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Extract of Purslane and Its Application

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Dedication

To those who race the words to express ideas of their own

To those who taught me and stand with me through all the things I suffered and difficulties I went through to get to where I am now.

To the one who is with me when worries take over me and she drowns me in the sea of tenderness to relieve my pain. My mother.

To the one I miss whom the world did not give me a chance of enjoying more of his tenderness. My dad.

To my brothers and family.

To anyone who taught me a letter and lit the way for me.

To all my colleagues and friends.

I dedicate this modest research to my lord. The almighty to find acceptance and success.

Declaration

I certify that this thesis is submitted for the degree of master, and it is the result of my own research, except where for some excerpts noted in the research, and that this thesis has not been submitted for the higher degree to any other university or institute.

Signed:……

Name: Maysaa Mahmoud Khalil Al-Halaseh

Date: 22/5/2019

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Abstract

Portulacaoleracea L. (Portulacaceae) is a plant rich in benefits which contains high levels of linolinic acid and has been used in many countries to treat fevers and antibacterial healing. It is also used in wound healing, an analgesic and as a muscle relaxant**.**

The aims of this research are to get thermodynamic stability, improved solubilization and bioavailability for purslane extracts by using microemulsion that can be employed in diabetic drugs, and to investigate the effect of purslane on diabetes type2.

Samples of purslane were collected from the local markets from Al-Eizariya, in the mid of April 2018. The samples were dried under shade for two weeks until it turned completely dry, then it was grinded and extracted by soxhlet. Following that, the antioxidant activity (AA) was studied using Ferric Ion Reducing Antioxidant Power Assay (FRAP) method. Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) tests were also conducted on the extracts. It was noticed that the (TPC) and (TFC) of the seeds were higher than the (TPC) and (TFC) of leaves, which represented 243.0±5.0mg/g, 58.8±8.0mg/g, respectively. The antioxidant activity of FRAP was investigated on oil and on leaves; the best result was found among the leaves which represents 1.30268±0.1mg/g.

Then this research aimed at formulating biocompatible microemulsion that can be used in pharmaceutical drug. This was done by using purslane seed extract as oil, Tween 80 and Tween 20 as a non-ionic surfactant, water and leaf extracts as aqueous phase. After that, five microemulsion systems were selected; (A, B, C, D, E) to find the widest range of microemulsion. In every system at least one component was changed. System A and system B were selected as the basic methods of extracting, yet the same solvent was used. For systems C, D, E leaves extract was selected as aqueous phase. However, we changed the surfactant and method of extracting. This way the biggest range of microemulsion was achieved , because oil and leaves extract from the same plant mixed .

The antibacterial effect of purslane leaves and seeds was studied on *bacillus bacteria* and *Pseudomonas bacteria*. The result showed that the extract of the leaves has an antibacterial activity against *Bacillus subtilis, Pseudomonas syringae*. On the other hand, the seedextract also showed a slight activity against *Bacillus subtilis*only.

Finally the effect of purslane on diabetic 2 was investigated through antiglycation test. The extract of leaves was examined by fluorescence, the result showed that the purslane gives inhibition against glycation, which is the main reason of diabetic diseases. That means it is

effective on diabetic disease. We conclude from this research that Purslane has a potential antioxidant, antibacterial application and antiglycation effect.

Table of Contents

Lists of Tables

List of Figures

List of Abbreviations

TPC: Total Phenolic Content

TFC: Total Flavonoids Content

FRAP: Ferric Ion Reducing Antioxidant Power Assay

Eth: Ethanol

RBF: Round bottom flask

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DM: Diabetic mellitus

CNS: Central nervous system

PNS: Peripheral nervous system

AGE: Advanced glycation end product

GAE: Gallic acid equivalence

HPLC: High-performance liquid chromatography

DW: Distilled water

PSWMeE: Purslane seed water methanolic extract

PSO: Purslane seed oil

(βCB): β-carotene bleaching

POB:*Portulacaoleracea* From Bulgarian

POG:*Portulacaoleracea* from Greek

AEAC: Ascorbic acid equivalent antioxidant activity

POFE: Effect of flavonoid *from Portulacaoleracea*

S. aures: Staphylococcus aureus

CPOP: Crude Portulacaoleracea

MC: Model group

NC: Normal group

FBG: Fasting blood glucose

CGF:Cocciniagrandisfruits

BSA: Bovine serum albumin

IC50: Half maximal inhibitory concentration

HLP: Hydrophilic-lipophilic balance

Chapter One:

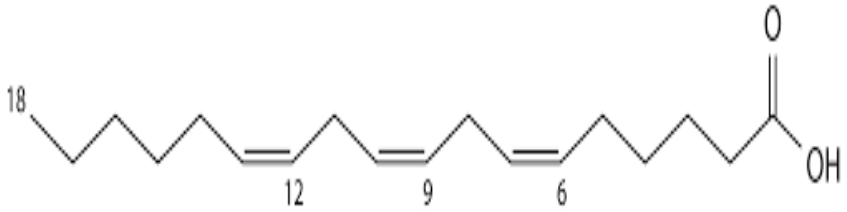
Introduction:

1.1. Medicinal plant

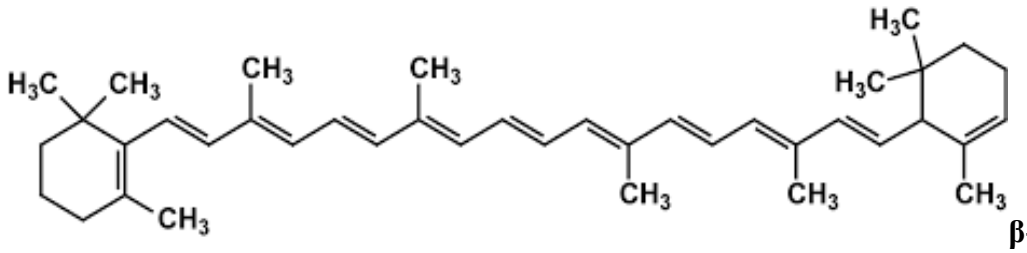
Recent studies have dealt with medicinal plants because they have so many benefits with several properties including: antioxidant and anti-bacterial. Medicinal plants contain high amounts of essential fatty acids that are not produced by the human body and are obtained from food, they are important to build healthy cells. In addition, it contains phenolic compounds which are associated with deeply linked antioxidants. Medicinal plants are the main source in the pharmaceutical industry and healthcare products (Ercisli *et al*.2008).

1.2. Purslane

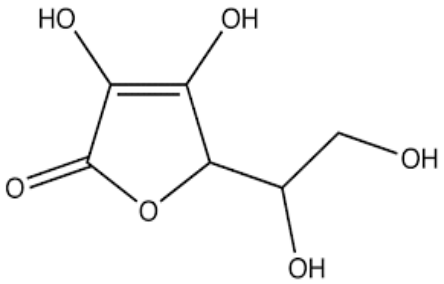
Purslane, in Arabic ‘Rejlah’, *(Portulacaoleraceae L.)* (Hussein *et al.*2010), it grows rapidly and produces large numbers of seeds that last a long time (Liu *et al*. 2000). Many types of Purslane grow in different regions and different weathers (Siriamornpun *et al.*2010).World Health Organization (WHO) classified purslane as one of the most widely used medicinal plants. Many studies have proven that this plant has many medicinal effects such as treating viral hepatitis, diabetes management, muscle relaxant activity, analgesic effect, anti-inflammatory effect, anti-oxidant effect, anticancer effect, an antibacterial effect and wound healing effect. Researches indicates that purslane has the best quality food among vegetables. It contains high amount of ß-carotene, ascorbic acid, and linolenic acid.Purslane is a strong source of alpha-linolenic acid, which is an omega-3 fatty acid, that have great importance in human growth and disease prevention (Kamal Uddin *et al*.2014)



Linolenic acid



β-Carotene



Ascorbic acid

Fig (1.1): Chemical structure of linolinic acid ,ascorbic acid and β-carotene.

Table (1.1) Fatty acid composition% of purslane (Ercisli *et al.* 2008)

Myristic acid (14:0)	5.04
Palmitic acid (16:0)	9.72
Stearic acid (18:0)	4.36
Oleic acid (18:1ω9)	8.83
Linoleic acid (18:2ω6)	14.01
Linolenic acid (18:3ω3)	56.33
Σ Fatty acid	98.29
Σ Saturated fatty acid	19.12
Σ Unsaturated fatty acid	79.17

Purslane can be found in different parts of the world, including : Asia, Africa, Europe, Australia, and North America(Liu *et al*. 2000). Moreover Portulacaoleracea was found to be as efficient as sulfa drugs; over 90% effectiveness in acute cases and 60% in chronic cases.*PortulacaOleracea* has been described as being traditionally used in inflammatory nipples, toncitis, astringent, heart, colic, dermatitis, diarrhea, dysentery, indigestion, dyspnea, eczema, haematuria, hyperglycemia and itching (Abdelaziz *et al*.2014).

1.3. Polyphenols

Most of the promising medicinal plants contain polyphenols. Polyphenols are the result of secondary metabolism and are natural compounds found in various plants, especially in fruits and vegetables, also it was found in cereals and beverages. Fruits like grapes, apple, pear, cherries and berries make the main groups of polyphenolic compounds. The flavonoids , stilbenes and lignans are shown in fig(1.2) that illustrate the various groups of polyphenols and their chemical structure, epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandy *et al.* 2009).

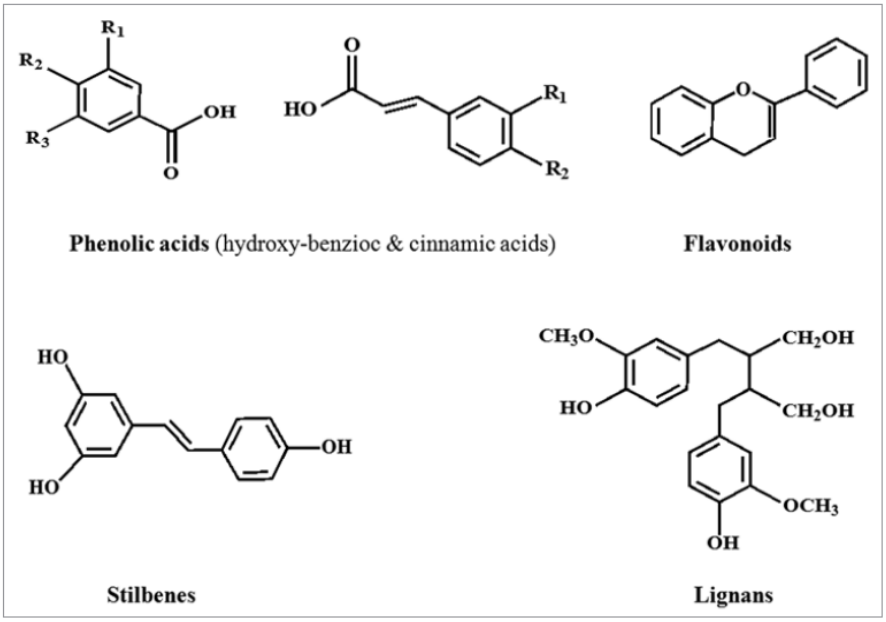


Fig. (1.2). Chemical structures of different classes of polyphenols. Where R1,R2,R3 are H,OH,OCH_3 respectively (Pandy *et al.* 2009).

1.3.1. Total Phenolic Acid (TPC)

Phenolic Quantification Assay is based on the Folin - Ciocalteu method. Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry . The Folin-Ciocalteu method is described in several pharmacopoeias, the reaction forms a blue chromophore constituted by a phosphotungsticphosphomolybdenum complex, where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds. This reaction gives a blue chromophore formed by a phosphotungstic phosphomolybdenum complex (Blainski *et al*. 2013).

1.3.2. Total Flavonoids Content (TFC)

Flavonoids are compounds of secondary metabolism also known as vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments. Furthermore, they react with in humans easily, and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities (Rebaya *et al*. 2014).

1.4. Chemical Assays of Plant Extracts

1.4.1. Total Antioxidant Capacity

Antioxidants are compounds that delay or prevent oxidation processes that occur under the influence of atmospheric oxygen or oxygen interactive; they have several uses. It can stabilize polymer and petrochemical products, foodstuffs, cosmetics and pharmaceuticals .Antioxidants have resistant to diseases which are associated with the attack of free radicals (Pioschi *et al.* 2012).

