Research Article

Anti-Angiogenic Activity of *Mentha piperita* Leaves Extracts

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Received: 10-03-2017; **Revised:** 18-04-2017; **Accepted:** 22-05-2017.

ABSTRACT

The objective of this study is to determine the probable anti-angiogenic activity of *Mentha piperita* leaves extracts. *Mentha piperita* leaves powder was extracted consecutively with chloroform, methanol and water applying the hot maceration method. The *ex vivo* rat aorta ring assay was employed to screen the extracts for propable anti-angiogenesis activity, and in this assay, serial dilutions was also prepared for the active sample extract(s) to reveal the dose–response effect. Free radical scavenging ability was done to the active sample extract(s) performing 1, 1 –diphenyl –2 –pecrylhydrazyl assay (DPPH Assay). The achieved data clarified that the three sample extracts demonstrated a significant inhibition of the blood vessels growth after they were compared to the DMSO data (negative control) ($P<0.001$), however, methanol and chloroform extracts exhibited the greatest percent for inhibition of the blood vessels growth. In response to the screening results, each of methanol and chloroform sample extracts were experienced for another investigation. Both of these extracts of *Mentha piperita* leaves showed a significant dose–dependent anti–angiogenesis action with IC_{50} of 22.126µg/ml for methanol extract and 30.608µg/ml for chloroform extract. In addition, methanol and chloroform sample extracts revealed a significant scavenging activity for the free radical ($P<0.05$) with IC_{50} (3.51 µg/ml and 3.7µg/ml) respectively. The outcomes disclosed that both of methanol and chloroform sample extracts of *Mentha piperita* leaves showed the most significant anti–angiogenesis action together with a significant scavenging activity for the free radical.

Keywords: Angiogenesis, *ex vivo* study, *Mentha piperita* leaves

INTRODUCTION

Angiogenesis is the development of new blood vessels arising from current vasculature. It happens all over lifetime in health as well as illness, starting from utero and ongoing throughout adulthood. This process is typically initiated within hypoxic tissues where additional new blood vessels are required to maintain oxygenation and nutritional supply [1]. As a therapeutic mode, the body is either Stimulate angiogenesis; as in peripheral arterial disease, ischemic cardiac diseases, wound healing, Ovulation, furthermore in corpus luteum formation, where there is a demand to augment blood supply [2]. Or lessening or stopping angiogenesis during cancer, ophthalmic problems, and rheumatoid arthritis. Blood capillaries production and regression in healthy tissues are regulated in response to functional needs The primary step of it is thought to be initiated by activation of endothelial cells of pre-existing vessels in response to angiogenic stimuli. Vascular Endothelial Growth Factor (VEGF) has an essential function in physiologic, pathologic, and developmental vascularization. Fibroblast growth factor (FGF), besides VEGF, is also recommended in wound healing process, and macrophages promote the release of these angiogenic molecules [3]. Physiologic angiogenesis lasts to few days, otherwise to weeks at more. But it can stay for a bout months to years in pathological states .Types of angiogenesis are two; the sprouting angiogenesis, that is the most common studied mechanism, includes disintegration of capillary basement membrane, and followed by endothelial cells migration, beginning with the tip cell which travels along a gradient of the pro-angiogenic factors. Subsequently, endothelial cells proliferate and migrate and following the tip cell. Then, these endothelial cells will form a lumen and recruit smooth muscle cells to encircle the vessel and ultimately the basement membrane will be produced [4]. The second type is the splitting (intus susceptive) angiogenesis, involves the expansion of the vessel wall to the lumen resulting in a single vessel that splits into two. This type of angiogenesis is supposed to be fast and more efficient in comparison with sprouting angiogenesis, since, initially, it just requires the reorganization of the current endothelial cells and there is no need for immediate proliferation or migration for the endothelial cells [5]. Some of the pathological disorders are resulted from extreme stimulation of angiogenesis as in cancer, psoriasis, and inflammatory conditions. In other states angiogenesis is inadequate, like in diabetic neuropathy and Alzheimer disease [6]. *Mentha piperita* is also peppermint. Mentha (mint) is a genus of the plants in the family Lamiaceae. The plant indigenous to Europe and the Middle East, but now widespread by cultivation in many lands of the world. *M. piperita* is the earliest and most popular herb which can be utilized in many forms (leaf, leaf water, leaf extract and oil). Peppermint is the most well-liked flavor that is often used in tea and intended for flavoring chewing gum, toothpaste, ice cream, and confectionery. Peppermint can also be found in particular soaps, skin

