Comparison of The Effect of Aqueous Extracts of two Plants, *Origanum Vulgare L.* and Fenugreek Seeds with Anticancer Drug Cis-Platin on the Growth of Cancer Cell Lines

*Jinan Hussain * Iman Hashim* Farooq Ibrahem mohammed***
Ashgan salman Noora saad**

Received 18, October, 2011
Accepted 26, June, 2012

Abstract:

This study involved the effect of the aqueous extracts of two plants, *Origanum vulgare L.(1)*, *Trigonella Foenum Graecum L*. (Fenugreek) seeds(2) on the growth of cancer cell lines. Rhabdomyo sarcomas (RD) of human cell line and female intestine cells of Albino mice (L20B) *in vitro* System. These extracts were compared with the known anticancer drug Cis-platinum(Cis-Pt) as a positive control. The phytochemical tests were used for screening the active compounds in plants. The inhibition activity assay was used as a parameter of the cytotoxic effect of these extracts. Cancer cell lines were treated with four concentrations of Cis-platin, 31.25, 62.5, 125 and 250 µg/ml for 72 hour exposure time. The same concentrations were used for the other extracts. This study found that the two aqueous extracts (1,2) have a cytotoxic effects on cancer cells as could be seen from their effects on inhibition percentage and the significant differences ($p<0.05$) which were observed for each extract (1,2) by the increased the inhibition percentage as the concentration was increased. The higher level of inhibition(51.63%) was obtained from 250 µg/ml of *Origanum vulgare* extract (1) on RD line and 51.41% on cell line L20B at the same concentration. The cytotoxic effects of extract 1 and 2 on cancer cell line L20B were similar to that on RD cell line.There are no significant differences between two cancer cell line in all used concentrations. The strong relationship which to be found between the concentrations and the two aqueous extracts (1,2) comparable with Cis-Pt drug.

Key words: Fenugreek, *Origanum vulgare*, Cis-platin,Cancer cell lines

Introduction:

According to the world health organization(WHO), cancer is a leading cause of death world wide. The most frequent types of cancer among women are breast, lung and stomach cancer [1]. Herbs were used as complementary medicine among women with cancer especially those with advanced cancer.The plant *origanum vulgare L.* belongs to the family Lamiacean that native to worm and Mediterranean region [2]. *Origanum vulgare* (*Origano)* contains naturally occurring substances such as phenols, carvacrol, terpinen and flavonoids (quercetin, apigenin). Origano has demonstrated an activity against cancer cells throught the inhibition of the development of induced colon cancer in rats [3]. The *origanum vulgare* extracts affect cancer proliferation and cell death on colon adenocarinoma($CaCO_2$) cells, and leads to growth arrest and cell death in a dose and time dependent manner [4].The fenugreek (*Trigonella Foenum Graecum L.*) belonges to the family Fabaceae and flowering annua (with autoganous white

*Chemistry Department , College of Science Women , Baghdad University
**Biotechnology Department , Al-Nahraine University

flowers), grows native in Asia and southern Europe [5]. Fenugreek seeds contain active ingredients like vitamins, flavonoids (apigenin, luteolin, quercetin, vitexin) saponins, glycosides, volatile oils and amino acids [6,7]. Several compounds extracted from Fenugreek plant were reported to have antitumour activity and ability to induce cell death and morphological changes indicative of apoptosis in leukemic cell lines CCRF-HSB-2 and HL-60,10.[8]. Cis platin or Cis-diammino dichloro platinum (Π) (CDDP) is a chemotherapy drug that is widely used to treat different types of cancer, including germ cell cancer, head and neek cancer and lung cancer [9]. At the centre of this drug is an atom of the metal platinum, that binds DNA throught cross linking and hence damaging the cancer cells [10]. The aim of this study is to compare between the cytotoxic effects of the aqueous extracts of two plants with that of the anticancer drug Cis-platin on growth cell lines (RD and L20B).