The antioxidative system contains both enzymatic and non-enzymatic systems. The non enzymaticsystem contains ascorbic acid (vitamin C); ά-tocopherol, curtains, etc. On the other hand, an enzymic system includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), polyphenol oxidase (PPO) and other. An antioxidant system works to remove the toxic free radicals during the oxidation process and thus helps plants survive (Kumar *et al*. 2014)

1.4.1.1. FRAP Method

The FRAP method depends on the ferric tripyridyltriazine (Fe (III) -TPTZ) complex to the ferrous tripyridyltriazine (Fe (II) -TPTZ) at low pH. Fe (II) -TPTZ has an intensive blue color and can be monitored at 593 nm (Kaushik *et al*. 2012).

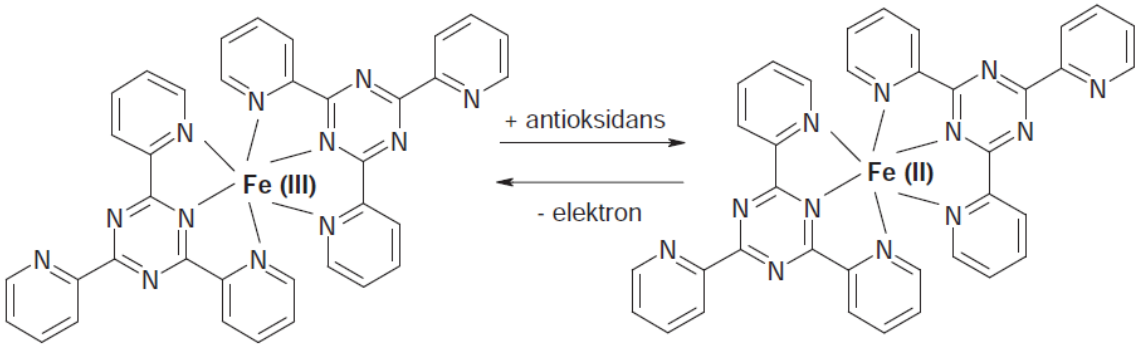


Fig (1.3) Chemical structures of reaction of yellow Fe^{+3} TPTZ complex (2, 4, 6-Tri (2 pyridyl) -1, 3, 5-triazine) with antioxidants is reduced to the blue Fe^{+2} TPTZ complex by electron-donating substance (Aldina *et al*.2015)

1.4.1.2. DPPH Method

The 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) method depends on the reduction of DPPH which is stable free radical. The DPPH radical with an unpaired electron affords a maximal absorption at 517 nm (violet color). When Antioxidants react with DPPH, which is a stable free radical, it becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as a consequence the absorbance decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow color) as shown in fig (1.3) (Shekhar *et al.*2014)

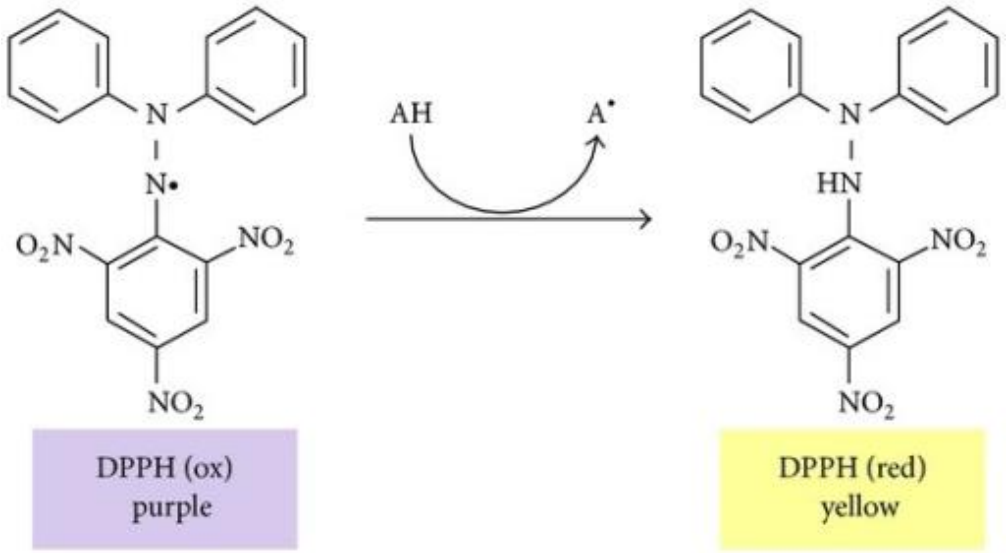


Fig (1.4): Chemical structure of DPPH (Teixeira *et al.*2013)

1.5. Microemulsion

Microemulsions are thermodynamically stable, liquid, visible clear dispersions of two non-miscible liquids such as oil and water. Microemulsions are mixtures of oil, water and surfactant, sometimes combined with cosurfactant. Surfactant or cosurfactant work on lowering the oil/water interfacial tension to ultra-few values (often less than 0.001 dynes/cm), allowing thermal motions to spontaneously disperse the two non-miscible phases (Klier *et al.* 2000).

1.6. Surfactant

(Surface-active-agent) a material which reduces interface activity. It is worth remarking that all amhiphiles do not show such activity. Only the amphiphiles with more or less equilibrated hydrophilic and lipophilic tendencies are likely to migrate to the surface or interface. It does not happen if the amphiphilic molecule is too hydrophilic or too hydrophobic, in which case it stays in one of the phases (Salager *et al*. 2002).

1.6.1. Classification of Surfactants:

a. **Anionic Surfactants** can dissociate in water in an amphiphilic anion, and a cation, which is in general an alkaline metal (Na+, K+) or a quaternary ammonium. They are the majority commonly used surfactants.

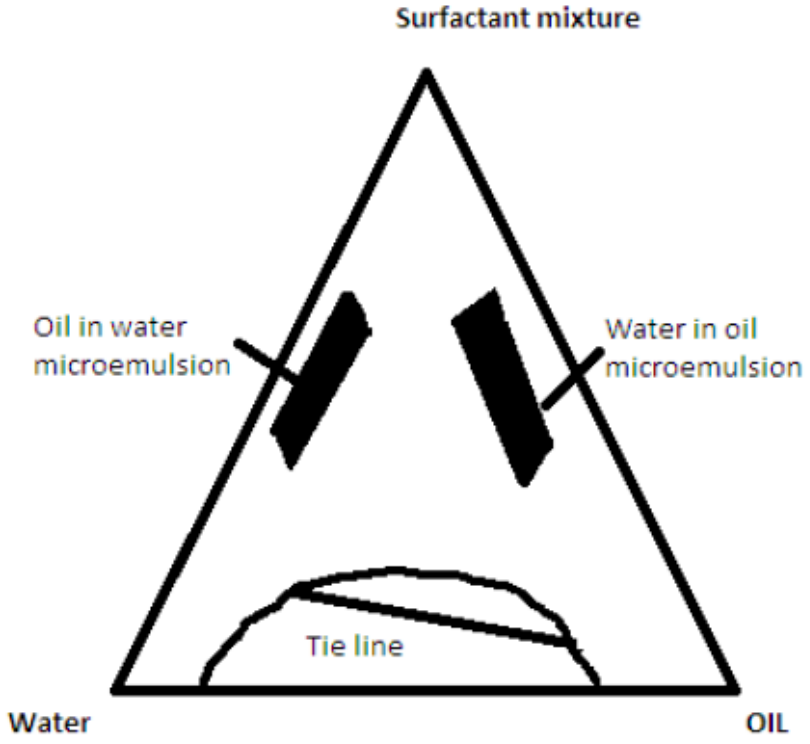
b. **Nonionic Surfactants** they come as a close second with about 45% of the overall industrial production. They do not ionize in aqueous solution, because their hydrophilic group is of a non dissociable type, such as alcohol, phenol, ether, ester, or amide. A large proportion of these nonionic surfactants are made hydrophilic by the presence of a polyethylene glycol chain, obtained by the polycondensation of ethylene oxide.

c**. Cationic Surfactants** are dissociated in water into an amphiphiliccation and an anion, most often of the halogen type.

d. **Amphoteric or zwitterionic** are molecules that exhibit both anionic and cationic dissociations (Salager *et al*.2002).

1.7. Phase behaviour

The phase diagram represents the relationship between the phase behavior of mixtures and its composition. It is possible to represent the phase behavior of microemulsion which compose of oil, water and surfactant assistant through pseudo ternary diagrams. In which each corner of the diagram represents 100% of the exact component. However, in most cases of microemulsions, they used extra component as cosurfactant and/or drug.In this case, four or more of components of phase diagram appear. Pseudoternary diagrams are built where a corner represents a binary mixture of two components as surfactant/co-surfactant, water/drug or oil/drug (Ritika *et al*.2012).



Fig(1.5) Pseudo –ternary phase diagram of oil, water and surfactant showing microemulsion region (Ritika *et al.*2012).

1.8. Diabetes Mellitus

Diabetes mellitus (DM) is marked by hyperglycemia is a common metabolic disorder. It is associated with central nervous system (CNS) and peripheral nervous system (PNS) neurological complication. DM in Long-term can cause a vast range of peripheral neuronal deficits, including reduced motor nerve conduction velocity, axonal shrinkage, impaired nerve regeneration and deficient axonal transport. STZ-induced diabetes is a well-defined experimental model for DM because the nerve damages are similar to the nerve degeneration that occurs in human diabetic neuropathy. Different kinds of diabetic neuropathies are some of the main complications of DM. It proposed that diabetes is one of the primary dangerous factors in the creation of senile dementia of the Alzheimer's type (SDAT), although the complete mechanisms by which diabetes could mediate these effects are not fully understood; it appears that hyperglycemia, oxidative stress and subsequent free radicals generation, lead to increasing the cell membrane damage (lipid peroxidation) and initiates death signaling pathways (Tabatabaeia *et a*l.2016).

Obesity and insulin resistance are conditions frequently associated with several complications such as hyperlipidemia, fatty liver, type II diabetes mellitus and cardiovascular diseases

(Osman *et al*.2015) (Oyedeji *et al*., 2013). Type II diabetes is a chronic metabolic disease that happens as a result of environmental and polygenic changes, that happens due to insulin secretion and/or insulin resistance. More than 90% of patients are the second type diabetes patients (Oyedeji*et al*. 2013). The World Health Organization predicts that the total number of people with diabetes will be reach 200 million in 2030. (Bai *et al*. 2016)

1.8.1 Advanced Glycation End Product(AGE)

Advanced glycation is consist through non-enzymatic reaction between carbonyl group and amino group; this process called glycation or Mallerd reaction. This reaction is divided into three main stages:

1. First stage : glucose (or other reducing sugars such as fructose, pentoses, galactose,

mannose, xylulose) react with a free amino group of biological amines, to produce an unsteady aldimine compound, the shiff base. Then during an acid-base catalysis, this labile compound undergoes a rearrangement to a more stable early glycation product known as Amadori product

2. Second stage: the Amadori product degrades by dehydration, oxidation and other chemical reactions to reactivate dicarbonyl compounds that are much more reactive than the initial sugars.They act as propagators of the reaction, again reacting with free amino groups of biomolecules.

