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Research Article
Anti-angiogenic and Antioxidant Activity of Iraqi *Cyperus rotundus* Ethanol Extract

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Abstract

Background and Objective: *Cyperus rotundus* (*C. rotundus*) has been used in medicine years ago. The aim of the study was to investigate the possible anti-angiogenic and antioxidant activity and to find the different phytochemicals present in *Cyperus rotundus* ethanol extract that may has anti-angiogenic activity. **Methodology:** Qualitative analysis of various secondary metabolites by specific chemical tests was carried out on the ethanol extract. The *ex vivo* rat aorta ring assay was used to screen the extract for possible anti-angiogenic activity, this assay was also used to determine the dose-response effect of the active extract. Six concentrations of crude extract were tested (100, 50, 25, 12.5, 6.25 and 3.125 μg mL^{-1}) on rat aortic rings to determine the dose response curve. Free radical scavenging activity of extract was determined by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay. **Results:** *Cyperus rotundus* ethanol extract showed significant dose-dependent blood vessels inhibition in comparison to the negative control ($p<0.05$). Ethanol extract showed significant free radical scavenging activity. Phytochemical investigation of alcoholic extract indicated the presence of various chemical constituents like alkaloids, glycosides, steroids, tannins, carbohydrates and flavonoids. **Conclusion:** The anti- angiogenic activity showed by the ethanol extract may be due to the presence of antioxidant compounds.

Key words: Anti-angiogenic, *Cyperus rotundus*, phytochemical analysis, natural products, 1, 1-Diphenyl-2-picrylhydrazyl assay

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cyperus rotundus, the Arabic common name is Saed, Sajal, Seil and in English it is called nut grass, purple nutsedge, Nagarmotha and in China known as Xiang Fu. *Cyperus rotundus* is widely distributed in tropical, subtropical and temperate climates such as in Iraq, Egypt, Tunisia, China and India[1]. The plant fruits were used as carminative, diuretic, tonic, stomachic, anti-bilious and refrigerant[2]. While the tubular part utilized for the treatment of dysmenorrhoea and irregular menstrual cycle. Different pharmacological and biological activities including anti-*Candida*[3], anti-inflammatory[4], antidiabetic[5], antidiarrhoeal, antioxidant[6], cytoprotective[7], antimutagenic[8], antimicrobial, antibacterial[9], cytotoxic and apoptotic, anti-pyretic and analgesic activities have been reported for *Cyperus rotundus*[10]. Angiogenesis is a process that involves the formation of new blood vessels from pre-existing ones, this process is typically initiated within hypoxic tissues where additional new blood vessels are required to maintain oxygenation and nutritional supply[11]. These processes are activated only under certain conditions when there is a physiological demand for increase in the blood supply as in wound healing or implantation of the fertilized egg in the endometrium[12]. Strict regulation of this system is very important for the human being, because both excessive and insufficient development of blood vessels lead to serious diseases[13]. Normal angiogenesis is limited to perhaps a few days or weeks. However, in a disease setting, angiogenesis can persist for months or years[14]. The aim of this study was assessing of anti-angiogenic effect, phytochemical investigation and the free radical scavenging activity of *Cyperus rotundus*.

MATERIALS AND METHODS

The study was carried out from March-June, 2017, in Al-Nahrain University, College of Pharmacy and in University of Baghdad.

Plant material: The *C. rotundus* collected from local market in Baghdad and authenticated by the National Herbarium at Abu-Graib, Baghdad.

Chemicals: Ethanol 98% (BDH), ascorbic acid (Sigma-Aldrich, USA), dimethyl sufoxide (DMSO) Romil, UK, aprotinin (Sigma-Aldrich, USA), fibrinogen (Sigma-Aldrich, USA), thrombin 100 IU vial (Sigma-Aldrich, USA), foetal bovine serum (Sigma-Aldrich, USA), L-glutamine (Sigma-Aldrich, USA), gentamicin 80 mg/2 mL vial (Al-Hikma, Jordan), aminocaproic acid (Sigma-Aldrich, USA), amphotericin B vial (Bristol Myers Squibb, England), Earls salt M199 solution (Sigma-Aldrich, USA).

Extraction of *Cyperus rotundus*: The dried and powdered whole plant parts (which includes roots, rhizomes, flowers and leaves) of *Cyperus rotundus* (200 g) extracted with 85% ethanol using Soxhlet apparatus until exhaustion for 16 h. Alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40°C using rotary evaporator to produce crude extract.

