher temperatures are required to obtain the same quality and quantity of the extracted oil[36].

M. ANDREAssl *et. al.* determined the efficacy of ɣ-linolenic acid in the treatment of patients with atopic dermatitis, a study was conducted for 60 patients with atopic dermatitis (15-30 years), equally divided by gender, 30 were treated with ɣ-linolenic acid of (C18:3 n-6) at a dosage of 274mg twice daily, the other 30 patients were given a placebo. The patients who received ɣ-linoleuic acid showed gradual improvements in symptoms, which were statistically significant compared with the control group ($P<0.001$); ɣ-linolenic acid was found to be effective and without side-effects for the treatment of atopic dermatitis[37].

R. Foster *et. al.* was recommended the borage oil in the treatment of atopic dermatitis by his study, in where he showed the effective role of EFAs in the skin structure and physiology, patients with atopic dermatitis have been reported to have a deficiency in EFAs and imbalance in their levels. This review identified 12 clinical trials of oral or topical borage oil for treatment of atopic dermatitis and one preventive trial. All studies were controlled and mostly were randomized and double-blind, but many were small and had other methodological limitations. The results were highly variable, five studies reported a significant effect of borage oil in the treatment of Atopic dermatitis, insignificant effect in other five studies, and mixed in remaining two studies, the majority of studies showed at least a small degree of efficacy or weren't able to exclude the possibility of an oil's effect in treatment. Ultimately, nutritional supplements with borage oil is unlikely to have a prominent clinical effect. However, it may be useful in some individual patients with less severe atopic dermatitis who are seeking an alternative treatment. Besides that, borage oil is well tolerated in the short term as data shown, but no long term tolerability data availability[31].

A. Miceli *et. al.* investigated the antibacterial activity of Borage Officials aqueous extracts which evaluated in vitro and in situ using different food model system,the antagonistic activity was examined and evaluated against several bacteria commonly associated with food borne diseases by paper disc diffusion method. The aqueous solution of B. Officials showed inhibition at minimum concentration (MIC)(10 mg/ml) for the majority of the sensitive strains, in addition it has been proven effective as natural antibacterial substance[38].

A. Borowy *et. al.* examined biological active compounds and antioxidant activity of Borage flowers and leaves, to talk about Borage Officinalis, it is an annual planet from Boraginaceae family natives back to the Mediterranean region, also found in other

countries such as parts of Europe like Poland, also its flowering season is from June to July. Its stems, leaves and calyx is covered with rough and dense hairs producing essential oils as an extraction. This experiment showed that borage flowers and leaves have a higher polyphenolics content in comparison with various extracts of many culinary and medical herbs grown in Maryland, besides it contains carotenoids, chlorophyll and vitamin C which are a potent antioxidants. In this study the antioxidant activity of borage leaves and flowers was evaluated by the FRAP assay, also using Folin's method in addition to the free radical method with DPPH reagent[39].

C. Soto *et. al.* investigated antioxidant content of oil and defatted meal obtained from borage seeds by an enzymatic - aided cold pressing process, Tocopherol content and polyphenols content were determined in borage oil and borage defatted meal, respectively, also the antioxidant activity of extracts obtained from borage defatted meal was evaluated. Several solvents were used during extraction of polyphenols from defatted borage meal , highly soluble solids and phenolic compounds with antioxidant activity as free radical-scavenging (DPPH) was extracted by using methanol. In addition, the enzymatic technology increases the antioxidant activity of the extract, as DPPH scavenging, also maintain the Tocopherol contents of the borage oil obtained by cold pressing, which is higher than the oil's tocopherol extracted by the solvent[40] .

Chapter three:

Materials, Methods and Experiments.

3.Materials, Methods and Experiments

3.1. Chemicals And Plant Materials

Borage (Borago officinalis L.) seeds and leaves were collected from Hebron -Palestine, and were certified to approve the botanical identification by Dr. Khaled Sawalha (Botanist & Associate professor at Biology department, faculty of science & technology. Al-Quds University- Palestine),Ethanol 95% (EtOH),distilled water, Tween 80, Tween 20, propylene glycol (PG), 1-Propanol, Sodium Acetate Anhydrous, Glacial Acetic Acid, Tpz (2,4,6-Tris(2-Pyridyl)-1,3,5-Triazine), Hydrochloric Acid, Sulfuric Acid, Ferric Chloride Trihydrate, Mueller Hinton Agar, *Staphylococcus aureus* as gram-positive bacteria, *Escherichia coli* as gram-negative bacteria and *candida albicans* yeast sterile

3.2. Instruments and Equipment

Seed grinder was used to minimize the size of the seed. A Soxhlet extractor has three main sections: a round bottom flask, condenser, extraction chamber, a thimble (porous bag made of strong filter paper) which holds over the solid to be extracted, heat mantel. Then, was used rotary evaporator to evaporate the solvent.

UV-Vis spectrophotometer and micropipette were used in antibacterial test and FRAP assay to measure absorbance.

Then, 10ml glass test tubes with screw cap, syringes, thermostatic water bath, four digit analytical balance, incubator cross polarizers and vortex were used in microemulsion. Plastic petridishes, Incubator, and, sterilizer cotton swab were used in antibacterial test.

3.3. Methodology

3.3.1.Borage leaves and seeds sample preparation

The fresh borage leaves and seeds were washed with water to remove dust, then the leaves were exposed to air drying at room temperature for 2 weeks. and the seeds were stored in dark and cool place until used for extraction.

dried leaves were crushed in order to decrease the particle size and increase the surface area, then the sample were stored in dark and cool place until used for extraction.

3.3.2. Soxhlet Ethanol Extraction of Borage leaves and seeds

Solvent Extraction is a process that aims to extract certain soluble components present in plant. In where it's the process of leaching which is a solid (plant)/ liquid (solvent) separation operation. The solid object is placed in contact with a fluid (suitable solvent) then the plant components of interest are solubilized and contained within the solvent, the obtained solution is the desired extract, eventually, the solvent will be eliminated to isolate the plant extract. This process is the safest form of extraction, in addition to its other features in providing similar results to other commonly used methods and one of the least expensive options available, and using a common laboratory equipment creates a smooth workflow that is fast, safe, and inexpensive, this is what makes it the most used in the essential oils and botanicals industries[43].

In this study ethanol 95% was used as a solvent , which is one of the most popular solvents, because it is safe to use in food products according FDA which labeled ethanol as a Generally Recognized as Safe (GRAS) substance, in addition, ethanol is the second most important solvent after water and the least toxic of alcohols, also it has distinctive

ability to dissolve both polar and non-polar molecules, which make it more suitable for use in different industries and customer products.

