Formulation and Applications of Borage (*Borago officinalis*) seeds oil and leaves extracts as Microemulsion

Abstract:

Aims: This study aims to investigate antioxidant and antibacterial activities of borage (seeds and leaves) extracts, and to prepare different topical microemulsion formulations using borage oil.
Study design: Borage specifically selected in this study due to its abundance in the Palestine mountains, and being the major botanical source of gamma-linolenic acid.
Methodology: The seeds were cultivated upon their ripening season in April of 2016 from the Halhul mountains in Hebron/Palestine. 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 found to be 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 for both borage seeds and leaves extracts using a well diffusion method against *Staphyloococcus aureus, Escherichia coli, &Candida albicans*.
Results: Results showed that the seeds extract has inhibition zone (12 mm) against *S. aureus* (gram positive bacteria) higher than inhibition zone that leaves extracts exhibited (7.5mm), but no significant effects observed for both extracts against *E. coli* and *C. albicans*. In addition, antibacterial activity for microemulsions formulation was measured against *S. aureus, E. coli, and C. albicans*. Results showed that there is minor activity against *S. aureus* when compared to PenicillinG and the pure seed oil or leaves extract. 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 reagent 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.
Conclusion:Microemulsions using borage seeds and leaves extracts wereprepared, and these microemulsions are transparent, stable, and have antibacterial and antioxidantactivities.

Key words:Borage, seeds, leaves, extract, microemulsion, antioxidant activity, antibacterial activity.

1. Introduction:

Borage oil is derived from the seeds of the *Borago officinalis*. Borage oil contains high levels of the ω-6 series essential fatty acids that are important in skin structure and function (1). The linoleic acid in borage oil contributes to its therapeutic actions in Atopic dermatitis (AD). Topical application of borage oil in infants and children with seborrheic dermatitis or AD has been shown to normalize skin barrier function (2).A double-blind, placebo-controlled clinical trial was performed to test clinical effects of undershirts coated with borage oil on children with AD (3). In the group treated with borage oil, Transepidermal Water Loss from the stratum corneum (TEWL)on the skin of back decreased. Additionally, no side effects were found in these subjects(3).

N. Grampurohit *et. al* chose the non-ionic surfactants in their 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 (4, 5), beside the co-surfactant like as propylene glycol (6).Non-ionic surfactants are commonly used to formulatemicroemulsion due to their good skin tolerance, low irritation potential and toxicity, environmentally friendly compatible, and being commercial inexpensive surfactant. In addition, they are less sensitive to water hardness and they foam less strongly (7,8,9).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 polyethoxylatedsorbitan and lauric acid and it has an HLB value16.7.

There has been a revolution in the last two decades in exploitation of microemulsion in a variety of chemical and industrial process, including the usein enhanced oil recovery, detergency, as coatings and textile finishing, cosmetics, food, biotechnology, analytical application, microemultion gel technique, liquid membranes and in

pharmaceuticalmicroemulsion application is considered 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(5,10). Borage officinalis from Boraginaceae family, as known as borage, bourrache, buglass, borage.Borage is a 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(11,12,13,14).Each flower produces a fruit;with 3-4 brownish nutlet after growing, these fruits are small brownish oval wrinklednutlet(11,15)as shown in Figure (1).

Figure. 1:small brownish oval wrinkled nutlet of Borage seeds.

P. Kotniket. 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(16).R. Foster *et. al*was recommended the borage oil in the treatment of atopic dermatitis by his study, where he showed the effective role of Essential fatty acids (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(17).A. Micel*iet. 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(18). A. Borowy*et. al* examined biological active compounds and antioxidant activity of Borage flowers and leaves(19).

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(20).The appropriate time to cultivate this plant is 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(13,21,22).

There are several fatty acids combinations 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 acids 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 who have a clinical condition caused by GLA deficiency, such as atopic dermatitis (23).

Borage oil also contains minor components which 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(11,24).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(25,26).Essential fatty acids abnormalities and defects could contribute to AD in two ways(11):through a direct effect on the skin structure and function, andby affecting maturation and sensitization of the immune system affecting the skin.

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(17)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(27,28,29).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(23).

The objectives of this study is therefore to extract borage seeds and leaves with ethanol as a solvent using Soxhlet extraction, and to determine antioxidant and antimicrobial activities of the extracts. The best ternary phase diagram for both extracts based on non-ionic surfactant will be also investigated.Finally, the effect of using 1-propanol and propylene glycol as solubilization enhancers will be also studied.

2. Materialsand methods

2.1. chemicals and 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). The following chemicals and materials were also used in this study: 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.

