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In Vitro Evaluation of Anticoagulant and Phytochemical Screening of Some Medicinal Plants in Syria

Malaz Albasha[1,*]
Abdullah Kattah[2], Imad Balid[3]

[1]*Postgraduate student (PhD) Faculty of Science, Aleppo University, Syria*
[2]*(Department of chemistry, Faculty of Science, Aleppo University, Syria)*
[3]*(Department of Laboratory Medicine, Faculty of Medicine, University of Aleppo, Syria)*

Abstract: In this study, the active components of the aerial parts, and roots of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*,plantswere extracted by using different solventsofmethanol 70%،ethanol 80%, methanol, ethanol، ethyl acetate and hexane.The extracts were used for anticoagulant assays.PT and APTT assays were carried out citrated plasma of healthy volunteer donors with different concentration of the extracts. The methanol 70% and ethanol 80% extracts of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*, prolonged the timetaken for blood clotting in all the tested methods. The activity was increasing as the concentration of extracts increased.Qualitative phytochemical screening ofcarbohydrates, comarins and tannins were observed in all plant extracts, while flavonoids and phenolic acids were found in extracts of methanol70%, ethanol 80% methanol and ethanol for aerial parts and roots of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L.*The alkaloids were found only in extracts of methanol70%, ethanol80% methanol and ethanol for the aerial parts of *Sonchus Oleraceus L***,** and their absence were observed entirely in *Melilotus Officinalis Land Anethum graveolens L,* Saponins were present only in extracts of methanol70%, ethanol80%methanol and ethanol for the aerial parts of *Melilotus Officinalis L* and their absence were observed entirely in *Sonchus Oleraceus L and Anethum graveolens L.*

Keywords:*Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*, anticoagulant, APTT, PT.

1. INTRODUCTION

Currently, the cardiovascular disease is a leading cause of death all over the world. These diseases are caused mainly due to the abnormal blood coagulation (clotting) in the arteries supplying blood to the heart.Blood clots that develop in the arteries can cause myocardial infarction. The clots disconnect blood flow to the heart [1]. The undesired blood clot interferes in the free flow of blood leading to dysfunction/permanent damage to the heart. The usual coagulation process is essential to avoid unnecessary blood loss through the injured blood vessels; but undesired blood coagulation results in several life threatening diseases. Blood clots formation in the arteries supplying blood to the heart or brain is the common cause of heart attack and stroke [2].

A number of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin and indomethacin have been used as antithrombotic agents. These drugs *in vitro* and *in vivo* cause inhibition of platelet aggregation and thromboxane formation [3].Anticoagulant drugs include; heparin and derivative substances e.g. low molecular weight heparin and coumarins (vitamin K antagonists) such as Warfarin, which acts via inhibition an enzyme vitamin K epoxide reductase, which recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. Reduced vitamin K must be regenerated from the epoxide for sustained carboxylation and synthesis of biologically competent proteins [4].

The prothrombin time test (also known as the PT test) is a useful screening procedure for the extrinsic coagulation mechanism including the common pathway. It detects deficiencies in factor II, V VII, and X. The prothrombin time test is frequently used to follow oral anticoagulant therapy that inhibit factors II, VII, IX and X.Thromboplastin activates the extrinsic coagulation system in plasma in the presence of calcium ions. The subsequent clotting time is dependent on the concentration of different factors II, V VII and X. Thus prolongation time indicates a deficiency of one or more of these factors [5,6].The normal prothrombin time is 11-15 seconds. Each prothrombin time within this range indicates that the person has normal amounts of clotting factors VII and X while prolongation in prothrombin time is considered abnormal [3].

Determination of Activated Partial Thromboplastin Time (APTT) helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII as haemophilia.The arrest of bleeding depends upon primary platelet plug formed along with the formation of a stable fibrin clot. Formation of this clot involves the sequential interaction of a series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues [5,6].

Medicinal plants have been identified and used throughout human history, this plants show widespread bioactivity with minimum side effects. In contrast to synthetic compounds, herbal products are safer and hence it is preferred for treatment of various elments[7] .Across the world, large segment population accept traditional remedial system that includes use of phytomedicines obtained from different medicinal plants drugs and cosmetics[8].

Reactive oxygen species (ROS) are free radicals naturally produced in the cells and are involved in many cellular biochemical activities such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity [9,10]. However, overproduction of free radicals or failure in endogenous antioxidant mechanisms can cause oxidative damage to biomolecules (lipids, proteins and DNA), eventually leading to many chronic diseases, such as atherosclerosis, cardiovascular diseases, cancer, diabetes mellitus, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, chronic inflammation, stroke,septic shock, aging and other degenerative diseases in humans [9,10]. Antioxidants are important substances that have the ability to protect the organism from the damage caused by the oxidative stress. Due to this ability, there is a special interest in the presence of natural antioxidants in medicinal plants that may help the organism to maintain the normal balance of ROS [11]. Plants are frequently reported as a good source of antioxidant components, such as phenolic compounds [10].

Heparin is commonly used in various surgeries. Beside the pharmaceutical properties such as myocardial infarction, inflammatory and allergic conditions, heparin shows serious side effect like hemorrhage and it is expensive [11].Therefore, it is necessity and demand of time to explore alternative anticoagulants. The plant are safer source of medicines hence, The recent study undertook the anticoagulation study of extracts and phytochemicals from Syrian Medicinal Plants such as,*Melilotus Officinalis L*, *Sonchus Oleraceus L*, *Anethum graveolens L.*

Melilotus officinalis (L.) Pallas, (*Fabaceae*) is an annual to biennial erect or decumbent plant, 30-250 cm tall. The plant is distributed in Pakistan, Kashmir, India, Tibet, Russia, China, Turkey, Syria, Middle and Southern Europe [12]. The herb has aromatic, emollient and carminative properties [13]. Traditionally it is use to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances and for the symptomatic treatment of bruises, sprains and insect bites[12], in liver diseases and as a diuretic.The aerial parts of plant are traditionally reported to use in hypertension and chronic venous insufficiency in Montenegro[14]. The dried leaves and flowers have

been used in Europe as a treatment of arthritis, bronchitis, hemorrhoids and rheumatism.The anticoagulant properties of this plant is due to the presence of sweet smelling coumarins[15].

Sonchus Oleraceus L. (*Asteraceae*)is an annual weed which can establish at any time of the year. The plant is distributed in native South Europe, West Asia, and widely spread in various Arab countries Syria, Iraq, Egypt and Sudan.It is eaten by some people as a vegetable[16].it can grow up to 1 m tall, it can be quite a competitive weed. its distinguishing features are that it has quite a succulent stem when it flowers, and the foliage ooze a milky sap when cut. Thought it is classed as a weed Sow Thistle is a medicinal plant native to Asia and Europe, The common name Sow thistle refers to its attractiveness to swine and the similarity of the leaf to the ear of a pig, while the *Oleraceus* portion of the Latin name refers to its delectable nature (*Sonchus* refers to the hollow stem)[17]. It is well known for its high content of antioxidant activity.