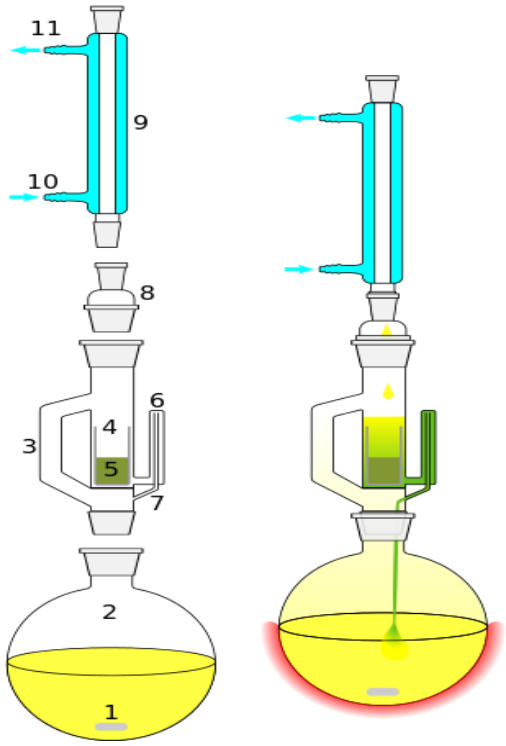
About 20 g of grinded air dried leaves material were put in "thimble" made from strong filter paper and interested into the broad central tube of the soxhlet extractor, and the same process was repeated for 30 g of dried seeds sample crushed by seed grinder. Sequentially, Borage leaves or seeds, after that 300 ml of the solvent (Eth95%)was added to 1000 ml round bottom flask.

Ethanol was heated using the heat mantel to reflux, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble, When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of the chamber siphon into flask, and cycle begin again.

The process should run for a total of 10 hours, the equipment can be turned on and off when overnight running is not permitted, and the time spilt over a number of days.

Once the process has completed, the ethanol was evaporated by using a rotary evaporator under reduced pressure at 40 C, the extract was stored in a fridge at 2-5 C.

Soxhlet extraction is depicted as a **Figure (3.1)**



Figure(3.1): Soxhlet apparatus for hot extraction

3.3.3.Constructionof Ternary Phase Diagram

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

1. For the microemulsion formulation each samples of borage leaves and seeds extracts were mixed with the surfactant (T20 or T80) at different weight ratios as shown in **Table (3.1)**, and were inserted in 10ml glass test tubes with screw caps.

Table (3.1): Surfactant/ Oil weight ratio.

Wight ratio	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	10
Surfactant	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Oil	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0

2. Then the mixture was mechanically shaken for 2-3 minutes by vortex for each sample due to the high viscosity of the surfactant and extracts in order to guarantee a homogenous dispersion.

3. After 48h a drop by drop titration of water phase (water or water: PG) with specific weights was injected as in **Table (3.2).**

4. The tubes then were left at rest for 24h to reach equilibrium before the next addition of water phase and analyzing.

5. The tubes temperature was controlled by placing it within the thermostatic water path at (25±1) ^{o}C if necessary.

Table (3. 2): Water phase percentage and Weight

%Water Phase	4%	8%	12%	16%	20%	24%	28%	32%	36%
Weight	0.0417	0.0453	0.0494	0.0541	0.0595	0.0658	0.0732	0.0817	0.0919
%Water Phase	40%	44%	48%	52%	56%	60%	64%	68%	72%
Weight	0.1042	0.1191	0.1374	0.1603	0.1894	0.2273	0.2773	0.3473	0.4466
%Water Phase	76%	80%	84%	88%	92%	96%			
Weight	0.5955	0.8337	1.2070	2.0833	4.1667	12.5			

5. Four microemulsion systems for each of borage leaves and seeds extract were prepared at room temperature; the composition of each system is detailed in **Table(3.3)**.

Table(3.3): Microemulsion composition of four different systems.

System #	Composition
System(1)	Water + Tween80 + extract
System(2)	Water + Tween20 + extract
System(3)	Water + Tween80: 1-Propanol + extract
System(4)	Water: PG(2: 1) + Tween80: 1-Propanol + extract

Microemulsion was identified by visual inspection after each addition of water phase as transparent, single phase and low viscous mixture. The anisotropy was detected by cross polarizers, and finally the phase diagrams were drawn using Origin 2017.

3.3.4.Antimicrobial activity

The antimicrobial activities of Borage leaves and seeds was tested against different types of microorganisms: Staphylococcus aureus, E. coli and Candida albicans by using well diffusion technique which depends on diffusion of the sample tested from a vertical cylinder through a solidified agar layer in a plate. the Positive control was used for bacteria (Penicillin for Staphylococcus aureus and ampicillin for E. coli). The Muller Hinton media was prepared by mixing 19g agar in 1 L distilled water, and then boiled to dissolve the media completely, then sterilized at 121°C for 15 minutes by autoclaving. After sterilization, the media was cooled to about 45°C, then the suspension of each microorganism added separately to the nutrient broth. Both gram negative (Escherichia coli) and gram positive (Staphylococcus aureus) bacteria and yeast (candida albicans) were tested using UV-Spectrophotometer until suitable concentration is reached. The

Muller Hinton media was then distributed on plastic petridishes (20-30 mL/plate) and allowed to solidify. After the media solidified, four holes with a diameter of 9mm were made using sterile cylinder and were filled with 50µl of the extract samples for each plate. As well a positive control disk was placed on the agar surface. The plates incubated at 37 ± 0.5°C for 24 hours. After incubation period, the zone of inhibition was measured from edge to edge of the clear area around the holes containing the samples. The measuring rule in millimeter (mm) was used to take the measurement.

3.3.5. Measurement of antioxidant activity by FRAP assay

The antioxidant activity of borage leaves and seeds extracts were calculated utilizing modified method of the assay of ferric reducing/antioxidant (FRAP) assay according to (Benzie and Strain 1996)[46]. FRAP Freshly prepared reagent (3.0 ml) were warmed at 37°C and mixed with 40 µl of the borage extracts of leaves and seeds. Thereafter the reaction mixtures were later incubated at 37°C. the absorbance was recorded at 593 nm with taking the reagent blank which containing distilled water as a reference point; which was also incubated at 37°C for up to 1 hour instead of 4 minute, which was the original time applied in FRAP assay. Aqueous solutions of known Fe (II) concentrations in the range of (2 - 5 mM) ($FeSO_4.6H_2O$) were used for calibration. This experiment was applied in duplicate to obtain more accurate results and the average absorbance for each extract was taken for calculations.

3.3.6. FRAP Reagent Preparation

FRAP reagent was prepared according to Benzie and Strain 1999, This reagent was prepared fresh just before the assay by the addition of:

1- FRAP reagent was prepared by the addition of 2.5 ml of a 10mM tripydyltriazine (TPTZ) solution in 40mM HCl plus 2.5 ml of 20mM $FeCl_3.6H_2O$ and 25 ml of 0.3M acetate buffer at pH 3.6.

2- Acetate buffer (0.3 M) at pH 3.6 was prepared according to British Pharmacopeia by dissolving 16.8g of acetic acid and 0.8g of sodium hydroxide in 1000 ml of water.

3- TPTZ (10mM, Mwt = 312.34 g/mol) was prepared by dissolving 0.312g TPTZ in 100ml HCl 0.04M HCl was prepared by diluting 21.8 ml of stock HCl solution (0.0917M) to 50 ml with water.

4- Ferric chloride hexahydrated (20mM, Mwt = 270.3 g/mol) was prepared by dissolving 540mg of it in 100ml of water.

5- 10% $AlCl_3$ was prepared by dissolving 10g of $AlCl_3$ in 100ml of water.