3. Late stage: in this stage, the glycation process through oxidation and dehydration, irreversible compounds, advanced glycation end product called (AGE) are formed(Odjakova *et al*.2012)

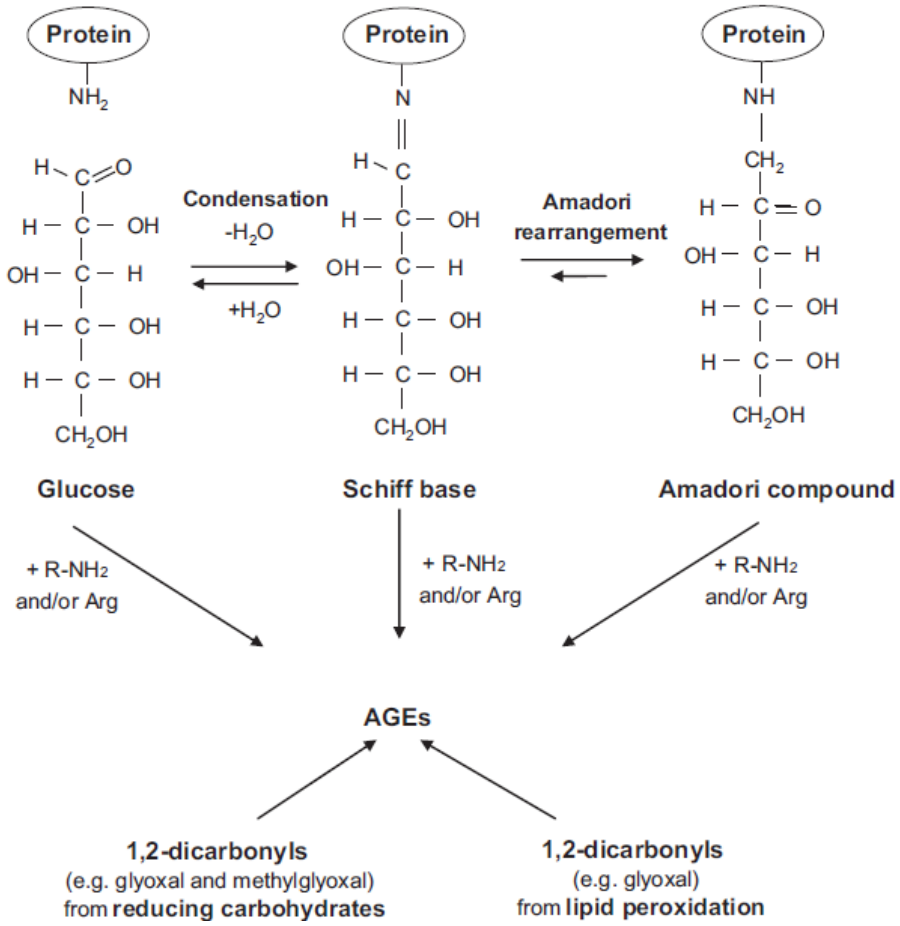


Fig (1.5) the synthesis of advanced glycation end products (AGEs) is a Nonenzymatic reaction where in its classical form there active carbonyl group of a sugar reacts with the nucleophilic amino group of an amino acid (e. g. arginine¼Arg) or a protein (R-NH2) (Striban *et al*.2014)

1.8.2. Relationship Between Mean Blood Glucose And Hemoglobin Glycated (HbA1c)

Measurement of hemoglobin A1c (HbA1c) is considered the gold standard for monitoring chronic glycemia of diabetes patients. Hemoglobin A1c indicates an average of blood glucose levels over the past 3 months. There is a correlation between HbA1c and mean blood glucose hemoglobin. This relationship permits the expression of HbA1c, as an equivalent mean glucose level. Also, it is beneficial for diabetics and treatment providers alike as the check of endemic glucose gain would be in the same units as the patients' self-monitoring.Since the

HbA1 test was submitted more than 25 years ago, it has become the most used scale of chronic glycemia in epidemiological studies, clinical trials and the management of diabetes.The concentration of HbA1c, shaped by the non-enzymatic annexed of glucose to hemoglobin is commonly considered to reflect the integrated mean glucose level over the previous 3 months, the time period being dictated by the 120 day lifespan of the erythrocyte. The relationship between mean blood glucose and HbA1c has been proposed by ancient studies which are employed at various measures of outpatient and inpatient plasma and capillary glucose concentrations. All of which suffered from relatively rare sampling of glucose levels, infrequent sampling of HbA1c, or both. The two most used studies measured from four to six fingerstick blood glucose levels per day for 6–8 weeks(Nathan *et al*.2007).

1.8.3. Relationship Between Antioxidant and Glycation

Anti oxidants safe guard against glycation-derived free radicals and have been proposed as a therapeutic agent. Diabetes can be tackled more effectively by the synergistic effect of compounds which contain antioxidant and anti-glycation properties. Thus, compound working against oxidation and against antiglycation offers a useful treatment of diabetes. The AGE in diabetes are growing, it has been proposed that inhibition of the configuration of AGEs may prevent the progression of diabetic complications and delay the ageing process. (Ramikisson *et al*. 2013)

1.9. Objective of the Research

1. To extract of purslane leaves and seeds by soxhlet extraction with ethanol 95%
2. To determine the total phenolic content of purslane.
3. To determine total flavonoids of purslane.
4. To evaluate the antioxidant activities of purslane.
5. Construction of ternary phase diagram for employing this in Pharmaceutical drug.
6. To investigate the antibacterial activity of extract of purslane and differ between two type of bacteria*:* Pseudomonas syringae is a bad bacteria and Bacillus subtilis is a good bacteria .
7. To evaluate the antiglycation activity of purslane to find solutions for diabetic disease.

Chapter Two:

Literature Review:

2.1. Drying Purslane Leaves:

A study by Gallo *et al.,* has determined the percentage of moisture of Purslane leaves before and after drying. The result represented 90% of moisture in Purslane leaves and 10% residual percentage(Gallo *et al. 2017).*

A second study conducted by Ercisli *et al*., has calculated the amount of moisture in purslane leaves, the moisture of P. Oleracea leaves were 84%.(Ercisli *et al*. 2008)

2.2. Extraction of Purslane

Zhu *et al*., investigated different methods of extraction on flavonoids from P. oleracea L. A, and those methods are:

1. Microwave-assisted extraction.

2. Ultrasonic extraction

3. Reflux extraction.

4. Soxhlet extraction.

5. Marinated extraction

These methods have been compared with different solvents to give high efficiency .

Three types of solvent were used and compared , including : methanol–water, ethanol–water, and acetone–water. The results show that extraction of flavonoids from *P. Oleracea L.* with ethanol–water gave the highest efficiency and the microwave extraction was most appropriate for the extraction of flavonoids from *P. Oleracea L.* due to its high efficiency and short extraction time (Zhu *et al*. 2011).

On the contrary, Chunmei *et al*., tested three extraction methods of polysaccharides in Purslane, by using water, ultrasonic and microwave. The result showed ultrasonic method to be the best one upon these three methods (Chunmei *et al*.2014).

Furthermore, Peksel *et al*., the antioxidant activities of the infusion and aqueous extracts of Purslane were investigated. The result showed antioxidants, total phenolic and total flavonoid contents of the infusion were found to be greater than those of the queous extract. (Peksel *et al*.2006),

2.3 Applications of Purslane

2.3.1 Phenolic and Flavonoid Contents of Purslane Compared with different solvent:

Kamal Uddin *et al*., examined the aqueous extract, methanolic extract and ethanolic extract to find out the best extraction solvent. The values of TPC and FRAP were calculated. The result was found that methanol is the best solvent in the extraction due to its ability to prevent oxidation of polyphenols and also it's quick evaporates compared to water. The lowest values of TPC and FRAP were 174.5 ± 8.53 mg GAE/100 g and 1.8 ± 0.1 mg GAE/g, respectively (Kamal Uddin *et al*. 2012).

In correlation, Sallam *et al.*, evaluated the antioxidants of irradiated Purslane ,with three solvent methyl alcohol , ethyl alcohol and distilled water. Antioxidant activities were investigated by (DPPH) radical scavenging activity), then calculated (TPC) and (TFC) by different solvent. The results came to represent that extraction by methanol (50%) had higher total phenolic contents (TPC) and (TFC) than other solvents. As for DPPH, the results found that the extracts by methanol gave higher effect than other solvents (Sallam *et al.* 2017).

Balabanova *et al*., evaluated the methanol-aqueous extracts of Purslane *aerial* parts from Bulgarian (POB) and Greek (POG) origin for their antioxidant potential using radical scavenging activity and ferric reducing power. In DPPH and ABTS assays, both purslane

extracts revealed equal IC50 values. DPPH scavenging (IC50) capacity ranged from 1.30 ± 0.04 to 1.71 ± 0.04 mg/ml, while (AEAC) values were 229.5 ± 7.9 to 319.3 ± 8.7 mg AA/100 g, (TPC) varied from 174.5 ± 8.5 to 348.5 ± 7.9 mg GAE/100 g *(*Balabanova *et al*. 2016).

In addition, Abd El-Aziz *et al*., investigated the phenolic compound and flavonoids in aqueous extract of fresh and dried powder of Portulaca Oleracea, *it* was found catechein is highest in phenolic compound while Rosmarinic is high in flavonoids (Abd El-Aziz *et al*.2014).

2.3.2. Total Phenolic of Purslane Seeds

Jalali *Mousavi et al.*, determined the antioxidant activity of Purslane Seed Water Methanolic Extract (PSWME), the result showed the DPPH radical scavenging activity of Purslane seeds at various concentration ranges from 15.41% to 79.06% and Total phenolic content in purslane seeds was 121.09 mg/kg (GAE) (Jalali *Mousavi et al.*2008).

In another important study, Guo *et al*., tested the antioxidant of purslane seeds by DPPH method , the result showed a linear relationship between concentration of oil and DPPH scavenging and given high values of DPPH radicals scavenging of PSO (Guo *et al*.2016),

A third study investegated the (TPC) and (TFC) of Purslane seeds for three time by El-sayed *et al*., the result found polyphenols is range between (40.6-41.8)% and flavonoids range between (26.7-27.9)% (El-sayed *et al*.2011).

2.4. Antimicrobial Activity

This study focused on the antimicrobial effect of Purslane extracts, the antimicrobial effect and its mechanism of extract from P. Oleracea. (POFE) were investigated by Dua *et al*., the results showed that antibacterial activity against Staphylococcus aureus (S. aureus).This is because its (POFE) entrances into the cytoplasm through membrane pores, causing two parallel reactions, namely an increase in the level of DNA fragmentation, which cause cell death. (Dua *et al*. 2017)

Moreover, Oraibi *et al*., studied the antibacterial activity of Portulacaoleracea L. In vitro using diffusion method ,the result shows the small inhibition zone of Pseudomonas

aeruginosa and Staphylococcus aureus but it was proved high efficiency as anti bacterial compared with greater concentration of the standard antibiotic (Oraii *et al*.2017).

Ercisli *et al*., investigated the antibacterial activities of methanolic extract from leaves of Purslane, the result showed antibacterial activities of methanolic extracts against some bacteria but water has no give antibacterial activities against bacteria (Ercisli *et al*.2008).

2.5. Effect of Purslane on Blood Glucose and Diabetic II

On the other hand, The impact of this plant on diabetes has been studied.There are many papers that report the effect of Purslane on blood glucose and diabetic 2, Hussein *et al*., used the purslane to cure for rats with diabetic. The rats were given different doses of purslane (150 and 300 mg/kg body weight) for 8 weeks with additional healthy diet system . The result was that the purslane with a health system improved insulin resistance, which could be used as a treatment for diabetes (Hussein *et al*.2010) .

— Another study, Bai *et al*., investigated the effect of a polysaccharide extract of purslane on rats infected with diabetes by oral administration to investigate the effect of it on fasting blood glucose (FBG) body weight. The diabetic rats were randomly divided into five groups based on the dose of purslane. After the overnight fast, blood sugar levels of rats that had been injected 20% sterile glucose solution in NC(normal control), MC(model control), CPOP crude polysaccharide of purslane (100,200,400)mg/kg of dose respectively , and glyburide groups, the highest dose of Purslane reached lowest values at 120 min(Bai *et al*. (2016).

At the same time, Zakizadeh *et al*., examined the impact of purslane seeds on biomarkers of oxidative stress in type 2 diabetic patients the result showed slight reducing in plasma total antioxidant capacity (Zakizadeh *et al*.,(2015).

2.6.Antiglygation Test

Harris *et al*.2010 analyzed ethanolic extracts from group of medicinal plants for inhibitory effects on in vitro AGE formation by fluorometric detection of fluorescent AGE. The result proved that the plant extracts prevent AGE formation, which share to vascular spoilage and development of diabetic servo (Harris *et al*.2010)

In another previous study (Beaulieu *et al*.2010) evaluated the inhibition of AGE formation by ethanolic extracts of the (Vacciniumvitis-idaea) with different concentration by fluorometric detection of fluorescent AGEs.

Another study, has evaluated a group of plant extracts (garlic, ginger, thyme, parsley, curry leaves, peppermint, turmeric, onion, green onion scallion and coriander) for antiglycation activity, The result showed these plants have antiglycation activity and suggested a glycation which a reason of diabetic disease (Ramkissoon*et al*.2013).

The antiglycation effect of Osbeckiaoctandra leaf decoction was investigated by Perera, et al., the result showed the extract of Osbeckiaoctandra leaf decoction has high antiglycation activity (Ramkissoon*et al*.2013).