Materials and Methods:

1- Cis- platin (0.1mg ⁄ ml) drug was provide by Ebew (Austria)

2- Aqueous extraction of these two plants was prepared as following method. 15 gm of each plant (*Origanum vulgare L.* and Fenugreek seeds) were put into the thumble of soxhelt apparatus which contain 100 ml of distilled water in a round flask and boiled at 100 C^o for 4 hours, then the mixture was evaporated by using the distillation apparatus to give weight of component for both *origanum vulgare* and fenugreek seeds) powder, then 10 mg of each powder extract was dissolved in 20 ml of normal salin as stock solution and stored at (2-8) c^o until used [11].

3- Phytochemical screening for both aqueous extracts of (*Origanum vulgare L.* plant and fenugreek seeds) was preformed using standard procedure (qalutative measurement) according to Katsoros [12]. 1-Test of Tannins: 2ml of aqueous extract was used in two test tubes,1ml of ferric chloride(1%) was added to tube one and 1ml of lead acetate(1%) was added to tube two then a positive result was gelationous ppt.,green-blue solution respectively. 2- Glycosides:Benedcit reagent was added to each extracts and put them in the boiling water bath for 5min ,then the red ppt.was a positive result .3-Flavonoids: Ethanol,Potassium hydroxide solution(10 %) were added in to extracts and mixing ,yellow solution was a positive test 4- Phenols: Ferric chloride (1%) was added,then the a greenish-blue ppt. was fomed .5- .Resins: Ethanol 95% was added and put them in boiling water bath for 2 min then, added 1ml of 4% HCL, the turbid solution was formed. .6-Saponins: The two extracts were mixing very well ,a froth was a positive result .7-Terpenoids:Chloroform,anhydrous acetic acid and Sulfuric acide were added in to extracts , the brown solution was a positive test.8-Alkaloids:Mayers reagent was added to each extracts ,white ppt. was a positive result . 9-Steroids: (The same of Trepenoids reagents after one day) ,the blueish solution was a negative result according to the method of Ayoola *et al* [13]method.

4- Study of inhibition percentage on growth cancer cell lines.

The cytotoxic effects were tested for the three solutions (*origanum vulgare* aqueous extract, fenugreek seeds aqueous extract and Cis-platin) on growth cancer cell lines L20B (female intestine of albino mice) and RD (Rhabdomyo sarcomase in human cell line) which were provided by the center Biotechnology research center of Al-Nahraine University. All solutions were prepared at the same and cultured tissues were studied *in vitro* under optimum conditions.The growth media

used in tissue culture technique was MEM (Minimum Essential Media) which contains fetal calf serum (10%) to form a confluent monolyer, then subcultured to discard the previous growth medium and the cells washed with sterilized phosphate buffer solution (PBS)(autoclave at l2l c^o for 15 min) then 2-3 ml of trypsin versene solution was added for 3-5 min with stirring.The trypsin- versene solution was discarded and the cells were incubated at 37c^o until the separation of the cells from the ground flask.

5- Cytotxicity assay

In this assay, the cell lines L20B and RD were treated with two aqueous extract (*Origanum vulgare*, fenugreek) and cis- platin using four concentrations (31,25, 62,5, 125, 250)µg ⁄ lml .Immediatly adding 25 ml of trypsin-versene solutions into culture bottle and 20 ml of culture medium which contains 10% of serum to provide the suspended cells, mixed very well and 0.2 ml was added to each microtiter. The plates were incubated at 37c^o for 24 hour to form monolayer, then the previous culture medium which presents in to the plates was discarded . 0.2 ml of the solutions under study were added and these three preparations repeated as negative control (cancer cell line L20B, RD with buffer solutions) were and incubated at 37c^o for 72 hour.The culture medium was discarded from microtiter plates, then 0.2 ml of crystal violet solution was added to wells and the plates were incubated for 20 min at 37 $c^{o.}$ The plates were washed gently with distilled water and left to dry. At the end of assay the plates were examined by ELISA reader at 492nm (transmitting wave length). Only viable cells able to take the stain while the dead cells were not. The inhibition percentage was measured according to Gao *etal* [14] and as follows:

$$\text{Inhibition percentage } \% = \frac{\text{Absorbance of negative control} - \text{Absorbance of test}}{\text{Absorbance of negative control}} \times 100$$

- Statistical analysis

The data were analyzed using analysis of variance ANOVA. Investigation of differences and correlation factor(R) between Cis-platin and the two extracts were determined using the statistical program (SPSS) within significant level ($P<0.05$) [15].