6- 7.5% Na2CO_3 was prepared by dissolving 7.5g of Na_2CO_3 in 100ml of water.

Chapter four:

Results and Discussion.

4.1. Ethanol Soxhlet extraction of Borage seeds and leaves oil

Plants have to be the backbone of medical treatments throughout much of human history [41]. Medicinal plant sources known as herbal or botanical medicine which refers to the use of plant seeds, leaves, roots, fruits and stem bark as treatment of diseases [42].

Borage seeds and leaves were extracted by Soxhlet method using ethanol 95% as an organic solvent, for total 10h. A dark yellow colored solution was obtained from seeds extraction after many cycles of the solvent, and a dark green solution also was obtained from leaves extraction by many cycles of the solvent, and the solvent was removed by utilizing the rotary evaporator technique, yielding a honey colored oil from seedsas shown in **Figure(4.1)**, besides a dark green semi-liquid extracted compound from leaves. The extraction yield percentage of the extracted seeds and leaves of borage was about 25.1% and about 20.5% respectively.

Figure(4.1):yielding oil from seeds.

4.2. Formation of microemulsion

Pseudo-ternary diagram of borage seeds and leaves extracts was constructed by determined appropriate surfactant, oil phase, and an aqueous phase with their concentration ranges that can result in certain existence area of microemulsion, this diagrams were developed by the water titration method.

This study aimed to formulate the phase behavior of microemulsion in the ethanolic extract of borage seeds and leaves, to achieve an environmentally, friendly, stable, biocompatible microemulsion between 95% ethanolic extracts of borage seeds and leaves, water, and non-ionic surfactant.

4.2.1 Phase Diagram of Tween 20 or 80/ Water/ Borage seeds oil or leaves extract.

Ternary Phase Diagram of the Borage seeds oil or leaves ethanolic extracts were obtained a different microemulsion reigns under the same formulation conditions, using two different non-ionic surfactants which are Tween 80 (polyoxyethelene (20) sorbitane monooleate) and Tween 20 (polyoxyethelene (20) sorbitane monolaurate) at 25°C.

4.2.1.1: Phase Diagram of Water / Tween 80 / oil seeds

Figure (4.2)shown ternary phase diagram of the system consists of oil seeds as oil phase, Tween 80 as a surfactant, and water as aqueous phase. The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% water from the point containing 60% oil seeds and 40% Tween 80. and extend to 32% towards the water apex Highly viscous anisotropic and shiny liquid crystals region was also appeared at point containing 28 wt.% of surfactant,14 wt.% of oil and 28wt% of water.

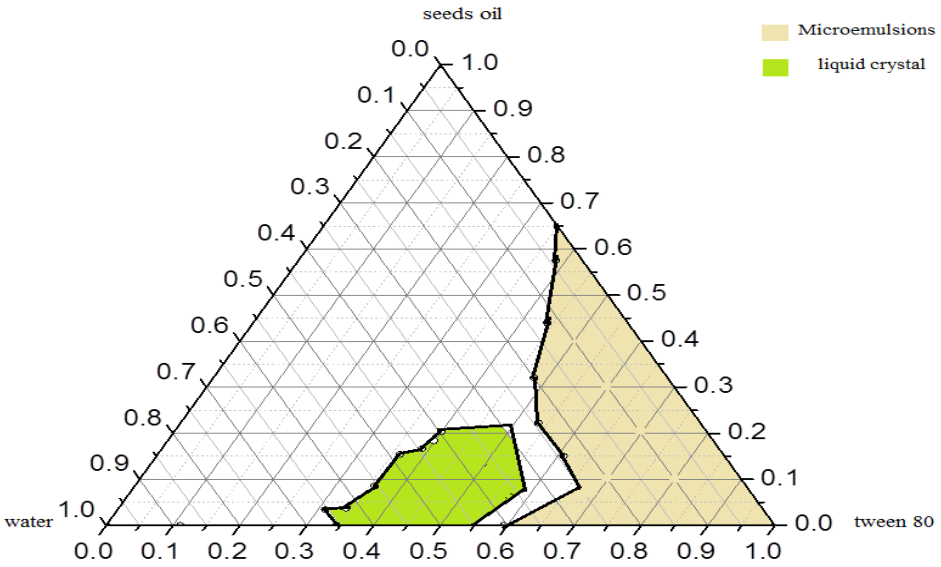
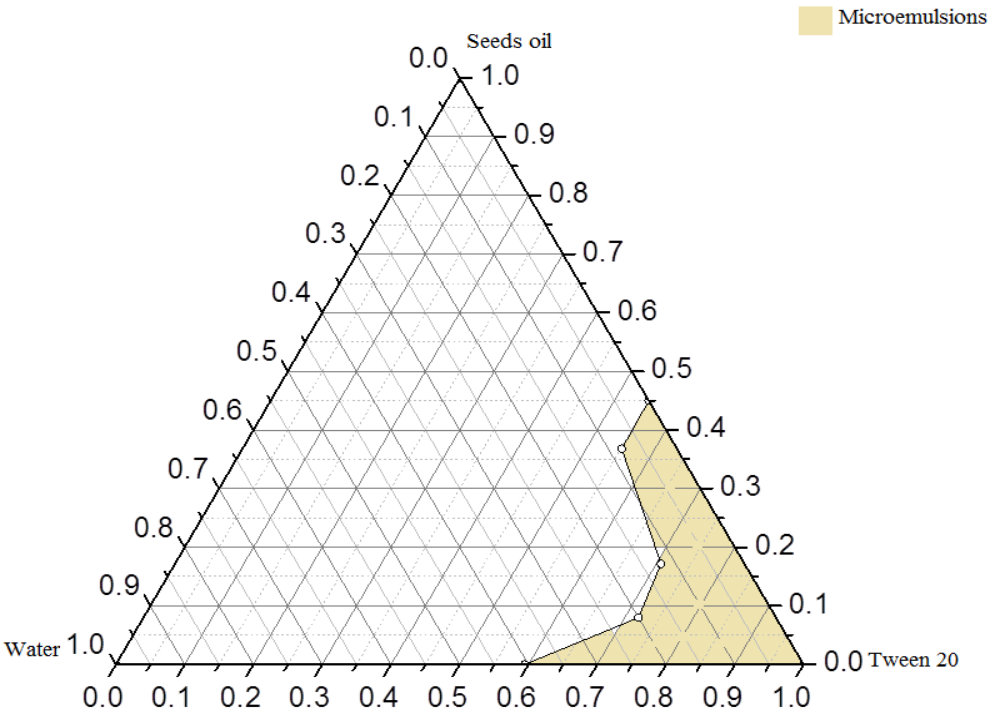


Figure (4.2): Ternary Phase diagram of system: Water/ oil seeds/ Tween 80 at 25°C. The microemulsion region is represented by beige color. The liquid crystals region is represented by light green.

4.2.1.2 : Phase Diagram of Water / Tween 20 / oil seeds

Figure (4.3) shown ternary phase diagram of the system consists of oil seeds as oil phase, Tween 20 as a surfactant, and water as aqueous phase. The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% water from the point containing 45% oil seeds and 55% Tween 20.and extend to 32% towards the water apex



Figure(4.3): Ternary Phase diagram of system: Water/ oil seeds/ Tween 20 at 25°C. The microemulsion region is represented by beige color.