Preliminary qualitative phytochemical analysis: Chemical tests were carried out using ethanol extracts of *Cyperus rotundus* and standard procedures[15] were carried out to identify the active constituents. Alkaloids identified by Mayer's test, 10 mL of ethanol extract was mixed with 5 mL of 1% HCL on a steam bath then added the reagent, the result is white precipitate indicating +ve test for alkaloids. Flavonoids identified by lead acetate: 5 mL of ethanol extract mix with 1 mL of 10% lead acetate to yield yellowish- white precipitate indicating the presence of flavonoids. Steroids Liebermann-Burchard identified via taking 3 mL of extract stirred with chloroform, acetic anhydride and few drops of sulphuric acid to yield dark pink or red colour indicate the presence of steroids. Anthraquinones identified by Borntrager's test through taking 3 mL of extract was shaken with 3 mL of benzene. Then, filtration was performed and 5 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken well, the development of a pink, red or violet colour in the ammonia (lower) phase indicates the presence of anthraquinones. Tannins been discovered by $FeCl_3$ solution (5% w/v) through taking 3 mL of distilled leaves extract (in water) mixed with 3 mL of $FeCl_3$ solution (5% w/v) to produce dark green or blue black precipitate indicate the presence of tannins. Terpenoids identification done by taking ethanol extract (2 mL) and dissolved in chloroform (2 mL) and evaporated to dryness. Concentrated sulphuric acid (2 mL) was then added and heated for about 2 min. A greyish colour was considered an indication for the presence of terpenoids.

Rat aorta ring anti-angiogenic assay: The rat aortic ring assay experiment was conducted after the experimental procedures were revised and approved by Ethics Committee of Al-Nahrain University, College of Medicine. The assay was performed according to the standard protocol[16]. Albino male rats were

humanely sacrificed via cervical dislocation under anaesthesia with diethyl ether. Thoracic aorta was excised, rinsed with serum free media, cleaned from the fibro-adipose tissue and was cross sectioned into thin rings of 1 mm thickness. A 300 µL of M199 growth medium (prepared by addition of fibrinogen 3 mg mL^{-1} and aprotinin 5 µg mL^{-1} to M199) was used as lower layer and loaded in 48-well plate and one aortic ring was seeded in each well. To each well, 10 µL of thrombin was added and then was incubated and allowed to solidify at 37°C in 5% CO_2 for 30-60 min. The top layer medium was prepared by adding 20% of heat inactivated fetal bovine serum (HIFBS), 1% L-glutamine, 0.1% aminocaproic acid, 1% amphotericin B and 0.6% gentamicin to M199 medium. A stock solution of *Cyperus rotundus* extract was prepared by dissolving the sample in dimethyl sulfoxide (DMSO) and diluted in M199 growth medium to make the final DMSO concentration 1%. Serial dilutions of the active extract were prepared in the following concentrations: 100, 50, 25, 12.5, 6.25 and 3.125 µg mL^{-1}. Wells without test samples were received medium with 1% DMSO used as the negative control. The tissue rings were incubated at 37°C, 5% CO_2 in a humidified incubator. The results examined on day 5 under inverted microscope and the extent of blood vessel growth was quantified under 10X magnification with aid of camera and software package. The magnitude of blood vessel growth inhibition was determined according to the technique developed by Nicosia *et al.*[17]. The experiment was repeated 3 times using six replicate per sample and the percentage of blood vessels inhibition was determined according to the following formula:

$$\text{Blood vessels inhibition (\%)} = 1 - \frac{A0}{A} \times 100$$

Where:

A0 = Distance of blood vessels growth for the test substance in mm

A = Distance of blood vessels growth in the control in mm. The concentration that inhibits 50% of the growing blood vessels "IC_{50}" was calculated using the logarithmic equation for the extract[18]