2.2 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.

2.3 Soxhlet Ethanol Extraction of Borage leaves and seeds

Ethanol 95% was used as a solvent. About 20g of grinded air-dried leaves material were put in "thimble" made from strong filter paper and inserted 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, and about 300 ml of the solvent (Ethanol 95%) 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.

2.4 Construction of Ternary Phase Diagram

The pseudo ternary phase diagrams consisting of oil, water, surfactant and co-surfactant mixture were constructed using water titration method.For the microemulsion formulation each sample of borage leaves and seeds extracts were mixed with the surfactant (T20 or T80) at different weight ratios as shown in Table1, and were inserted in 10ml glass test tubes with screw caps.

Table1: Surfactant/ borage Oil weight ratio used in the preparation of microemulsions in this study.

Wight ratio	Surfactant	Oil
1:9	0.1	0.9
2:8	0.2	0.8
3:7	0.3	0.7

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

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. After 48h, a drop-by-drop titration of water phase (water or water: PG) with specific weights was injected. The tubes then were left at rest for 24h to reach equilibrium before the next addition of water phase and analyzing. The tubes temperature was controlled by placing it within the thermostatic water path at (25±1) ^{o}C if necessary.

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(2).

Table(2): 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.

2.5 Antimicrobial activity

The antimicrobial activities of Borage leaves and seeds extracts was tested against different types of microorganisms: *Staphylococcus aureus, E. coli and Candida albicans* by using well diffusion method which depends on diffusion of the sample tested from a vertical cylinder through a solidifiedMuller Hinton agar layer in a plate.Positive control (antibiotic discs) was used for bacteria (Penicillin for *Staphylococcus aureus* and ampicillin for *E. coli*and nystatin for *C. albicans*). The suspension of each microorganism was added separately to a tube containing nutrient broth. Gram negative *(Escherichia coli)*, gram positive *(Staphylococcus aureus)* bacteria and yeast *(candida albicans)*turbidity was compared to that of MacFarland nephlometer tube no. 0.5 using UV-Spectrophotometer at 625nm until suitable concentration is reached. After thatfour holes with a diameter of 5.8 mm were made in the agar using sterile cylinder, each hole was filled with 50µl of the extract sample.

A positive control disc was selected and placed on the agar surface referring to the type of the tested bacteria, as well as a hole containing a solvent as a negative control. The plates were incubated at 37 ± 0.5°C for 24 hours. After incubation period, the zone of inhibition diameter was measured from edge to edge of the clear area around the holes containing the different samples.

2.6 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) (30). 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 minutes, which was the original time applied in FRAP assay. Aqueous solutions of known Fe (II) concentrations in the rangeof (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. Results and Discussion

3.1 Ethanol Soxhlet extraction of Borage seeds and leaves oil

Medicinal plant sources known as herbal or botanical medicine refer to the use of plant seeds, leaves, roots, fruits and stem bark as treatment of diseases (31). 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(2), besides a dark green semi-liquid extracted compound from leaves. The extraction yield of the seeds and leaves of borage was about 25.1% and 20.5%, respectively.

Figure(2): The oil resulting from seeds extraction.

3.2Formation 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, these diagrams were developed by the water titration method.

This study aimed to formulate the phase behaviour 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.

3.3 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 regions under the same formulation conditions, using twodifferent non-ionic surfactants which are Tween 80 (polyoxyethelene (20)sorbitanemonooleate) and Tween 20 (polyoxyethelene (20) sorbitanemonolaurate) at 25°C.

3.4 Phase Diagram of Water / Tween 80 / oil seeds

Figure (3) shows 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, 14wt% of oil and 28wt% of water.

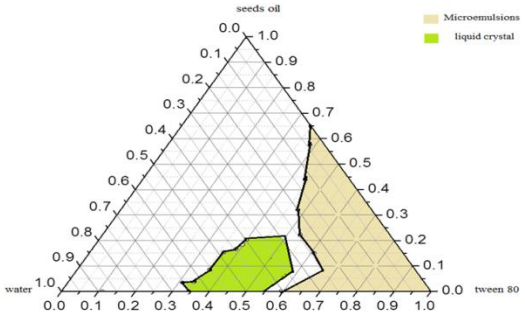
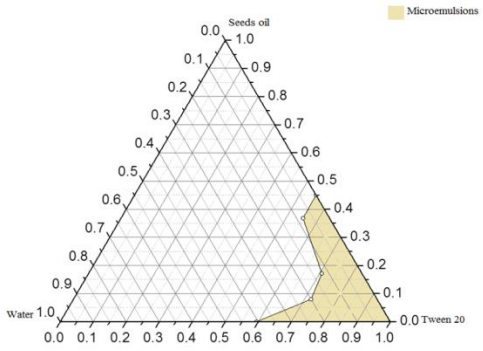


Figure (3): Ternary Phase diagram of the system: Water/ oil seeds/ Tween 80 at 25°C. The microemulsion region is represented by beige color. The liquid crystal region is represented by light green.