4.2.1.3 :Phase Diagram of Water / Tween 80 / leaves extract

Figure (4.4) shown ternary phase diagram of the system consists of leaves extract as oil phase, Tween 80 as a surfactant, and water as aqueous phase. The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% water from the point containing 60% leaves extract and 40% Tween 80. and extend to 32% towards the water apex Highly viscous anisotropic and shiny liquid crystals region was also appeared at point containing 34 wt.% of surfactant,14 wt.% of oil and 28wt.% of water .

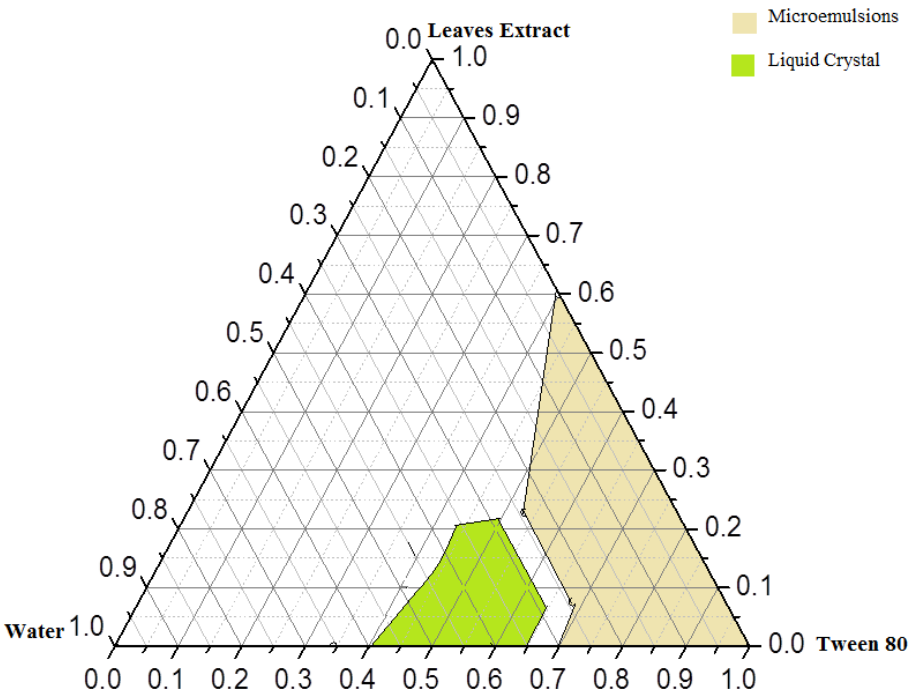


Figure (4.4): Ternary Phase diagram of system: Water/ leaves extract / Tween 80 at 25°C. The microemulsion region is represented by beige color. The liquid crystal region is represented by light green.

4.2.1.4 : Phase Diagram of Water / Tween 20 / leaves extract

Figure (4.5) shown ternary phase diagram of the system consists of leaves extract as oil phase, Tween 20 as a surfactant, and water as aqueous phase. The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% water from the point containing 45% leaves extract and 55% Tween 20, and extend to 44% towards the water apex.

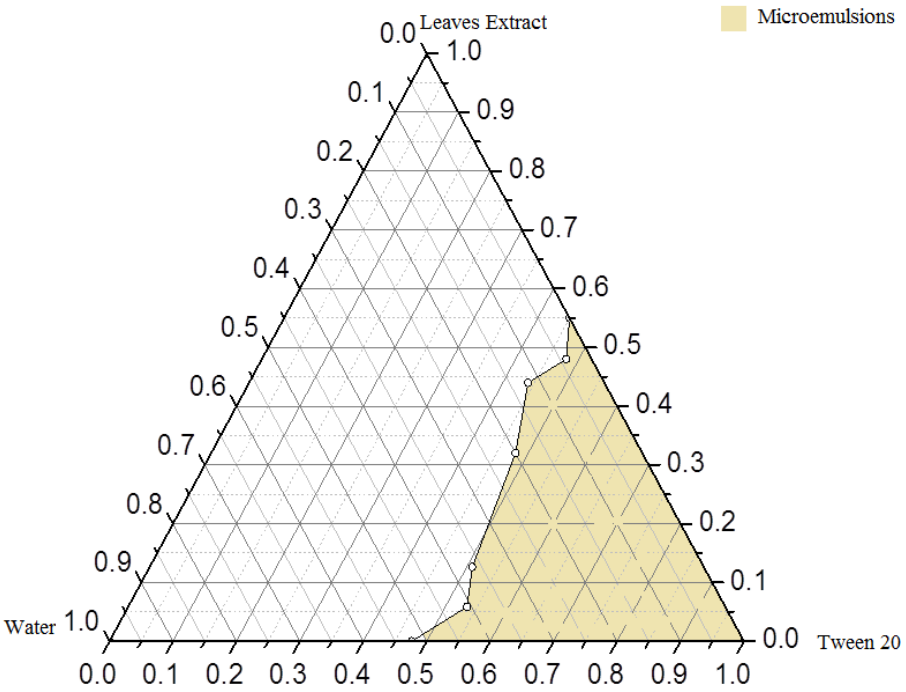


Figure (4.5): Ternary Phase diagram of system: Water/ leaves extract / Tween 20 at 25°C. The microemulsion region is represented by beige color.

The above figures showed that each of borage seeds and leaves extracts formulated in microemulsions using Tween 80 exhibited larger solubilization regions compared with those formulated with Tween 20, so Tween 80 seems like a better choice as it can act O/W emulsifier compare to Tween 20, this attributed to the ethylene oxide subunits in

Tween 20 which are responsible for the predominance of hydrophilic nature of this surfactant, while the hydrocarbon chain provides the hydrophobic environment, so it miscible the minimum oil phase (borage seeds and leaves oil), in contrast Tween 80 is more hydrophobic surfactant so it miscible the maximum oil in the aqueous surfactant phase[44].

That’s mean that the solubilization of each borage seeds and leaves extracts is sensitive to the hydrocarbon chain length of the surfactant, and it is favored with the larger carbon chain length as in Tween 80, this referred to the fact that interaction between the interface and oil decreased with decreasing of the surfactant hydrocarbon chain length.

4.2.2.1 : Phase Diagram of Tween 80: propanol (2:1) / Water / Borage seeds oil or leaves extract .

Ternary Phase Diagram of Borage seeds oil or leaves ethanolic extracts was studied upon addition of propanol as co-surfactant, which was grouped together with the Tween 80 at a fixed ratio(2:1)(Phase Diagram of Water / Tween 80: propanol (2:1) / oil seeds

Figure (4.6) shown the ternary phase diagram of the system water/ seeds oil / Tween 80: propanol (2:1) at 25°C.The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% of water from the point containing 22 % seeds oil and 78 % Tween 80: propanol (2:1) point, and extend to 88% towards the water apex.

