RESEARCH ARTICLE

Anti-angiogenic Activity of *Rosa canina* Extracts, an *Ex-vivo* and *In-vivo* Study

Zaman M. Jasim[1]*, Ghaith A. Jasim[1], Ibrahim S. Abbas[2]

[1] *Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Iraq*
[2] *Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Mustansiriyah University, Iraq*

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ABSTRACT

Angiogenesis, known as blood vessel growth from preexisting vasculature, occurs when proangiogenic overcomes the angiogenic factor. The most important angiogenic factors (promotors) of angiogenesis were vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), angiogenin and tumor necrosis factor (TNFα). *Rosa canina,* commonly known as rose hip or dog rose, widely distributed in Europe, Asia and North America, belongs to the Rosacea family. Different biological compounds were found in *R. canina,* such as high phenolic composition (specifically flavonoids), vitamins as vitamin C, carotenoids, and fatty acids as (linolenic acid). Polyphenolic compounds are very important due to its efficacy as antioxidants and antiangiogenic. The dried powder of *R. canina* was extracted by using successive solvent extraction according to polarity (hexane, ethyl acetate and ethanol). The yield% of hexane, ethyl acetate and ethanol extracts were (0.8, 1.2 and 2.4 g per 100 g dried powder). Rat aorta assay (*ex-vivo*) was done to investigate antiangiogenic activity and choose the most bioactive extract. IC_{50} of avastin (positive control), ethanol, ethyl acetate and hexane were 20.281, 33.582, 61.744 and 94.537, respectively. The results revealed that ethanol extract (EE) was the most biologically active extract. Chorioallantoic membrane assay (CAM) *in-vivo* was done using *R. canina* ethanol extract. eosinophilic esophagitis (EE) has a greater percentage of inhibition of blood vessels when used in two concentrations 250 and 500 mg/20 mL. The zone of inhibition as mean ± SD was 19.921 ± 4.048 mm and 30.302 ± 2.805 mm, respectively. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), was used to identify the phytochemical constituents of the EE. In the present study, EE of *R. canina* revealed six flavonoid compounds (rutin, quercetin, catechine, astragalin, hyperoside and gallic acid) that could be responsible for the biological activity of the ethanol extract. In conclusion, the EE of *R. canina* reported a promising antiangiogenic activity both *ex-vivo* and *in-vivo* that may be attributed to the flavonoid content of the extract. The purpose from the study in order to investigate the antiangiogenic activity of *R. canina* extracts *ex-vivo* and *in-vivo*.

Keywords: Keywords

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INTRODUCTION

Angiogenesis is a process of new blood vessel formation from preexisting vasculature. It is considered a strongly regulated process, stimulated when the proangiogenic overcomes the antiangiogenic molecules.[1] The angiogenesis term belongs to two Greek words: angio which means blood vessel, and genesis initiation or beginning.[2] Several endogenous angiogenic factors (promotors), including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), Transforming growth factor (TGF), platelet-derived growth factor (PDGF), tumor necrosis factor (TNFα), angiogenin, fibroblast growth factor (FGF), protease and protease inhibitors and endogenous modulators such as angiopoietin-1, angiostatin-11. Inhibition of angiogenesis occurred by several factors such as tissue inhibitors metalloprotease, p53, angiopoietin-2, angiotensin, and interleukin 10, 12 and interferon alpha.[3]

Types of Angiogenesis

Sprouting angiogenesis a process of angiogenesis occurred when there is tumor-induced hypoxia. The basic mechanisms are first the local stimulation of endothelial cells, second basement membrane degradation, third proliferation and migration of endothelial cells and finally sprouting stabilization and tubulogenesis, tube formation. VEGF is an important

*Author for Correspondence: pharmacyelegance1@gmail.com

factor known as vascular permeability factor produced by many cells such as tumor cells, platelets, macrophages, renal mesangial cells and keratinocytes. The VEGF and its receptors (VEGFR-1 and VEGFR-2) function are not only restricted to the physiological function, but also have a role in multiple pathological angiogenesis such as tumor angiogenesis. VEGF-A is a key driver of sprouting angiogenesis formation that was overexpressed in several solid tumors. Therefore, VEGF-A inhibition could suppress tumor growth in animal models.[4] The most important physiological functions include bone formation, wound healing and hematopoiesis. Therefore, anti-VEGF strategies that are used for the treatment of cancer, target the proangiogenic VEGF function throughout inhibition of neovascularization.[5,6]

