Iraqi Journal of Biotechnology, May 2019, Vol. 18, No. 2, 77-87

Extraction and Evaluation the Activity of *Urtica dioica* as Bleeding Stop Material

Hashim M. Zehraw , Zynab Muotaz , Muamer Taleb , Bydaa Mukhlif , Saad Abed Al-Jabbar

Ibn Al-Betar Research Center / Corporation of Research and Industrial Development / Ministry of Industry and Minerals / Al-Jadiriah / Baghdad / Iraq.

Received: February 17, 2019 / **Accepted:** April 7, 2019

Abstract: *Urtica dioica* From the medicinal herbs group, with many uses, the extract of the plant was prepared in order to study effect on the process of stopping hemorrhage of various pathological causes, collection of plants in the pre-blooming phase of the Jadiriyah area, Baghdad, Iraq. Prepare a sample of it by way of alcohol extraction for all parts of the plant. The substances and active groups were identified using chemical analysis. The results showed the presence of the necessary phytol in the blood clotting process as well as the glycosides, tannins, proteins, flavonoids and others. Some antioxidant, anti-inflammatory and anticancer compounds were found using gas chromatography technique. High-performance liquid chromatography was used as the important morphine was identified in the manufacture of a number of vitamins. The trace sample showed a high concentration of sodium and iron and a low concentration of zinc. The tested sample passed the toxicity test carried out on laboratory animals and proved its non toxic in the doses used. The results of the treatment of blood hemorrhage in mice found that the plant extract had an effect on reducing the duration of bleeding cessation to 1.14 seconds compared to the non-treated group (3.21) seconds, about 60% less, the hemoglobin level in rabbits was reduced to a significant level ($P < 0.01$) to 13.2 g / dl compared with the control (14.5) g / dl and decreased packed cell volume (PCV) to 41 ml compared to 46 ml with control. It is noted that the number of white blood cells decreased with a significant value ($P < 0.01$) to 5900×10^3 µl compared with control 8200×10^3 µl and found a decrease ($P < 0.01$) at the time of bleeding by 50% after treatment with the extract and reduced the coagulation time by one third and with a significant value $P < 0.01$).

Keywords: *Urtica dioica*, Hemorrhage, phytol, Morin.

Corresponding author: (Email: hzehraw@gmail.com).

Introduction:

Plants of the ages have been used as the main source of medicine, whether for therapeutic purposes or as industrial products. Among these plants is the plant of stinging or nettle scientifically known as *Urtica dioica*, which is a perenial floral plant and belongs to the Urticaceae family. In Iraq there are three species of *Urtica dioica*; The first type is located in northern and central Iraq and can be cultivated during the month of October to March (1). It is considered to be one of the most widely used medicinal herbs in the world. It has many uses, including but not limited to the use of drenched leaves as a treatment for various diseases including diabetes, dermatological dermatitis, and internal / external bleeding (2). Because nettle leaves contain a high amount of vitamin K (also known as blood clotting), they are used to stop nose bleeds and various types of wounds and during surgery to control bleeding hemorrhage and to treat hemorrhage during pregnancy. The nettle is rich in

many different active substances such as chlorophyll, so it is used to treat anemia, as well as its used in food coloring. It also contains vitamins such as vitamin C, vitamin B, panthotene acid, carotene, Tannin, essential oils and proteins where the leaves contain 21-23% crude protein and 9-21% raw fiber as well as minerals (iron, copper, manganese, nickel, potassium) (3). Some studies have shown the presence of acetylcholine and histamine on the filaments of the nettle leaves (2) while the leg and roots found flavonoids (4,5). On the other hand, studies conducted on animals have shown that the extract of nettle leaves can prevent blood cotting during thrombocyt-openia it can lower cholesterol levels as well as stimulate liver function (6-8).

Durak explained that the extract has effectively and accurately inhibited the effect of adenosine deaminase in prostate tissue for patients with prostate cancer (9). Some researchers also confirmed that the extract of nettle roots showed a counter-effect to multiply prostate cancer cells in humans (10). The addition of dry powder from nettle to chicken diet led to an important increase in egg production, which proved its effect in modifying and stimulating the immune system factors, (11) and found an effect in lowering total cholesterol and triglycerides concentration (12).