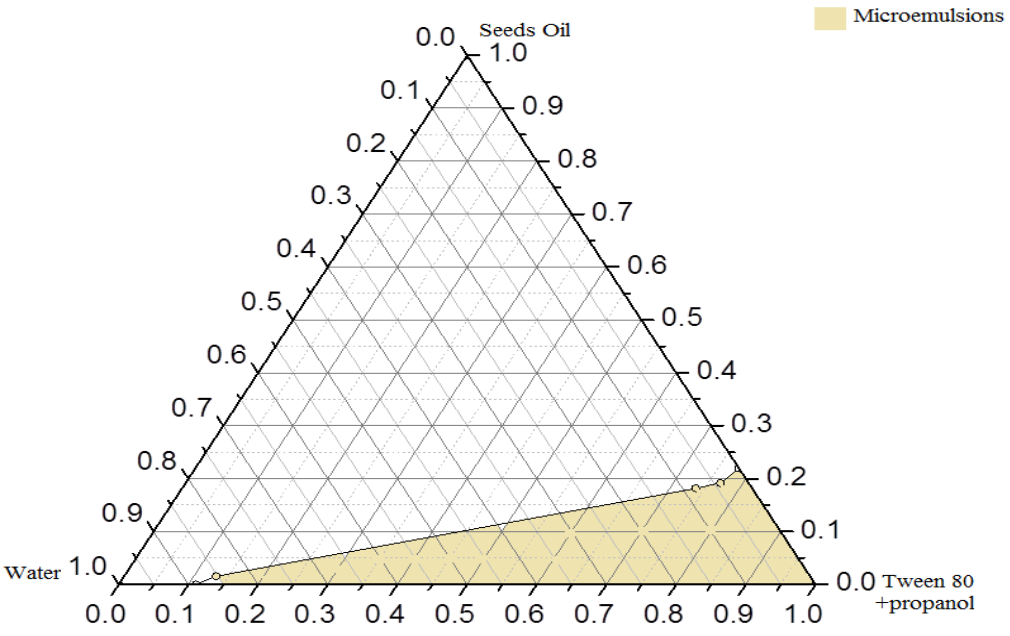


Figure (4.6) Ternary Phase diagram of system: Water/ seeds oil / Tween 80: propanol (2:1) at 25°C. The microemulsion region is represented by beige color.

4.2.2.2:Phase Diagram of Water / Tween 80: propanol (2:1) / leaves extract

Figure (4.7) shown the ternary phase diagram of the system water/ leaves extract / Tween 80: propanol (2:1) at 25°C.The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% of water from the point containing 33% leaves extract and 67% Tween 80: propanol (2:1) point, and extend to 84% towards the water apex.

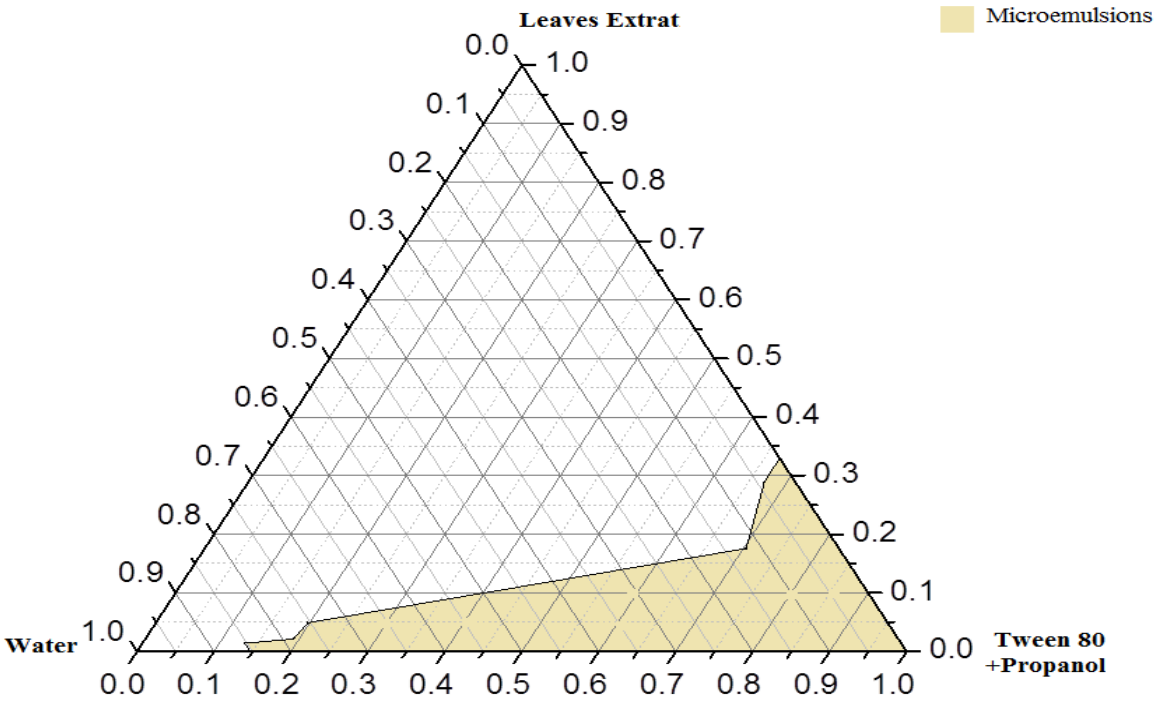


Figure (4.7):Ternary Phase diagram of system: Water/ leaves extract / Tween 80: propanol (2:1) at 25°C. The microemulsion region is represented by beige color.

4.2.3:Phase Diagram of Tween 80: propanol (2:1) / Water: Propylene glycol (2:1) / Borage seeds oil or leaves extract.

Ternary phase behavior of Borage seeds oil or leaves ethanolic extracts was studied upon addition of Propylene glycol as solubilization enhancer, which was grouped together with the water phase at a fixed ratio (2:1), and propanol as co-surfactant, which was grouped together with the Tween 80 at a fixed ratio(2:1).

4.2.3.1:Phase Diagram of Tween 80: propanol (2:1) / Water: Propylene glycol (2:1) / Borage seeds

Figure (4.8) shown the ternary phase diagram of the system water: Propylene glycol (2:1) / seeds oil / Tween 80: propanol (2:1) at 25°C.The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% of water from the point containing 20% seeds oil and 80% Tween 80: propanol (2:1) point, and extend to 32 % towards the water apex.