Anethum graveolens L. or dill, belonging to *Apiaceae (Umbelliferae*) family, is an annual aromatic herb known for culinary andmedicinal use since ancient times. It is cultivated in the most parts of Europe and the United States of America. A variant called east Indian dill orsowa (Anethum sowa Roxb.) is cultivated in India, Egypt Syria and Japan. The chemical composition of the essential oil of the two chimio types of dill and sowa differs mainly by the dillapiole content. The typical flavour of herbdill oil is due to α-phellandrene, limonene and dill ether (anethofuran). Forflavouring purposes the herb oil with low content of carvone is preferred. the dill seed oil contains a small quantity of dillapiole up to 3% when grown in tropical climate [18, 19]. In the east Indian dill (sowa) the content of in recent years the scientific literature reports pharmacological effects of dill such as antibacterial [20], antimycobacterial, antioxidant cancer chemopreventive [21]. The well-known properties of dill from the traditional medicine, such as carminative, stomachic, diuretic havebeen reported [21, 22]. The dill essential oil has hypolipidemic activity and could be a cardioprotective agent [22].

2. MATERIALS AND METHODS

2.1. Chemicals and Equipment

Chemicals:
From Merck: Aluminum Chloride,Ferric Chloride, Chloroform, Ethanol, Methanol, Ethyl Acetate, Hexane, Sodium Hydroxide, DMSO. From BDH:α Naphthol, Sulphuric Acid, Potassium Sodium Tartrate, Sodium Hydroxide, Copper Sulphate, Potassium, Lead Acetate, Ammonia Solution 30%, Dichloromethane, n-Heptane, tri-sodium citrate, bismuth nitrate, tartaric acid and potassium iodid.
Equipments: Ultrasonic Bath (Hwashin، Power Sonic 405), Rotary evaporator (Heidolph Laborata 4000, Germany), UV Detecting Chamber 254 nm, and 365 nm lamps.

2.2. Plant Material:

Fresh plants from *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*, were collected, during the period between May and June 2016, from the public gardens in Aleppo city, north Syria, where the studied plants were divided into two parts. The first section includes the aerial parts (legs, leaves and flowers). The second section includes the roots of the plant, Previous samples were dried in laboratory atmosphere by placing it in shade at 25° C for one week, powdered using mechanical grinder and kepted in airtight glass container until use.

Figure 1:*Melilotus Officinalis L*. Flowers, Leaves, Roots.

Figure 2:*Sonchus Oleraceus L*. Flowers, Leaves, Roots.

Figure 3:*Anethum graveolens L*. Flowers, Leaves, Roots.

2.3. Phytochemical Screening:

2.3.1.Extracts preparation:

Plant extractions were performed with methanol70%, ethanol 80%,methanol,ethanol,ethyl acetate and hexane. The plants were drvided into aerial parts and roots sections. Extractions were performed in glass bottles for one hour in a sonicator bath using 10 ml solvents per gram ground plant material. The extracts were filtered using Whatman No. 1 filter paper. The residual aerial parts and roots powder were re-extracted twice applying the same procedure. Finally, the combined extracts were evaporated under reduced pressure in a rotary evaporator at room temperature. All extracts were freeze-dried and stored at 40°C in the dark until testing [23,24].

2.3. 2.Carbohydrates:

Molisch's Test: 2 ml of solution was added to each of the following extracts methanol70% ethanol 80%,methanol,ethanol,ethyl acetate and hexane, for aerial parts and roots of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*,in test tubes, then a few drops of Molisch reagent (1% methanolic alpha-naphthol) was added with stirring and concentrated sulphuric acid was added into the sides of the test tube to form a layer. A violet ring indicates the presence of carbohydrates [25].

Fehling's Test: 2 ml of solution was added to each of the following extracts methanol70% ethanol 80%,methanol,ethanol,ethyl acetate and hexane, for aerial parts and roots of previous extracts in test tubes, and heated with equal amount of both of Fehling's A and B solutions in boiling water Formation of Cu_2O brick red precipitate indicates the presence of reducing sugars [25].

2.3. 3. Coumarins:

UV Test: 2 ml of solution was added to each of the following extracts methanol70%, ethanol 80%,methanol,ethanol,ethyl acetate and hexane for aerial parts and roots of *Melilotus Officinalis LSonchus Oleraceus L and Anethum graveolens L*,in test tubes, 0.5 ml of 10 % ammonia was added. The occurrence of an intense fluorescence under UV light (λ365 nm) is a positive test for the presence of coumarins and their derivatives[41]. TLC Tests: Optimal selectivity for investigated compounds was obtained on silica layers using: ethyl acetate:dichloromethane:n-heptane 40:30:30 v/v respectively as a mobile phase. It made complete separation of coumarins [39]. It was treated with 1 N NaOH solution and examined at 365 nm UV light a yellow or blue fluorescence indicates the presence of coumarins [26].

2.3. 4. Flavonoids:

Aluminum Chloride Test: 2 ml of solution was added to each of the following extracts methanol70%, ethanol 80%,methanol,ethanol,ethyl acetate and hexane, for aerial parts and roots of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*, in test tubes, with 1 ml 5% methanolic aluminum chloride in a test tube. Formation of yellow colour indicates the presence of Flavonoids [26,27].

2.3. 5.Saponins:

Froth Test: 2 ml of solution was added to each of the following extracts, methanol70%, ethanol 80%,methanol,ethanol,ethyl acetate and hexane, for aerial parts and roots of *Melilotus Officinalis LSonchus Oleraceus L and Anethum graveolens L*,in test tubes,with 5 ml of distilled boiling water, and shaken vigorously; a stable persistent froth indicates the presence of saponins [28].

Emulsion Test: 2 ml of solution was added to each of the following methanol70%, ethanol 80%,methanol,ethanol,ethyl acetate and hexane, for aerial parts and roots of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*,in test tubes, with 3 drops of olive oil and shaken vigorously; the formation of emulsion indicates the presence of saponins [27].

2.3. 6.Tannins:

Ferric chloride Test: 2 ml of solution was added to each of the following extracts methanol70% ethanol 80%, methanol, ethanol,ethyl acetate and hexane, for aerial parts and rootsof*Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*, in test tubes, and two drops ethanolic ferric chloride 1% was added. Development of dark blue green or violet colour or precipitate indicates the presence of tannins [26].

Lead acetate Test: About 0.05 g of the different dry extracts was dissolved in 10 ml d. water and filtered into a test-tube, its pH must be controlled into the range 6-8; and three drops of 9.5% lead acetate solution was added. Development of white or brown precipitate indicates the presence of tannins [27].

2.3. 7.Alkaloids:

20 ml of the previous plant extracts were evaporated to thick syrup, and added 4 ml 0.1N aqueous Sulphuric Acid and 5 ml Chloroform. Then the aqueous phase was made alkaline with ammonia and alkaloids were extracted using 5 ml chloroform followed by re-extracting them with 2 ml 0.1N aqueous Sulphuric Acid. Finally, the acid layer was added 0.25 ml of Dragendorff's, reagent (solution of potassium bismuth iodide prepared from basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodid). a precipitate of the colors orange, is considered positive [27].

2.3. 8.Phenolic Acids:

A few drops of ferric chloride solution were added to 2ml of the previous plant extracts in a test tubs, the appearance of bluish green colour indicated the presence of phenoicl acids[26].

2.4. Anti coagulant assay

2.4.1. Preparation of Pool of Plasma and Red Blood Cell (RBC) Suspension:

Blood samples were collected from healthy volunteers, using a disposable polypropylene syringe, and then anti-coagulated using 3.8% tri-sodium citrate in a polypropylene container (9 parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at 4000 × g for 15 min and plasma was separated and pooled. The freshly prepared plasma was stored at 4ºC until its use [29].