Antioxidant activity (DPPH radical scavenging assay): Free radical scavenging activity of the active extract was measured by using the DPPH method. Two hundred microliters of 0.1 mM DPPH dissolved in methanol was added to 100 µL of the active extract in the following concentrations (200, 150, 100, 50 and 25 µg) and incubated for 30 min. This procedure was executed using 96 well plates and each concentration was tested in triplicate, then the absorbance was measured at 517 nm using an ELISA reader. Ascorbic acid (Vitamin C) was used as a positive control and methanol alone as blank. The negative control was made of 100 µL of methanol and 200 µL DPPH. The percentage of antioxidant activity (AA) was calculated according to the formula[19]:

$$\text{Antioxidant activity (\%)} = 1 - \frac{\text{AS-AB}}{\text{AC-AB}} \times 100$$

Where:

AS = Absorbance of sample

AB = Absorbance of blank

AC = Absorbance of control

Statistical analysis: Statistical analysis was performed using Statistical Package for the Social Sciences SPSS 20, IBM, Armonk, NY, United States of America Software (version 20.0) data presented were Mean±SD from three different experiments. Statistical significance between different groups was determined using paired t-test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Dose response curve of extract on rat aortic ring model: Six serial concentrations of extracts (100, 50, 25, 12.5, 6.25 and 3.125 µg mL^{-1}) was prepared and added to the embedded rat aortic rings to determine the dose response curve. Extract showed significant dose dependent inhibition of blood vessels growth when compared to the negative control (DMSO 1%) ($p<0.05$) at 5 days of the experiment as found in Table 1. Also the dose response effect of each extract on blood vessels growth is shown in Fig. 1. The IC_{50} was determined from the logarithmic equation that is shown in Fig. 2 and it was found to be 15.39 µg mL^{-1}.

Table 1: Qualitative analysis of *Cyperus rotundus* extract

Tested component	Type of test	Result
Alkaloids	Mayer	Present
	Wagner	Present
Flavonoids	Lead acetate	Present
Steroids	Liebermann-Burchard	Present
	H_2SO_4	Present
Tannins	$FeCl_3$	Present
Anthraquinones	Borntrager	Not present
Terpinoids	Keller-kiliani	Present

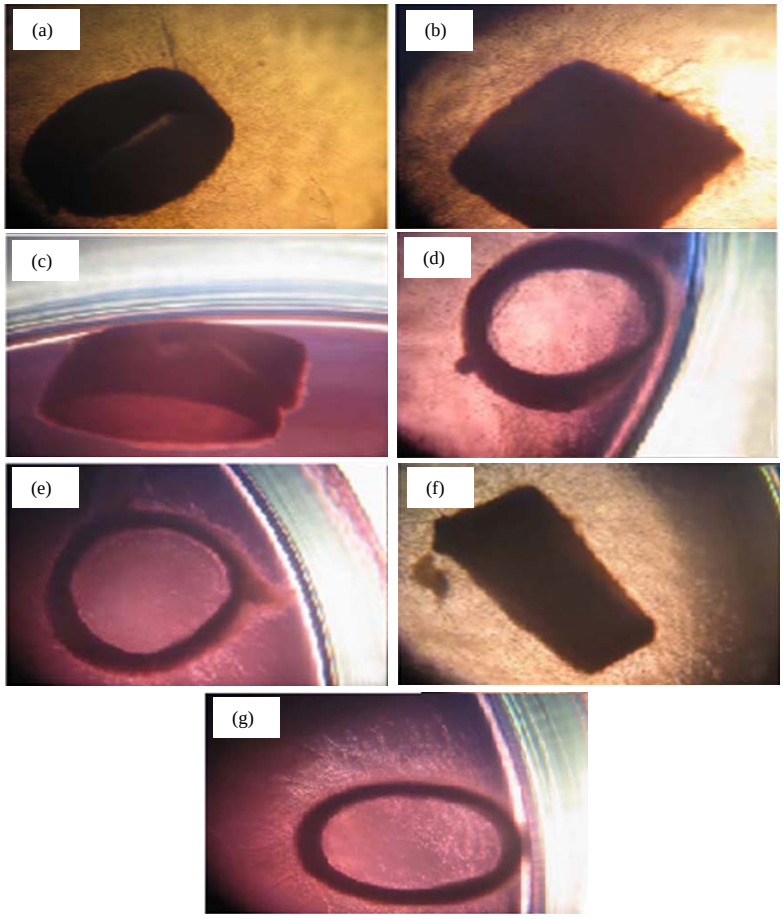

Fig. 1(a-g): Dose response effect of *Cyperus rotundus* ethanolic extract in rat aortic rings assay, represent the blood vessels growth inhibition which treated with (a) 1% DMSO, (b) 12.5, (c) 100, (d) 6.25, (e) 50, (f) 3.125 and (g) 25 μg mL^{-1}, respectively