care products as well as shampoos. Peppermint oil is generally used to relieve or treat symptoms such as nausea, vomiting, morning sickness, anorexia, abdominal pain, indigestion, and flatulence. Skin preparations having peppermint oil are used for treatment of headache, toothache, muscle pain, nerve pain, joint conditions, allergic rash, pruritus, and repelling of mosquitos [7]. Peppermint leaf, as well as peppermint oil commonly have been employed internally (upper-gastrointestinal tract and bile ducts) to relieve diarrhea, irritable bowel syndrome, Crohns disease, and ulcerative colitis, catarrh of the respiratory tract, and inflammation of the oral mucosa [8]. *M. piperita* leaves were found to be a good source of volatile oils (menthol, menthone, menthofuran, menthyl acetate, cineol, and limonene), phenolic acids (caffeic, chlorogenic and rosmarinic acid), flavonoids (menthoside, isorhoifolin, flavonones and luteolin), and tannins. Other reported constituents, azulene and minerals [9]. The Aim of this study is to identify which extract(s) of *M. piperita* leaves have the best anti–angiogenic activity.

MATERIALS AND METHODS

Extraction process

Four hundred grams of the leaves powder of the *M. piperita* were obtained from a farm in Baghdad city. The leaves were washed with tap water and then allowed to open air dry. The dried leaves were grounded into a fine powder. The powder extracted consecutively with (chloroform, methanol and water), applying cold maceration method. The mixture then filtered throughout whatman No.1 filter paper to obtain the extract which was concentrated by the rotary evaporator (Buchi, Switzerland)., the dried extract was stored in refrigerator till the time of experiment [10]

Ex vivo Rat Aorta Ring Anti –angiogenic Assay [RAR]

RAR study was performed after the experimental techniques were approved by Ethics Committee of Al-Nahrain University in College of Medicine. The experiment was conducted corresponding to the standard protocol delivered by Brown and coworkers [1], but with slight modifications. Fourty Sprague dawley male rats with twelve to fourteen weeks age were used in the study. They were taken from the animal house of the college of medicine/Al-Nahrain University. The animals were anesthetized in a jar containing chloroform soaked cotton piece. Then they were sacrificed via cervical dislocation. Just thoracic aorta was excised, it was dipped in serum free media, gently wiped from any fibro-adipose tissues and remaining blood clots. The cleaned aorta was cut into slim rings of 1mm in thickness. M199 medium containing 3mg/ml of fibrinogen and 5µg/ml of aprotinin was prepared for the lower layer. Each well of 48-well plate was injected with 300 µl of the prepared medium, and seeded with one aortic ring. 10 µl of thrombin solution (50 NIH U/mL in NaCl of 0.15M) was then added to each well. Next, the plate was incubated for 30-45 min at 37°C and 5% CO_2 allowing the lower layer to solidify. The upper layer medium was formulated from M199 growth medium, 20% HIFB serum, 1% L- glutamine, 0.6% gentamicin, 1% amphotericin B, and 0.1% aminocaproic acid. A concentration of 100µg/mL of the plant extract was added to the upper layer and each sample extract was applied in six replicates. Dimethyl sulfoxide (DMSO) was used to prepare the stock solutions for the plant extracts. Further dilution of the stock solutions in M199 medium to reach the final DMSO concentration of 1%. The plates containing the aortic rings were cultured in a humidified incubator (using deionized water for humidification) at a temperature of 37°C and 5% CO_2.

On day four, the top layer was replaced with fresh upper medium. Acetyl salicylic acid is an anti-angiogenesis agent, it was applied as positive control (in a concentration of 100µg/mL), while DMSO (1% v/v) was used as a negative control. The zone of blood vessel growth was observed on day 5 under 40 x magnifications employing an inverted microscope with the help of camera and software package. The value of blood vessel growth inhibition was assessed corresponding to the technique established by Nicosia and his friends [11]. The lengths of the minute blood vessels that outgrew from the parent vessel rings were measured. The outcomes are represented as mean percent inhibition in relation to the negative control ± SD. Briefly, the experiment was repeated six times applying six replicate per sample. Ultimately, percentage of blood vessel inhibition was established according to the following formula:

Blood vessel inhibition = $1- (A_0/A) \times 100$, where

A_0 = distance of the blood vessel growth in the test sample in mm.

A = distance of the blood vessel growth in the negative control in mm.