Results and Discussion:

1-Screening of plant materials.

The phytochemical screening of the*Origanum vulgare* and Fenugreek was studied, and results the presented in table (1):

Table (1): The phytochemical screening of the *Origanum vulgare* (extract 1) and Fenugreek (extract 2)

Active compounds	Reagents	Indicators	Results of extract (1)	Results of extract (2)
Tannins	Lead acetate, ferric	Gelationous ppt. green-blue solution	+	+
Glycosides	Bendict	Red ppt.	+	+
Flavonoids	Ethanol,potassium hydroxide	Yellow solution	+	+
Phenols	Ferric chloride	Greenish-blue ppt.	+	+
Resins	Ethanol95%→boiling→4%HCl	Turbid solution	+	+
Saponins	Convulse solution	Froth	+	+
Terpenoids	loroform, anhydrous acetic acid and sulfuric acid	Brown solution	+	+
Alkaloids	Mayers reagent	White ppt	_	+
Steroids	The same of trepenoids reagents after one day	Blueish solution	_	_

(+) positive test result
(-) negative test result

According to the results showed in table (1), the two aqueous extracts of *Origanum vulgare* plant and Fenugreek seeds contain phenols, flavonoids, trepenoids and tannins and others. The increased inhibition percentage when cancer cell lines (L20B and RD) were treated with extract (1) at different concentrations could be attributed to the phenolic compounds such as carvacrol and thymol commonly found in most plants which posses a wide spectrum of biological activities including effect on cell proliferation, differentions and apoptosis(a programmed cell death) [1]. It was reported that tannins compounds in aqueous extract led to apoptosis and stop one of the cell cycle phases (G, S, G2) on cancer cells [16]. *Origano* is widly used in natural medicine due to its many effective antioxidants such as Rosmarinic acid, caffeic acid and various flavonoids such as luteolin enderiodictyol [17]. The main chemical constituents of fenugreek were reported to be flavonoids, polysaccharidcs, tannins, phenols and saponins. The increased inhibition rates when cell lines (L20B and RD) were treated with extract (2) at different concentrations may be related to flavonoids including quercetin, apigenin , anthocanidine, flavonoids have antioxidant activity and thus protect against cancer [8].

2- Study of cytotoxic effects on growth cell lines

The cytotoxic effects of the aqueous extrat of *origanum vulgare* (extract 1) and aqueous extract of fenugreek (extract 2) were studied on two cancer cell lines and compared with anticancer drug Cis-Pt.

- Rhabdomyo sarcomas (RD) in human cell line

The results showed in table (2) and (Fig 1) indicat the significant differences ($P<0.05$) as the concentrations increased when the cancer cells were treated with each extract (1,2) comparable with the positive control Cis-Pt after 72 exposure time. The higher inhibition percentage reached 51.63% at high concertration (250 µg/ml).