Antioxidant activity: The free radical scavenging activity of extracts was measured using the DPPH assay. Five serial concentrations were used to determine the scavenging activity as shown in Table 2. Ascorbic acid had significantly higher scavenger activity compared to *Cyperus* extract at concentrations 25, 50, 100 and 150 μg mL^{-1}. However, at concentration 200 μg mL^{-1} there was no significant difference as shown in Fig. 3. The IC_{50} equal to 129 mg mL^{-1} according to Fig. 4.

Phytochemical analysis: Phytochemical analysis indicates the presence of alkaloids, flavonoids, anthraquinones, tannins and steroids as shown in Table 3. The presence of these groups provides a scientific rational for the supposed etheno-medical uses of *Cyperus rotundus* in the treatment of indicated diseases. The presence of tannins, flavonoids, alkaloids and terpenoids seems to be significant.

Table 2: Serial concentrations and their respective percentage of blood vessels inhibition

Concentration (μg mL^{-1})	Inhibition (%)
100	91.40
50	66.54
25	56.56
12.5	45.10
6.25	21.40
3.125	6.55

Table 3: Percentage of DPPH free radical scavenging activity for ethanol extract of *Cyperus rotundus*

Concentration (mg mL^{-1})	Sample	Ascorbic acid	p-value
25	66.27±2.74	95.70±0.36	<0.01 [Sig.]
50	68.70±0.56	90.70±0.41	<0.01 [Sig.]
100	78.22±0.50	100.00±0.00	<0.01 [Sig.]
150	92.50±2.45	100.00±0.00	<0.01 [Sig.]
200	100.00±0.00	100.00±0.00	>0.05 [NS]
p-value	<0.001	<0.01	

Scavenger activity presented as mean±SD, Sig.: Significant difference, NS: Not significant

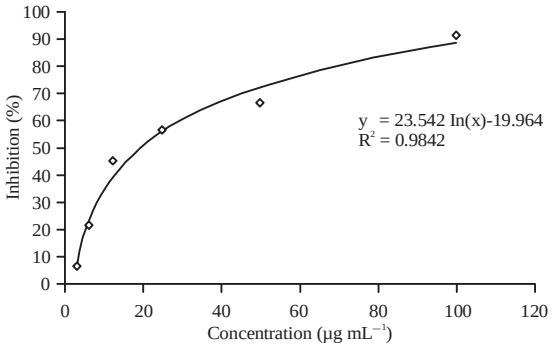

Fig. 2: Dose response curve of ethanol extract of *Cyperus rotundus* in rat aortic rings model

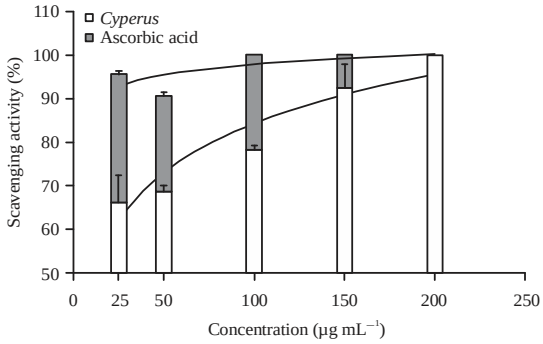

Fig. 3: Relationship between scavenger activity and concentration of both agents

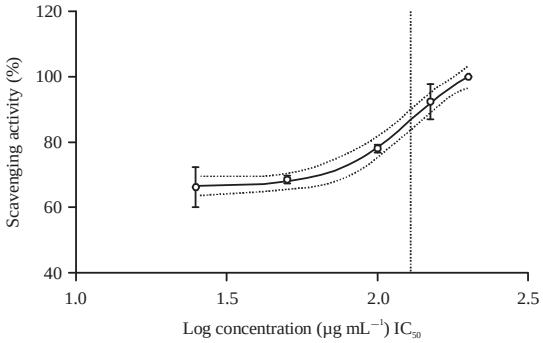

Fig. 4: Semilog of the relationship between scavenger activity of *Cyperus* extract and log concentration