2.7 Fatty acid Determination

KamalUddin *et al*., determine the lipid Content and Fatty Acid Composition. All fractions contained very low lipid content with0.47%in stems,0.51% in leaves, and 0.54% in the flowers (KamalUddin*et al*.2014)

Osman *et al.*, analyzed Purslane seeds fixed oil revealed the presence of 7 components by GC-MS the result 9,12,15-octadecatrienoic acid methyl ester was the primary composed of purslane seeds fixed oilconstitute (41.18%) followed by, 9, 12-octadecadienoic acid methyl ester (27.23%) (Osman *et al.* 2015).

Liu *et al* ., determined fhe fatty acid profile and b-carotene content of a number of Australian categories of purslane by GC and HPLC the result The total fatty acid content ranged from 1.5 to 2.5 mg/g of fresh mass in leaves, 0.6 to 0.9 mg/g in stems and 80 to 170 mg/g in seeds. a-Linolenic acid (C) formed about 60% and 40% of the total fatty 18:3 v3 acid content in leaves and seeds, respectively. The b-carotene content ranged from 22 to 30 mg/g fresh mass in leave (Osman *et al.* 2015).

Chapter Three

Materials, Methods and Experiments

3.1. Plant Materials Collection

Fresh Purslane leaves were collected from local markets from Al-Eizariyaarea in (April, 2018) and the seeds of purslane were collected from Attar shops.

3.2. Sample Preparation

Fresh green leaves were washed with distilled water and dried in shadow at room temperature for 15 days until it become completely dry .The dried samples and purslane seeds were crushed until a fine powder , then extracted it by soxhlet extraction with 95% ethanol .

3.3. Materials

3.3.A. Materials for soxhlet extraction.

a) 95% Ethanol Biotech For Medical Supplies
b) Purslane leaves local market
c) Purslane seeds Attar shops

3.3.B. Materials for microemulsion.

a) Deionized water (D.W) Biotech For Medical Suppliess
b) Tween80 Sigma –Aldrish company
c) Tween20 Sigma –Aldrish company

3.3.C. Materials for TPC content and TFC Content

All common chemicals were purchased from Sigma –Aldrich company .

a) Gallic acid.
b) Catechin.
c) Folin-Ciocalteu reagent.
d) Sodium carbonate.
e) 5%Sodium nitrate.
f) Aluminium chloride hexa hydrated.
g) Sodium hydroxide.
h) Distilled water

3.3.D. Materials for FRAP test

All common chemicals were purchased from Sigma –Aldrich company.

a) Sodium Acetate Anhydrous
b) Glacial Acetic Acid
c) Tpz (2,4,6-Tris(2-Pyridyl)-1,3,5-Triazine),
d) Hydrochloric Acid
e) Sulfuric Acid
f) Ferric Chloride Triahydrate.

3.3.E. Materials for AGE test

All common chemicals were purchased from Sigma –Aldrich company**.**

a) Bovine Serum Albumin
b) Glucose
c) Fructose
d) Sodium Phosphate Monobasic Monohydrate Buffer

3.4. Instruments and Equipment

A Soxhlet extractor has three main sections: a round bottom flask, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism. Then , rotary evaporator was used to evaporate the solvent .

UV-Vis spectrophotometer was used in TPC and TFC to measure absorbance. Samples of extract were put in volumetric flask to measure its by UV-Vis spectrophtometer.

Seed grinder was used to minimize size of seed, then, test tubes, syringes,four digit analytical balance, incubator cross polarizers and vortex were used in microemulsion. Plastic petridishes and sterilizer were used in antibacterial test. In antiglycation test the samples were put in 1.5 ml eppendorf tubes then, its put in incupator with shaker instrument, finally its measure by fluorescence.

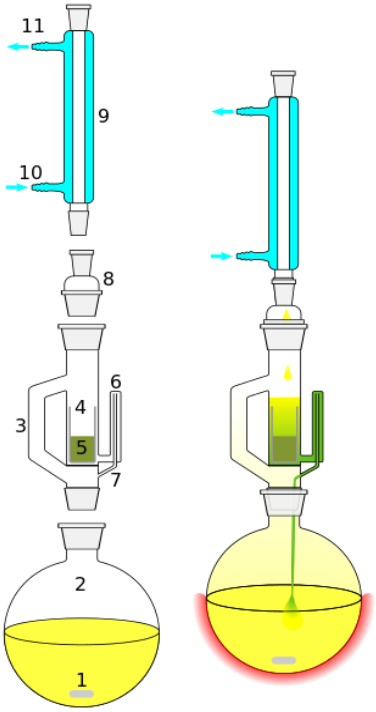
3.5. Methodology

3.5.1. Soxhlet Ethanol Extraction

25gm of Purslane leaves and seeds sample were put in "thimble" made from strong filter paper and interested into the broad central tube of the soxhlet extractor. Sequentially, purslane leaves or seeds, after that 250 ml of the solvent (Eth95%)was added to 1000 ml RBF .

Ethanol was heated by heating mantle to reflux, ethanol was condense, then distills into the reservoir include 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. This process is continuous.(Handa,*et al*,.2008)

The process was run for a total of 8 hours (leaves),but in seeds was run for 6 hours . 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 depectid as a figure (3.1) below



Fig(3.1): Soxhlet apparatus for hot extraction

3.5.2. Determination of Total Phenolic Contents (TPC)

The total phenolic compounds in the extracts were analyzed using the Folin-Ciocalteureagent(Singleton *et al*,. 1999). 40µl were put in the test tubes; 1.8 ml of Folin-Ciocalteu's reagent and 1.2ml of sodium carbonate (7.5%) was added. The tubes were mixed and tell to stand for 90 min. Absorption at 765 nm was measured using (UV-vis spectrophotometer). Gallic acid is used as the reference standard compound. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram of dry material.

3.5.3. Determination of Total Flavonoid Content (TFC)

Total flavonoids were analyzed using the Aluminum chloride (Zhishen *et al*,1999). 1.0 ml of extract of Purslane was added in volumetric flask containing 4ml of distilled water and 0.3ml portions of 5% Sodium nitrate was added. Followed by the addition of 0.3 ml portions of 10% Aluminium chloride solution and permitted to stand for 6 min after adding 2 ml solution of 1M Sodium hydroxide sequence. Total flavonoids were calculated from a catechin standard curve and reported as mg catechin hydrate equivalents (CAE) /g of dried weight. The absorbance of this reaction mixture was measured at 510 nm on UV spectrophotometer.

3.5.4. Measurement of Antioxidant Activity by FRAP Assay

The antioxidant activity of Purslane extracts (leaves and seeds) were calculated employing a modified method of the assay of ferric reducing/antioxidant (FRAP) assay of (Benzie and Strain 1996) .Preparation fresh FRAP reagent (3.0 ml) then heated to 37◦C and mixed with 40 µl of the purslane extracts of leaves and seeds , then the reaction mixtures were later incubated at 37◦C. Absorbance at 593 nm was recorded with reference to a reagent blank containing distilled water which was also incubated at 37 ◦C for up to 1 hour instead of 4 min, which was the original time applied in FRAP assay . Aqueous solutions of known Fe (II) concentrations in the range of (2 - 5 mM) ($FeSO_4$.6 H_2O) were used for calibration.

FRAP Reagent Preparation

1. 0.25M Acetate buffer (pH 3.6): Prepared by dissolving 4.10mg of sodium acetate (Mwt. 82.03) and 3.0mg of acetic acid in 100ml of distilled water. The solution was then made up to 200ml mark with distilled water, pH was adjusted to 3.6ml with NaOH or HCl.

2. 10mM 2,4,6-Tripyridyltriazine (TPTZ): 624.66mg of TPTZ ($C_{18}H_{12}N_6$, Mwt. 312.33) (Sigma-Aldrich) was dissolved in about 100ml of 0.25M acetate buffer and made up to 200ml with same.

3. 20mM Ferric chloride: Prepared by dissolving 1.0812g of ferric chloride ($FeCl_3.6H_2O$, Mwt. 270.3) in 0.25M acetate buffer and made up to 200ml with same.

4. Preparation of FRAP reagent: Prepared by mixing 10mM TPTZ and 20mM ferric chloride in 0.25M acetate buffer (pH 3.6). This reagent was prepared fresh just before the assay.

5. 1mM Ferroussulphate solution (working standard): Prepared by dissolving 27.8mg of $FeSO_4.7H_2O$ (Mwt. 278.02) in distilled water and made up to 100ml with same.

3.5.5. Antimicrobial Activity

The antimicrobial effects of the plant extracts was evaluated using antimicrobial tests which was carried out by well diffusion method on Mueller –Hinton Agar .

Muller- Hinton agar was prepared by adding 19 g to 1 L of distilled water, and then boiled to dissolve the medium completely , then it was sterilized by autoclaving at 121°C for 15 minutes . After that it was cooled to about 45°C , an amount of 25 ml of Muller- Hinton agar was poured on plastic petridishes and allowed to solidify. Agar plates were streaked using cotton swabs with reference bacterial strain which are : *Bacillus subtilis, Pseudomonas syringae*under aseptic condition to prevent contamination. Wells(diameter= 9mm) were filled with 50µl of the test sample (leaves and seeds of purslane extract,95% ethanol as a negative solvent), and incubated at 37°C for 24 h.

After the incubation period , the diameter of the growth inhibition zones was measured from edge to edge of the clear area around the wells containing samples. No measurement was taken if no clear zone of inhibition was observed .

3.5.6. Construction of Ternary Phase Diagram

The pseudo ternary phase diagrams consisting of oil, water, surfactant and co-surfactant mixture of different HLB (**hydrophilic-lipophilic balance**) values were constructed using water titration method.

1) The samples of extracting oil and nonionic surfactant were inserted into 10ml glass test tubes with screw caps at room temperatures at different weight ratios as in Table (3.1)

Table(3.1) the ratio between oil extract and nonionic surfactant

Weight ratio	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
Surfactant	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
Oil	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**	**0.4**	**0.3**	**0.2**	**0.1**

2) Mechanical shaking for 1 min. on the vortex for each sample.

3) A drop by drop titration with water is injected into each test tube with specific weights as in Table (3.2).

4) Vigorous mechanical shaking for 5 min. on the vortex ,due to the high viscosity of the surfactant and oil in order to guarantee a homogenous dispersion.

5) Temperature was control by placing the tubes within a thermostated water bath.

6)Left at rest during 24h to reach equilibrium before the next addition of water or aqueous phase then analyzing.

Table (3.2): Water phase percentage and Weight.

%	4%	8%	12%	16%	20%	24%	28%	32%	36%
Weight	**0.0417**	**0.0453**	**0.0494**	**0.0541**	**0.595**	**0.658**	**0.0732**	**0.817**	**0.919**
%	**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**
%	**76%**	**80%**	**84%**	**88%**	**92%**	**96%**			
Weight	**0.5955**	**0.8337**	**1.207**	**2.0833**	**4.1667**	**12.5**			

Several micro emulsions systems for each essential oils will be prepared at room temperature (25±1), the composition of each system is detailed in table(3.3).

Table (3.3): the composition of each system

System	Composition
System A	Oil (soxhlet) Tween 80 H_2O
System B	Oil(soak)Tween 80 H_2O
System C	Oil(soxhlet)Tween 80 ethanolic extract of leaves
System D	Oil(soxhlet) Tween 20 ethanolic extract of leaves
System E	Oil(soak)Tween 20 ethanolicextract of leaves

Note: Oil from seeds ofPurslane ,ethanolic extract from leaves of Purslane .

3.5.6.1. Determination of Phase Behavior

In order to study the behavior of oil, water, surfactant/co-surfactant emulsions, visual observation, anisotropy tests will be carried out.

1) Visual observation: the first step for determining the phase behavior, once the emulsion formed, is to make a visual inspection after each addition of water, different result will be obtained in color, texture and transparency. Microemulsions will identified as transparent, single phase, low viscous mixture.

2) Anisotropy: is detected by the cross polarizers. Clear, isotropic, one-phase systems were designated as microemulsion systems.

3) Draw the phase diagram using Origin Pro 8.1software.

3.5.7 AGE Test

Samples extracted from the Purslane leaves with 95% ethanol are processed, Bovine serum albumin (1 mg/mL) was incubated with 100mMglucose/100mMfructose in 100mM sodium phosphate monobasic monohydrate buffer (pH 7.4) with 95% ethanol vehicle (negative control) in eppendorf, with extract or compound (experimental treatment), or with quercetin (positive control) to reach 1000 microlitres of components by using microplates. Repeat of each treatment without BSA were included to control for analyte autofluorescence and repeat with BSA and vehicle (no sugar) was included to control for the fluorescence of BSA and as a negative control for immunochemical assessments (BSA control). Different concentrations of each extract or compound were tested in quadruplicate in eppendorf and incubated for 7 days at 37°C in darkness on a mechanical shaker with some modification .