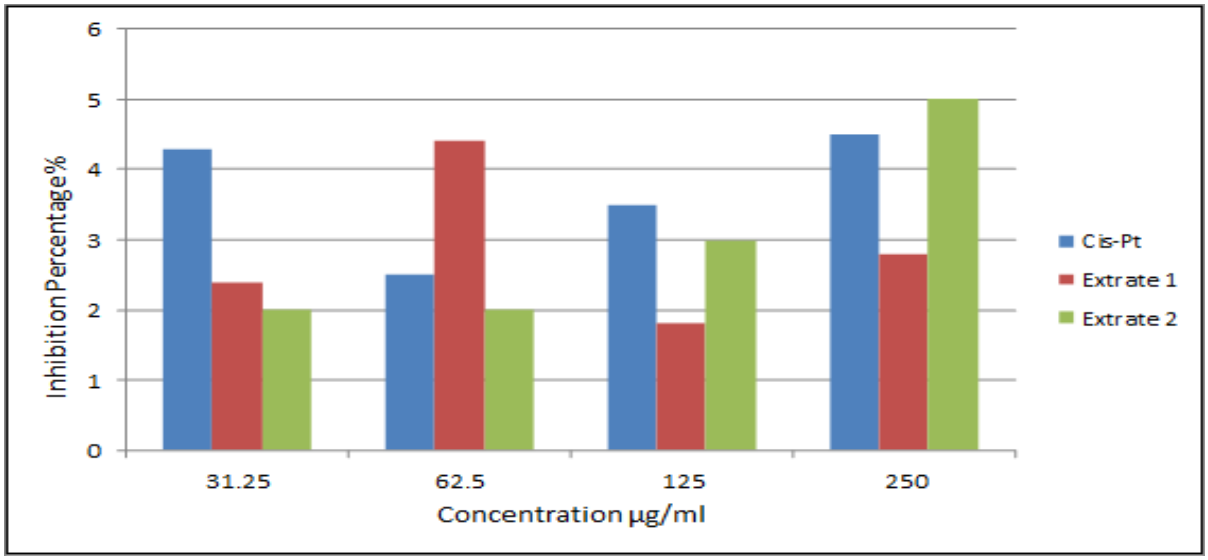

Figure (1): Inhibition percentage of human cancer cell line (RD) with different concentrations of two aqueous extracts (1,2) and Cis-Pt after 72 hour exposure time.

- Females intestine of albino mice (L20B) cell line.

The data of inhibition percentage of cancer cells treated with two extract (1,2) in L20B line are summarized in figure (2). The inhibition rates of the two aqueous extracts (1,2) have increased as the concentrations increaced.

.

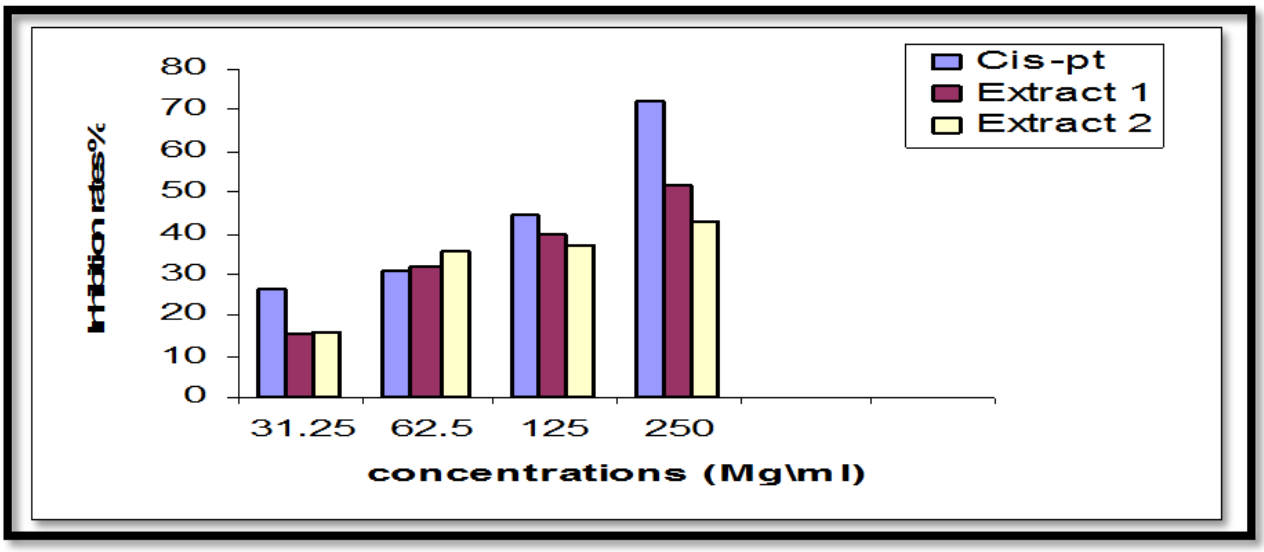

Figure (2): The inhibition percentage of females intestine of albino mice cell line(L20B) with different concentrations of two aqueous extracts (1 , 2) and Cis-Pt after 72 hour exposure time.