3.5 Phase Diagram of Water / Tween 20 / oil seeds

Figure (4) shows 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 20and extend to 32% towards the water apex.



Figure(4): Ternary Phase diagram of system: Water/ oil seeds/ Tween 20 at 25°C. The microemulsion region is represented by beige color.

3.6 Phase Diagram of Water / Tween 80 / leaves extract

Figure (5) shows 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.

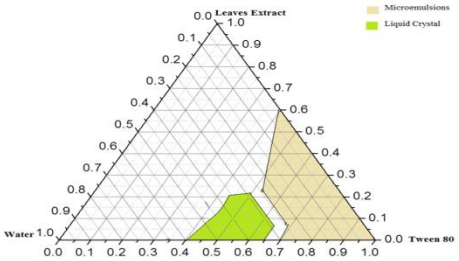


Figure (5): Ternary Phase diagram of system: Water/ leaves extract / Tween 80 at 25°C. The microemulsion region is represented by beige colour. The liquid crystal region is represented by light green.

3.7 Phase Diagram of Water / Tween 20 / leaves extract

Figure (6) shows ternary phase diagram of the system which 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.

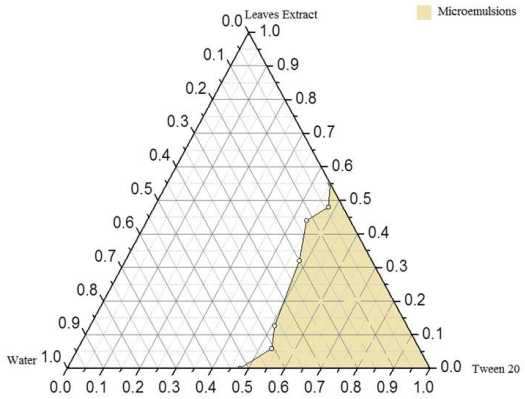


Figure (6): Ternary Phase diagram of system: Water/ leaves extract / Tween 20 at 25°C. The microemulsion region is represented by beige color.

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 as O/W emulsifier compare to Tween 20.This is 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(32).

This result means 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.

3.8 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 (7)shows 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.

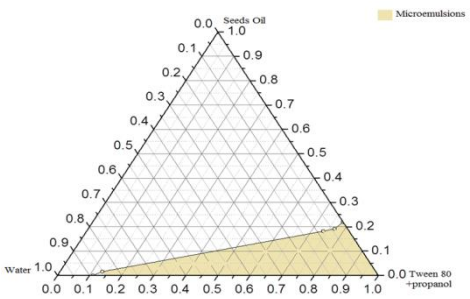


Figure (7) Ternary Phase diagram of system: Water/ seeds oil / Tween 80: propanol (2:1) at 25°C. The microemulsion region is represented by beige color.

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

Figure (8) shows 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.

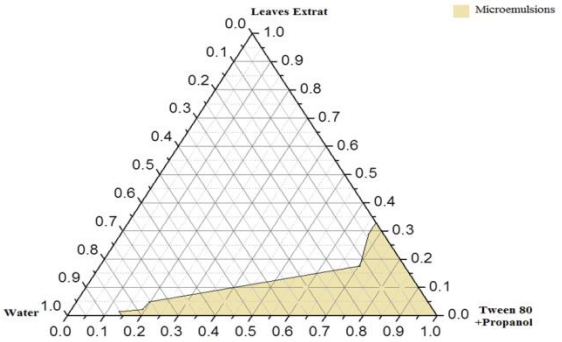


Figure (8):Ternary Phase diagram of system: Water/ leaves extract / Tween 80: propanol (2:1) at 25°C. The microemulsion region is represented by beige color.

3.10 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 solubilisation 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).

3.11Phase Diagram of Tween 80: propanol (2:1) / Water: Propylene glycol (2:1) / Borage seeds

Figure (9) shows 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 .