(Beaulieu and Harris et al.,2012)

3.5.7.1 Fluorescence-based Quantitation of AGE Formation

After incubation 7 days, fluorescence was recorded by employing a micro plate reader (SpectraMax M5, Molecular Devices) at excitation and emission wavelengths of 355 nm and 460 nm, respectively. (Beaulieu and Harris et al.,2012)

Chapter Four:

Result and Discussion:

4.1. Percentage Yield of Moisture in Purslane

Table (4.1) Determination of some values on the leaves of Purslane before and after drying

Purslane leaves	Value
Initial weight of the leaves	3000gm
The weight of the leaves after dried	300gm
Percentage of moisture	90%
Residual percentage	10%

Table(4.1) represents the percentage of purslane moisture.

Moisture is the presence of a liquid, especially water, when the percentage of moisture was calculated, it was given the high percentage of moisture around 90% that means it contain high amounts of water. Comparing the previous studies, these results are similar to those reported by (Gallo,et al.2017)

4.2. Percentage Yield of Extracts

Extracts of plants are starting to have a lot of interest as a potential source of medicinal plant. After the extraction of chemical compounds from Purslane (leaves, seeds) by soxhlet method by 95% ethanol. The yield of ethanolic extract was calculated from the mass of the extract before and after evaporation in relation amount of plant powder, after evaporation was obtained a green liquid extract because it has small amount of ethanol .

Table (4.2) presents the yield of extracts obtained from purslane (leaves, seeds) by soxhlet method with ethanol 95%. We used the same solvent in two extracts (leaves, seeds). The yield was compared from extracts and it was found the best extraction yield is seeds, an extract of purslane that was chosen the soxhlet extraction because given the best percentage yield and ethanol was used as a solvent because is the best solvent and it more safe than methanol. Not of all content of Purslane dissolve in water so the ethanol was chose as the best solvent.

Table(4.2) percentage yield of purslane (seeds and leaves)

Purslane	**Solvent**	**% percentage yield**
Leaves	Ethanol 95%	20.4
Seeds	Ethanol 95%	24.0

4.3.Total Flavonoid

This method was used to calculated the total flavonoid contents of various extracts of Purslane leaves and seeds. Total flavonoid contents was calculated using the standard curve of Catechin ($y = 0.0048x + 0.0034$; $R^2 = 1$) as shown in Figure(4.1) and was expressed as Catechin per gram of the plant extract. The (TFC) in seeds is higher than leaves see table (4.3)

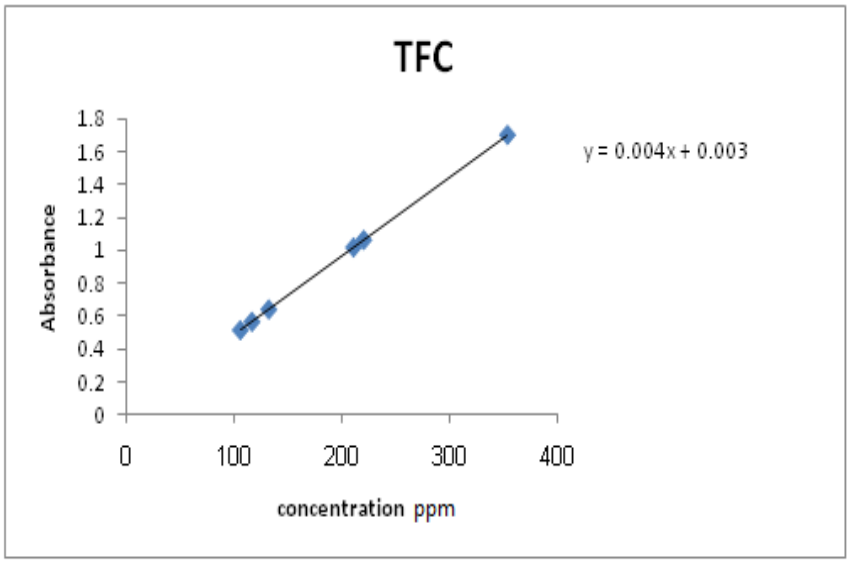


Fig (4.1): Calibration curve Catechin standard.

Table (4.3) Flavonoids content in mg Catechin / g sample

Purslane	**Total flavonoids content mg Catechin/g of purslane±SD**
Leaves	46.6±7.00
Seeds	58.8±8.00

Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities (Crozier et al., 2006). The (TFC) of Purslane extract was determined using aluminium chloride method the result found in table (4.3).TFC of seeds is higher than leaves.

In previous studies, (TFC) was reported to be 41.3±4 mg rutin equivalent/g ethanol by using ethanol as a solvent (Kamal Uddin, et al. 2012). Also (Sallam, et al. 2017) tested (TFC) give be between 42-60 (mgQE/100 g). The (TFC) in this study is within these limits

4.4. Ferric Reducing Antioxidant Power (FRAP)

This method evaluated the free radical scavenging activity of plant extracts. FRAP is simple, fast, inexpensive, and robust method, and does not require specialized equipment. In the FRAP method the yellowFe^{+3}TPTZ complex (2, 4, 6-Tri (2-pyridyl) -1, 3, 5-triazine) is reduced to the blue Fe^{+2} TPTZ complex by electron-donating substances (such as phenolic compounds) under acidic conditions (Benzie & Strain, 1996).

The Antioxidant Activity (AA) of solvent plant extracts of Purslane was evaluated by FRAP method and were expressed as moleFe^{+2} per gram of plant extract. It was calculated based on a standard curve of concentration ofFe^{+2} Linear equation was generated y = (0.2019x – 0.1766) with high coefficient of determination, R^2 = 0.9822 Figure(4.2).

Table (4.4) showed FRAP of plant extracts. As shown in this table, leaves extract was found higher than the seeds but not high difference .

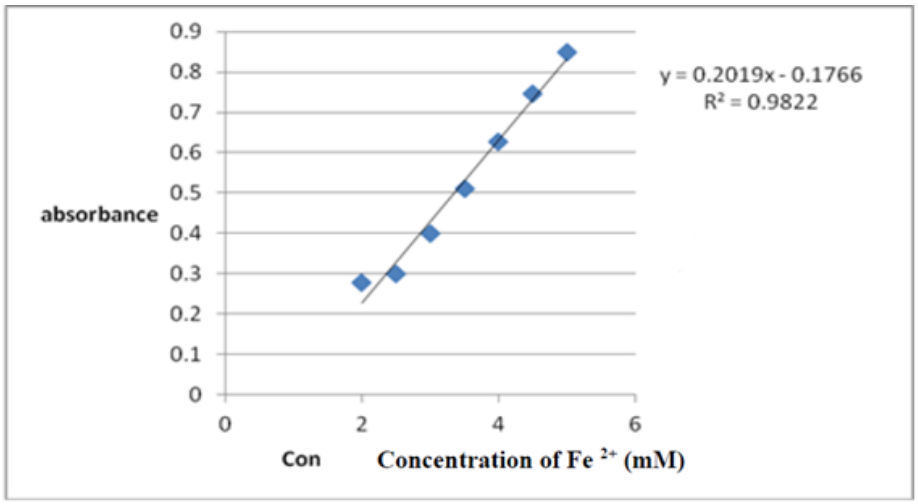


Fig (4.2): Calibration curve of Fe^{+2} standard

Table (4.4) FRAP content in mMFe^{+2}/ g of dry sample of ethanolic extract .

Purslane Plant	**MgFe^{+2}/ g sample±SD**
Seeds	0.8865± 1.51
Leaves	1.30268± 1.32

In this study, the analysis of the antioxidant activity was performed using FRAP method in table (4.4). The FRAP result of leaves is higher than leaves and it is similar to previous study was reported by (Kamal Uddin *et al*. 2012)

4.5. Total phenolic content

Total phenolic content of the different extracts of purslane leaves and seeds were calculated by employing the Folin-Ciocalteu reagent and were expressed as mg Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were counted using the standard curve of Gallic acid ($y = 0.0027x + 0.1172$; $R^2 = 0.9988$) Figure (4.3).

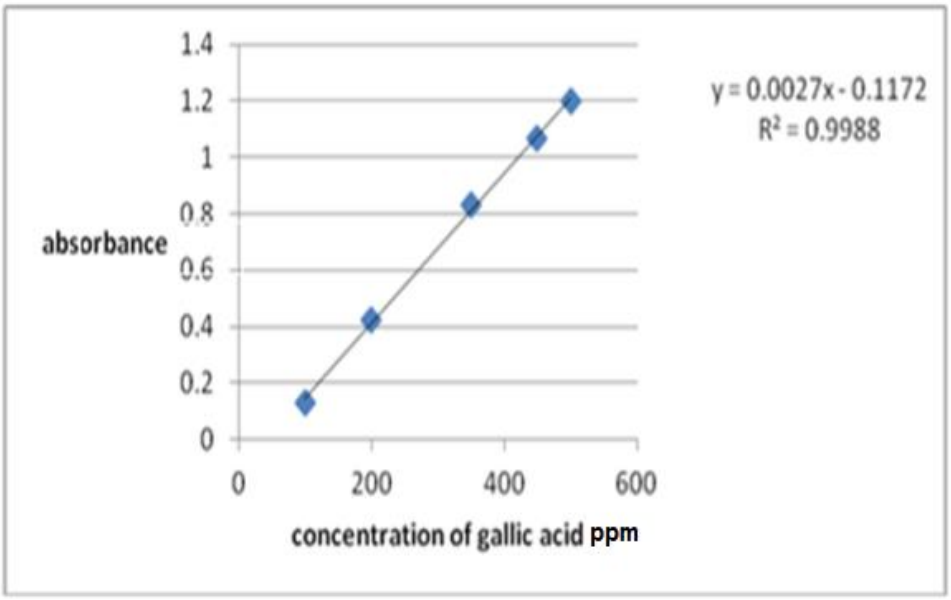


Figure (4.3): Calibration curve of Gallic acid

To calculate the mg of Gallic acid/ g of sample.

Table (4.5): Total phenolic content in mg Gallic acid/ g sample

Purslane	**TPC mg of GA/ g of purslane**
Leaves	209.3±5.00
Seeds	243.0±5.00

Polyphenols are the result of secondary metabolism and are natural compounds found in various plants such as Purslane. The present study indicated that Purslane leaves is a rich in polyphenols (209.3±5.00) mg of GA/g of Purslanedry, this result is simiar to previous study that reported by (Golovinsky,*et a.l*,2016) and polyphenols of seed (243.0±5.00)mg of GA/g of Purslaneseeds that's mean it contain high amout of (TPC).

4.6 Antimicrobial Effect

The extracts from the leaves showed antibacterial activities against *Bacillus subtilis, Pseudomonas syringae,* but extract from seeds showed slightly activities against *Bacillus subtilis* only, the zone of inhibition for each sample was measured using a ruler in millimeter (mm). The data in the table are average diameter for the duplicate repeated tests.