3- Comparison of the inhibition percentage between L20B and RD cell lines.

As shown in table (2), the inhibition percentage of both aqueous extracts (1) and (2) on growth cell line L20B was similar to that of RD cell line comparable with anticancer drug Cis-platin using different concentrations after 72 hour exposure time.The higher level of inhibition percentage was 72.13 % at 250 µg ⁄ml

Table (2): Comparison of the inhibition percentage between the two aqueous extracts (1,2) and Cis-Pt on L20B and RD cancer cell lines

Treatment / Concentration µg/ml	Inhibition rates%(mean±standard deviation SD)					
	Cis-platin		Extract (1)		Extract (2)	
	RD	L20B	RD	L20B	RD	L20B
31.25	a 10.50±6.99	b 26.33±6.65	a 14.55±4.03	a 15.42±4.32	a 11.29±3.55	a 15.81±4.43
62.5	a 23.72±3.10	a 30.92±8.34	a 24.74±3.04	a 31.88±3.92	a 26.49±5.14	a 35.76±6.50
125	a 29.99±4.81	a 44.56±7.99	a 36.11±4.49	a 39.86±7.04	a 33.11±1.74	a 37.21±5.23
250	a 46.64±6.99	a 72.13±7.96	a 51.63±6.83	a 51.41±8.33	a 43.12±6.06	a 42.97±7.42

* differences a,b are significant (P<0.05) to comparison row.

4- The correlation factor(R) of the extracts and the concentrations.

The results present in table (3) show the strong correlation rang from medium to high between different concentrations and two aqueous extracts (*Origanum vulgare* 1 and Fenugreek 2). Comparable with anticancer drug Cis-Pt by using two cancer cell lines: RD and L20B, when cancer cells treated with Cis-Pt, extract 1 and 2 at different concentrations.

Table (3): The correlation factor (R) between the concentrations and each groups (*Origanum vulgare* extract 1, Fenugreek extract 2) and Cis - Pt and between the same groups.

Groups	Conc. µg /ml	R (RD)	Cis- Pt	Extract 1	R(L20B)	Cis-Pt	Extract 1
Cis-platin	31.25,62.5,125,250	0.920			0.781		
Extract 1	31.25,62.5,125,250	0.982	0.974		0.928	0.954	
Extract 2	31.25,62.5,125,250	0.971	0.984	0.992	0.999	0.752	0.915

R= correlation factor

The results in table 2 ,3 and figure 1,2 show an evidence that the two extracts (1,2) have a cytotoxic effects on cancer cell lines (L20B and RD) as shown by the elevated inhibition percentage with the increased concentrations (31.25, 62.5, 125, 250) µg /ml compared with that of anticancer drug cis-platine . In this study, we suggest that *Origanum* extract have cytotoxic effect, this effect was similar to Srihari *et al* [3] that show the *Origanum* extract have antimutagenic, antigenotoxic, and antiproliferation properties.*Origanum* extract protect cells from oxidative stress, mitogen, and radition induced DNA damage. Carvacrol and thymol have been reported to protect DNA from variety damaging agents and suppress proliferation of cancer cells [18]. Tatjana [1] show the relationship activity between extract and two human breast cancer cell lines (MDA-MB-361) and (MDR-MB-453), which exhibited significant antiproliferation after treatment with the extract. The elevated inhibition percentage with increased concentration incrased when cell lines (L20B and RD) were treated with fenugreek extract . This effect attributable to the fenugreek seeds components such as phenols, saponins and alkaloids, flavonoids, trepenoids, these extracts active ingredients contribute to its prevention effects on cancer [19].

Alarcon *et al* [20] studied the effect of fenugreek extract on human neoplastic cells, their results showed the cytotoxic effect of the extract against this cell line which resulted in growth inhibition, cell death and apoptosis.Flavonoids such as quercetin and taxifolin have antiproliferative effects on growth cancer cell lines (squamous cell carcinoma and leukemia HL-60) [21].Thomson *et al*[10] have studied the effect of the cisplatin binding and cross linking of DNA which ultimately triggers apoptosis .