Intussusceptive angiogenesis is also known as splitting angiogenesis because the vessel wall reaches into the lumen, leading to splitting the vessel wall. Intussusceptive angiogenesis has recently been discovered by (Caduff et al., 1986; Burri and tarek 1990). Intussusceptive process make new capillaries to develop when capillaries are already present was depended on the existence of trans-capillary pillars. This type of angiogenesis was discovered in the post-natal lungs of both rats and humans. This type is faster in comparison with sprouting angiogenesis so it is more prominent in the embryo during vascular development.[7-9]

Angiogenesis Inhibition

VEGF antibodies such as humanized anti-VEGF monoclonal antibodies, act as an antiangiogenic strategy.[10] Angiogenic inhibitors are categorized into direct inhibitors that target endothelial cells found in the growing vessels such as angiostatin, endostatin, canstatin or indirect inhibitors that inhibit the expression or block the activation of angiogenic inducers, such as bevacizumab. Antiangiogenic therapy can be used as either monotherapy or in combination with other anticancer medications.[11,12]

Natural Antioxidants in the Treatment of Cancer

Natural antioxidants such as polyphenols, vitamins, and plant-derived bioactive substances that acts as physiological enzymes have been used, for example, superoxide dismutase, catalase and glutathione peroxidase. Natural antioxidants have anti-inflammatory and antioxidant characteristics found in many vegetables and species. Flavonoids, vitamins, curcumin, quercetin, carotenoids and others, have been used as complementary therapies for cancer treatment.[13]

Rosa canina

Rosa canina belongs to the rosacea family.[14] *R. canina* Has an anti-inflammatory effect and therefore indicates osteoarthritis treatment. The bioactive ingredients of *R. canina* are vitamin C, polyphenolic compounds, flavonoids, organic acids, poly saturated and unsaturated fatty acids, carotenoids and tocopherol and tannins.[14]

METHODOLOGY

Experimental Animals

Four adult male albino rats, weighing 200–300 g were used in the rat aorta antiangiogenesis assay. The animals were kept in a well-ventilated cage with providing food and water. The tests were approved in the college of pharmacy, Mustansiriyah University/Baghdad–Iraq.

Rat Aorta Ring Antiangiogenesis Assay (*ex- vivo*)

The angiogenesis assay tested in this study depends on the study developed by Brown and Coworkers with a slight adjustments.[15] MEM 500 mL, fetal bovine serum 50 mL, penicillin and streptomycin 10 mL, EGF 10 ng/mL of MEM media.[16,17] The stock solution was prepared by taking 200 mg from each extract and dissolved in 2 mL of dimethyl sulphur oxide (DMSO). Using serial dilution from each extract of *R. canina*, 200 mg of each extract was obtained. Ethanol, ethyl acetate and hexane were used in triplicate well plates. To obtain serial dilution. 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/mL of media.[18,19] Phosphate buffer saline was used for washing of an aorta. Euthanize four rats and dissect the thoracic aorta by eliminating the lung, esophagus and heart. Then forceps and a blade were used to take the aorta from the spine and washed in cold PBS. The surrounding tissue and fat were removed by using of the blade. The aorta was sectioned into approximately 20 rings of about 1 mm in width.[20] 100 mL of media was added in 96 flat well plates. The ring was added to each well plate above the media. Incubate them overnight to provide rest to the aorta. The 2nd day, 100 mL from each extract, positive control and negative control that diluted with media in 96 well plate above was added to the ring in the following concentration (200, 100, 50, 25, 12.5 , 6.25, 3.125 and 1.56 mg/mL). Each concentration was repeated 3 times for each extract. Incubate them for 5 days and the media were replaced each 3 days.[21-23]