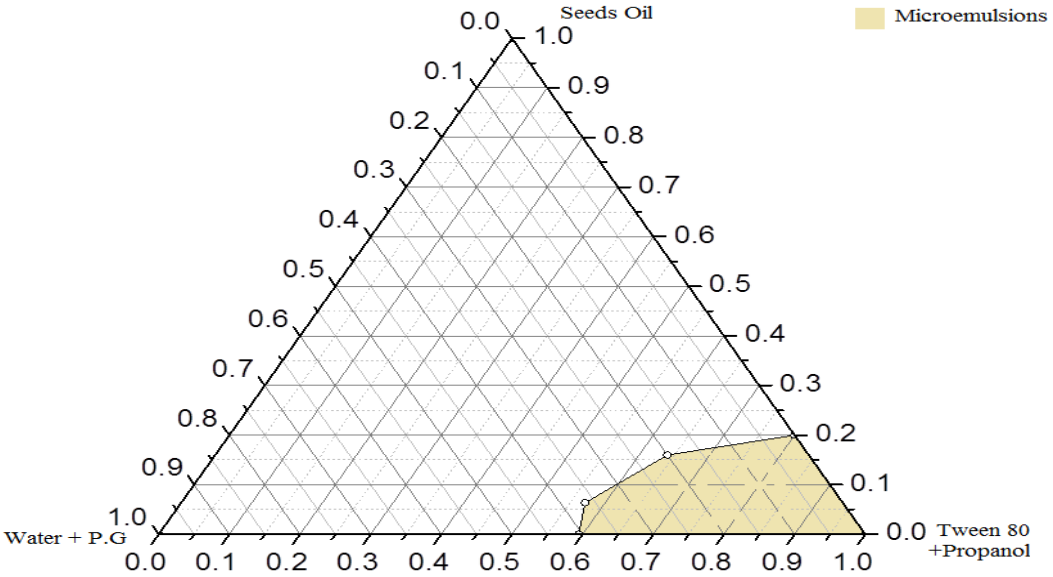


Figure (4.8) Ternary Phase diagram of system: Water: PG (2:1)/ seeds oil / Tween 80: propanol(2:1) at 25°C. The microemulsion region is represented by beige color.

4.2.3.2:Phase Diagram of Tween 80: propanol (2:1) / Water: Propylene glycol (2:1) / Borage leaves.

Figure (4.9) shown the ternary phase diagram of the system water: Propylene glycol (2:1) / leaves extract / Tween 80: propanol (2:1) at 25°C.The microemulsion region starts as a single clear isotropic and not shiny solution upon the addition of the first 4% of water from the point containing 30 % seeds oil and 70% Tween 80: propanol (2:1) point, and extend to 40 % towards the water apex.

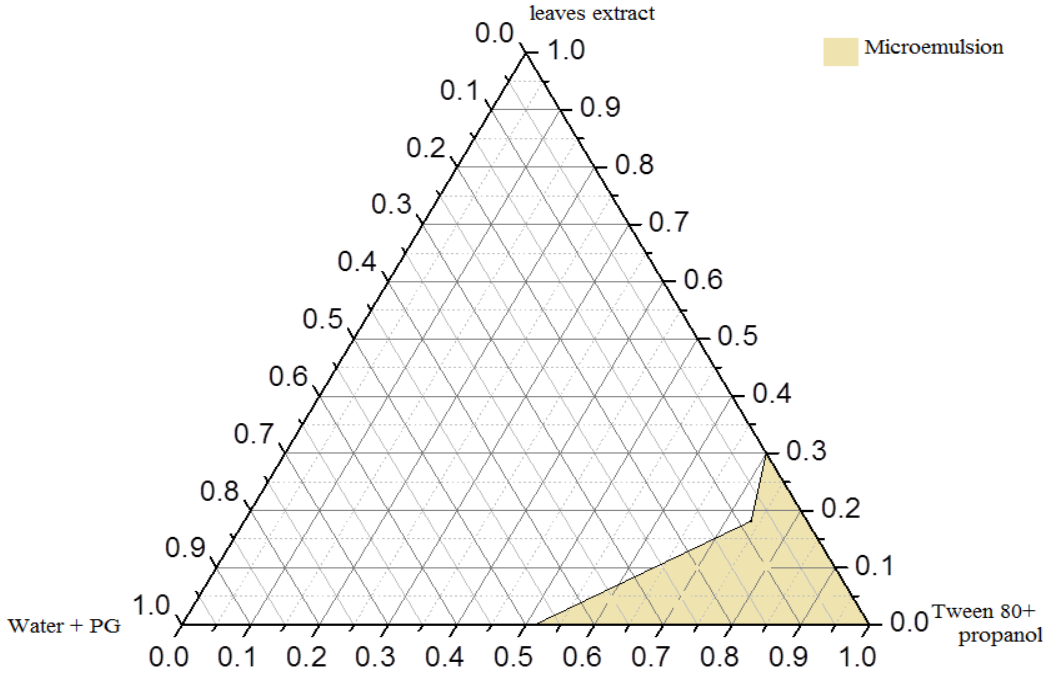


Figure (4.9):Ternary Phase diagram of system: Water: PG (2:1)/ leaves extract / Tween 80: propanol (2:1) at 25°C. The microemulsion region is represented by beige color.

Figures (5.6.7.8.4) showed a significant decreasing in the one phase microemulsion region for both leaves and seeds oil extracts , by adding propanol as a co-surfactant and

propylene glycol as a co-solvent . It has been reported that propanol and propylene glycol have not a significant role in increasing microemulsion region.

4.3. Antimicrobial activity test.

The antimicrobial activity of borage seeds and leaves extracts was studied against Streptococcus aureus as a gram positive bacteria, Escherichia coli as a gram negative bacteria, and Candida albicans as yeast, in addition to the microemulsion formulation of borage seeds oil (32% seeds oil + 48% Tween 80 + 20% Water) and microemulsion formulation of borage leaves extract (50% Tween 80+ 33% leaves extract + 16% Water) by using the well diffusion method.

The test was performed in duplicate for each species to insure the accuracy of the results.

Results showed that borage seeds and leaves extracts have an activity against S. aureus with a zone inhibition 12mm , 7.5mm for seeds oil and leaves extract respectively, and 9.5mm, 6mm for seeds oil and leaves extract microemulsion respectively, in where the seeds oil inhibition zone was clearer as shown in **Figure(4.10)**.

Whereas no effect was observed against E. coli and C. albicans which means they have resistance to borage seeds and leaves extracts as shown in **Figure(4.11)** and **Figure(4.12)**.

The sensitivity difference between the two types of bacteria was explained because gram negative bacteria have an outer membrane and a unique periplasmic space not found in gram positive bacteria which has a less complicated cell wall.

Penicillin, Ampicillin, and Gentamicin were employed as a positive controls for S. aureus, E. coli and C. albicans respectively, in addition to use Tween 80 as negative control.

There was an obvious difference when comparing the results with positive control (Penicillin G.), this significant difference could be due to the minor antibacterial effect of borage seeds and leaves extracts. More in detail, using the positive control "disc" by placing it superficially to the surface of agar plate, whereas the plant extracts solution inserted within a tiny hole created by sterile cylinder. In that the experimental conditions for each of test and control should be as close as possible so that the conclusion of results can be valid.

A. R. Łyko, *et. al*. [45] tested seedcake extracts at different concentrations against S. aureus, Enterobacter spp. and L. monocytogenes by using disc diffusion testing, all concentrations exhibited inhibition activity against S. aureus with zone of inhibition varied between 7-10mm, the negligible difference in zone inhibition diameter between seedcake extract and borage seeds oil extract attributes to the reality of seeds oil containing more raw materials, and it could be due to the non-unified experimental conditions.

The antibacterial activity of microemulsion formulation for borage seeds leaves against S. aureus showed a lower inhibition zone than its extract formulation, mind that the concentration percentage of oil in the extract was 100% and was about 32% in the seeds oil microemulsion formulation, and 33% in the leaves extract microemulsion formulation.