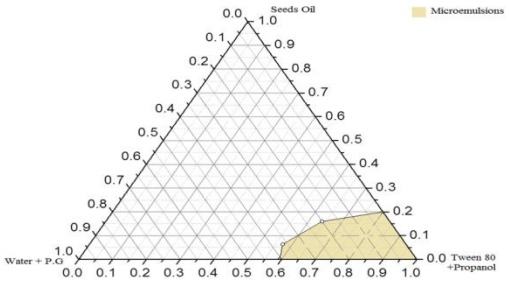


Figure (9): Ternary Phase diagram of system: Water: PG (2:1)/ seeds oil / Tween 80: propanol(2:1) at 25°C. The microemulsion region is represented by beige color.

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

Figure (10)shows 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.

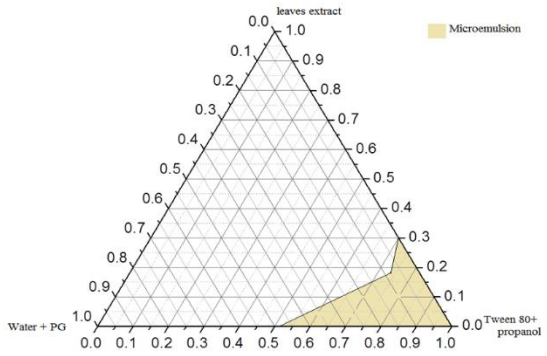


Figure (10):Ternary Phase diagram of system: Water: PG (2:1)/ leaves extract / Tween 80: propanol (2:1) at 25°C. The microemulsion region is represented by beige color.

Figures6-9 showed a significant decrease 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.

3.13 Antimicrobial activity test:

The antimicrobial activity of borage seeds and leaves extracts was studied against *Staphylococcus aureus* as a gram-positive bacterium, *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 triplicates 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 aninhibition zone diameter 12 mm for seeds oil and, 7.5 mm for leaves extract. While for microemulsion the results showed that 9.5 mm diameter for seeds oil microemulsion, and6mm diameter for leaves extract microemulsion. It was noticed that the seeds oil inhibition zone is more clear.

On the other hand, results showed no effect against *E. coli* and *C. albicans* which means they have resistance to borage seeds and leaves extracts. Thedifferent sensitivity of the two types of bacteria and *Candida* may referred to the difference in structure of their cell walls.

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

A. R. Łyko,*et. al*(33)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.

Table(3): Antibacterial activity of leaves and seeds oil extracts and their microemulsion formulation.

System #	Composition	Bacteria	Inhibition zone(mm)
1	LeavesExtract	*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	LeavesExtract microemulsion	*Staphylococcus aureus*	6(±0.5)
		Escherichia coli	0.0
		Candida	0.0
4	Seed oil Microemulsion	*Staphylococcus aureus*	9.5(±0.5)
		Escherichia coli	0.0
		Candida	0.0

3.14 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, R2 = 0.9822, Figure(11).

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 (Table 4), an average ferric reducing ability of seeds was 1.21±0.1 mgFe+2/g, in comparison to that of 0.50±0.2 mgFe+2/g determined for leaves, this averages for the triplicate 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.

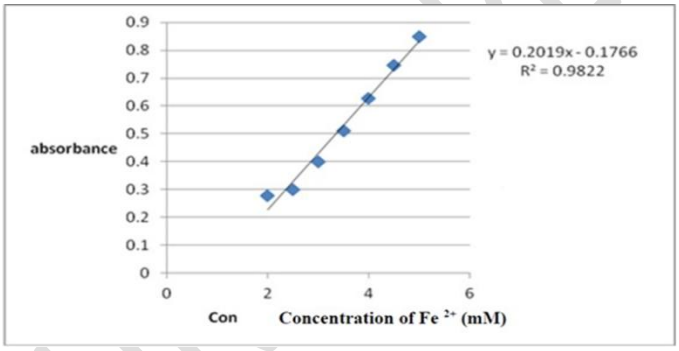


Figure (11): Calibration curve of Fe^{+2} standard used for FRAP study.

Table (4) showed FRAP of plant extracts. As shown in this table, seeds oil was found to be higher than the leaves with significant difference.

Table (4): Antioxidant activity (FRAP) of plant extracts (seeds and leaves).

Borage Plant	**mg Fe^{+2}/ g sample±SD**
Seeds	1.21±0.1
Leaves	0.50±0.2

Conclusion:

Borage Officials plant which is the major botanical source of several essential fatty acid particularly gamma-linolenic acid is 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 in microemulsion regions was obtained when propylene glycol is used as a co-solvent and propanol as a co-surfactant to enhance the solubilization.

Based on the findings of the present study, borage seeds extract has 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 has preferable antibacterial and antioxidant activities than leaves extracts.In conclusion, borage seeds and leaves microemulsions are transparent, stable, considered as antibacterial and antioxidantagent, rather than the chemical activity of their individual components.

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