2.4.2. Prothrombin Time PT Test:

The action in extrinsic pathway was evaluated by PT test, as previously described in literature with a few modifications [29,30]. The test was carried out using commercial reagent kits (Diagnostica Stago, France). Plasma (90 µL) was mixed with 10 µL of samples (10,20,30,40µg/mL) in saline containing a DMSO concentration of 2% (v/v) and incubated at 37°C for 5 min at 37°C. Then, 200 µL of PT assay reagent (rabbit brain extract and calcium chloride) pre-warmed at 37°C for 10 min.The tube was shaken to mix the contents and it was tilted gently back and forth and the stopwatch was stopped as soon as the clot formation began. Plasma alone was used as control (absence of anticoagulant activity).Heparin 10 µg/mL (Chandra Bhaga, Pharma pvt. Ltd, Mumbai, India) was used as positive control, and DMSO 2%was used in place of the extracts for the negative control.

2.4.3. Activated Partial Thromboplastin Time APTTTest:

The action in intrinsic and common pathways was evaluated by APTT test, as previously described in literature, with a few modifications [28,30, 31]. The test was carried out, using commercial reagent kits kits (Diagnostica Stago, France). Plasma (90 µL) was mixed with 10 µL of samples (10, 20, 30, 40 µg/mL)in saline containing a DMSO concentration of 2% (v/v) and incubated at 37°Cfor 5 min at 37°C, before the addition of pre-warmed APTT reagent (rabbit brain extract and ellagic acid) and incubation at 37°C for 2 min. Pre-warmed 37°C,25 mM calcium chloride was then added .The tube was shaken to mix the contents and it was tilted gently back and forth and the stopwatch was stopped as soon as the clot formation began. Plasma alone was used as control (absence ofanticoagulant activity Heparin 10 µg/mL(Chandra Bhaga, Pharma pvt. Ltd, Mumbai, India) was used as positive control, and Normal saline water 0.9% was used in place of the extracts for the negative control.

3.RESULTS AND DISCUSSION

3.1.Phytochemical Screening Results:

Methanol70%, ethanol 80%,methanol,ethanol,ethyl Acetate and hexane extracts of *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*of aerial parts and roots, were tested forphytochemical constituents of the plants studied was investigated for the following metabolites: Carbohydrates, Coumarins, Flavonoids, Saponins, Tannins, Alkaloids and Phenolic acids.

Table 1: Phytochemical screening of the *Melilotus Officinalis L*using methanol70%, ethanol 80%, methanol, ethanol, Ethyl Acetate andhexane extracts.

***Melilotus Officinalis L* extracts**													
The Extract		**Methanol 70%**		**Ethanol 80%**		**Methanol**		**Ethanol**		**Ethyl acetate**		**Hexane**	
Part used		**A.P**	**R**	**A. P**	**R**	**A. P**	**R**	**A.P**	**R**	**A.P**	**R**	**A.P**	**R**
Carbohydrates	**Molisch's**	++	++	++	++	++	++	++	++	++	++	++	++
	Fehling's	++	++	++	++	++	++	++	++	++	++	++	++
Coumarins	**Fluorescence**	++	+	+	++	+	+	+	+	+	+	+	+
	TLC	++	+	++	+	++	+	++	+	+	+	+	+
Flavonoids	**$AlCl_3$**	++	+	++	+	++	+	++	+	±	-	-	-
Saponins	**Froth test**	+	-	+	-	+	-	+	-	-	-	-	-
	Emulsion test	+	-	+	-	+	-	+	-	-	-	-	-
Tannins	**$FeCl_3$**	++	-	++	-	+	-	+	-	+	-	+	-
	Lead acetate	++	-	++	-	+	-	+	-	+	-	+	-
Alkaloids	**Dragendorff's**	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic Acids	**Ferric chloride**	++	+	++	+	++	+	++	+	±	-	-	-

- Indicates the absence of phytochemicals, ± couldn't determine their presence,+ Indicates trace of phytochemicals, ++ Indicates the presence of phytochemicals, A.P: Aerial Parts, R: Roots.

All the extracts Showed that the carbohydrate were exist in the *M. Officinalis L*, extracts in both aerial parts and roots. All the extracts of *M. Officinalis L* contained coumarins, which indicated the presence of hydroxyl and glycoside coumarins that dissolve only in more polar solvents. The methanol 70%, ethanol 80%, methanol andethanol extracs of *M. Officinalis L* in both aerial parts and roots contained Flavonoids and phenolic acids, While their absence was observed in ethyl acetate and hexane extracts. The results of methanol70%,ethanol 80%,methanolandethanol extracts, of *M. Officinalis L* in aerial parts contained the saponins, While the absence of saponins were observed in all extracts of rootsof*M. Officinalis L*. All of the *M. Officinalis L* extracts of aerial parts contained the tanins, While The extracts of *M. Officinalis L*, of roots their result were not present. The absence of alkaloids were observed in all extracts of *M. Officinalis L*.

Table 2: Phytochemical screening of the *Sonchus Oleraceus L*using methanol70%, ethanol 80%, methanol, ethanol, Ethyl Acetate andhexane extracts.

***Sonchus Oleraceus L* extracts**												
The Extract		**Methanol 70%**		**Ethanol 80%**		**Methanol**		**Ethanol**		**Ethyl acetate**		**Hexane**	
Part used		**A. P**	**R**	**A. P**	**R**	**A. P**	**R**	**A. P**	**R**	**A. P**	**R**	**A.P**	**R**
Carbohydrates	**Molisch's**	++	++	++	++	++	++	++	++	++	++	++	++
	Fehling's	++	++	++	++	++	++	++	++	++	++	++	++
Coumarins	**Fluorescence**	+	+	+	+	+	+	+	+	+	+	+	+
	TLC	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	**$AlCl_3$**	++	+	++	+	++	+	++	+	-	-	-	-
Saponins	**Froth test**	-	-	-	-	-	-	-	-	-	-	-	-
	Emulsion test	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	**$FeCl_3$**	+	-	+	-	+	-	+	-	+	-	+	-
	Lead acetate	+	-	+	-	+	-	+	-	+	-	+	-
Alkaloids	**Dragendorff's**	+	-	+	-	+	-	+	-	-	-	-	-
Phenolic Acids	**Ferric chloride**	++	+	++	+	++	+	++	+	-	-	-	-

- Indicates the absence of phytochemicals,+ Indicates trace of phytochemicals, ++ Indicates the presence of phytochemicals, A.P: Aerial Parts, R: Roots.

All the extracts Showed that the carbohydrate were exist in the *S. Oleraceus L*, extracts in both aerial parts and roots.All the extracts of *S. Oleraceus L* contained coumarins.The methanol 70% ethanol 80%, methanol and ethanol extracs of *S. Oleraceus L* in both aerial parts and roots contained Flavonoids and phenolic acids, While their absence was observed in ethyl acetate and hexane extracts. The results of methanol70% and ethanol 80% extracts, of *S. OleraceusL* in aerial parts contained the alkaloids, While the absence of alkaloids were observed in all extracts of *S. Oleraceus L* roots. All of the *S. Oleraceus L* extracts of aerial parts contained the tanins, While The extracts of roots of *S. Oleraceus L*, their result were not present. The absence of saponins were observed in all extracts of *S. Oleraceus L*.