Dose Response Study for the effective sample extracts in Rat Aorta Ring Assay

Serial dilutions for the effective sample extract were done to get the concentrations of 100, 50, 25, and 12.5 µg/ ml from the first stock solution of the samples which was dissolved in DMSO, and then diluted in the M199 medium to reach the final DMSO concentration 1%. The wells that without sample extract were filled with medium having 1% DMSO to use them as the negative control. The data was presented as mean ± SD . The concentration of the test sample which inhibits 50% of the blood vessel growth" IC_{50}" was estimated by employing the linear regression equation, as well as the logarithmic equation for the sample. Where Y= percentage of inhibition, and X= the concentration.

1, 1-diphenyl-2-picrylhydrazyl [DPPH] scavenging activity

The antioxidant activities of *M. piperita* leaves extracts were evaluated through measuring free radical scavenging activity by DPPH method. Serial dilutions of

chloroform and methanol extracts of *M. piperita* leaves in methanol were made to obtain the concentrations of 0.007, 0.015, 0.031, 0.062, 0.12, 0.5 mg/ml. 2ml of each concentration was transferred into 96-plate wells, each concentration was repeated for three wells. 1ml of 0.1 Mm DPPH solutions in methanol was then added. Ascorbic acid is a familiar antioxidant agent; it was used as positive control in this assay. The DPPH solution (0.1 Mm) in methanol without the leaves extract was used as the negative control and just methanol to represent the blank. All the tests were accomplished in triplicate. After 30 min incubation, the absorbance was assessed at 490 nm by means of ELISA plate reader. Percentage of DPPH scavenging activity (Q) was measured through performing the formula below[12]:-

Q = (A0 - A) / A0 × 100%

Where:

A0: the absorbance of the control

A: the absorbance of the sample.

Statistical Analysis

The experimental design which applied for this study was Rationalized Complete Block Design [RCBD]. Results were presented as means ± SD. The differences between groups were matched by the one way ANOVA and then followed by Tukey Post-hoc test [t-test]. It was considered significant if *(P* < 0.05, 0.01, 0.001). The concentration that caused 50% growth inhibition of blood vessels and reduction of free radicals [IC_{50}] was measured by means of logarithmic equations and the statistical analysis was performed depending on SPSS edition 17.0 [1].

RESULTS

Extraction Process

Three solvents were used in the extraction of 400gm of *M. piperita* leaves powder. These solvents are chloroform, methanol and water. Among the three extracts, methanol extract displayed the best yield percentage (9.59) as shown in table (1)

Table 1: Weight and yield percentage attained from *Mentha piperita* leaves crude extracts.

Type of extract	Weight (g/400g)	Yield (%)
Chloroform	24.07	6.017
Methanol	38.37	9.59
Water	27.89	6.97

400gm of *M. piperita* leaves powder used in the extraction process.

Ex vivo rat aorta ring growth inhibition by chloroform, methanol and water extracts of *Mentha piperita* leaves

The pictures viewed that all the extracts significantly inhibited blood vessels growth at day five of the experiment, there was a significant difference in blood vessels growth inhibition among each of the three extracts of *M. piperita* and the negative control (DMSO) (*P<0.05*).

It was found that a significant difference between the positive control and water extract is exist in the inhibition of blood vessel growth at (P<0.001). While methanol extract and chloroform extract exhibited a significant difference in the growth inhibition in relation to the positive control at (P<0.03) and (P<0.01) respectively. In addition, there is no significant difference between methanol and chloroform extract at (P=0.628).

Among these three extracts, the methanol extract presented the highest anti-angiogenesis activity when matched with the other two extracts; table (2), figure (1) , image (1).

Table 2: The percentage of inhibition blood vessels growth produced by the sample extracts, positive, and negative controls.

Compound	% of inhibition ± SD
Negative control "DMSO 1%"	0
Positive control "aspirin"	93.29 ± 0.24
Methanol extract	84.96 ± 0.37
Chloroform extract	79.26 ± 1.79
Water extract	23.17 ± 1.02

The results are presented as mean percent inhibition to the negative control ± SD. The experiment was repeated three times using six replicate per sample (n=18).