Imaging and Measurement of Sprouting of the Aorta Rings

Visualizing of rings by using an inverted microscope under 4X magnification on 7th and 8th day of the experiment by the presence of the camera and laptop.[24] The maximal sprouting was observed at 9th day.[25] The tiny blood vessel length was measured by using a micro capture program. The calculation was represented as mean ± standard deviation (SD). The test was repeated 3 times. Blood vessel inhibition percentage was calculated depending to the following formula.[26]

$$\text{Blood vessel inhibition} = 1-(A0/A)*100$$

Where

A0 = blood vessel growth distance in mm

A = blood vessel growth distance of the control in mm

Rat Aorta Antiangiogenic Dose-response Study of the Ethanol, Ethyl Acetate, Hexane Extracts and Positive Control (Avastin) of *R. canina*

Serial dilution from each extract and positive control of *R. canina* were provided in the following concentrations. (1.56,

3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/mL) The samples were diluted in the media to prepare the final DMSO concentration of 1%. Negative control was prepared by adding DMSO to media alone without adding sample from any extract. 200 mg/mL of aspirin was used as positive control. The calculations were found as mean ± SD. The IC_{50} is the inhibiting concentration of the blood vessel growth by 50% was measured by the linear regression equation for the ethanoic extract.

Y= the inhibition percentage

X= the concentration

Chick Chorioallantoic Membrane Assay (CAM assay)

The CAM is an extraembryonic membrane formed on day four from the incubation period of fertilized eggs. CAM is characterized by the rapid growth of capillaries that are responsible for the supply of oxygen and nutrients.[27] CAM assay used to detect the antiangiogenic activity of RC ethanol extract. Fertilized eggs were obtained from Agricultural Research Department in Abu Ghraib, Baghdad, Iraq, cleaned by using antiseptic alcohol spray 70% and povidone-iodine 10% solution, then incubated at 37°C with relative humidity associated with egg movement around the axis of north, south of the egg.[28] The fertilized eggs were incubated at 37°C under constant humidity. 2–3 mL of albumin were drawn on day three of incubation so the CAM can be separated from the shell using syringe gage.[18] The window is then closed with sterile adhesive tape and incubated until time of experiment[29] on 9th day of incubation, the sample was prepared as 100 mg/mL of *R. canina* ethanol extract. 500 and 250 mg of extract were used. 20 mL was used on round disk of filter paper; transfer the sample after drying to the CAM. After that, the window was reclosed and returned to an incubator for further 3 days. On days 10, 11, 12, the pictures were taken in order to calculate the zone of inhibition.[30-33]

Quantification and Imaging of CAM Assay

The eggs were grouped to control and ethanol extract groups. Each one consist of 12 eggs. Calculating the area covered by several veins was utilized as initiation and termination points.[34] The control group represents eggs without treatment; tested group received two concentrations of *R. canina* ethanol extract 500 mg/20 mL and 250 mg/20 mL. The zone of inhibition was calculated by using image analyzer that called (micro capture 6.9.12) according to these scores 3–6 mm means (+), 7–9 mm means (++) and more than 10 mm means (+++).

Identification of the Phytoconstitutes

Thin Layer Chromatography (TLC)

Is a technique used to isolate nonvolatile mixtures? This analysis is performed on an inert sheet as plastic, aluminum or glass, that is covered with a thin layer of adsorbent material such as silica gel, cellulose or aluminum. The thin layer is called the stationary phase. The solvent mixture used in this test was called mobile phase.

Ultraviolet light was used to visualize the spots raised with the mobile phase.[35,36] TLC was done on crude extract of *R. canina* in the laboratory of pharmacognosy in Mustansyrhea University/College of pharmacy. The spots of sample can be noticed by using UV light chamber.[37,38] TLC was developed by Izmailov in 1938 including Mikhail Tswetts description on chromatography.[39]

High-performance Liquid Chromatography (HPLC)