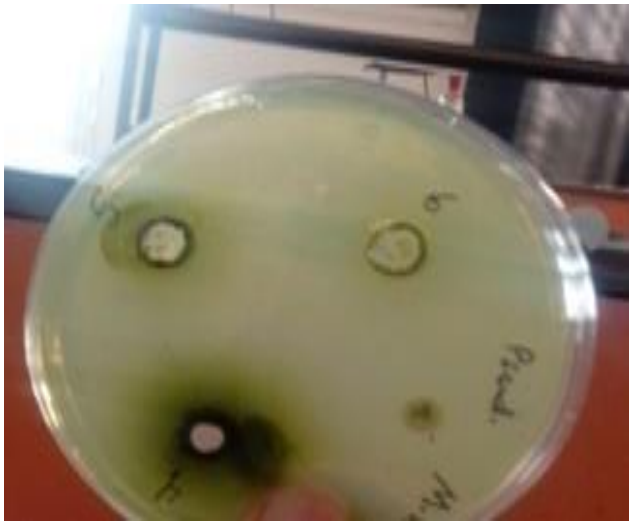
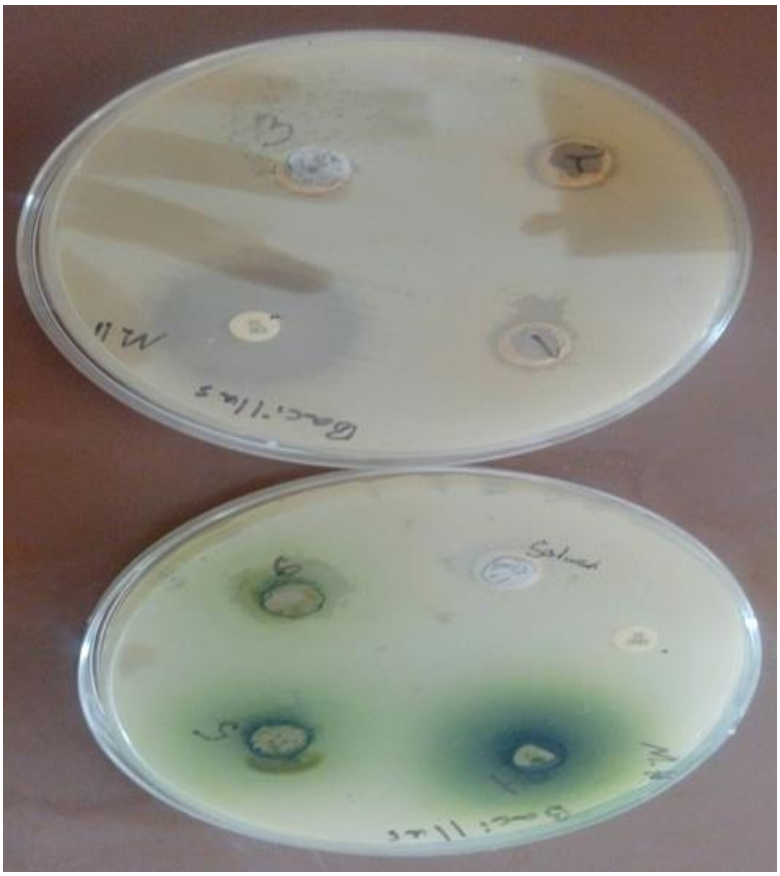


Figure (4.4) : Effect of ethanolic extract of purslane leaves on *Pseudomonas* bacteria .

Zone (4) :concentrated extract , zone (5)1:1 extract:ethanol95%,zone(6) 1:2 extract :ethanol95%, antibiotic

Fig (4.5): Effect of ethanolic extract of Purslane on *bacillus* bacteria

Zone (4) :concentrated extract, zone (5)1:1 extract:ethanol95%,zone(6) 1:2 extract :ethanol95%, antibiotic ,zone of solvent &finally antibiotic



Fig(4.6) Effect of ethanolic extract of seeds on *Bacillus*

Zone (1,2,3) of seeds ,zone (1) is concentrated (2) 1:1,(3)1:2 , extract:ethanol95% , zone (4,5,6)of leaves ,Zone (4) :concentrated , zone (5)1:1 ,zone(6) 1:2 extract :ethanol95%, antibiotic ,zone of solvent &finally antibiotic

Table (4.6) The inhibition zone (mm) of extract

Extract	Concentration	Bacteria	Inhibition zone(mm)
Leaves	**Concentrated** **Diluted 1:2** **Diluted 1:3**	**Bacillus**	**7** **6** **5**
Leaves	**Concentrated** **Diluted 1:2** **Diluted 1:3**	**Pseudomonas**	**5** **4** **2**
Seeds	**Concentrated** **Diluted 1:2** **Diluted 1:3**	**Bacillus**	**2** **1** **1**
Seeds	**Concentrated** **Diluted 1:2** **Diluted 1:3**	**Pseudomonas**	**No zone**

Result show in figure (4.5) and figure (4.6) show that no inhibition zone of ethanol. Thats mean no effect of ethanol on bacteria, thats means the inhibition zone from extract, the ethanolic extracts from the leaves showed as figure(4.4) and figure (4.5) antibacterial activities against *Bacillus subtilis and Pseudomonas syringae*, previously study that reported by (Ercisli,*et al*.2008) show the methanolic extract give antibacterial effect against *Bacillus subtilis* and *Pseudomonas syringae*, but ethanolic extracts of seed show as figure(4.6) that gives antibacterial effect against *Bacillus subtilis* only. When the concentration of extracts are increased it gives higher inhibition zone this mean more concentration kill more bacteria .

4.7 Constructing of Phase Behavior

Microemulsion formation of oil extracted from purslane leaves and seeds at ambient conditions (temperature and pressure) is the challenging at best**.** Ternary phase diagram was built by selecting oil phase, surfactant and an aqueous phase. It was constructed by performing aqueous titration.

We wanted to formulate the phase behavior of microemulsion in the ethanolic extract of seeds and leaves of purslane, to achieve an environmentally friendly, stable, biocompatible microemulsion between 95% ethanolic extract of purslane seeds and ethanolic extract of purslane leaves and water and nonionic surfactant.

This formulation may help in microemulsion formation, the work was studied by two sugar based surfactants namely Tween 80, Tween 20 at 25C and We integrated seed oil of Purslane with ethanolic extract of Purslane leaves to differ between them and choose the best of all.

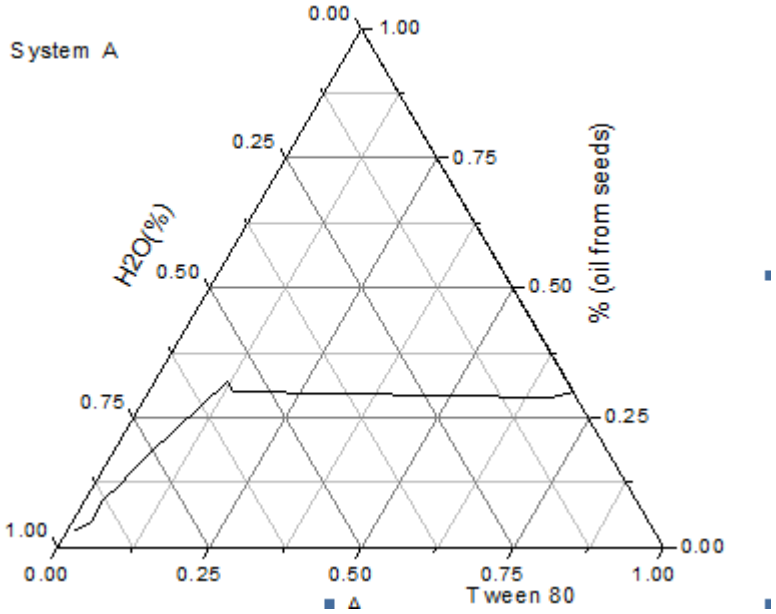
five systems of microemulsion were examined by changed the (surfactant or aqueous phase or oil phase) then studied the work of two sugar based surfactants namely Tween 80, Tween 20 at 25C, and again the extraction methods were changed and Purslane leaves with its seeds were mixed together to compare the best and which of them gives the best microemulsion.

The percentages by weight of the aqueous phase, surfactant and oil that gave a clear, transparent and isotopic was represented as a point in the phase diagram. The border line that passed through these points and the area on its right hand represented a total monophasic microemulsion while the region on the left hand side represented a cloudy dispersion or multi phase domain.

Pseudo-ternary phase diagram of system A

The pseudo-ternary phase diagram of microemulsion system A consists of oil from seeds of Purslane as oil phase, Tween 80 as a surfactant, and water as aqueous phase are shown in figure(4.7)

The oil from seeds of purslane extracted by soaking method as the same solvent ethanol, we compare between soaking method and soxhlet method in systems (A&B), and the same solvent, show the result in figure (4.7) & (4.8)

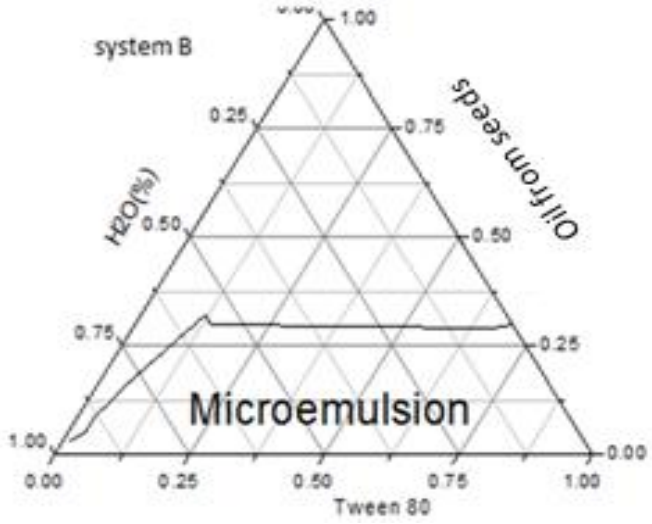


Fig(4.7) pseudo-ternary phase diagram of (oil of seeds of purslane, Tween 80, H_2O)

Pseudo-ternary phase diagrams of system B

The pseudo-ternary phase diagram of microemulsion system B consists of oil from seeds of porcelain as oil phase, Tween 80 as a surfactant, and water as aqueous phase

The difference between system A and B that the oil in system A extracted by soaking method, but system B by soxhlet method.



Fig(4.8) pseudo-ternary phase diagram of (oil of seeds of purslane, Tween 80, H_2O)

As can be seen from the above figures (4.7) & (4.8) that there is an identical between the two areas of microemulsion that means there is no difference between the soaking method and soxhlet method this is meant the method no effect on microemulsion and in two systems the area of microemulsion started from a higher percentage of surfactant at 70% of surfactant so we need high amount of surfactant.

The pseudo-ternary phase diagram of microemulsion system C composed of oil from seeds as oil phase, Tween80 as surfactant and ethanolic extract of the leaves as aqueous phase are shown in figure(4.9)

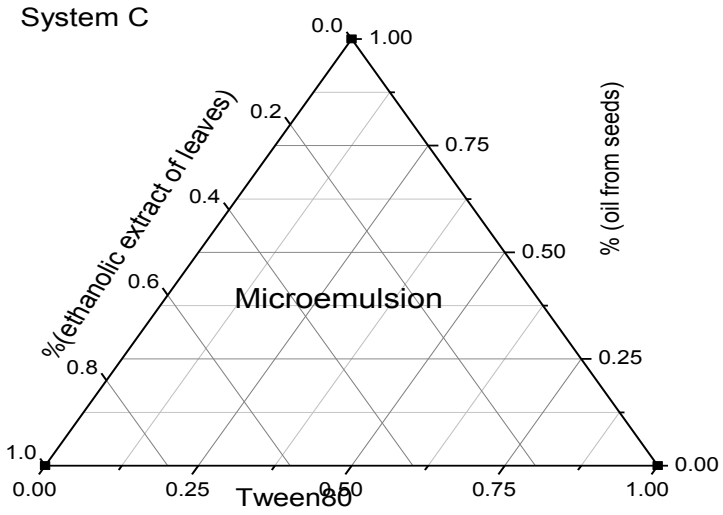


Fig (4.9) pseudo-ternary phase diagram of system C composed of (oil of seeds of purslane, Tween 80, the ethanolic extract of the leaves)

The microemulsion region starts as a single clear isotropic and not the shiny solution before addition ethanolic extract and it continues to finally microemulsion.

The pseudo-ternary phase diagram of the microemulsion system D composed of oil from seeds of purslane as oil phase, Tween20 as surfactant and ethanolic extract as aqueous phase as shown in fig (4.10)

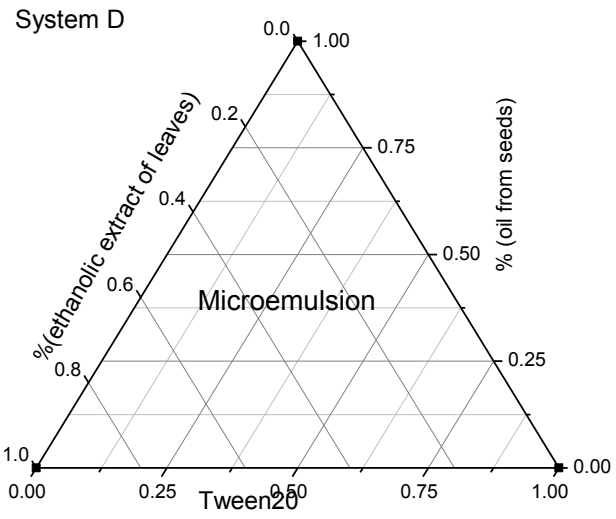


Fig (4.10)pseudo-ternary phase diagram of system C composed of (oil of seeds of purslane, Tween 20,ethanolic extract of leaves)

Here in system C and D we compared the effect of surfactant on system C we used Tween80 but in system D we used Tween20, in two systems give us microemulsion from beginning titration to end of titration.