DISCUSSION

This study showed that *Cyperus rotundus* ethanol extract has robust anti-angiogenic activity, because angiogenesis process linked to many important and serious diseases and linked to important physiological process as well many scientists have focused on this process and targeting its steps as mean to stop or control it[20]. Further investigations were mandatory to understand the possible mechanism of action which may elucidate the pharmacologic action and the possible interaction may have as well. Free radical scavenging activity was tested for the extract in comparison to vitamin C which approved as antioxidant drug, the data showed that this extract has significantly scavenge the free radicals and this finding agreed with previous study conducted by Soumaya *et al.*[21], moreover it was important to identify the possible bioactive constituents and the results showed that there are many agents with potent antioxidant activity. Several studies demonstrated that antioxidants were able to inhibit angiogenesis process both, *in vitro* and *in vivo*[22]. These compounds include natural substances (catechins, resveratrol, polyphenols, flavonoids, isoflavones, lycopene, pigment epithelium-derived factor, glutathione), nutritional components (vitamins C, D, E, β-carotene and selenium) and semi-synthetic and synthetic compounds (Nacetylcysteine, L-NAME, L-NIO, sodium pyruvate, pyrrolidine dithiocarbamate and organoselenium compounds)[23]. In this concern, agent with antioxidant activity strongly inhibit vascular endothelial growth factor VEGF expression and H_2O_2-induced release of VEGF in vascular smooth muscle cells in concentrations that are likely to be achieved in blood after moderate consumption of antioxidant activity[24]. Moreover, antioxidant agents showed ability to scavenge ROS such as hydroxyl radical and superoxide anion to inhibit the expression of xanthine oxidase to diminish adhesion and invasion of tumoural cells induced by ROS from hypoxanthine/xanthine oxidase system and to increase activity of catalase and glutathione peroxidase[24]. This study considers the first step in identifying the pharmacological activity, further study required to identify the responsible agents by using high performance liquid chromatography. Moreover, *in vivo* study very important to start the formulation prior conducting clinical trial.

CONCLUSION

Phytochemical investigation of Iraqi *Cyperus rotundus* was done and the results revealed the presence of alkaloids, flavonoids, steroids, tannins and terpenoids in the whole plant extract and the absence of anthraquinones in this plant parts, also this study demonstrates the positive anti-angiogenic effect of *Cyperus rotundus*, ethanol extract showed significant reduction of free radicals by the DPPH assay and in a concentration-dependent manner. Free radical scavenging activity was very important in order to better understand the

possible mechanism of action behind their ability to suppress blood vessels growth.

SIGNIFICANCE STATEMENT

This study discovers the significance anti-angiogenic activity of *Cyperus rotundus* ethanol extract-that can be beneficial for targeting many pathological process such as rheumatoid arthritis, tumor and psoriasis. This study may help researchers in future to formulate it and test it clinically to be prescribe later on. This study will help the researchers to uncover the critical areas of fighting tumor and other angiogenesis related disease and possibly other combinations may be arrived at. Regarding to the novelty of this study no one has conducted such study on this extract.