Several studies have been published on the possibility of extracting nettle as an antioxidant, antimicrobial, antiulcer, and analgesic activity (13). It is worth mentioning that this plant is a major and important use in conjunction with some other plants such as thyme, licorice and grape vine, which is known as the commercial level named Ankaferd blood stopper (ABS), which plays an important role in the blood clotting haemostatic agent and as an impact factor on Epithelial cells, blood cells, angiogenesis, cellular proliferation, dynamics, as well as activation of cell mediators (14,15). Studies and research are ongoing on key mechanical knowledge in the work of this product as a thrombolytic substance that is not known exact (16). From this point of view, the aim of this study was to arrive at the preparation of a extract from the plant and its description and knowledge of its basic compounds which have an effect on the process of stopping bleeding of various kinds and on the process of blood clotting using laboratory animals.

Material and Methods:

(1) Plant material:

1- Plant collection and classification: - The plant was collected in April 2015 and during the pre - flowering phase of the gardens of AL-Jadiriyah area Baghdad and the plant was classified at the University of Baghdad / Faculty of Science / the herbivores department of biology sciences under the supervision of Dr. Ali Al- Musawi.
2- Dry the plant at room temperature for two weeks to get rid of moisture and to maintain as much as possible the highest proportion of oils and active substances and avoid volatilization
3- After drying the plant, the contents of the plant (roots, leaves and stems) were crushed by the ceramic mortar until it became a fine powder, then stored in a dark plastic container at room temperature until extraction.

(2) Extraction:

(1-2) The method below was adopted with some modification in the extraction process(17):

1- Use the alcohol extraction method by mixing the prepared plant powder in the previous step with methanol alcohol by 1: 10. Weight (50 g) of plant powder was placed in a 1000 ml glass flask and then mixed with alcohol in 500 ml With 70% concentration.
2- Incubated in the Shaker Incubator at 150 cycles / minute and at room temperature for one hour.
3- Use the cooled centrifuge to filter the model at 4000 cycles / minute at 4 c^o for 20 minutes.
4- Neglected the deposit and the collection of leachate after it was filtered again using filter paper type Wattman No. (1)
5- The filtrate was concentrated using rotary evaporator and 40 c^o for 2 hours.
6- Collect the concentration form and spread in a metal plate and put in oven with temperature 40 c^o until the dry form.
7- The dry model was collected and grinded using ceramic mortar until it became a fine powder and placed in a dark container at room temperature until the required tests.

(2-2) Prepare a second model consisting only of leaves of the plant for the purpose of comparing its components with the model of the alcoholic extract recorded in paragraph (1-2) where after drying at room temperature for two weeks was grinded by ceramic mortar and kept inside container dark at room temperature until tests are conducted.

(3) Chemical tests:

For the purpose of identifying the main effective chemical groups in the plant, the following tests were performed:

1- Detection of proteins: Use the Bayoret solution to detect the presence of proteins, where mix one ml 10% of the reagent with water copper sulphate 80%. The appearance of violet indicates the presence of protein.
2- Detection of tannins: Added (1) ml of water lead acetate at the concentration of 1% to (1) ml of the extract. The result is positive when a yellow deposit appears.
3- Detection of the glycosides: Fehling solution used for this purpose and Appearance of the red deposit indicated presence of the glycosides.
4- Detection of phenols: mixed (0.1) grams of the extract in (1) ml distilled water and then added a drop of solution ferric chloride, the appearance of green or blue indicates the presence of phenols.
5- Flavonoids detection: 1 ml of alcoholic potassium hydroxide with 5 Normality to 1 ml of extract. The presence of a yellow deposit indicates the presence of flavonoids.
6- Detection of saponine: Attended by adding (1) ml of mercury chloride reagent at the concentration of (5%) to (1) ml of the extract, and when the white deposit is positive result and indicate the presence of saponine.
7- Detection of alkaloids: - using wagners reagent by adding several drops of reagent to (1) ml of the extract and appearance of the deposit brown indicated the result positive.
8- Resins test: (1) ml of lead acetate (1%) added to (1) ml of the extract. When white precipitation is found, the result is positive and indicates the presence of resins.