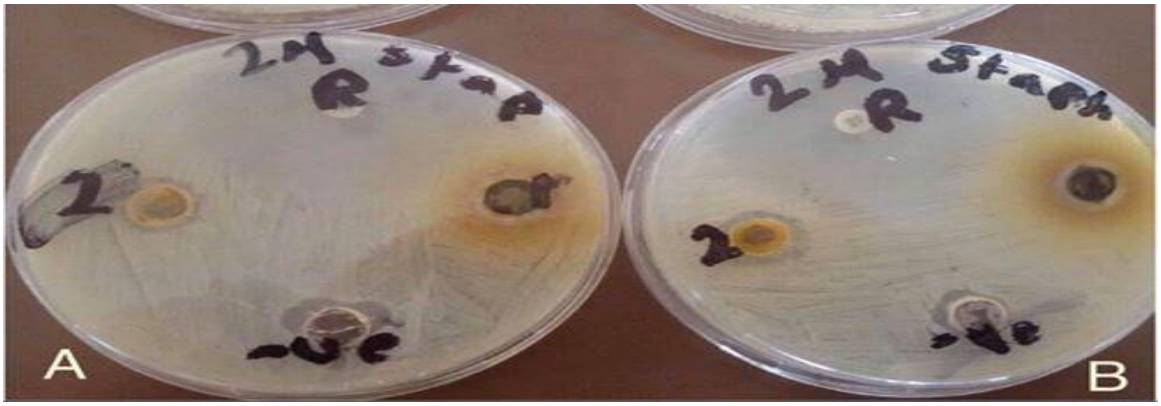


Figure (4.10) Zone of inhibition for seeds and leaves extract and microemulsion, against Staphylococcus aureus **(A)** 1-leaves extract microemulsion 2- seeds oil microemulsion, **(B)**1-leaves extract 2- seeds oil.

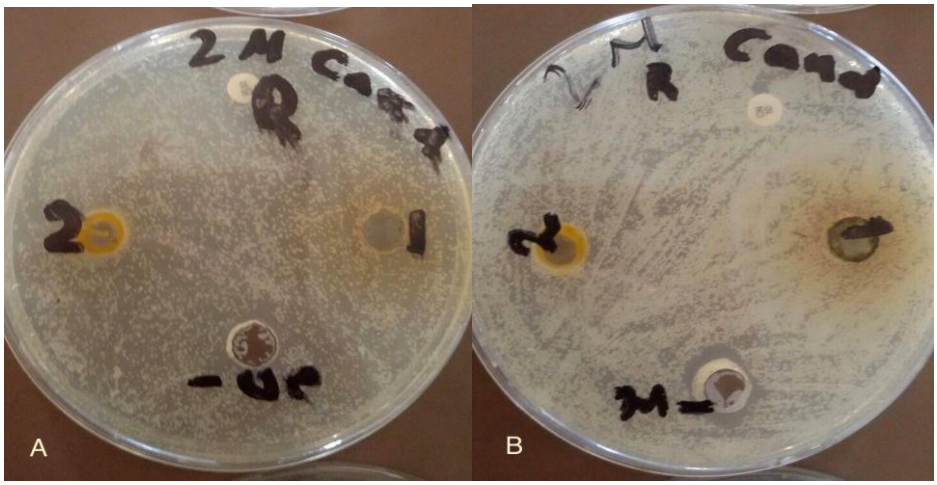
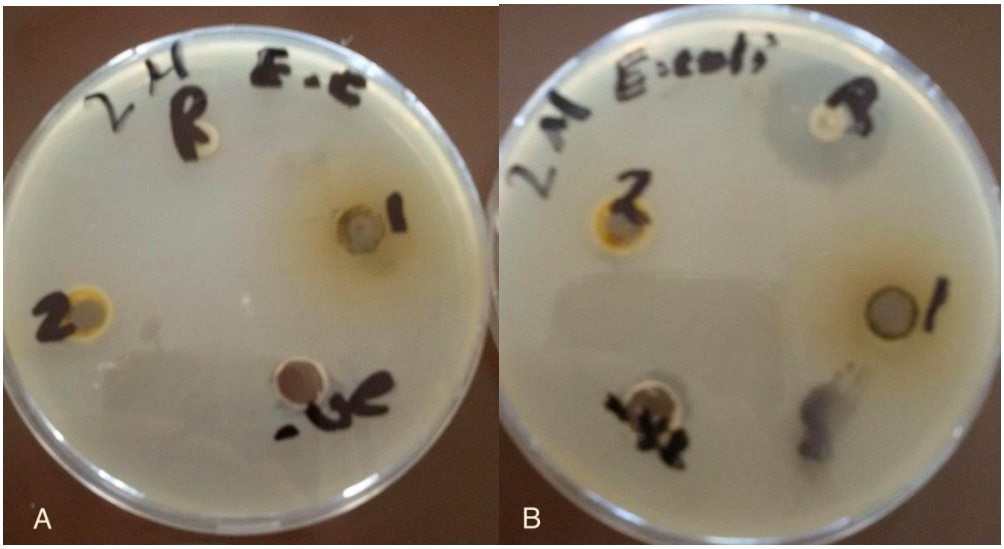


Figure (4.11): no effect was observed against C. albicans**(A)** 1- leaves microemulsion2- seeds oil microemulsion**(B)** 1- leaves extract 2- seeds oil.



Figure(4.12): no effect was observed against E. coli **(A)**1- leaves microemulsion 2- seeds oil microemulsion**(B)** 1- leaves extract 2- seeds oil.

Table(4.1): Antibacterial activity of leaves and seeds oil extracts and their microemulsion formulation.

System #	Composition	Bacteria	Inhibition zone(mm)
1	**Leaves Extract**	***Staphylococcus aureus***	7.5(±0.5)
		Escherichia coli	0.0
		Candida	0.0
2	**Seeds oil**	***Staphylococcus aureus***	12(±0.5)
		Escherichia coli	0.0
		Candida	0.0
3	**Leaves extract**	***Staphylococcus aureus***	6(±0.5)
		Escherichia coli	0.0
		Candida	0.0
4	**Microemulsion**	***Staphylococcus aureus***	9.5(±0.5)
		Escherichia coli	0.0
		Candida	0.0

4.4 Ferric Reducing Antioxidant Power (FRAP)

Ferric Reducing Antioxidant Power Assay (FRAP) is a quantitative assay for measuring the antioxidant potential within different samples, this method is simple, quick, inexpensive, doesn't require specialized equipment, and suitable for using with serum, plasma, biological fluids, and purified food and drug extracts.

In the FRAP method the reduction of ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) occurs by antioxidants present in the sample, the kit colorimetric probe of FRAP develops a blue color which is read colorimetrically at 540-600nm. The antioxidant potential of the given sample is selected based on an iron standard curve of concentration of Fe^{+2} Linear equation was generated y = (0.2019x – 0.1766) with high coefficient of determination, R^2 = 0.9822.**Figure(4.13).**

The antioxidant activity of borage seeds and leaves extracts were evaluated by FRAP method and were expressed as mgFe^{+2}per gram of plant extract.