Conclusion: The study showed that the two aqueous extract (*Origanum vulgare* and Fenugreek) have a cytotoxic effects on that two cancer cell line (L20B and RD) at different concentrations after 72 hour exposure time, these effects were similar to the effect of anti-cancer drug Cis-platin.

References:

1.Tatjana,P.S.; Aleksandra,k.r.;Zorica,D.J. and Milka, J.2010. Cytotoxic and cell cycle effects induced by two herbal extract on human cervix carcinoma and human breast cancer cell lines,J. Medicinal Food. 13 (2) : 445-450.

2.Faleiro,l.2005. Antibacterial and antioxidant activites of essential oils isolated from thymbra capitata L. (cav) and origanum vulgare L., Agric. Food.chem.53,8162-8168.

3. Sriharia,T.*et al*.2008. Role of oregano on bacterial enzymes in 1,2-dimethyl hydrazine-incluced experimental colon carrinogenesis,Can J. Physio P harmacol, 86 (10), 667-674.

4. Isebella,S; Rosaria,A.;M aria,v.and Luciana ,A. 2009. Origanum vulgare induced apoptosis in human cancer $CaCO_2$ cells nutrition and cancer, Nut.and Cancer, 61(3), 381-389.

5. Ahmed,H.;Amr,F.and khalid,F.2002. Effect of extraction methods on the chemical activity of Egyption,Flav.Frag.9,54-61.

6.Blumethal, M.; Goldberg, A. and brinkman,J.2003. Herbal medicine: expanded commission E Monographs, American botantical council. Publ. by integrative medicine communications, USA, pp 130-133.

7.Shang,M.; Cai,s.; Han,J.l.; Zhao,y.;Z heng, J.;Namba,T.;kadota,S.; Tezuka,y.and Fan,w.1998. Studies on flavonoids from fenugreek (Trigonella Foenum Graecum L.) zhongguo zhong yao za zhio,23 (10): 614-630.

8. Shaban,A.;S eyed,A.and Ali,R.2009. Antineoplastic effect of Fenugreek (Trigonella Foenum Graecum) seed extract against acute myeloblastic leukemia cell line (KG-1), Iranian.J. Blood and cancer, 1(4),139-146.
9. Pruefer, F.G.;Lizarraga,F.;Malonado,V.and Melendes-zajgla,J.2008. Participation of omi htra 2 serine-portease activity in the apoptosis induced by Cisplatin on SW480 Colon Cancer Cells, Chem. 20, 348-354.
10. Thomson,A.J.; Christine,D.A.and Tansey,E.M.2007. The discovery, used and impact of platinum salts as chemotheraphy agents for cancer, welcome trust witnesses to Twentieth Century Medicine, 130, 6-15.
11. Salmman,I.2008. Effect of crude silybum marianum on cancer and normal cell lines,Thesis, Education college for women, Iraq. pp239-259.
12. Katsaros,N.2009. Rhodium and its compounds as potential agents in cancer treatmen, Oncology Hem.42 (3), 297-300.
13. Ayoola, G.; Coker,H.; Adesegun,S;Adepoju-bello,A.;Ezennia,E.and Alangbayila,T. 2008.Photochemical screeing and antioxidant activities of some selected medicinal plant used malaria therapy in South Western Nigeria,Topi. Pharm. Res . 7, 1019- 1024.
14. Gao, X.; Xu,X.; Janak iraman, N.and Gautam, S.2002. Disparate *in vivo* antileukemic effect of reseveratol a natural polyphenolic compound found in Grapes,Nutv. 132, 2076- 2081.
15. Al-Mohammed, N.T.; Al-Rawi,k.M.; Younis, M.R; Al-Morani,w.k.1986. principles of Statistics, Al-mousl. Univ.7,50-52.
16. Birt, F.D.; Hendrich, S. and Wang,W."Dietary Agents in Cancer Prevention Flavonoids and Iso Flavonoids Pharmacology and Therapeutics, USA; pp 157- 177.
17. Victor, B.; Guadalupe, D. and Jose, p.2010. Effects of Aqueous extract of origanum vulgare L. (lamiaceae) on the preimplantational mouse emberyos, Rev. Peru. Boil. 17 (3), 381- 384.
18. Savini, I. *et al*.2009. Origanum vulgare induced apoptosis in human Colon Cancer $CaCO_2$ cells, Nutr. Cancer, 61 (3) , 381- 389.
19. Abuharfeil, N.M.; salim, M. and Von, K. 2001.Augmentation of natural killer cell activity in vivo against tumor cells by some wild plants from Jordan, phytother. Res. 15, 109-113.
20. Alarcon, F.J.; Roman, R.; Perez, S.; Aguilar, A.; Contreras, C. and Flores, J.L1998. Study of the anti-hyperglycemic effect of plants used as antidiabetics, J. Éthnopharmacol, 61, 101- 110.
21. Piantelli, M.; Rinelli, A.; Macri, E.; Maggiano, N.; Larocca, L.M.; Scerrati, M.; Roselli,R.; Laconngeli, M.; Seambi,G.; Capelli, A.and Ranelletti, F.O.1993. Type II estrogen binding sites and antiproliferative activity of quercetin in human meningiomas, J. Cancer, 71, 193- 198.