(4) Diagnosis of ingredients of the extract:

Most of the important components of the two models were diagnosed using GC mass, using (Agilent 5977 MSD system) Column type Hp5 ms ultra inert (30 × 0.25µm ID × 0.25 µm df) and flow rate 1.2784 ml / min by pressure (11.2) psi, injection of the 2-microliter column from the model and in the injection method of Splitless type, AuxHeaters 275, Rang Mass 50-600. Used the helium gas (99.9%), the injection temperature 250 c^{o}, the oven temperature is set at 60 c^{o}(3) minutes, up to 280 c^{o} for10 minutes at 8c^{o} / min, total time (40) minutes.

(5) Diagnosis of phenols using HPLC:

The test was conducted according to (18) as follows:

(0.5) g of crushed ground sample was solved in 5 ml distilled hot water for 2 hours, then transfer to ultrasonic bath for 20 minutes to ensure that all extract is dissolved under 60 c^{o}. Then, the extract was filtered using filtration papers No. (1), and 0.5 mL was taken in preparation for the HPLC analysis. In this system, reverse phase C-18 and column specifications (50 × 4.6 mm ID), particle size 3 µm, mobile phase 0.1% acetic acid-methanol (40: 60 size / volume), flow rate 1.2 Ml / min. The UV Detector was used at wavelength (275) nm.

(6) Determination of Trace Element Level:

(1) g of dry plant leaves mixed with 2 ml of hydrogen peroxide and 8 mL nitric acid in a glass flask then digest the mixture in microwave oven according to the following data:

Heat c^{o}	Power (Wat)	Tim (min)
100	1000	15
120	1000	15
150	1000	15

After that, it is allowed to cool down to the second day, after which it is filtered and supplemented to (200) ml of distilled water and measured the level of the elements by the Atomic Absorption Spectrophotometer.

(7) Toxicity Test:

The toxicity test was conducted in accordance with the OECD Guidelines for the Testing of Chemicals (19),in Al-Razi Center for Research and Production of Medical Diagnostic / Department of Animal Welfare and Care. The albino-white mice were used for this purpose and provided the recommended conditions according to the above source, where the animal house temperature was 22 c^{o} and relative humidity was 30% and provided light for 12 hours / day with food and water. Average weight 25 grams of non-pregnant females. The animals were prevented from eating for 3 hours before giving doses according to the following:

1- The first dose (50) mg / kg of animal weight, the number of animals tested 3.

2- Second dose: (300) mg / kg of animal weight, number of animals tested (3).
3- Third dose: (2000) mg / kg of animal weight, number of animals tested (3)

Each dose was dissolved in 1 ml of distilled water and injected into the intraperitoneal. The observations, signs and abnormal symptoms were then recorded during 4 hours and then to the next day.

(8) Hematological Test:

(8-1) Test the treatment of hemorrhage in mice:

The mice were divided into two groups: the first consisted of three mice as a control group, which caused hemorrhage in the tail area by cutting it by scissors and at a distance (3) cm from the end of the tail and leave without treatment until the bleeding stops and record the time required. The second group also consists of three mice with the same specifications of the first group except using 0.5 mg of extract sample mixed with (1) ml of distilled water. The cotton dipping was treated with a prepared extract and then placed on the tail area which was cut until the bleeding stopped and the time was recorded.

(8-2) Blood tests in rabbits:

This test was conducted at the Al-Razi Center inside the Animal House using two sets of white New Zealand rabbits. Each group has three rabbits weighing (1-2) kilograms and one to two years old, male and female. Equipped with water and normal green food, temperature (20 c^o-25 c^o). Group 1: Mix the ground dried form with food by 5 g / kg / day for 2 weeks. Group 2: leaving without treatment (control agent). The animals are monitored during this period and observations are recorded from the body temperature, Heart beat and after the time required for the test, blood samples were taken by direct withdrawal from the heart and placed in test tubes containing an anticoagulant (EDTA) in preparation for the next test i.e. Hematological tests: red blood cells (RBCs), white blood cells (WBCs), hemoglobin ratio, Packet Cell Volume PCV, Bleeding time, Clotting time).