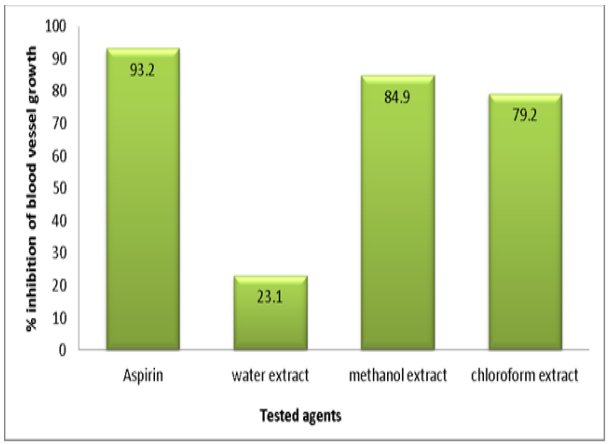

Figure 1: Anti – angiogenesis activity of 100µg/ml of each of chloroform extract (CE), methanol extract (ME) and water extract (WE) in ex vivo aortic ring model. DMSO as negative control and aspirin was used as positive control.

Dose response curve of methanol and chloroform extracts of *Mentha piperita* leaves in rat aortic ring assay

Sequential dilutions of the methanol and chloroform extracts of *M. piperita* were added to the blood vessel rings. Six concentrations were used (6.25, 12.5, 25, 50, and 100µg/ml). Methanol extract at these concentration displayed a significant inhibitory activity in dose response manner (*P<0.05*); (table3).The IC50 value was obtained from the logarithmic equation: y=22.32lnx-19.124, where: x = concentration, y = the percentage of inhibition, and was equal to 22.126µg/ml; figure(2), image(2). Furthermore, Chloroform extract also displayed a

significant inhibitory activity in dose response manner (*P<0.05*);table(4). The IC_{50} value was obtained from the logarithmic equation y=22.10lnx-25.61, and was equal to 30.608µg/ml; figure(3), image(3).

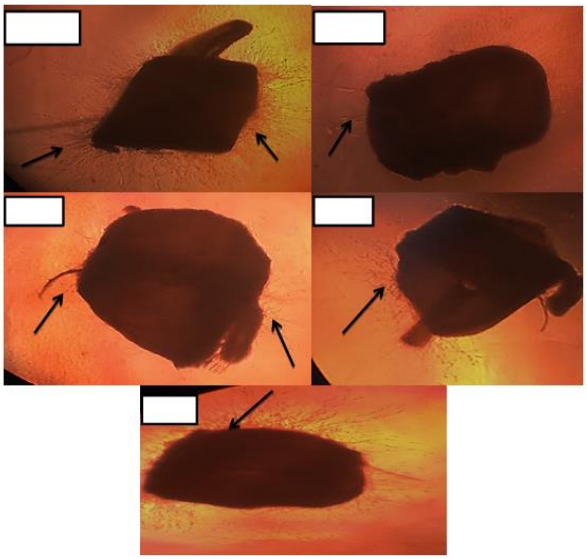

Image 1: A picture showing the Anti– angiogenesis effect of 100µg/ml of *Mentha piperita* leaves extracts on the growth of blood vessels. Acetyl salicylic acid as the positive control and DMSO as the negative control.

Table 3: Serial concentrations and their inhibition percentage for methanol extract of *M. piperita* leaves.

Concentration (µg/ml)	% of inhibition ± SD
6.125	19.11±1.35
12.5	26.42±1.08
25	50.41±0.85
50	74.39±0.75
100	84.959±0.37

Table 4: Serial concentrations and their respective inhibition percentage for chloroform extract of *M. piperita* leaves

Concentration (µg/ml)	% of inhibition ± SD
6.125	11.78± 1.27
12.5	23.17± 1.21
25	37.8± 1.14
50	67.07± 0.42
100	79.27± 0.45

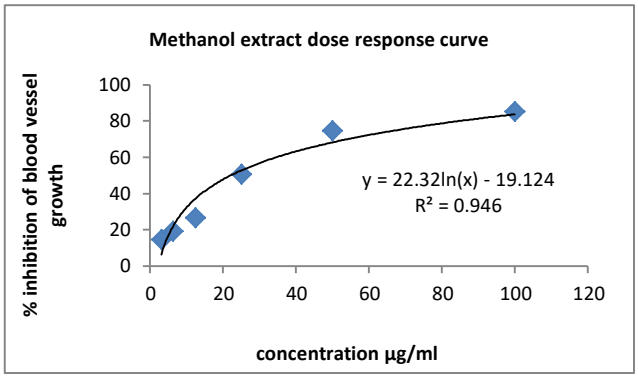

Figure 2: Dose response curve of chloroform extract of *M. piperita* leaves on rat aortic rings model

The experiment was repeated three times using six replicate per concentration (n=18).