Table 3: Phytochemical screening of the *Anethum graveolens L*using methanol70%, ethanol 80%, methanol, ethanol, Ethyl Acetate and hexane extracts.

***Anethum graveolens L* extracts**												
The Extract		**Methanol 70%**		**Ethanol 80%**		**Methanol**		**Ethanol**		**Ethyl acetate**		**Hexane**	
Part Used		**A. P**	**R**	**A. P**	**R**	**A. P**	**R**	**A. P**	**R**	**A. P**	**R**	**A. P**	**R**
Carbohydrates	**Molisch's**	++	++	++	++	++	++	++	++	++	++	++	++
	Fehling's	++	++	++	++	++	++	++	++	++	++	++	++
Coumarins	**Fluorescence**	+	+	+	+	+	+	+	+	+	+	+	+
	TLC	+	+	+	+	+	+	+	+	+	+	+	+

Flavonoids	**$AlCl_3$**	++	+	++	+	++	+	++	+	-	-	-	-
Saponins	**Froth test**	-	-	-	-	-	-	-	-	-	-	-	-
	Emulsion test	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	**$FeCl_3$**	+	-	+	-	+	-	+	-	+	-	+	-
	Lead acetate	+	-	+	-	+	-	+	-	+	-	+	-
Alkaloids	**Dragendorff's**	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic Acids	**Ferric chloride**	++	+	++	+	++	+	++	+	-	-	-	-

- Indicates the absence of phytochemicals, + Indicates trace of phytochemicals, ++ Indicates the presence of phytochemicals, A.P: Aerial Parts, R: Roots.

All the extracts Showed that the carbohydrate were exist in the *A. graveolens L*, extracts in both aerial parts and roots.All the extracts of *A. graveolens L* contained coumarins.The methanol 70%, ethanol 80%, methanol and ethanol extracs of *A. graveolens L* in both aerial parts and roots contained flavonoids and phenolic acids, While their absence was observed in ethyl acetate and hexane extracts. All of the *A. graveolens L* extracts of aerial parts contained the tanins, While The extracts of *A. graveolens L*, of roots their result were not present. The absence of both saponins and alkaloids were observed in all extracts of *A. graveolens L*.

3.1. Prothrombin Time PT Test:

Methanol 70%،ethanol 80%, methanol, ethanol، ethyl acetate and hexaneextracts from three medicinal plants, *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*,of aerial parts and rootswere tested for blood coagulation effects in normal human plasma and found to be significantly prolonged the prothrombin time (PT) of normal human plasma.

Table 4: Effect of *Melilotus Officinalis L* Extracts on Prothrombin Time (PT) of Normal Human Plasma

Extracts of *Melilotus Officinalis L*	**Conc (µg/ml)**	**Prothrombin time (PT) (Second)**	
		Aerial parts	**Roots**
Methanol 70%	10	40.6	30.3
	20	43.3	33.6
	30	50.8	36.9
	40	54.7	42.3
Ethanol 80%	10	45.5	32.8
	20	50.4	35.3
	30	56.1	39.1
	40	65.5	44.4
Methanol	10	22.4	18.2
	20	25.7	21.7
	30	29.4	23.6
	40	32.7	26.2
Ethanol	10	22.8	19.5
	20	26.2	22.4
	30	34.7	25.6
	40	38.4	28.4
Ethyl acetate	10	17.5	16.3
	20	19.2	18.7
	30	20.2	20.5
	40	22.6	22.2

Hexane	10	15.3	14.0
	20	17.1	16.2
	30	18.4	18.8
	40	20.8	20.6
Heparin	10	>>100	
DMSO 2%	10	15	

The methanol 70%، ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts of aerial parts and roots of *M. Offecinale L* were studied for the *in vitro* anticoagulant activity using the prothrombin time assay. Observed from Table 2, the Ethanol 80% aerial parts extracts of *M. Offecinale L* showed the highest blood clotting time compared to other solvent extracts,65.5 sec, 56.1sec, 50.4sec, 45.5secat40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml concentrations respectively, Followed by a methanol 70% extracts, methanol, ethanol respectively, Whereas ethyl acetate and hexane extracts exhibited lower activity, when were comparable with polar extracts.

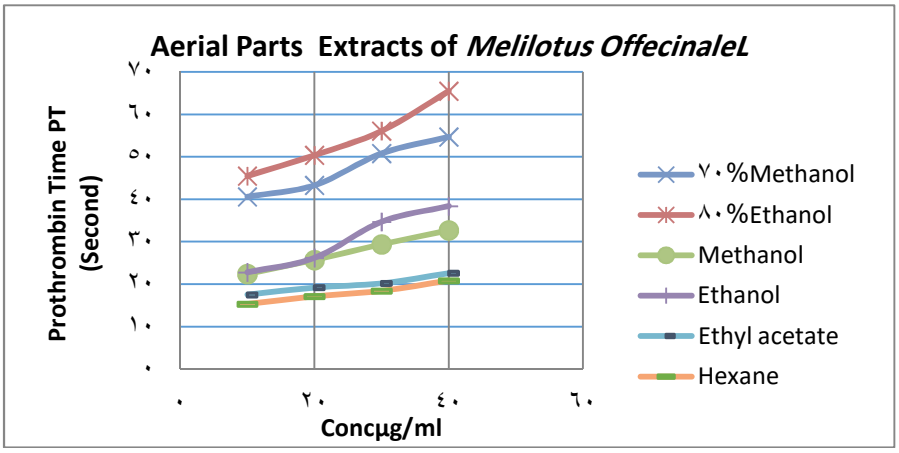

Figure 4: Effect of *Melilotus Officinalis L* Aerial Parts Extracts on Prothrombin Time (PT) of Normal Human Plasma.

Increasing concentrations of the extracts of aerial parts of *Melilotus Officinalis L* lead to increasingly prolonged of clotting time.

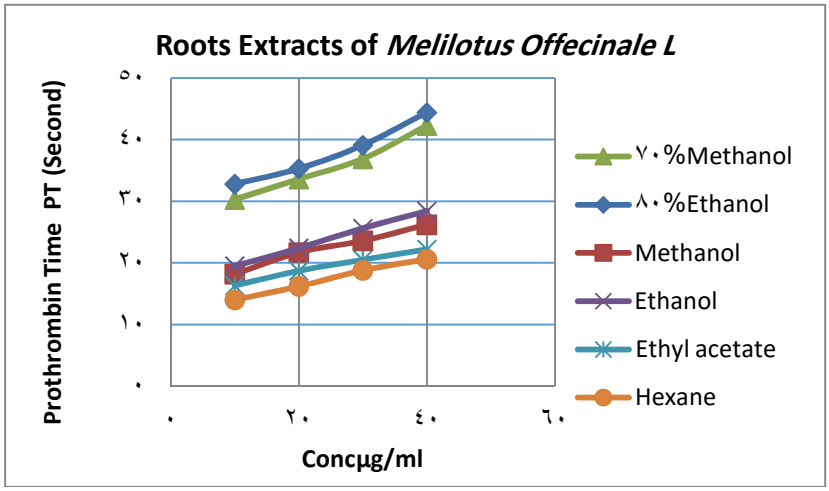

Figure 5: Effect of *Melilotus Officinalis L* Roots Extracts on Prothrombin Time (PT) of Normal Human Plasma.