Results and Discussion:

The alcohol extraction method is preferred because it is the best in extracting many active substances but it may affect the nature of some other substances such as fatty acids and others. Warm extraction may also lead to the loss of the effectiveness of some substances that are affected by heat such as proteins and minerals such as calcium, magnesium and vitamins (especially vitamin K) which is important in the process of blood clotting, so this method was excluded. In addition, the method of extraction of cold water was not chosen because some of the necessary substances for the process of stopping bleeding is not water-soluble, such as vitamin K, Fatty oils that do not dissolve in water are distinct from water-soluble vitamins, which is why alcohol extraction is chosen. The results of the chemical tests of the alcohol extract plant showed the presence of (glycosides, tannins, proteins, flavonoids and various phenolic compounds, alkaloids, saponins), while free of resins and steroid (Table 1). This result agree with result of (20).

Table (1): Chemical compounds of the extract of the plant extract *Urtica dioica*

No.	Compounds	Indicator	Notes	Result
1	Toasts	Lead acetate 1%	-	+
2	glycosides	Fehling test	Red deposit	+
3	phenols	1% water solution of ferric chloride	Green color	+
4	Risen	Ethanol + boiling	Lack of turbidity	-
5	flavonoids	Alcoholic potassium hydroxide	Yalow color	+
6	saponine	Mercury chloride	White color	+
7	alkaloid	Wackner detector	Brown deposit	+
8	proteins	Bayoret detector	Purple color	+

The results of the analysis of the gas chromatography of the alcohol extract appearance of several chemical compounds of medical and therapeutic importance, such as antioxidants, anti-inflammatory, anti-carcinogenic anti-aging, (Table 2).

Table (2): Phytochemical compounds of the extract of methanol for *Urtica dioica* using gas chromatography method.

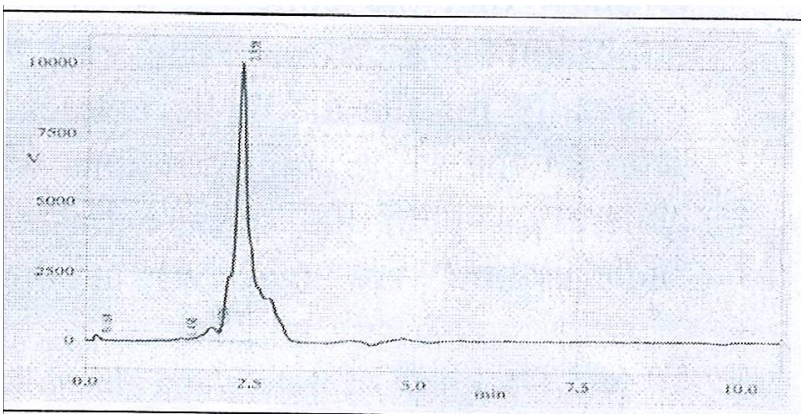
Nature of the origin of the composite	GC-MS Probability%	Name of compound
Palmitic acid	99	Hexadecanoic acid, methyl ester
Stearic acid	99	9-Octadecenoic acid,mthyl ester
Phenolic compound	96	Phenol,2,2-methylenebis 6-1,1-dimethylethyl)-4-methyl
Phenolic derivative	90	Diisooctyl phthalate
Fatty acid Derivative	47	2-Myristynoyl-glycinamide
-	99	Squalene
-	91	Methyl stearate
Alcan hydrocarbon	87	Undecane
-	97	2,6-Diisopropylnaphthalene
Fatty acid	90	Tetradecanoic acid

The result of the examination of the second model recorded in paragraph (2-2) with the gas chromatography showed that there are many different chemicals shown in (Table 3). Important chemicals include Phytol, which can play an important role as a precursor for the manufacture of vitamin E and vitamin K1, essential for blood clottining.

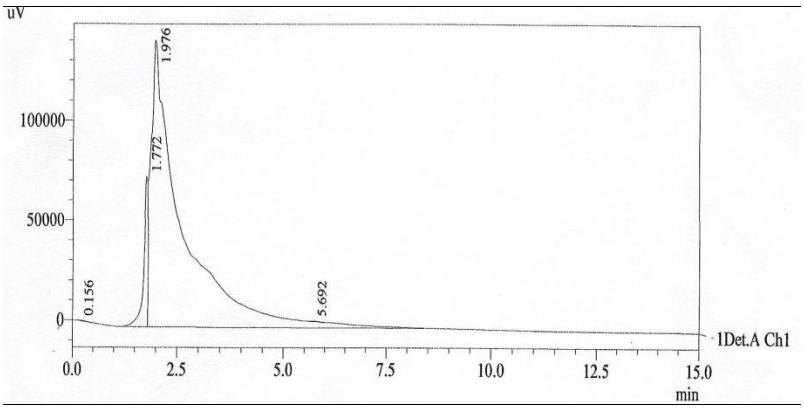
Table (3): Phytochemical compounds of leaves nettle grinded using gas chromatography.