The pseudo-ternary phase diagram of the microemulsion system E composed of oil from seeds as oil phase by soak, Tween20 as surfactant and ethanolic extract of the leaves as aqueous phase are shown in figure (4.11)

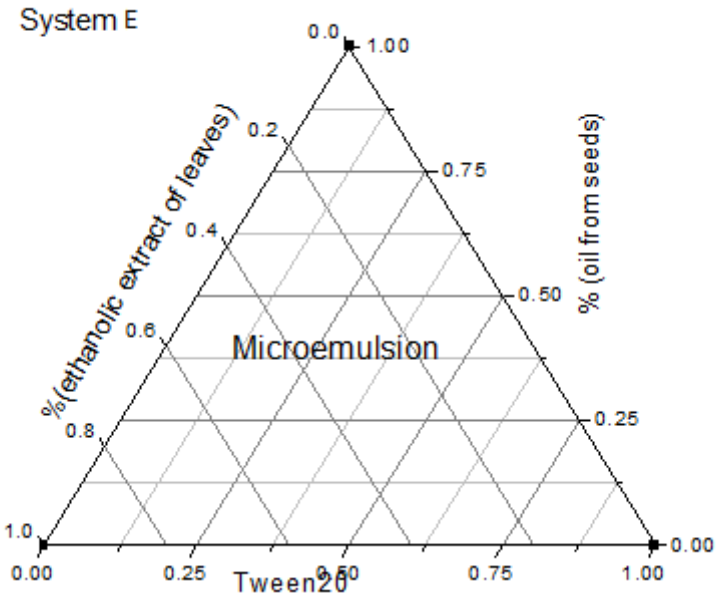


Fig (4.11)pseudo ternary phase diagram of system E composed of (oil of seeds by soak ,Tween20 and ethanolic extract)

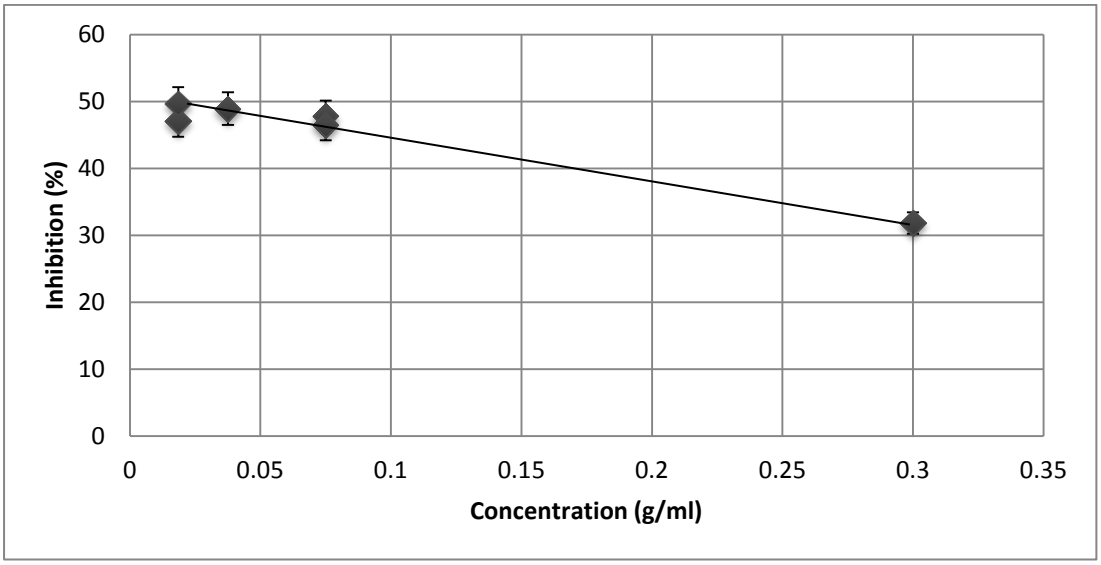
We showed the result in system D confirm and support system A and B this is meant the method of extraction (soak or soxhlet) didn't effect on the area of microemulsion in this case when combine seeds and leaves from the same plant

As for system C, D, E give us microemulsion for all percentages of titration that's a mean component of seeds and leaves are similar and compatible, so we didn't find the effect of surfactant when changed from Tween80 to Tween20 in this case.

Microemulsions are clear, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a cosurfactant. Microemulsions act as potential drug carrier systems for oral, topical, and parenteral administration. They offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, and improved drug solubilization and bioavailability. In this study extract of leaves and extract of seeds were mixed and give higher region of microemulsion as shown in figures(4.9),(4.10) and (4.11) there high compatibility between oil from seeds and ethanolic extracts of leaves that's very good and can be used to deliver drugs to the patients via several routes, when the method of extraction was changed the origion of microemulsion isn't change is still as the same as shown in figure (4.7) and (4.8) .

4.8.Antiglycation Test

The ethanolic extract of purslane leaves was determined in the fluorescence-based on test of AGE formation in triplicated experiments detected a log-correlation between inhibition of fluorescent AGE crosslink shaping and the concentration of the purslane extract. The purslane leaves give 50% inhibition at concentration lower than 0.05g/ml and 31% at concentration 0.3 g/ml, then noted from figure (4.12) the inhibition values is convergent at different concentration fig(4.12)



Fig(4.12): Inhibition of the formation of fluorescent AGE crosslinks by ethanol extract of Purslane leaves

In vitro antiglycation activity of the spices was examined by testing the ability of the extracts to inhibit the methyl glyoxal mediated development of fluorescence of bovine serum albumin (BSA)(Matsuda,*et al*.2003). AGEs contribute to diabetic complications through a series of pathological changes such as increased atherogenicity of LDL, increased basement membrane permeability and decreased insulin binding to its receptors. AGEs play, as well, an important role in diabetic micro- and macroangiopathy where it deposits under endothelial cells(Daroux *et al*. 2010) and (Park *et al.* 2010). The extracts of Purslane leaves tested displayed good antiglycation ability. There inverse relationship between concentration of extract and inhibition of formation AGE that's explain the extract of purslane leaves work as antiglycation at low concentration.

4.9. Conclusion

1. TFC and TPC results found that Purslane contains a high amount of phenolic and flavonoids compounds.

2. Antibacterial test results found that inhibition zone is positively increased when concentration of purslane increased.

3. Construct of phase behavior test found that mixing purslane seeds and leaves gives a large area of microemulsion.

4.The antiglycation test found that the purslane can be used to reduce or inhibit the blood glucose.

5. In microemulsion system , the same results were found when the method of the extract changed between system A and B.

6. large region of microemulsion was achieved in system C,D and E at low concentration of surfactant.

4.10. Future work

In this study, we have reached the impact of diabetes on the Type II diabetic by antiglycation test, but we couldn't get the concentration of leaves of purslane because lack of time. This study is a path for researchers to complete the antiglycation test and know what is the concentration of purslane leaves that can be used to reach the highest inhibition. Microemulsion system can be used in medicinal application diabetic as disease.

References

1.Abd El-Aziz,H.A.,Sobhy, M.H., Ahmed,K.A, Abd El hameed,A.K., Rahman,Z.A.And H

assan, W.A. (2014). Chemical and remedial effects of purslane (portulaca oleracea) plan. Life science journals; 11 (6): 31-43

2.Akbari ,M., Hajinezhad M.R., Zadeh,E.R.AndJamshidian ,A.(2014). Effects of purslane seeds extract on japanese quail blood glucose, serum lipoproteins and abdominal fat pad weigh. International journal of analytical pharmatical and biomedical science; 60:65

3.Aldina Kesić,A. , Mehmedinović,N.I. And Šestan,A. (2015).Phytochemical Profile of Honey. Phytochemicals - Isolation, Characterisation and Role in Human Health.Ch#4

4. Al Sayed, M.A. (2011). Effect of portucala seeds in treatment of type 2 diabetic mellituts patient as adjunctive and alternative therapy .Journal of ethnopharmocology;643:651

5. AmirulAlam,MD., Juraimi,A.S., Rafii,M.Y., AbdulHamid,A., Aslani,F., Hasan,M.M., Zainudin,M.A.M. And KamalUddin, MD.(2013). Evaluation of Antioxidant Compounds, Antioxidant Activities, and Mineral Composition of 13 Collected Purslane (Portulacaoleracea L.)Accessions.Hindawi Publishing Corporation BioMed Research International,p 10.

6. Azuka, O.I., Mary, A. And OrachuLovina Abu, O.L. (2014).A review on Portulacaoleracea (Purslane) plant – Its nature and biomedical benefits.International journal of biomedical research; 76:80.

7. Bai,Y., Zang,X.,Ma,J. And Xu, G. (2016).Anti-Diabetic Effect of Portulacaoleracea L. Polysaccharideandits Mechanism in Diabetic Rats.International journal of molecular science; 2:14.

8.Beaulieu,L.P. , Harris,C.S., Saleem,A.,Cuerrier,A., Haddad,P.S, Martineau,L.C., Steffany A.L. Bennett,S.A.L. And Arnason,J.T.(2010). Inhibitory Effect of the Cree Traditional Medicine Wiishichimanaanh (Vacciniumvitis-idaea) on Advanced GlycationEndproduct Formation: Identification of Active Principles ;741-747

9. Benzie, I. F. And J. J. Strain (1996). "The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power": the FRAP assay." Analytical biochemistry" 239(1): 70-76

10.Blainski, A., Lopes, G.C. And de Mello, J.C, P. (2013). Application and Analysis of the FolinCiocalteu Method for the Determination of the Total Phenolic Content from LimoniumBrasiliense L. ,molecules; 18:6852-6865.

11. Chunmei ,G.U.And Shu, P.A.N. The Comparison and Analysis of Three Extraction Methods for Polysaccharides in Purslane. Journal of Food and Nutrition Research; 2(7): 401-405

12. Crozier, A., Ashihara, H. And Clifford, M.N. (2008). Plant Secondary Metabolites: Their Role in the Human Diet. Planta Medi;.p353

13.Daroux ,M., Prévost, G., Maillard-Lefebvre, H., Gaxatte,C., D'Agati, V.D., Schmidt, A.M.And Boulanger, E.(2010). Advanced glycation end-products: Implications for diabetic and non-diabetic nephropathies. Diabetes & Metabolism. ;36:1–10.

14. Dkhil,M.A., Abdel Moniem,A.E., Al-Quraishy,S. And Saleh, R.A. (2011).Antioxidant effect of purslane (Portulacaoleracea) and its mechanism of action. Academic journal; 5 (9): 1590-1595.

15. Dua,Y.K., Liua,J., Li a,X.M., Pan b,F.F., Wen ,Z.G ., Zhang, T.C. And Yang, P.L. (2017).Flavonoids extract from Portulacaoleracea L. Induce Staphylococcus aureus death by apoptosis-like pathway.International Journal Of Food Propertie;S534:S542.

16. Ercisli, S. Coruh, I. Gormez, A.And Sengul, M. (2008).Antioxidant and antibacterial activities of p ortulacaoleracea l. grown wild in turkey. Ital. J. Food Sci; (4): 533-542.

17. Farhadpour, F, Albany, A., Khakpour, B., Ahmadi, R. And Mahdavi, E. (2014).The Effects of Portulacaoleracea Seed Hydroalcoholic Extract on Pain Threshold.International Conference on Food, Biological and Medical Sciences; 80:81.