Increasing concentrations of the extracts of roots of *Melilotus Officinalis L* lead to increasingly prolonged of clotting time.

Table 5: Effect of *Sonchus Oleraceus L*Extracts on Prothrombin Time (PT) of Normal Human plasma

Extracts of *Sonchus Oleraceus L*	Conc (µg/ml)	Prothrombin time (PT) (Second)	
		Aerial parts	Roots
Methanol 70%	10	33.2	28.7
	20	39.5	30.7
	30	44.8	33.4
	40	46.4	38.3
Ethanol 80%	10	32.8	26.6
	20	38.4	29.5
	30	43.2	32.6
	40	45.3	36.2
Methanol	10	16.8	16
	20	20.9	19.3
	30	23.1	21.6
	40	26.3	23.5
Ethanol	10	16.5	15.4
	20	20.4	18.4
	30	22.2	20.7
	40	24.5	22.4
Ethyl acetate	10	14.7	14.4
	20	16.6	16.3
	30	19.2	18.6
	40	21.2	19.5
Hexane	10	12.5	12.7
	20	14.9	14.4
	30	17.6	16.3
	40	19.1	17.2
Heparin	10	>>100	
DMSO 2%	10	15	

The methanol 70%، ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts of aerial parts and roots of *S. Oleraceus L* were studied for the *in vitro* anticoagulant activity using the prothrombin time assay. Observed from Table 3, the Methanol 70% aerial parts extracts of *S. Oleraceus L* showed the highest blood clotting time compared to other solvent extracts,46.4 sec, 44.8 sec, 39.5 sec, 33.2 secat40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml concentrations respectively, Followed by a ethanol 80% extracts, methanol, ethanol respectively, Whereas ethyl acetate and hexane extracts exhibited lower activity, whene were comparable with polar extracts.

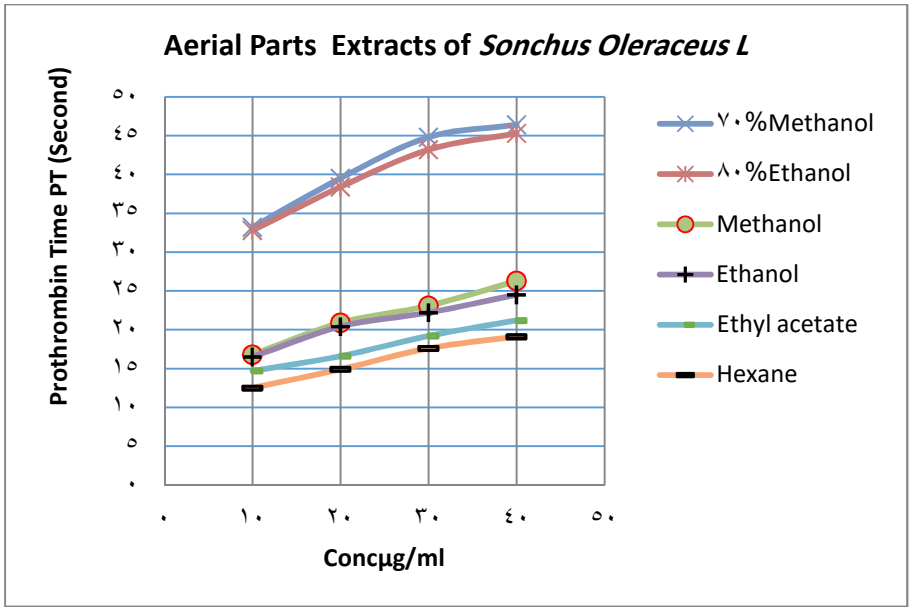

Figure 6: Effect of *Sonchus Oleraceus L* Aerial Parts Extracts on Prothrombin Time (PT) of Normal Human Plasma

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Increasing concentrations of the extracts of aerial parts of *S. Oleraceus L* lead to increasingly prolonged of clotting time.

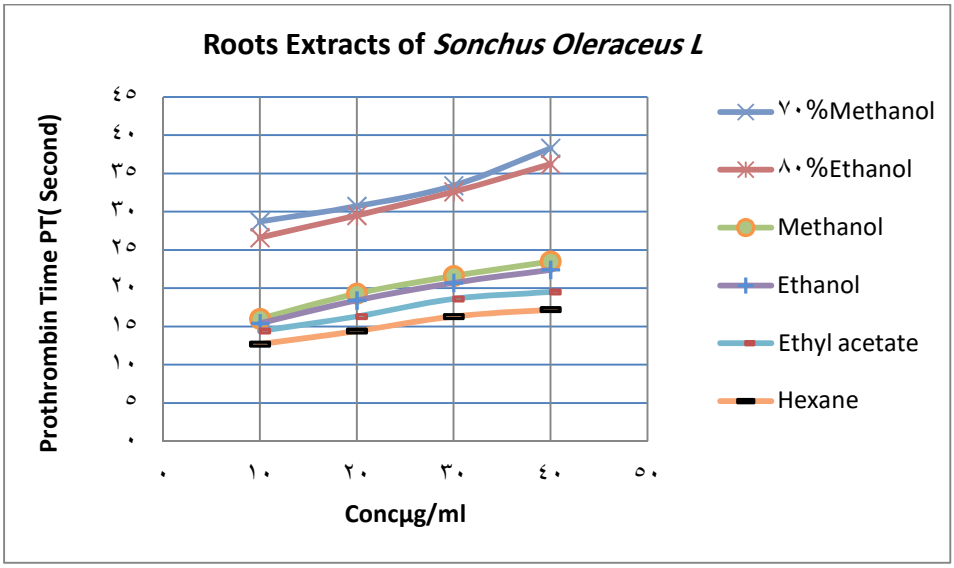

Figure 7: Effect of *Sonchus Oleraceus L* Roots Extracts on Prothrombin Time (PT) of Normal Human Plasma.

Increasing concentrations of the extracts of roots of *S. Oleraceus L* lead to increasingly prolonged of clotting time.

Table 6: Effect of *Anethum Graveolens L* Extracts on Prothrombin Time (PT) of Normal Human Plasma

Extracts of *Anethum Graveolens L*	Conc (µg/ml)	Prothrombin time (PT) (Second)	
		Aerial parts	Roots
Methanol 70%	10	32.6	26.7
	20	35.7	28.4
	30	39.1	30.3
	40	42.5	33.4
Ethanol 80%	10	35.4	29.4
	20	40.8	31.9
	30	46.4	34.8
	40	50.9	40.1
Methanol	10	16.5	14.1
	20	18.3	16.2
	30	22.3	18.8
	40	27.6	20.9
Ethanol	10	17.8	16.7
	20	21.8	19.6
	30	24.5	21.9
	40	28.4	25.8
Ethyl acetate	10	14.4	13.1
	20	16.1	15.2
	30	18.2	17.8
	40	22.7	20.9
Hexane	10	12.7	11.7
	20	14.2	13.6
	30	16.8	15.2
	40	20.4	19.1
Heparin	10	>>100	
DMSO 2%	10	15	

The methanol 70%، ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts of aerial parts and roots of *A.Graveolens L* were studied for the *in vitro* anticoagulant activity using the prothrombin time assay. Observed from Table 4, the Ethanol 80% aerial parts extracts of *A.Graveolens L* showed the highest blood clotting time compared to other solvent extracts,50.9 sec, 43.4.1sec, 40.8sec, 35.4secat40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml concentrations respectively, Followed by a methanol 70% extracts, methanol, ethanol respectively, Whereas ethyl acetate and hexane extracts exhibited lower activity, whene were comparable with polar extracts.