Nature of the origin of the composite	GC-MS Probability%	Name of compound
Diterpene alcohol	92	Phytol
-	93	1-Heneicosanol
Aromatic dicarboxylic acid	90	Phthalic acid, di(2-propylpentyl) Ester
Alkan	91	Tritetracontane
-	91	Octacosan
Fatty acid	78	Olic acid
Phenolic derivative	14	Diisooctyl phthalate
Higher alkans	90	Pentacosan
Saturated fatty acid	98	n-Hexadecanoic acid

(Figure 1) shows the high-performance HPLC of the standard morin and the sample of nettle extract. Morin was identified in the prepared extract according to the retention time obtained from the standard model under similar conditions, where detected in 2.5 and 2.9 minute. Moraine is a substance that comes under the class of flavonol (a task that enters the process of manufacturing a number of vitamins and plays an important role in the protection of chemicals (chemoprevention), cancer treatment, Systems of anti-oxidation protection (21,22).



(A)



(B)

Figure (1): High Performance Liquid Chromatography (HPLC) A- Standard Morin (20). B - Sample of leaves nettle extraction

The concentration of trace elements in leaves nettle is shown in (Table 4), which shows a high concentration of sodium, iron, zinc and there is no lead or copper elements. This result is similar to what Farhan *et al* (20) found in some element concentrations as sodium 34ppm As compared to the absence of the elements of lead and iron, while differed in some other elements such as iron and zinc 27pmm and 8.2pmm respectively. This may be due to several reasons, including the time for the plant picking. Some studies have confirmed that the content of the young leaves of the elements and phenolic materials are higher than the mature leaves in addition the Preparation way and analysis may be different.

Table (4): Concentration of trace elements in leaves of *Urtica dioica*.

Element	Symbols	Concentration pmm
Sodium	Na	27.9
Iron	Fe	2.1
Zink	Zn	.35
Lead	Pb	nil
Copper	Cu	nil

The toxicity test carried out on laboratory animals proved that no abnormal symptoms were recorded in the animals after they were doses of the extract prepared by three concentrations during the first four hours of the test and there were no losses after 24 hours . It is known that the leaves of the nettle have some substances such as alkaloids with a toxic effect, as some studies have shown (23-25). This effect was not shown for the alcohol extract because alkaloids have a higher concentration in dry leaves that are not exposed to any treatment while their concentration is less when extracted with alcohol.

As for the treatment of blood hemorrhage in mice, paragraph (1-8), as shown in (Table 5), the prepared plant extract has an effect on the cessation of blood hemorrhage in mice less than the non-treated group. In the first group it took (1.14) seconds while it took (3.21) in the control group, which is about 60% less.

Table (5): Effect of plant extracts on coagulation time in mice.

	Coagulation time (sec)	Average
The first group Control without treatment (Three replicates)	3.25	3.21
	3.28	
	3.10	
the second group Plant Extract (Three replicates)	1.10	1.14
	1.17	
	1.15	

As for the results of blood tests conducted on rabbits (paragraph 8-2), it was found that the value of hemoglobin decreased with a significant value at the level of P <0.01) after two weeks of treatment with plant extract to 13.2 g\dl compared to non-treated control 14.5 g\dl . The level of packet cell volume was decreased at a significant level (P <0.02) after treatment to 41 compared to control 46. It was also observed that the number of white blood cells was decreased at a significant level (P <0.01) compared to control, (Table 6). These results are consistent with the results of (25) but differ on the other hand in measuring the time of bleeding and coagulation time). In this study, there was a significant decrease (P <0.01) in bleeding time by 50%. The extract also reduced the coagulation time by about one-third and at a significant level (P <0.01) as shown in (Table 6), This is due perhaps to the difference in the method of nutrition used by researchers for rabbits, where the use of soft paper in the first stage and dry papers in the second phase. Researchers have explained the increase in time to the presence of Dicoumarol, which interferes with vitamin K in the gut of rabbits, while in this study the alcohol extract was used in the feeding of rabbits and did not show this material and therefore vitamin K perform its functions without any overlap and worked to reduce the time of bleeding and time of coagulation.