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REFERENCES

1. Cirujeda, A., A. Anzalone, J. Aibar, M.M. Moreno and C. Zaragoza, 2012. Purple nutsedge (*Cyperus rotundus* L.) control with paper mulch in processing tomato. Crop Prot., 39: 66-71.
2. Ates, D.A. and O.T. Erdogrul, 2003. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J. Biol., 27: 157-162.
3. Duarte, M.C.T., G.M. Figueira, A. Sartoratto, V.L.G. Rehder and C. Delarmelina, 2005. Anti-*Candida* activity of Brazilian medicinal plants. J. Ethnopharmacol., 97: 305-311.
4. Sundaram, M.S., T. Sivakumar and G. Balamurugan, 2008. Anti-inflammatory effect of *Cyperus rotundus* Linn. Leaves on acute and subacute inflammation in experimental rat models. Biomedicine, 28: 302-304.
5. Raut, N.A. and N.J. Gaikwad, 2006. Antidiabetic activity of hydro-ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats. Fitoterapia, 77: 585-588.
6. Kilani, S., R.B. Ammar, I. Bouhlel, A. Abdelwahed and N. Hayder *et al*., 2005. Investigation of extracts from (Tunisian) *Cyperus rotundus* as antimutagens and radical scavengers. Environ. Toxicol. Pharmacol., 20: 478-484.
7. Zhu, M., H.H. Luk, H.S. Fung and C.T. Luk, 1997. Cytoprotective effects of *Cyperus rotundus* against ethanol induced gastric ulceration in rats. Phytother. Res., 11: 392-394.
8. Kilani, S., I. Bouhlel, R.B. Ammar, M.B. Shair and I. Skandrani *et al*., 2007. Chemical investigation of different extracts and essential oil from the tubers of (Tunisian) *Cyperus rotundus*. Correlation with their antiradical and antimutagenic properties. Ann. Microbiol., Vol. 57. 10.1007/BF03175369.
9. Dhillon, R.S., S. Singh, S. Kundra and A.S. Basra, 1993. Studies on the chemical composition and biological activity of essential oil from *Cyperus rotundus* Linn. Plant Growth Regul., 13: 89-93.
10. Pal, D.K. and S. Dutta, 2006. Evaluation of the antioxidant activity of the roots and rhizomes of *Cyperus rotundus* L. Indian J. Pharm. Sci., 68: 256-258.
11. Al-Zubaidy, A.A., H.B. Sahib and M.H. Sadiq, 2016. Anti-angiogenig activity of *Fenugreek* extracts: *In vivo* and *ex vivo* study. Int. J. Pharm. Sci. Rev. Res., 36: 184-189.
12. Jasim, G.A., A.A. Alzubaidy, S.M. Hussain and H.B. Sahib, 2015. The antiangiogenic activity of *Commiphora molmol* oleo-gum-resin extracts. Int. J. Pharm. Sci. Rev. Res., 33: 83-90.
13. Sahib, H.B., A.F. Aisha, M.F. Yam, M.Z. Asmawi and Z. Ismail *et al*., 2009. Anti-angiogenic and anti oxidant properties of *Orthosiphon stamineus* benth. Methanolic leaves extract. Int. J. Pharmacol., 5: 162-167.
14. Swift, M.E., H.K. Kleinman and L.A. DiPietro, 1999. Impaired wound repair and delayed angiogenesis in aged mice. Lab. Invest.: J. Tech. Meth. Pathol., 79: 1479-1487.
15. Sarker, S.D. and L. Nahar, 2012. An Introduction to Natural Products Isolation. In: Natural Products Isolation, Sarker, S.D. and L. Nahar (Eds.)., Springer, Germany, pp: 1-25.
16. Brown, K.J., S.F. Maynes, A. Bezos, D.J. Maguire, M.D. Ford and C.R. Parish, 1996. A novel *in vitro* assay for human angiogenesis. Lab. Invest., 75: 539-555.
17. Nicosia, R.F., Y.L. Lin, D. Hazelton and X. Qian, 1997. Endogenous regulation of angiogenesis in the rat aorta model. Role of vascular endothelial growth factor. Am. J. Pathol., 151: 1379-1386.
18. Sharma, O.P. and T.K. Bhat, 2009. DPPH antioxidant assay revisited. Food Chem., 113: 1202-1205.
19. Stiffey-Wilusz, J., J.A. Boice, J. Ronan, A.M. Fletcher and M.S. Anderson, 2001. An *ex vivo* angiogenesis assay utilizing commercial porcine carotidartery: Modification of the rat aortic ring assay. Angiogenesis, 4: 3-9.
20. Sahib, H.B., N.A.H. Harchan, S.A.M. Atraqchi and A.A. Abbas, 2010. The role of medicinal herbs in angiogenesis related diseases. Int. J. Pharmacol., 6: 616-623.

21. Soumaya, K.J., G. Zied, N. Nouha, K. Mounira, G. Kamel, F.D.M. Genvieve and G.C. Leila, 2014. Evaluation of *in vitro* antioxidant and apoptotic activities of *Cyperus rotundus*. Asian Pac. J. Trop. Med., 7: 105-112.
22. Carmeliet, P., 2003. Angiogenesis in health and disease. Nat. Med., 9: 653-660.
23. Sahib, H.B., A.A. Al-Zubaidy, S.M. Hussain and G.A. Jassim, 2014. The anti angiogenic activity of *Vitex agnus castus* leaves extracts. Int. J. Pharm. Pharm. Sci., 6: 863-869.
24. Prauchner, C.A., 2014. Angiogenesis inhibition by antioxidants. Int. J. Biomd. Sci. Eng., 2: 7-19.