Sample was analyzes on agilent 1200 system with delivery system of binary pumpLC-20 AT. Diode array SPD-M20 A, in addition to UV-vis detector. 10 mL of sample was injected on an EclipseXDB-C18. Flow rate was 0.5 c. the chromatograms were noticed at 280, 340, and 520 nm, respectively. The identification and peak assignment of compounds depended on their retention time, UV-vis spectra, and these results were compared with standard data. The measurements were done in the positive mode with the voltage of ion spray about 3000 V. while the capillary temperature about 350°C. This test is used to identify flavonoids, polyphenolic compounds and anthocyanins dependent on molecular mass determination.[40-42]

RESULTS

Rat Aorta Assay

The growth of blood vessel inhibition was found as mean percentage ± SD as in Table 1. There was a significant difference in the inhibition of blood vessels between hexane, ethyl acetate and ethanol ($p < 0.05$). Significant differences in blood vessel inhibition also found between each of three

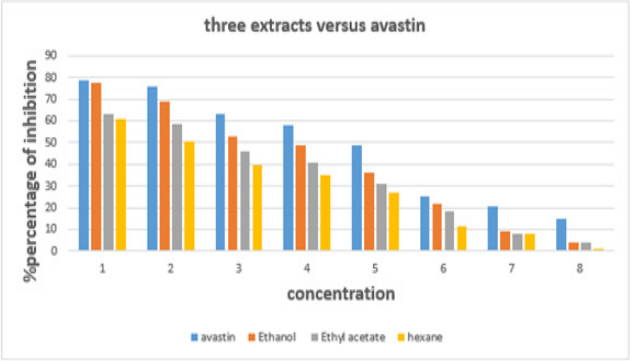


Figure 1: the effect of each extract in addition to positive control (Bevacizumab).

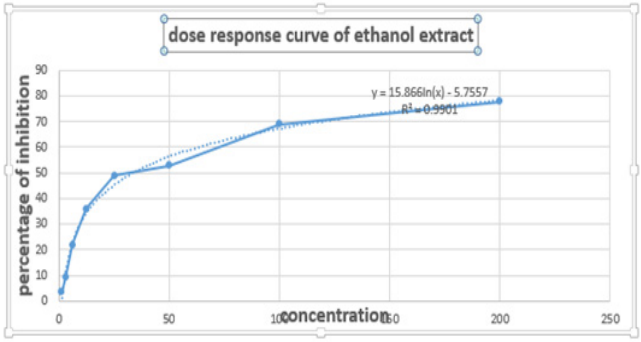


Figure 2: dose-response curve of *R. canina* ethanol extract on rat aorta ring assay from the above figure, the IC_{50} was calculated as (IC_{50} = 33.582)

Table 1: Inhibition of blood vessel growth stimulated by tested samples.

Extract	Mean percentage % +- SD of three extracts , Avastin & DMSO							
	1.56	3.125	6.25	12.5	25	50	100	200
DMSO								zero
bevacizumab	14.627± 1.175	20.69± 0.845	25.16± 0.954	48.696± 1.21	57.768± 1.222	62.893± 1.0004	75.696± 1.077	78.514 ±1.078
Hexane	1.069± 0.02	7.95± 1	11.632 ± 1.005	26.883± 0.995	35.117± 1.005	39.637± 1.01	50.25± 1.005	60.95± 0.99
Ethyl acetate	3.883± 1.004	8.109± 1	18.109 ±0.999	30.692± 1.01	40.788± 0.98	45.687± 1.10	58.408± 1	63.011 ±1.001
Ethanol	3.65± 0.839	8.98± 1	21.76± 0.995	35.87± 0.951	48.97± 1.01	52.65± 0.759	68.87± 1.05	77.76± 1.005

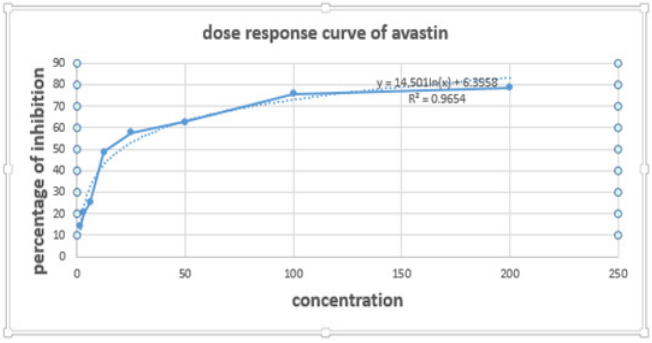


Figure 3: Dose-response curve of Avastin on rat aorta ring assay (IC_{50}= 20.281)