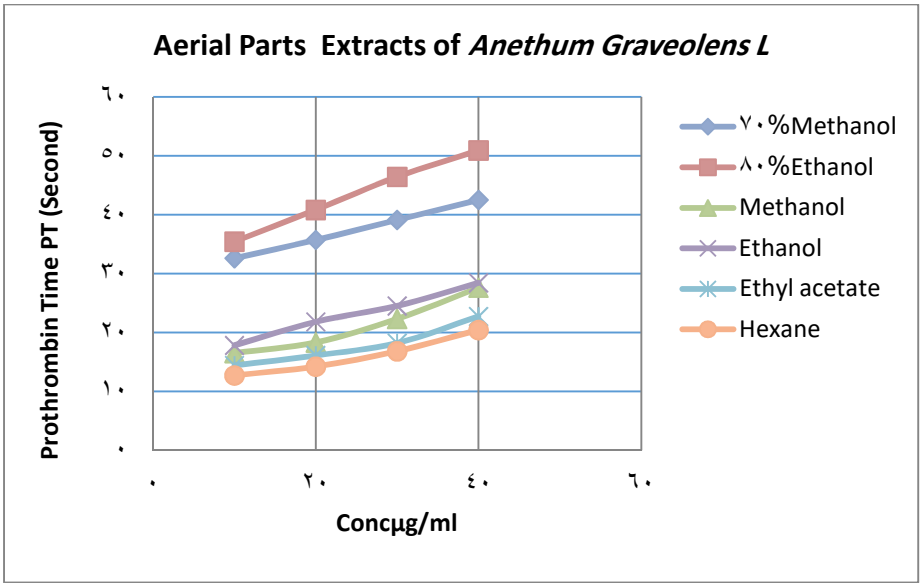

Figure 8: Effect of *Anethum Graveolens L* Aerial Parts Extracts on Prothrombin Time (PT) of Normal Human Plasma.

Increasing concentrations of the extracts of aerial parts of *A. Graveolens L* lead to increasingly prolonged of clotting time.

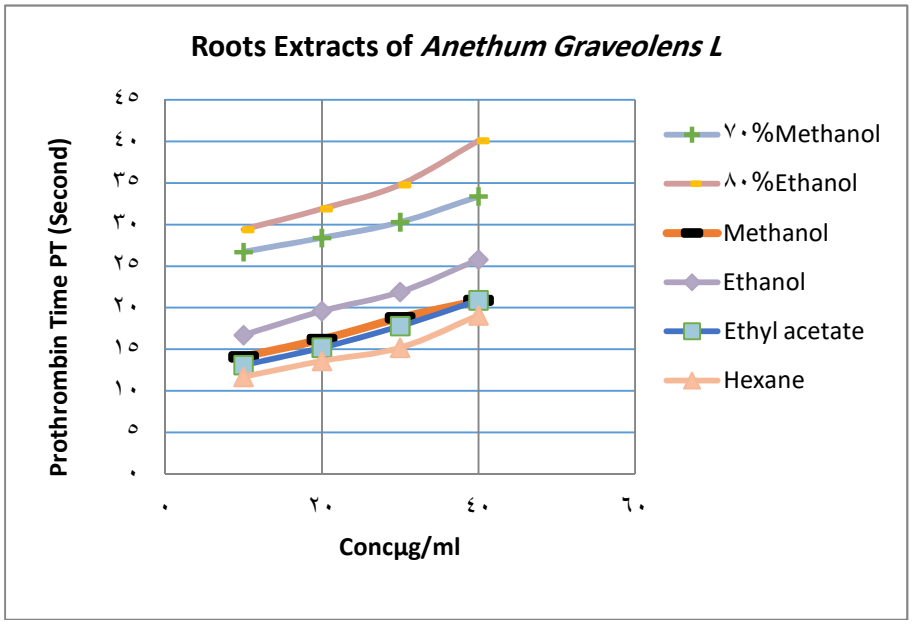

Figure 9: Effect of *Anethum Graveolens L*RootsExtracts on Prothrombin Time (PT) of Normal Human Plasma.

Increasing concentrations of the extracts of roots of *A. Graveolens L* lead to increasingly prolonged of clotting time.

3.2.Activated Partial Thromboplastin Time APTT Test:

Methanol 70%،ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts from three medicinal plants, *Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*,of aerial parts and roots, were tested for blood coagulation effects in normal human plasma and found to be significantly prolonged the activated partial thromboplastin time (APTT) of normal human plasma.

Table 7: Effect of *Melilotus Officinalis L* Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma

Extracts of *Melilotus Officinalis L*	Conc (µg/ml)	Activated Partial Thromboplastin Time APTT (Second)	
		Aerial parts	Roots
Methanol 70%	10	90.6	64.2
	20	94.3	66.5
	30	98.4	69.7
	40	102.7	72.2
Ethanol 80%	10	95.4	72.5
	20	100.1	75.7
	30	107.6	78.1
	40	115.8	80.6
Methanol	10	75.2	43.2
	20	79.7	45.4
	30	82.4	49.7
	40	84.9	52.8
Ethanol	10	79.4	48.3
	20	82.6	52.2
	30	85.8	55.4
	40	89.8	58.3
Ethyl acetate	10	45.4	33.6
	20	47.3	35.9
	30	49.7	37.3
	40	52.2	40.5
Hexane	10	42.8	30.4
	20	45.3	32.1
	30	48.1	35.7
	40	50.9	37.6
Heparin	10	>>240	
DMSO 2%	10	45	

The methanol 70%، ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts of aerial parts and rootsof*M. Offecinale L* were studied for the *in vitro* anticoagulant activity using the activated partial thromboplastin time (APTT) assay. Observed from Table 5, the Ethanol 80% aerial parts extracts of *M. Offecinale L* showed the highest blood clotting time compared to other solvent extracts,115.8 sec, 107.6 sec, 100.1.4sec, 95.4 secat40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml concentrations respectively Followed by a methanol 70% extracts, methanol, ethanol respectively, Whereas ethyl acetate and hexane extracts exhibited lower activity, when were comparable with polar extracts

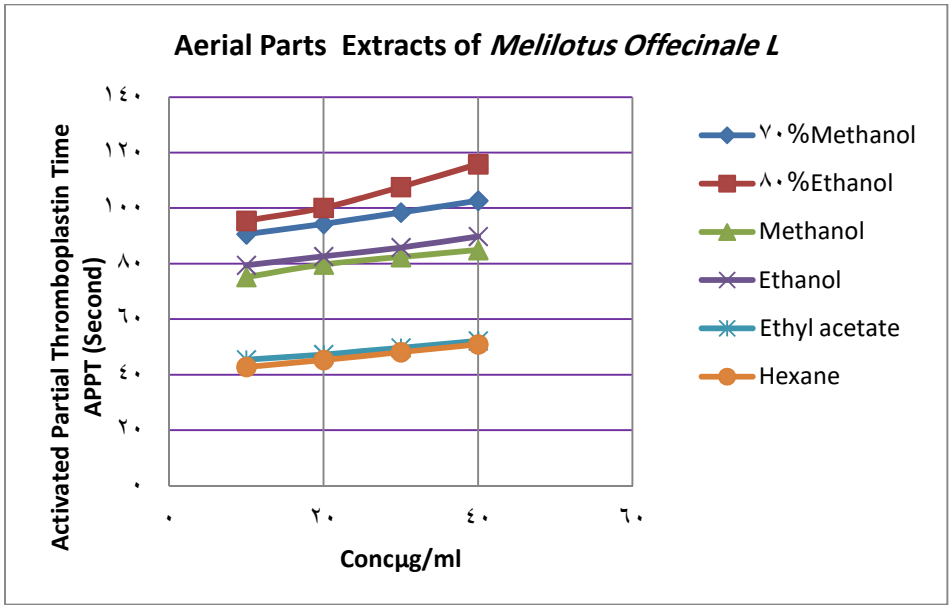

Figure 10: Effect of *Melilotus Officinalis L* Aerial Parts Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma.