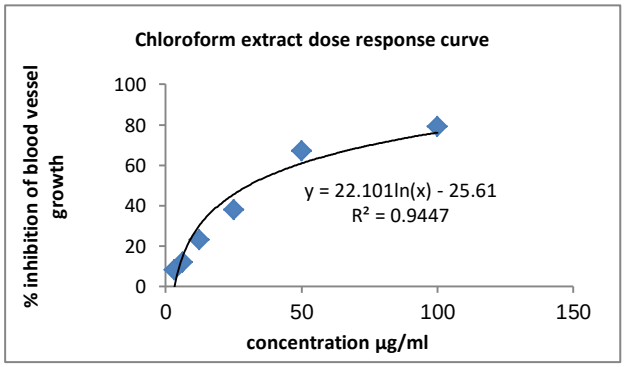

Figure 3: Dose response curve of methanol extract of *M. piperita* leaves on rat aortic rings model The experiment was repeated three times using six replicate per concentration (n=18).

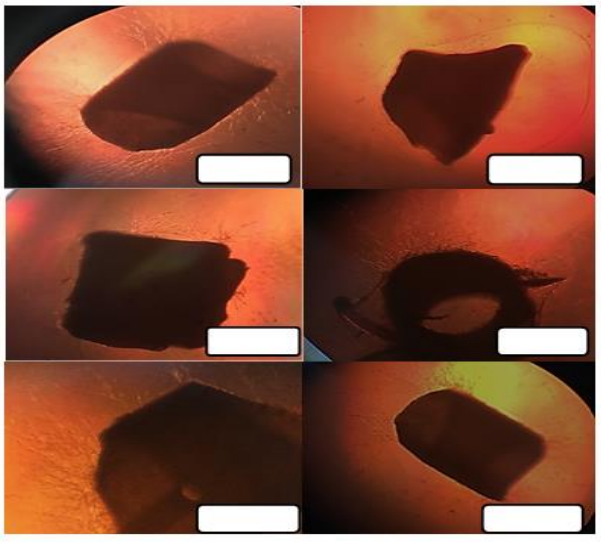

Image 2: The dose response effect of the serial dilutions of methanol extract of *M. piperita* leaves on rat aortic rings model. DMSO was used as negative control. The results were taken at day five of the experiment.

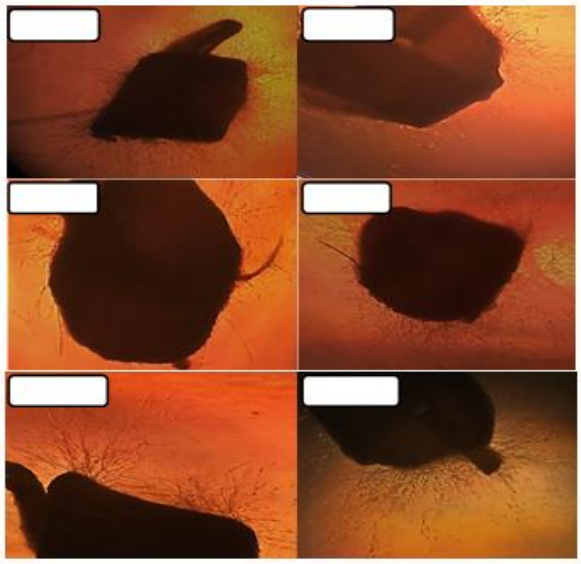

Image 3: The dose response effect of the serial dilutions of chloroform extract of *M. piperita* leaves on rat aortic rings model. DMSO was used as negative control. The results were taken at day five of the experiment.

Scavenging activity of methanol and chloroform extracts of *Mentha piperita* leaves for the free radical

The antioxidant activity of methanol and chloroform extracts of *M. piperita* leaves were measured through applying DPPH scavenging activity assay. Serial dilutions of 15.62, 31.25, 62.5, 125, 250 and 500µg/ml for each extract were done; table (5). The outcomes showed that DPPH scavenging activity of the two extracts of *M. piperita* leaves and ascorbic acid are dose dependent. IC50 of DPPH scavenging activity of the positive control (ascorbic acid), methanol and chloroform extracts were measured by using the logarithmic equations (Y=6.2588ln(x)+56.668), (Y=18.272ln(x)-19.467) and (Y=18.424ln(x)-50.945) respectively, where y= percentage of DPPH scavenging activity and is substituted by 50% to obtain IC50 which was 0.34, 3.51, 3.7µg/ml respectively.