Table (6): Hemoglobin blood tests, cell size, white blood cells, time of hemorrhage and coagulation time after treatment with plant extract.

P value	week			Type of test
	2	0		
0.01%	13.2	14.6	Treatment	Hemoglobin ratio g / dl
	14.6	14.5	control	
0.02%	41	46	Treatment	Packet Cell Volume PCV
	46	46	control	
0.01%	5900	8400	Treatment	Total W.B.C µl /x10^3
	8200	8400	control	
0.01%	1.9	3.2	Treatment	Bleeding Time (min)
	2.1	3.5	control	
0.01%	4.3	6.2	treatment	Clotting Time (min)
	6.6	6.2	control	

The nettles extract induces the formation of an encapsulated complex protein web with vital erythroid aggregation covering the entire physiological hemostatic process). This induced depends primarily on the interactions between nettle extract and blood proteins, particularly with fibrinogen-gamma. This aggregation and adhesion occurs by binding with receptors of Spectrin and Ankryin on the erythrocyte membranes (21). Some studies have also shown that a mixture of plants, including nettle, stimulated the formation of a network of proteins *in vitro* for separate samples of whole blood, plasma and serum, which formed clotting at a record time. Red blood cells are affected by a number of factors, such as the dynamics of microvascular flow. Some plant extracts can affect the rheological characteristics of the red blood cells. They also affect the penetration of their membranes by altering the interference and interaction of the lipoprotein layer of the membranes. A close relationship between the concentration of proteins and the aggregation of erythrocytes (23).

Conclusions:

1- The alcoholic extract of the nettle plant consider non-toxic and safer than soft or dry leaves that are used without any treatment.
2- Alcohol extract can be used as substance for bleeding and help to reduce the duration of coagulation and at the same time can be used in contrast (dry leaves) according to the method of extraction of active substances from the plant.
3- Active substances are more concentrated in young leaves than in mature leaves and before the process of flowering. Therefore, based on the above, we recommend:
   a- Not to use the leaves of the plant as soon as possible without any treatment due its containing concentrated toxic substances and resort to at least thermal or alcoholic treatment to avoid toxic effects.
   b- Employment leaves of nettle with some plants that similar effects in the act as effective ingredients to produce active mixture lead to stopping blood bleeding and reduces the time of coagulation

c- Studying the other effects of the plant on the red blood cells, platelets and protein factors that affect the blood clotting process and the rest other cells with knowledge of the mechanism work of Physiology plant.

References:

1. AL-Rawy, Ali. (1986). Medicinal plants in Iraq. Conference of medicinal plants in the Arab world and the prospects of its development (24-28) November, Baghdad.
2. Tuakov, J. (1997). Lecenje biljem, Rad, Beograd, 405.
3. Stankovic,M. (1995). Cajne mesavine posebne namene, Tehnoloski fakultet u Leskovent, Leskovac, 39.
4. Akbay, P.; Basaran, A.; Undeger, U. and Basaran, N. (2003). In vetro immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. *Phytother Res*., 17(1) :34-37.
5. Chaturvedi, S.K. (2001). A new flavones from *Urtica dioica* roots. *Acta Cienc. Indica. Chem*., 27: 17.
6. El Haouari, M.; Bnouhm, M.; Bendahou, M.; Aziz, M. and Mekhfi, H. (2006). Inhibition of rat plateletaggregation by *Urtica dioca*. Leaves. Extracts *phytotherapy Res*., 20(7): 568-572.
7. Daher, C.F.; Baroody, K.G. and Barody, G.M. (2006). Effect of *Urtica dioica* extract intake upon blood lipid profile in the rat. *Fitoterapia*, 77: 183-188.
8. Nassiri-Asl, M.; Zamansoltani, F.; Abbasi, E.; Daneshi, M.M. and Zangivand, A.A. (2009). Effects of *Urtica dioica* extract on lipid profile in hypercholesterolemic rats. *J. of Integrative Medicine. V*., (7): 428-433.
9. Durak, I.; Biri, H.; Devrim, E.; Sozen, S. and Avci, A. (2004). Aqueous extract of Urtica dioica makes significant inhibition on adenosine deaminase activity in prostate tissue from patients with prostate cancer. *Cancer Biol. Ther*., 3(9): 855-857.
10. Konrad, L. and muller, H. (2000). Antiproliferative effect on human prostate cancer cells by a stinging nettle root (*Urtica dioica*) extract. *Planta Med*., 66: 44-47.
11. Mansoub, N.H. (2011). Effect of nettle *(Urtica dioica)* on performance, Quality of egg and blood parameter of laying hens. Adv. *Environ. Biol*., 5(9): 2718-2721.
12. Mansoub, N. (2011). Comparison of effects of using nettle *(Urtica dioica)* and probiotic on performance and serum composition of broiler chickens. *Global Veterinaria*, 6(3): 247-250.
13. Gulcin, I.; Kufrevioglu, O.I.; Oktay, M. and Buyukokuroglu, M. (2004). Antioxidant,antimicro--bial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J. Ethnopharmacol*, 90: 205-215.
14. Matsuda, H.; Ando, S.; Kato, T.; Morikawa, T. and Yoshikawa, M. (2006). Inhibition from the rhizomes of *Alpinia officinarum* on production of nitric oxide in lipopo-lysaccharide-activated macroph--age and the structural requirements of diarylhetanoids for the activity. *Bioorg. Med. Chem*., 14(1): 138-142.
15. Barkaa, E.A.; Gognies, S.; Nowakc, J.; Audran, J.C. and Belarbi, A. (2002). Inhibitory effect of endophyte bacteria on Botrytis cinerea and its influence to promote the grapevine growth. *Biol. Control*, 24(2): 135-142.
16. Goker, H.; Haznedaroglu, I.C.; Ercetin, S.; Kirazli, S.; Akman, U.; Ozturk, Y., *et al.* (2008). Haemostatic action of the folkloric medicinal plant extract ankaferd blood stopper. *J. Int. Med. Res*., 36(1): 163-170.
17. Aires, A.; Dias, C.; Zenao, S.; Rosa, E.; Fernandes, C. and Saavedra, M.J. (2013). Antibacterial screening of active ethanolic fractions of *Urtica dioica* L. against pathologenic bacteria from gastrointestinal and respiratory tract. In 5th Joint national congress of Microbiology & Biotechnology: Microbiotec13. Aveiro.
18. Nicolas, R.; Morris, P. and Judith. K. (2005). Phenolic Compounds: Extraction and Analysis. *Lotus japonicus Handbook, Chap*., 7: 349-355.
19. OECD. (2000). Guidance Document on Acute Oral Toxicity. Enviromental Helth and Safety Monograph Series on Testing and Assessment No 24.
20. Farhan, S.A.; Faraj, M.; Al-Shemari, H.H. and Jassim, A.M. (2012). Study of some *Urtica dioica* L. leaves components and effect of their extracts on growth of pathogenic bacteria and identify of some flavonids by HPLC. *Al-Mustansiriya J. Sci*., 23: 79-86.

21. Berat, Z.H.; Yavuz, B.; Sharon, L.W. and Ibrahim, C.H. (2012). Pleiotropic cellular, hemostatic and biological action of Ankaferd hemostat. *J of Oncology Hemato--logy*. 83: 21-34.
22. Sivaramakrishnan, V. and Shilpa, P.N. (2007). Attenuation of N-nitrosodiethylamin-induced hepatocellular carcinogenesis by anovel Biol. *Interact*, 10 (1): 79-88.
23. Beyazit, Y.; Kurt, M.; Kekilli, M.; Goker, H. and Haznedaroglu, I.C. (2010). Evaluation of hemostatic effect of Ankaferd as an alternative medicine. *Alterm. Med. Rev*., 15(4): 329-336.
24. Al-Khafaji, N.J. and Al-Zubaedi, R.M. (2013). The clinical and hematological changes in rabbits exposed to leaves of *Urtica dioica* under experimental conitions. *Kufa Journal for Veterinary Med. Sci*., 4: 51-62.
25. Abou Zeid, Shehata Nasr. (1968). Medicinal plants and herbs. Dar El-Behar, Beirut.