Increasing concentrations of the extracts of aerial parts of *Melilotus Officinalis L* lead to increasingly prolonged of clotting time.

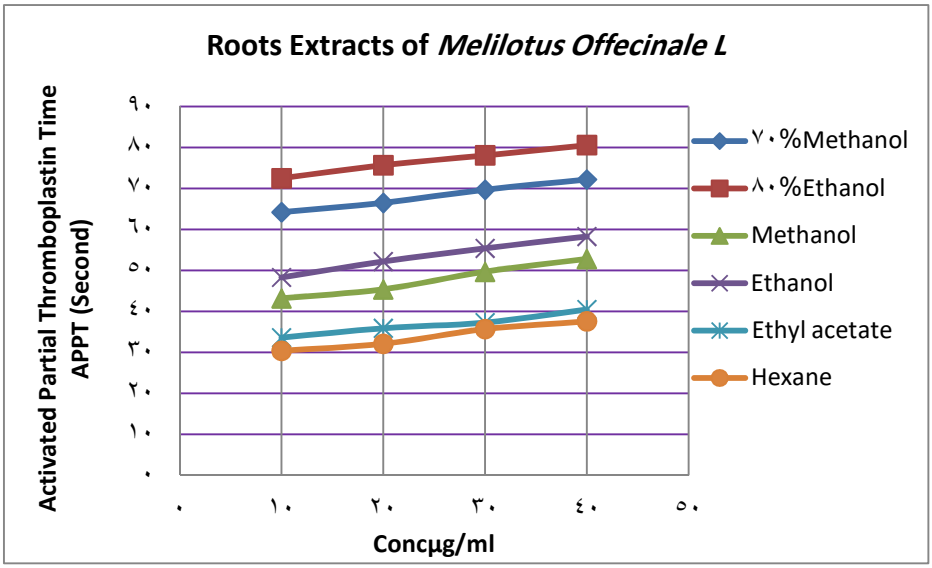

Figure 11: Effect of *Melilotus Officinalis L* Roots Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma.

Increasing concentrations of the extracts of roots of *Melilotus Officinalis L*lead to increasingly prolonged of clotting time.

Table 8: Effect of *Sonchus Oleraceus L*Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma

Extracts of *Sonchus Oleraceus L*	Conc (µg/ml)	Activated Partial Thromboplastin Time APTT (Second)	
		Aerial parts	Roots
Methanol 70%	10	85.4	61.8
	20	89.5	64.4
	30	93.3	68.3
	40	99.4	71.4
Ethanol 80%	10	81.7	55.4
	20	83.3	57.8
	30	86.9	60.9
	40	89.6	63.4
Methanol	10	65.2	37.4
	20	68.6	40.6
	30	72.3	43.5
	40	74.8	45.8
Ethanol	10	69.5	42.7
	20	72.9	44.1
	30	75.4	47.3
	40	77.7	50.2
Ethyl acetate	10	39.8	28.4
	20	41.9	31.2
	30	43.3	33.6
	40	45.4	35.9
Hexane	10	35.5	24.8
	20	37.8	27.7
	30	39.6	29.7
	40	41.2	31.3
Heparin	10	>>240	
DMSO 2%	10	45	

The methanol 70%،ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts of aerial parts and rootsof*S. Oleraceus L*were studied for the *in vitro* anticoagulantactivity using the activated partial thromboplastin time (APTT) assay.Observed from Table 6, theMethanol70% aerial parts extracts of *S. Oleraceus L* showed the highest blood clotting time compared to other solvent extracts,100.6 sec, 97.1 sec, 39.5 sec, 93.7 sec, 87.3secat40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml concentrations respectively,Followed by a ethanol 80% extracts, methanol, ethanol respectively, Whereasethyl acetate and hexane extracts exhibited lower activity,whene were comparable with polar extracts.

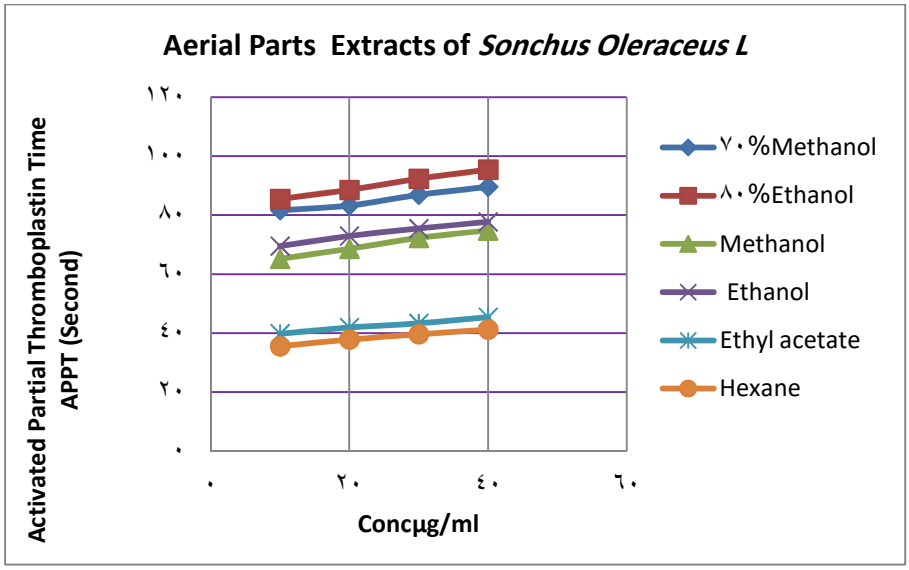

Figure 12: Effect of Aerial Parts *Sonchus Oleraceus L* Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma.

Increasing concentrations of the extracts of aerial parts of *S. Oleraceus L*lead to increasingly prolonged of clotting time.