The results disclosed that both of methanol and chloroform extracts significantly reduces DPPH free radical by a concentration dependent way (P<0.05). In addition, it was observed that there is a significant difference between methanol and chloroform extracts in their ability for scavenging DPPH free radical, that methanol sample presented more effective scavenging activity than that of chloroform one (P<0.05).

DISCUSSION

Extraction process

Extraction includes the separation of the medicinally active components of the plant tissue that is used from those that may be inert by employing suitable solvents and the proper techniques of extraction. In this study, grinding of the dried plant leaves aids in producing a homogenous sample and increasing the area of sample contact with the solvent system. The extraction process was performed consecutively with the three solvents, beginning from the non-polar one, and ending with highest polarity to make sure that most of the plant leaves constituents were extracted in relation to their polarity [13]. Methanol extract gave the greatest yield of the crude extract, and water extract came after, while chloroform extract gave the lowest yield of the crude extract (table 1).

Table 5: The percentage of DPPH free radical scavenging activity for chloroform and methanol extracts of *Mentha piperita* leaves in comparison to that of ascorbic acid.

Concentration (µg/ml)	DPPH free radical scavenging activity (%)		
	Ascorbic acid	Chloroform Extract	Methanol Extract
15.62	71.64±0.002	2.21±0.01	17.46± 0.02
31.25	78.84±0.003	11.44±0.01	46.55±0.03
62.5	84.06±0.005	23.7±0.02	68.39±0.08
125	88.66±0.012	31.84±0.03	76.83±0.01
250	91.57±0.017	58.63±0.04	81.79±0.06
500	93.49±0.003	61.81±0.04	83.37±0.11

Ascorbic acid used as positive control, each concentration has been triplicated (n=3)

It shows that there are a number of factors that may affect the difference in the yield and the constituent of phytochemicals in every extract, these involve: particle size of the plant leaves powder, solvent to sample ratio, mode of the extraction method, extraction process length, water bath temperature, agitation type of solvent and its pH, polarity and concentration. It was also observed that the way for drying the part of the plant that is used highly affects the yield as well as the composition of the constituents in any extract which will be tested for its activity later in the research trial. The extraction process used was the cold maceration method to avoid destruction of thermo-labile compounds caused by high temperature [14].

Effect on *ex vivo* RAR anti–angiogenesis assay

Angiogenesis assay is valuable for discovering the potential angiogenic drugs and looking for pharmacologic inhibitors. Rat aorta ring (RAR) assay is most widely used assay to study angiogenesis and its mechanisms since it is reproducible, effective, and easy and has a good correlation with *in vivo* assay [15]. This model is based on the capacity of rat aortic rings to produce new blood vessels. *M. piperita* leaves has been reported to possess antioxidant activity and cytotoxic effect [16], but till now, there are no anti-angiogenesis activity reports about *M. piperita*. In the present study, screening the three extracts in RAR anti – angiogenesis assay was very essential to reveal if these extracts have anti–angiogenesis effect, and subsequently to select the best anti–angiogenic extracts for additional investigations. Aspirin was used as a positive control in this experiment since its approval to have anti–angiogenesis activity through COX –dependent pathway, or through blocking

NF-KB which is closely associated with angiogenesis and inflammation [17]. The outcomes clarify that methanol extract and chloroform extract had the best anti–angiogenic ability and both of them had a comparable effect toward the blood vessel growth inhibition. Each extract was employed in further investigations. Furthermore, WE inhibited angiogenesis about some degree, but the inhibition extent was not comparable to that created by methanol and chloroform extract (figure 1). Anti-angiogenesis quantification on this system involves measuring of the length and number of branching micro blood vessels [1]. A phenolic compound in peppermint leaves possess numerous health benefits and anti–angiogenesis characteristics [18]. Different types of polyphenols found in this plant leaves can exhibit diverse polarities, therefore, the temperature and the type solvent applied throughout the extraction process are highly influence the polyphenols in the plant extracts [19]. SO, the outgrowth inhibition of the micro blood vessels caused by ME and CE in this assay may be resulted from the presence of the different phenolic constituents in each extracts. Chloroform extract contains an anti-angiogenic monoterpenoid constituent that is D-limonene [20]. Other phytochemicals like vitamin C (ascorbic acid) which also was found to possess anti–angiogenic effect [21]. These findings were established by the phytochemical analysis of both extracts. As a result of the significant anti–angiogenesis activity for chloroform extract and methanol extract in the screening assay, the dose response assay was done for each of them.