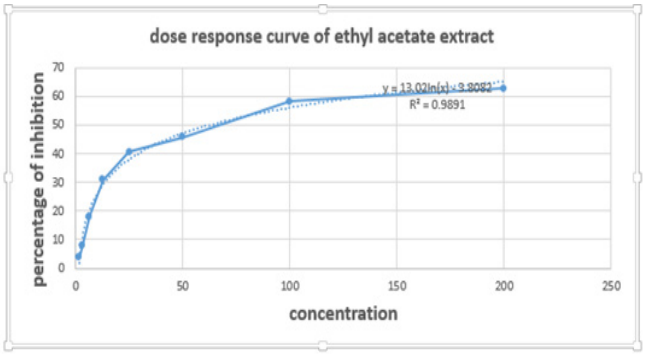


Figure 4: Dose-response curve of *R. canina* ethyl acetate extract on rat aorta ring assay. (IC_{50} = 61.744).

extracts and negative control DMSO (a compound used in order to dissolve sample) ($p < 0.05$). In addition to there is no significant difference between ethanol extract and positive control (Avastin) as an antiangiogenic. The ethanol extract was more biologically active than hexane and ethyl acetate, inhibiting blood vessel growth as antiangiogenic as in Table 1 and Figure 1.

Dose-response Curve of *R. canina* Ethanol Extract on Rat Aorta Rings

The serial dilution of ethanol extract of *R. canina* were added to the aorta rings. Eight concentrations were utilized (1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 mg/mL). According to the table below, there was a significant difference in the inhibition of blood vessel growth in all concentrations of ethanol extract in comparison with DMSO. IC_{50} for ethanol, avastin, ethyl acetate and hexane extracts were calculated from the linear regression equation as in the Figure 2-5.

Where y = inhibition percentage and x= the concentration.

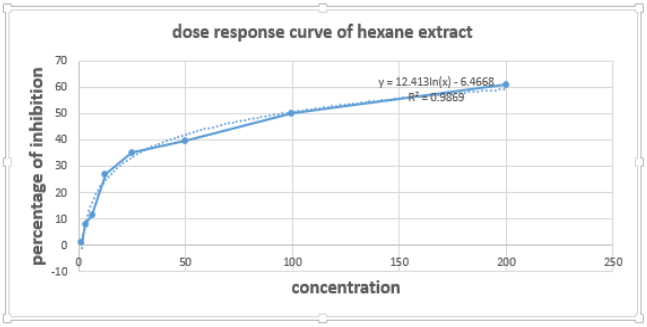
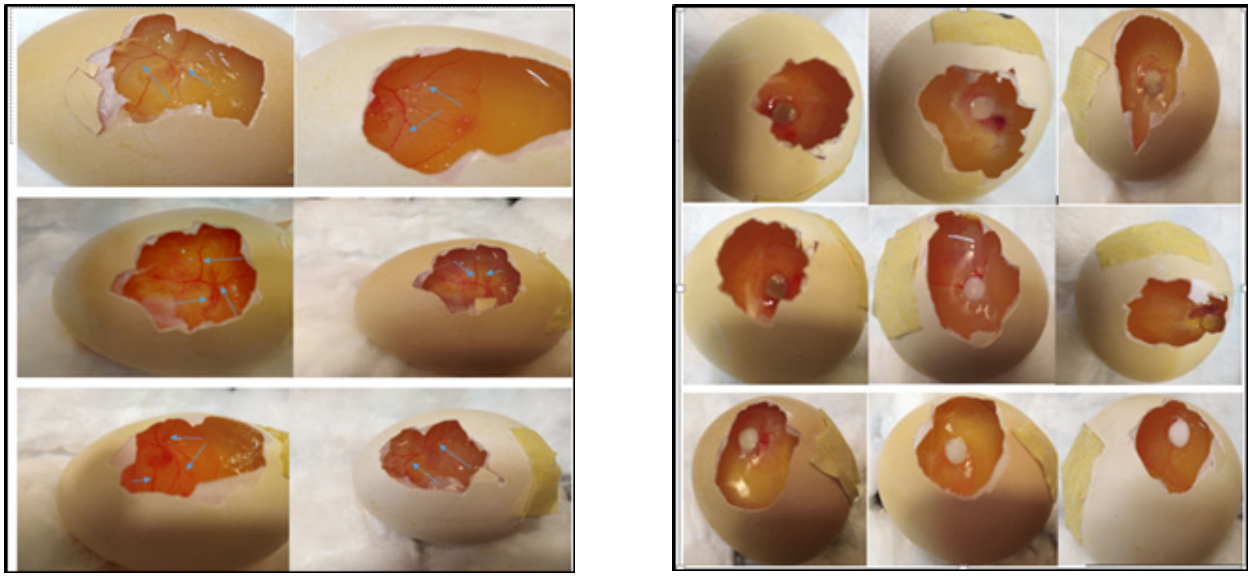