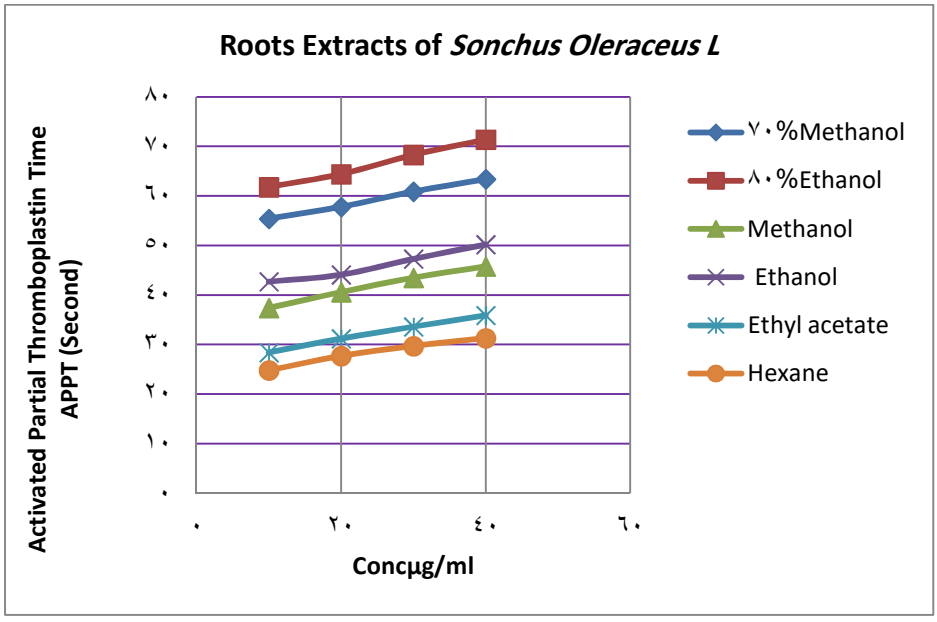

Figure 13: Effect of Roots *Sonchus Oleraceus L* Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma.

Increasing concentrations of the extracts of roots of *S. Oleraceus L*lead to increasingly prolonged of clotting time.

Table 9: Effect of *Anethum Graveolens L*Extracts on Activated Partial Thromboplastin Time APTT of normal human plasma

Extracts of *Anethum Graveolens L*	Conc (µg/ml)	Activated Partial Thromboplastin Time APTT (Second)	
		Aerial parts	Roots
Methanol 70%	10	84.8	60.5
	20	88.4	62.1
	30	92.9	64.3
	40	98.2	66.7
Ethanol 80%	10	87.3	64.2
	20	93.7	68.4
	30	97.1	72.9
	40	100.6	78.5
Methanol	10	72.5	44.4
	20	75.8	47.9
	30	78.3	51.2
	40	80.7	54.1
Ethanol	10	70.3	40.3
	20	72.4	42.7
	30	75.7	44.6
	40	78.8	48.9
Ethyl acetate	10	41.5	30.2
	20	43.8	32.9
	30	46.1	34.3
	40	48.3	37.7
Hexane	10	38.5	28.5
	20	40.7	30.4
	30	42.1	32.3
	40	44.3	34.4
Heparin	10	>>240	
DMSO 2%	10	45	

The methanol 70%،ethanol 80%, methanol, ethanol، ethyl acetate and hexane extracts of aerial parts and roots of *A.Graveolens L* were studied for the *in vitro* anticoagulantusing the activated partial thromboplastin time (APTT) assay. Observed from Table 7, theEthanol 80% aerial parts extracts of *A.Graveolens L* showed the highest blood clotting time compared to other solvent extracts,95.4 sec, 92.3 sec, 88.5sec, 85.4secat40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml concentrations respectively Followed by a methanol 70% extracts, methanol, ethanol respectively, Where asethyl acetate and hexane extracts exhibited lower ,whene were comparable with polar extracts.

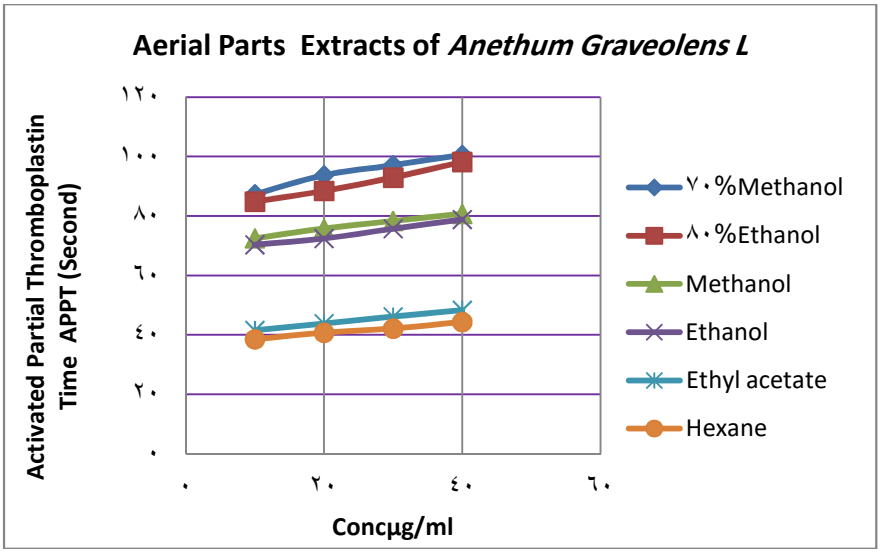

Figure 14: Effect of Aerial Parts *Anethum Graveolens L* Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma.

Increasing concentrations of the extracts of aerial parts of *A. Graveolens L*lead to increasingly prolonged of clotting time.

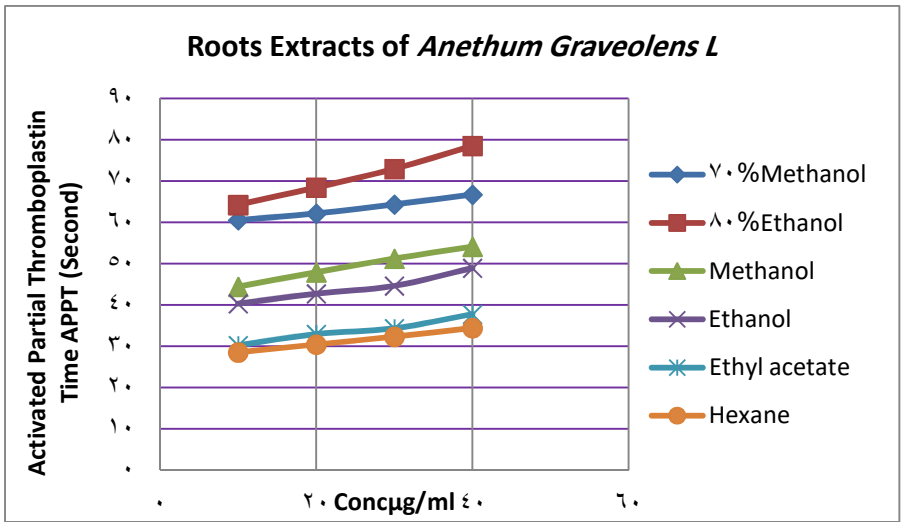

Figure 15: Effect of Roots *Anethum Graveolens L* Extracts on Activated Partial Thromboplastin Time APTT of Normal Human Plasma.

Increasing concentrations of the extracts of roots of *A. Graveolens L*lead to increasingly prolonged of clotting time.

CONCLUSION

The results of this study indicated that plants of*Melilotus Officinalis L*, *Sonchus Oleraceus L and Anethum graveolens L*are richwithcarbohydrates, coumarins, flavonoids, tannins and phenolic acids.Ethanol 80% and methanol70% extracts of *Melilotus Officinalis L* of aerial parts and roots showed the highest blood clotting time comparablewith*Sonchus Oleraceus L and Anethum graveolens*

L in APTT and PT assays.The intrinsic and extrinsic anticoagulant activity may be due to the presence of coumarins. The coumarins are known as strong anticoagulant agents[31,32].

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