Methanol and chloroform extracts 1 , 1-diphenyl-2-picrylhydrazyl [DPPH] scavenging activity

In pathological situations, generation rate of reactive oxygen species (ROS) is overweigh the elimination rate. Consequently, various health disorders like cancer, diabetes mellitus, and inflammatory and neurodegenerative illnesses could be attributed to the free radicals. ROS have the ability to stimulate angiogenesis by augmenting VEGF expression in many types of cells, such as macrophage, smooth muscle cells, and endothelial cells, and this result in the evolution of various angiogenesis associated disorders [22]. Test of scavenging activity for DPPH was done for each methanol and chloroform extracts of *M. piperita* leaves. This test is of a great interest to identify the probable mechanism of action behind their suppression to the blood vessels growth. Methanol extract and chloroform extract displays a significant concentration-dependent reduction for the free radicals in the DPPH assay. This might be due to the existence of phenolic constituents in each extract. Peppermint leaf is a source of antioxidant phytochemicals such as flavonoids (menthoside, isorhoifolin, luteolin and its 7-glucoside) [23], phenolic acids (caffeic, chlorogenic and rosmarinic acid) [24], taninns [25]. Methanol extract exhibited better antioxidant activity than that created by chloroform extract but at a very little bit due to the IC_{50} of the methanol extract was significantly less than that of chloroform extract (3.51µg/ml for ME and 3.7µg/ml for CE). The antioxidant activity produced by ME and CE of *M. piperita* leaves was not only due to the presence of polyphenols, but also the terpenoid D-limonene and ascorbic acid were found in the these extract respectively [26]; these were established by the phytochemical analysis. Ascorbic acid (vitamin c) known for its potent antioxidant activity. In fact, all identified physiological and biological functions of ascorbic acid is related to its ability to donate one or two electrons that make it an excellent reducing compound and antioxidant, and the rate of oxidation is PH dependent [27]. Vitamin c was found to possess anti–angiogenic activity that was proven in a study done by Yeom and his co-workers, which showed that ascorbic acid suppressed blood vessels growth in mice implanted with cancer cells.

CONCLUSION

All extracts of *M. piperita* leaves revealed a significant anti–angiogenesis action. However, methanol and chloroform extracts demonstrated the best anti–angiogenesis activity as well as significant dose – dependent anti –angiogenic effect. Furthermore, each of methanol and chloroform extracts exhibited a significant free radical scavenging activity in DPPH assay in concentration–dependent order.

Acknowledgement: This work is confirmed by the College of medicine Al-Nahrain University.

REFERENCES

1. Hayder B. Sahib, Adeeb A Al-Zubaidy, Shallal M Hussain, Ghaith Ali Jassim. The Anti Angiogenic activity of *Vitex agnus castus* leaves extracts Int J Pharm Pharm Sci, 6, 2014, 863-869.
2. Hayder B Sahiba*, Adeeb A Al-Zubaidya, Ghaith A Jasimb. Anti Angiogenic Activity of *Vitex Agnus Castus* Methanol Extract *In Vivo* Study. Iranian Journal of Pharmaceutical Sciences 12 (1), 2016 59-68.
3. Stephan MD, Harold MD and Olivera MD. 'Clinical application of growth factors and cytokines in wound healing' Wound Rep. and Regen. 22, 5, 2014, 569-578.
4. Adeeb A Alzubaidy Hayder B Sahib, Zena Ayad. The anti – angiogenic activity of phoenix dactylifera seeds extarcts *International journal of pharmacy and pharmaceutical sciences* 8, 1, 2016, 311-315
5. Djonov VG, Baum O and Burri PH. Vascular remodeling by intussusceptive angiogenesis. Cell Tissue Res, 314, 2003, 107-117
6. Carmeliet P. Angiogenesis in health, disease and medicine. Nat. 438, 2005, 932-936.
7. Ali B., AL-Wabel N.A. and Anwar F. Essential oils used in aromatherapy: A systemic review 5(8), 2015, 601-611.
8. Hasani M., Nouri M., Hakimzadeh V., Maleki M. Chemical composition and antimicrobial activity of the essential oil of menthe piperita endemic in Khorasan-Iran. Isl. Aza. Univ. 11(5), 2015, 197-200.
9. Kuzmenko,A.N.,Pashkova,E.B., Pirogov, A.V., Razzhivin, R.V., Reshetnyak, V.Yu. Study of a composition of offincinal herb

mixtures using gas-liquid chromatography with mass-spectrometric detection. Moscow Univ. Chem. Bull. 65, 2010, 106.