Figure 5: Dose-response curve of *R. canina* hexane extract on rat aorta ring assay (IC_{50}= 94.537)

The calculations represent significant dose related inhibitions that associated with 50% inhibition the concentration equals to (33.582 mg/ mL)



A B

Figure 6: (A) Images of Chick chorioallantoic membrane assay of control eggs while (B) represents treated eggs with EE.

Table 2: results of zone of growth inhibition described as mean ± st dev for different concentration of *R. canina* in CAM assay. Score of inhibition zone are {+} for 3–6 mm. {++} for 7–9 mm and {+++} for mean that was more than 10 mm

Scoring zone of inhibition in mm								
RC ethanol extract	concentration	Egg1	Egg2	Egg3	Egg4	Egg5	Egg6	Zone of inhibition (mean± St dev)
Group 1	250microgram/20microliter	17.2±2.553/ +++	24.933±4.261/ +++	24.966±4.384/ +++	15.933±1.582/ +++	19.366±1.15/ +++	17.133±3.967/ +++	19.921±4.048/ +++
Group 2	500 microgram / 20 microliter	31.433±7.947/ +++	29.7±4.158/ +++	25.566±5.095/ +++	29.25±8.558/ +++	33.333±7.516/ +++	32.533±9.194/ +++	30.302±2.805/ +++

Dose-response Curve of Avastin (Positive Control)

Serial dilution of avastin was used (1.56, 3.125, 6.25, 12.5, 25, 50,100 and 200 µg/mL) as positive control. IC_{50}= 20.281 mg/mL.

Dose-response Curve of Ethyl Acetate Extract

Serial dilution of ethyl acetate extract was used (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL). The table 2 below shows the mean percentage of inhibition ± SD of ethyl acetate extract. IC_{50} = 61.744 mg/mL

Dose-response Curve of Hexane Extract

Serial dilution of hexane extract was used (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL) mean percentage of inhibition ± SD as in the Figure 5. IC_{50}= 94.537 mg/mL.

Chick Chorioallantoic Membrane Assay *in-vivo*

The images below represent the results of CAM assay procedure that was obtained at day 13 of experiment. The inhibition zone for the growth of blood vessels was shown. The images in Figure (6-A) represent the control group while Figure (6-B) represents the tested *R. canina* ethanol extract group at 250,500 mg/20 mL concentration. The arrow in the control group shows the growth of the blood vessel before using the tested material. The tested group represents the inhibition zone for these vessels after using the disc containing tested *R. canina* ethanol extract. According to the pictures below, the inhibition zone after treatment includes not only the vessel that was treated but also the surrounding vessels as shown in