The measurements of iron ion reduction ability and polyphenols content showed the good antioxidant activity of borage seeds and leaves with the indication of higher seeds activity, an average ferric reducing ability of seeds was 1.218±0.1 mgFe^{+2}/g, in comparison to that of 0.5026±0.2 mgFe^{+2}/g determined for leaves, this averages for the duplicate repeated tests, the seeds extract contains amounts of polyphenol, Tocopherol, and Vitamin C greater than leaves extract, this explains the observed variation within FRAP averages.

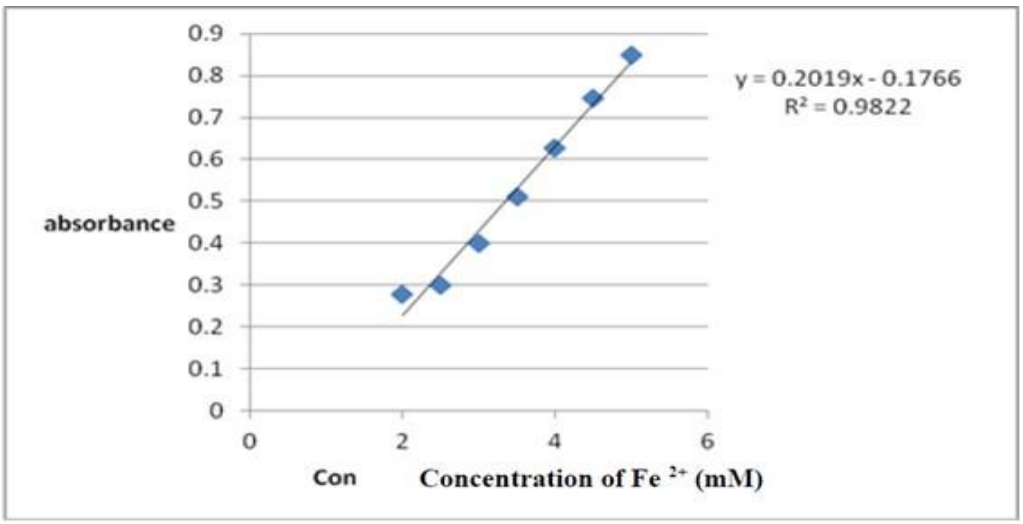


Figure (4.13):Calibration curve of Fe^{+2} standard.

Borage Plant	**$MgFe^{+2}$/ g sample± SD**
Seeds	1.218±0.1
Leaves	0.5026±0.2

Table (4.2): showed FRAP of plant extracts. As shown in this table, seeds oil was found higher than the leaves but not high difference.

Chapter five:

Conclusion

5. Conclusion

Borage Officials plant the major botanical source of several essential fatty acid particularly gamma- linolenic acid, which administered in the treatment of Atopic Dermatitis has both a sound theoretical basis and is justified from a clinical practical point of view.

Soxhlet method was chosen for the borage oil extraction process using 95% ethanol as a solvent. This research derives insights for the preparation of microemulsion with using minimum concentrations of Tweens which used as a surfactant, in addition to a short chain alcohol used as co-surfactant that is propylene glycol. Significant decrease obtained in microemulsion regions when we used the propylene glycol as a co-solvent and propanol as a co-surfactant to enhance the solubilization.

Based on the findings of the present study, results showed that borage seeds extract has an antibacterial activity against S. aureus higher than the borage leaves extract activity against the same species, The antibacterial activity of microemulsion formulation for borage seeds and leaves against S. aureus showed a lower inhibition zone than its extract formulation, in addition to the magnificent antioxidant activity of borage oil extract and microemulsion was indicated due to its content of polyphenols, tocopherol, and Vitamin C, which are the most abundant natural antioxidants, furthermore they could act synergistically as an effective antioxidant. In general, the borage seeds oil have preferable antibacterial and antioxidant Activities than leaves extracts.

In conclusion, borage seeds and leaves microemulsions are transparent, stable, considered as antibacterial and antioxidant agent, rather than the chemical activity of their individual components.

6. Future work

1. In light of the animals' inability to produce gamma- linolenic acid by themselves we support the idea that Borage be included in the general nutritional pattern.
2. It is recommended to determine total phenolic and flavonoids content of both leaves and seeds and to correlate it with antioxidant activity.
3. It is also recommended to do free radical scavenging ability of leaves and seeds extract of Borage e.g. using DPPH assay method.
4. It is recommended to test the microemulsion of the seed for Eczema treatment.
5. It is recommended to investigate and study the flowers, stems, and roots of borage with their microemulsion and extracts formulation as an antibacterial and antioxidant agents.

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صياغة وتطبيقات مستخلصات بذور وأوراق الحمحم (Borago Officinalis) ومستحلبه

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الملخص

تهدف هذه الدراسة إلى البحث في تكوين مستخلص النبتة العشبية "الحمحم" واستخداماته المتعددة بالإضافة إلى دوره كبديل في الطب التكميلي لمعالجة الاكزيما ، ينبت هذا النبات في جبال فلسطين عموماً ، والخليل خاصة ، تنضج بذور هذه النبتة في نيسان من كل سنة .

تم الحصول على مستخلص بذور وأوراق نبتة الحمحم بواسطة طريقة " soxhlet " باستخدام المحلول الكحولي " الايثانول ٩٥%".

قمنا باستخدام نوعين من المؤثرات السطحية في الحصول على المستحلب الخاص ببذور و أوراق نبات الحمحم وهما توين ٢٠ وتوين ٨٠ ، حيث أثبت توين ٨٠ ملائمته بشكل أكبر في إذابة كل من مستخلص البذور والأوراق مقارنة بتوين ٢٠ إلى جانب استخدام مذيبات مساعدة مثل مذيبات ذات سلسلة قصيرة من الكحول (propylene glycol and propanol).

وتم تقييم دور كل من مستخلص و مستحلب البذور والأوراق كمضادات للبكتيريا باستخدام طريقة الانتشار من حفرة عمودية عميقة (well diffusion method) حيث أظهر كل منها فاعليته ضد البكتيريا موجبة الجرام وهي:(streptococcus aureus) ، في حين لم تظهر أي فاعلية تُذكر ضد كل من البكتيريا سالبة الجرام (Escherichia coli) و فطريات (Candida albicans) .

كما وتم بحث فعالية هذه المستخلصات و المستحلبات الدقيقة كمضادات أكسدة باستخدام طريقة (FRAP) ، والتي أظهرت دورها كمضادات طبيعية للأكسدة ويعود ذلك إلى كونها غنية بعناصر تعتبر أشهر مضادات أكسدة طبيعية وهي (polyphenol, tocopherol, Vitamin C).

في النتائج ، يمكننا أن نوصي بإدراج مستخلص بذور و أوراق نبات الحمحم كمكون فعال في معالجة مرضى الاكزيما وتحديداً لدى المرضى أصحاب الدرجة الطفيفة من المرض والذين يسعون إلى إيجاد علاج بديل ، بالإضافة إلى إمكانية استخدامه كعنصر فعال في تطبيقات مضادات الأكسدة و الأكسدة ومضادات البكتيريا.