مقارنة تأثير المستخلصات المائية لنباتي المردقوش *Origanum vulgare L.* وبذور الحلبة *Fenugreek* seeds مع العقار المضاد للسرطان السز بلاتين على نمو الخطوط الخلويه السرطانيه

جنان حسين* ***ايمان هاشم**** ***فاروق ابراهيم محمد*****
اشكان سلمان* ***نورا سعد****

*قسم الكيمياء- كلية العلوم للبنات - جامعة بغداد
** قسم التقنيات الاحيائيه , جامعة النهرين

الخلاصة:

في هذه الدراسة تم مقارنة تأثير المستخلص المائي لنباتين الاول (1) هو نبات المردقوش *origanum vulgare* L. والثاني (2) هو نبات fenugreek (*Trigonella foenum Graecum* L.) على نمو الخطوط الخلوية السرطانية، الخط الخلوي السرطاني للعضلة البشرية في الأنسان (RD) والخط الخلوي السرطاني لأمعاء اناث الفئران L20B في نظام خارج جسم الكائن الحي *in vitro* وقورن تأثير المستخلصين مع العقار المعروف المضاد للسرطان السزبلاتين Cis-Pt كسيطرة موجبه، لقد أجريت الفحوصات الكيمياوية للكشف عن المركبات الفعالة الموجودة في النباتات وأختبار الفعالية التثبيطية كمقياس للتأثير السمي الخلوي لتلك المستخلصات. تم معاملة الخلايا السرطانية بأربع تراكيز من عقار السزبلاتين Cis-Pt وهي 31.25,62.5,125,250مايكروغرام /مل وخلا ل فترة تعريض 72 ساعة، وقد أستخدمت نفس التراكيز الاربعة للمستخلصين المائيين.أظهرت هذه الدراسة أمتلاك المستخلصين المائيين (1و 2) تأثيرات سمية خلوية على الخلايا السرطانية تم ملاحظتها من خلال معدلات التثبيط ووجود الفروق المعنوية ($p<0.05$) لكل من المستخلص 1 و 2 من خلال ازدياد معدلات التثبيط مع تزايد التركيز. بلغ أعلى مستوى للتثبيط 51.63% للمستخلص المائي للمردقوش (1) عند 250 مايكروغرام/ مل على الخط RD و 51,41% على الخط الخلوي L20B عند نفس التركيز. التأثيرات السمية الخلوية للمستخلص 1و 2 على الخط الخلوي L20B كانت مشابهة للتأثيرات الحاصلة على الخط الخلوي RD حيث لم تظهر فروق معنوية بين الخطين الخلويين عند جميع التراكيز المختلفة، وهنالك علاقة قوية بين التراكيز والمستخلصين المائيين (1و2) مقارنة بعقار السزبلاتين.