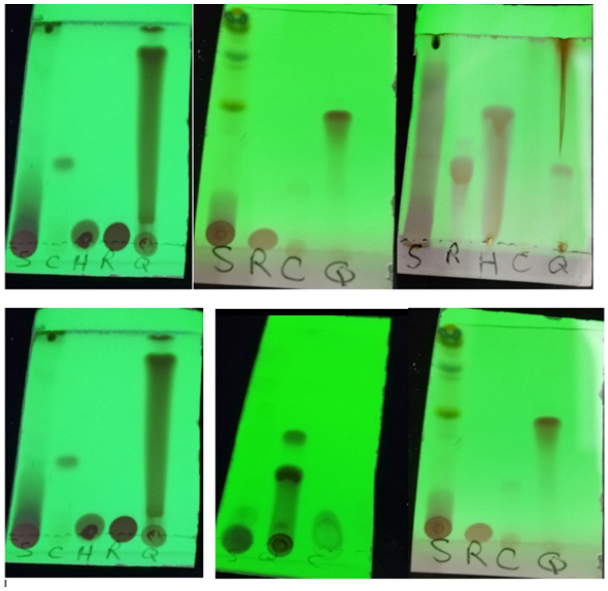


Figure 7: Thin layer chromatography of EE of *R.canina*

Figure 6 (A and B) by using the two concentrations of *R. canina* ethanol extract. The zone of inhibition in this result was more than 10 mm, therefore classified as {+++}. Zone of inhibition was calculated as in Table 2.

Identification of the Phytoconstitutes

Thin Layer Chromatography

Thin layer chromatography was done on the crude extract of *R. canina* in order to find the ratio of the distance of sample transferred by the mobile phase to the distance of solvent. The higher the RF value, the lesser the polarity of this substance. TLC discovers rutin, quercetin, hyproside, and catechin. As in Figure 7 below.

High-performance Liquid Chromatography (HPLC)

HPLC was used for phytochemical identification of *R. canina* crude extract to identify polyphenolic compounds, flavonoids, and anthocyanin. Catechin (flavan-3-ols) is the most important flavanol that present in *R. canina* (vagri et al., 2012). Rutin is an important flavonoid quercetin also found in *R. canina* crude extract that act as an antioxidant. Hyperoside, astragalin and gallic acid were identified in crude extract of *R. canina*. The flavonoids discovered by HPLC were catechin, astragalin, hyproside, rutin and quercetin at the retention time 7.445, 4.194, 14.691, 2.837 and 15.225 minutes, respectively. Gallic acid showed at the retention time 4.060 minutes as Table 3.

Table 3: Result of HPLC

Active ingredients of EE of R. canina	*Retention time(minutes)*
Rutin	2.837
Quercetin	15.225
Catechin	7.445
Astragalin	4.194
Hyproside	14.691
Gallic acid	4.060

DISCUSSION

Extraction and Extract Yield

The cold extraction method was used in this study to avoid degradation of active ingredients by increased heating. The yield percentages of hexane, ethyl acetate and ethanol were (0.8, 1.2 and 2.4 g/100 g dried powder) re The difference in the yield of extract belong to many factors such as extraction periods, solvent type, and temperature, origin and extraction method. According to previous studies, the yield of *R. canina* supported approximately was between 2.75–12.90% depending on the type of solvent[43] in another study, ethanol *R. canina* extract was 14.46 mg/g of dried powder (i.e., 1 g/100 g). However, the results disagreed with another study which extraction yield of *R. canina* using maceration in acetone/water was 197.24 mg of 100 g of dried *R. canina*. That means, many factors may affect the extraction.[44] Another study the yield of extract of *R. canina* by addition of dried leaves to boiled water for 30 minutes, 24.81%.[45]

Rat Aorta Ring Assay (*ex-vivo*) the Inhibition of Blood Vessel Growth by Hexane, Ethyl Acetate and Ethanol *R. canina* Extract in Comparison to Control