18.Gallo ,M., Conte,E. And Naviglio,D.(2017). Analysis and Comparison of the AntioxidantComponent of PortulacaOleracea Leaves Obtainedby Different Solid-Liquid Extraction Techniques.Antioxidants;(6):64

19. Guo,G ., Yue,L., Fan,S., Jing,S. And Liang-Jun Yan.(2016). Antioxidant and Antiproliferative Activities of Purslane Seed Oil. Journal of Hypertension;(5):2

20. Gevrenova,R., Dimitrova,D.Z., Balabanova,V., Lazarova,I., Ruseva,S.And Valentin Lozanov.(2016). A Phytochemical Study And Antioxidant Potential Of Portulaca Oleracea L. (Purslane) Grown Wild In Bulgaria And Greece. Biologie Botanique;7:865-870

21. Gonella, M. ,Charfidine, M. , Converse, G. And Santamaria, P. (2014). purslane :A review of its potential for health and agriculture aspects.TheEuropien journals of plant science and biotechnology;(1):131-136

22.Handa,S.S.,Khanuja, S.P.S.,Longo,G. And Rakesh,D.D.(2008).Extraction Technologies for medicinal and Aromatic Plants. International centre for science and high technologyics-unido,AREA Science Park Padriciano99,34012 Trieste,Italy ;21-259

23.Harris,C.S., Beaulieu,L.P., Marie-Hélène Fraser,M.H., McIntyre,K.L., Owen,P.L., Martineau,L.C., Cuerrier,A. ,Johns,T., Haddad,P.S., Bennett,S.A.L. and Arnaso,J.T.(2010) Inhibition of Advanced Glycation End Product Formation by Medicinal Plant Extracts Correlates with Phenolic Metabolites and Antioxidant Activity. PlantaMed;(77):196-204

24. Hussein, M.A.(2010). Purslane Extract Effects on Obesity-Induced Diabetic Rats Fed a High-Fat Diet. Mal J Nutr;16(3):419-429.

25 .Jalali Mousavi,S.R ,Niazmand,R. And M. Noghabi,M.(2008). Antioxidant Activity of Purslane (Portulacaoleracea L.) Seed Hydro-alcoholic Extract on the Stability of Soybean Oil.J. Agr. Sci. Tech (17):1480-1473.

26. Jouybari,M.F., Arkhazlue,P.F. And Hashemvarzi, S.A. (2014). The effect of 8 weeks aerobic exercise with Purslane seeds consumption on peroxidant and antioxidants indicators in women with type2 diabetes. International Journal of Sport Studies; 4 (2): 238-245

27. KamalUddin,MD., Juraimi,A.S.,MdSabirHossain,MD.S., Nahar,M,A.U., Ali,MD.E. And Rahman, M.M. (2014).Purslane Weed (Portulacaoleracea): A Prospective Plant Source of Nutrition, Omega-3 Fatty Acid, and Antioxidant Attribute. Hindawi Publishing Corporation journal; p6

28. Kamal Uddin ,M.D.,Juraimi ,A.S., EaqubAli,M.D. And Ismail, M.R. (2012). Evaluation of Antioxidant Properties and Mineral Composition of Purslane (Portulacaoleracea L.) at Different Growth Stages.International Journal of Molecular Sciences; (13): 10257-10267

29. Kaur, M. And Nitika. (2016). Review on Sea purslane.Journal of Pharmacognosy and Phytochemistry; 3 (5): 22-24

30.Kaushik, A., Jijta, C., Kaushik, J.J. ,zeray, R., Ambesajir, A. And Beyene, W.(2012), FRAP (Ferric reducing ability of plasma) assay and effect of Diplaziumesculentum (Retz) sw. (A green vegetable of north India) on central nervous system, Indian journal of natural products and resources:3(2):228-231.

31.Klier, J. , Tucker, C.J. ,Kalantar,T.H. And D. P. (Patrick) Green. (2000).Properties and Applications of Microemulsions: A Review.WILEY-VCH Verlag GmbH; 1751:1757

32. Kumar, s. (2014).The importance of antioxidant and their role in pharmaceutical science-A Review. Asian Journal of Research in Chemistry and Pharmaceutical Sciences; (1)1:27-44.

33. Liu,L., Howe,P., Zhou,Y.F., Xu,Z.Q. And Hocart, C. , (2000). Fatty acids and b-czrotene in Australian purslane (Portulacaoleracea) varieties. Journal of chromatography; 208:215

34.Matsuda H., Wang T., Managi H. And Yoshikawa,M.(2003). Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. Bioorg Med Chem;11:5317–5323

35. Najafabadi, E. A., Dehghani, A. ,Behradmanesh, S. And Najarzadeh, A. (2015).The effect of purslane seed on fasting blood sucrose and serum liver enzymes in patient of nonalcoholic fattylivers.Iranian Journal Of Diabetes And Obesit; (7): 163-172

36.Nathan,D.M., Turgeon,H. And Regan,S.(2007) . Relationship between glycatedhaemoglobin levels and mean glucose levels over time . Diabetologia;50:2239-2244

37.Odjakova,M. ,Popova,E. , Al Sharif,M.A. And Mironova,R.(2012). Glycosylation .Plant-Derived Agents with Anti-GlycationActivity.Ch(10)

38. Oliveira, I., Valentão, P., Lopes, R., Andrade, P.B., Bento, A. And Patrecia, J.A. (2009). Photochemical characterization and radical scavenging of portulaca leaves and stems.microchemical journal; (92): 129-134

39. Oraibi,A.G., AlShammari,A.A., Mohsien,R.A. And Obaid,W.J.(2017). Investigation the Antibacterial Activity of Portulaca oleracea L. Tissue Cultures in vitro. Journal of Pharmaceutical Research International; 18(5):1-7.

40. Osman, S.M. and Hussein, M.A. (2015).Purslane Seeds Fixed Oil as a Functional Food in Treatment of Obesity Induced by High Fat Diet in Obese Diabetic Mice.Journal of Nutrition & Food Sciences; 5:1.

41.Pandey, K.B. And Rizvi, S.I (2009).Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity; 2 (5): 270-278

42. Park ,C.H., Noh, J.S., Yamabe, N., Kang, K.S.And Tanaka T, Yokozawa, T.(2010) Beneficial effect of 7-O-galloyl-D-sedoheptulose on oxidative stress and hepatic and renal changes in type 2 diabetic db/db mice. European Journal of Pharmacology; 640:233–242.

43. Peksel,A. ,Arisan,I,A.T.A.C.And Yanardag,R.(2006). Antioxidant Activities Of Aqueous Extracts Of Purslane (Portulaca Oleracea Subsp. Sativa L. Ital. J. Food Sci;3:18

45.Perera,P.R.D., Ekanayake,S. And Ranaweera,K.K.D.S.(2013). In vitro study on antiglycation activity, antioxidant activity and phenolic content of Osbeckiaoctandra L. leaf decoction .Journal of Pharmacognosy and Phytochemistry;(4):198-201.

46.Pisoschi, A.M. And Negulescu, G.P. (2012).Methods for Total Antioxidant Activity Determination: A Review.Biochemistry and Analytical Biochemistry; 1 (1): 106

47.Ramkissoon,J.S., Mahomoodally,M.F., Ahmed,N. and bratty,A.H.(2013).Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs.Asian Pacific Journal of Tropical Medicine;561:569.

48.Rebaya,A., Belghith,S.L., Baghdikian,B., Leddet ,V.M., Mabrouki,F., Olivier,E., Cherif,J.K. And Ayadi, M.D. (2014). Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of Halimiumhalimifolium (Cistaceae). Journal of Applied Pharmaceutical Science; 5 (1): 52-57

49.Ritika, Harikumar S.L. And Geeta,A.G.(2012).Microemulsion System In Role Of Expedient Vehicle For Dermal Application. Journal of Drug Delivery and Therapeutics;2(4):23-28.

50.Salager, J.L. (2002). Surfactants - Types and Uses.FIRP BOOKLET,# E300-A

51. Sallam, E.M. And Anwa, M.M. (2017).. Antioxidant Activity of some Extracts from Gamma irradiated Purslane (Portulacaoleracea) Plant. International Journal Of Agriculture &Biology; (19): 48-52

52.Shalaby, E. A. And Shanab, S.M.M. (2013). Assays of determination and mode of action, African Journal of Pharmacy and Pharmacology; 7 (10): 528-539.

53.Shekhar, T.C. And Anju, G.(2014).Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. American Journal of Ethnomedicine; 1 (4): 244-249

54.Singleton,V.L., Orthofer,R. And Lamuela-Ravent,R.M. (1999).Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Oxidants and Antioxidants Part A Methods in Enzymology;(299):152–178.

55. Siriamornpun., S. And Suttajit, M. (2010).Microchemical Components and Antioxidant Activity of Different Morphological Parts of Thai Wild Purslane (Portulacaoleracea),. Weed Science; 58:182-188.

56. Sultana, A. And Rahman, K. (2013). Portulaca Oleracea Linn: A Global Panacea With Ethnomedicinal And Pharmacological Potentiae.International journal of pharmacy and pharmatical science; 5 (2) :33-39.

57.Stirban,A., Gawlowski,T. And Roden,M.(2014). Vascular effects of advanced glycation end products: Clinical effects and molecular mechanisms .Molecular Metabolism;3:94-108.

58. Tabatabaeia,S.R.F., Rashnob ,M. Ghaderib, S. And Askaripour, M. (2016). The Aqueous Extract of PortulacaOleracea Ameliorates Neurobehavioral Dysfunction and Hyperglycemia Related to Streptozotocin-Diabetes Induced in Ovarierctomized Rats.ShaheedBeheshti University of Medical Sciences and Health Services; 561-571.

59. Teixeira, J., Gaspar A., Garrido, E.M., Garrido, J. And Borges, F.(2013). Hydroxycinnamic acid antioxidants: an electrochemical overview. BioMed Research International;p11.

60. Oyedeji, K.O. And Bolarinwa, A.F. (2013). Effects Of Crude Extracts Of Portulaca Oleracea On Male Reproductive Functions In Albino Rats. IOSR Journal of Pharmacy and Biological Sciences. Rs;213: 218.

61. Zhisen,j.,Mengcheng,T. And Jinaming,W.(1999).The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals .Food chemistry,64(4)(,555-559

62. Zhu,H., Wang,Y., Liu.,Y ., Xia,Y., And Tang,T.(2011).Analysis of Flavonoids in Portulacaoleracea L. by UV–Vis Spectrophotometry with Comparative Study on Different Extraction Technologies . Advanced Materials Research.;3:90-97.

استخلاص نبتة البقلة وتطبيقاتها

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إشراف: د.إبراهيم الكيالي

الملخص:

نبتة البقلة تشتهر بأسماء عديدة منها الفرفحينة , الرجلة , الفرفح, و تعتبر البقلة نبات صحي بسبب خصائصها الغذائية العالية وتحتوي على نسب عالية من الاوميغا 3 والكاروتين والمغنيسيوم ومضادات الأكسدة وقد وصفت كدواء تقليدي وشعبي في الصين لعلاج انخفاض ضغط الدم والسكري االهدف من هذا البحث هو البحث عن فوائد تلك النبتة خاصة أنها استخدمت لمرض السكري سابقا ولها اثر على هذا المرض عن طريق microemulsion والحصول على اكبر region of microemulsionباستخدام Tween20 Tween80, لتوظيفها كدواء لعلاج السكري .

عينات من البقلة تم جمعها من الأسواق المحلية في منتصف ابريل 2018من فلسطين ، تم تجفيفها في الظل لمده أسبوعين حتى أصبحت جافه تماما ، تم طحنها ،ومن ثم استخراجها من قبل جهاز soxhlet. تمت دراسة النشاط المضاد للأكسدة باستخدام طريقة FRAP و حساب كميات الفينول والفلافونويد التي توجد في هذه النبتة محتوي الفينول الكلي (TPC) وإجمالي محتوي الفلافونويد (TFC). ولاحظنا أن (TPC) و (TFC) من البذور هو اعلي من (TPC) و (TFC) من الأوراق.

ثم عمل system for microemulsionالصديقة للبيئة التي يمكن استخدامها في المستحضرات الصيدلانية. وقد تم ذلك باستخدام استخراج بذور البقلة واستخدام surfactant non ionic as Tween80,Tween20 تم إنشاء خمس أنظمة ليتم المقارنة بينهم واختيار الأفضل منهم من حيث وجود منطقة اكبر من . microemulsion الذي يستخدم في كثير من الصناعات الدوائية .

ودرست تأثير هذه النبتة على البكتيريا من الأوراق والبذور على نوعين من البكتيريا pseudomonas bacteriaو bacillus bacteriaوأظهرت النتيجة استخراج من أوراق له أنشطه مضادة للبكتيريا ضد bacillus bacteria، pseudomonas bacteria ، ولكن استخراج من البذورأظهرت تأثيرا ضدbacillus bacteria فقط .

وأخيرا تم دراسة antiglycation test لمعرفة إذا كانت نبتة البقلة تعطي أثرا ضد السكري ولقد أظهرت النتائج أنها تعطي تثبيط عالي ونحن نستنتج من هذه ذلك ان لهذه النبتة تأثير على المرض السكري كما وجد في الدراسات السابقة.