10. Zainab Khalid Ali, Haitham1, Mahmood Kadhim1, Hayder B Sahib. The Anti-proliferative Activity of Vitis vinifera Leaves of Methanol Extract Alone and in Combination with Doxorubicin against Liver Cancer Cell Lin Int. J. Pharm. Sci. Rev. Res., 41(2), 66, 2016, 363-366.
11. Nicosia RF, Lin YJ, Hazelton D & Qian X. Endogenous regulation of the angiogenesis in the rat aorta model. Role of vascular endothelial growth factor. Am. J. Pathol, 151, 1997, 115-122.
12. Oktay M., Gulcin I. and Kufrevioglu, O.I. Determination of *in vitro* anti -oxidant activity of funnel (*Foeniculum vulgare*) seed extracts. Leb.-Wiss. U.-Techn. 36, 2003, 263-271.
13. Singh D., Sachan A., Singh H., Nath R. and Dixit R.K. Extraction, isolation and characterization of phytochemicals. Wld j. of pharm. Res. 4(5), 2015, 2703-2717.
14. Shams K.A., Abdel-Azim N.S., Saleh I.A., *et al*. Green technology: Economically and environmentally innovative methods for extraction of medicinal &aromatic plants (MAP) in Egypt. J. Chem. Pharm. Res.2015; 7(5): 1050-1074.
15. Ahmed R., Hayder B. and Muneer M. 'Anti-angiogenic activity of Zizyphus spinachristi Leaves Extracts', Int. J. Pharm. Sci. Rev. Res. 35L: 1, 2015, 169-174.
16. Pereira E., Pimenta A. I. and Ferreia I.C.F.R. 'Infusions of gamma irradiated *Aloysia citrodora* L. and *Mentha piperita* L.:Effects on phenolic composition, cytotoxicity, antibacterial and virucidal activities'. Indus. Crop.and Prod. 97, 2017, 582-590.
17. Battinelli EM, Markens BA and Italiano JE Jr. Release of angiogenesis regulatory proteins from platelet alpha granules: modulation of physiologic and pathologic angiogenesis. Blood, 118, 2011, 1359–1369.
18. Das K, Tiwari RKS and Shrivastava DK. 'Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends'. J. of Med. Plants Res, 4, 2010, 104-111.
19. Al–Rawahi AS, Rahman MS, Guizani N and Essa MM. Chemical composition, water sorption isotherm, and phenolic contents in fresh and dried pomegranate peels. Drying Tec, 31, 2013, 257–263.
20. Murthy K. N. C., Jayaprakasha G. K. and Patil B.S. 'D-limonene rich volatile oil from blood oranges inhibits angiogenesis, metastasis and cell death in human colon cancer cells' Life Sci. 91(11), 2012, 429-439.
21. Shanmugam MK, Dai X, Kumar AP, Tan BK, Sethi G and Bishayee A. Oleanolic acid and its synthetic derivatives for the prevention and therapy of cancer: preclinical and clinical evidence. Cancer Lett, 346, 2014, 206–16.
22. Wang Y, Zang QS, Liu Z, Wu Q, Maass D, Dulan G, Shaul WP, *et al*. Regulation of VEGF-induced endothelial cell migration by mitochondrial reactive oxygen species. Am J Physiol. Cell Physiol, 301, 2011, 695-704.
23. Agati.G., Azzarello E. and Tattini M. 'Flavonoids as antioxidants in plants:Location and functional significance'. Plant Sci. 196, 2012, 67-76.
24. Sato Y., Itagaki S., and Iseki K.'In vitro and in vivo antioxidant properties of chlorogenic acid and caffiec acid' Inter. Jour. Of Pharm. 403(1), 2011, 136-138.
25. Espinoza F.M.C., Zafimahova A. and Legrand P.C. 'Grape seeds and apple tannins: Emulsifying and antioxidant properties' P.C. Food Chem. 178,2015, 38-44.
26. Bacanli M.,Basaran A.A. and Basaran N.The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin. Food and Chem. Toxi. 81, 2015, 160-170.
27. Du, J., Cullen J.J. and Buettner G.R. 'Ascorbic acid: Chemistry, biology and treatment of cancer'. Bioch. Bioph. Act. Rev. on Canc. 1826(2), 2012, 443-457.

Source of Support: Nil, **Conflict of Interest:** None.