The percentage of blood vessel inhibition for hexane, ethyl acetate, ethanol of *R. canina* extract and positive control (Avastin) was 60.95, 63.011, 77.76 and 78.514, respectively. From the presented study, the ethanol extract had the highest biological activity compared to hexane and ethyl acetate, therefor ethanol extract have been used for further investigations. However, in comparison to avastin, hexane and ethyl acetate *R. canina* extract shows significant difference. This could be related to the lower concentrations of active ingredients that act as antiangiogenics. Antiangiogenic activity of *R. canina* extract may be due to the antioxidant activity (suppress reactive oxygen species formation) because of the presence of vitamins C and E and quercetin in the *R. canina* extract. High levels of vitamin C (ascorbic acid) decrease the production of nitric oxide (which was considered an important modulator in the expression of VEGF and FGF, therefore NO involved in tumor angiogenesis) in dose-dependent manner.[45] In addition to due to presence of large quantities of phytochemicals such as flavonoids and polyphenols.[46] Such as rutin, which acts as antiangiogenic due to its capability in suppressing the expression and production of VEGF and IL-B and stimulation of TNF-alpha.[47] *R. canina* also contains astragalin that inhibits migration and invasion and tube formation stimulated by VEGF in a concentration-dependent manner.[48] Gallic acid also inhibits tube formation and VEGF levels by inhibiting ADAM17 associated with downregulation of P13K/Akt, Ras/ MAPK pathway. Catechin found in *R. canina* inhibits angiogenesis by regulating pro and antiangiogenic factor production. Such as nitric oxide, IL2, and VEGF.[49] All three extract has antiangiogenic properties at 200 mg/mL in compared with DMSO but in different efficacy. Bevacizumab (Avastin) was approved as an antiangiogenic agent due to its

ability to inhibit blood vessel growth by VEGF inhibition.[50] The resulted IC_{50} of hexane, ethyl acetate, ethanol and avastin were 94.537, 61.744, 33.582 and 20.281, respectively. According to the national cancer institute of plant screening program, the cytotoxicity of crude extract is considered nontoxic if IC_{50} is greater than 20 mg/mL.[51] The presented study was supported by previous studies of *R. canina* as antioxidant, anti-cancer such as colorectal carcinoma and anti-inflammatory.[52,53]

Chick Chorioallantoic Membrane (CAM) Assay

The percentage of blood vessel inhibition that occurred by using ethanol extract 250 and 500 mg/20 mL was 19.921 and 30.302%, respectively. The both percentage had antiangiogenic activity but the highest antiangiogenic effect was shown by 500 mg/20 mL. From the picture presented in the Figure (7 A and B), the antiangiogenic effect extends not only the treated area but also the surrounding area of blood vessels. The study support the antiangiogenic activity of ethanol extract of *R. canina* according to the score (+++) refer to more than 10. Therefore, the presented study agree with the literature studies that show using of *R. canina* with different parts in colon cancer. Antiangiogenic mechanism of *R. canina* due to active ingredients, specifically flavonoids such as rutin that act as anti-angiogenic by suppressing expression and production of VEGF.[54] Second mechanism of antiangiogenic effect was antioxidant due to vitamin C and E composition of *R. canina*. The interpretation for the correlation between antiangiogenic and antioxidant effect belong to the presence of ascorbic acid (vitamin C) by antioxidant against reactive oxygen species as well as hydrogen peroxide. High concentration of ascorbic acid affect migration proliferation and tube formation. Because first high concentration of ascorbic acid changes the metabolic activity by lowering ATP levels about 20% at 300 mg/dl. Leading to prevention of proliferation without affecting cell viability. Second cell migration suppression. Third tube formation suppression after 24 hours of cell exposure to ascorbic acid.[55]

Identification of the Phytoconstitutes

Thin Layer Chromatography (TLC)

In the presented study, ethanol extract of *R. canina* contains flavonoids and phenolic acids such as rutin, quercetin, hyperacid, astragalin and catechin. The presented study agree with the previous studies of extraction and characterization of flavonoids from *R. canina*.[56, 57]

High-performance Liquid Chromatography (HPLC)

The HPLC result of the ethanol extract of *R. canina* presents the study exhibiting phenolic acids and flavonoids that support the result of thin layer chromatography of the same extract. The phenolic and flavonoid compounds that were present were rutin, quercetin, hyperoside, astragalin, catechin and Gallic acids. The presented study agree with the previous studies of *R. canina* extract.[56-58] Rutin and astragalin are inhibitors of VEGF that make them active against cancer cell.[59]

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