Research Article

The Antiangiogenic Activity of *Commiphora molmol* oleo-gum-resin Extracts

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ABSTRACT

The study aimed to investigate the possible antiangiogenic activity of *Commiphora molmol* oleo-gum-resin extracts. The oleo-gum-resin was grounded to fine powder, and extracted using sequential extraction, using the following solvents in increasing order of polarity: chloroform, methanol and de-ionized distilled water. *Ex-vivo* rat aorta antiangiogenic assay was used to identify the most antiangiogenic extract, active extract has been chosen for dose response study, and the active extract was tested on human umbilical ventricular endothelial cell line as *in vitro* study. Free radical scavenging activity has been tested by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) to detect which extract has the highest free radical scavenging activity to help determine the possible mechanism of extract action. Methanol extract was the most biological active extract in terms of percentage of blood vessels growth inhibition in comparison to chloroform and water extracts ($P< 0.05$). Also, there were significant differences between methanol, chloroform and water extract ($P< 0.05$). Methanol extract concentrations showed significant dose dependent inhibition activity ($P<0.05$) on rat aorta assay and IC_{50} was (20.78µg/ml). IC_{50} value was calculated from the linear regression equation. Methanol extract indicates significant dose related inhibitions against human umbilical ventricular endothelial cells (HUVEC) and IC_{50} was (77.59µg/ml). Methanol extract had the highest free radical scavenging activity in comparison to chloroform and water extract. The IC_{50} of DPPH for methanol extract was (109.76 µg/ml). *Commiphora molmol* extracts showed potential antiangiogenic activity and this activity may be due to the high activity in free radical scavenging capability. Highest effect was observed by the methanolic extract.

Keywords: Antiangiogenesis, *Commiphora molmol*, free radical scavenging activity (DPPH), Human umbilical ventricular endothelial cells line (HUVEC).

INTRODUCTION

Angiogenesis defined as the growth of blood vessels from the existing vasculature, and it is the creation of new blood vessels. The term comes from two Greek words: angio, which means "blood vessel," and genesis, means "beginning."

Normally, this process considered as a healthy process, because new blood vessels, help the body heal wounds and repair damaged tissues. But in cancer, the same process creates new, very small blood vessels which give a tumor its own blood supply and allow it to grow[1].

Angiogenesis occurs throughout life in both health and disease, when begins in uterus and continues on through age, oxygen plays a vital role in this process[2]. Control of angiogenesis could have therapeutic value has stimulated great interest.

Stimulation of angiogenesis can be therapeutic in ischemic heart disease, peripheral arterial disease and wound healing. The inhibition of angiogenesis can be therapeutic in cancer, ophthalmic conditions, rheumatoid arthritis, and other diseases[3].

The angiogenesis is a complex, highly regulated system like most processes in homeostatic cellular systems. A large number of proangiogenic growth factors have been identified, one of these factors is a protein known as vascular endothelial growth factor (VEGF)[1].

Antiangiogenesis as a Strategy against Cancer

Since 1970, Dr. Judah Folkman suggested inhibiting the formation of new blood vessel as a way to fight cancer[4]. The oxygen and nutrient supply would be depleted, as well as the malignant tissue to be unable to eliminate metabolic wastes. This in turn would inhibit tumor growing and metastatic progression that accompanies most advanced cancers. These are the main steps of the angiogenic process that can be interrupted:

1. Inhibiting endogenous angiogenic factors, such as bFGF and VEGF.
2. Inhibiting degradative enzymes MMP, which are responsible for the degradation of the basement membrane of blood vessels.
3. Inhibiting endothelial cell proliferation.
4. Inhibiting endothelial cell migration.
5. Inhibiting the activation and differentiation of endothelial cells[5].

Commiphora molmol (Myrrh)

Commiphora molmol (Myrrh) is an oleo-gum resin, obtained from the stem of various species of genus *Commiphora* of family Burseraceae, which grow in north-east Africa and Arabia. *C. molmol* Myrrh consists of water-soluble gum, alcohol-soluble resins and volatile oil. The gum contains polysaccharides and proteins, while the

volatile oil is composed of steroids, sterols and terpenes[6,7]. *C. molmol* is used in Chinese medicine to treat wounds, relieve painful swelling, and to treat menstrual pain, *C. molmol* tincture is used in mouthwash preparations aimed at treating mild inflammation in oral cavity and pharynx. *C. molmol* is also used for the common cold, to relieve nasal congestion, and coughing, *C. molmol* have antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria and *Candida albicans*[8-10].

MATERIALS AND METHODS

Plant Materials and Extraction

The oleo-gum-resin of *Commiphora molmol* (Myrrh) was collected from local herbal apothecary in Baghdad and authentication was done in department of botany.

The dried powder of *C. molmol* oleo-gum-resin (500 gm) was extracted using successive solvent extraction (SSE) the extraction process was performed using the following solvents in increasing order of polarity: chloroform, methanol and distilled water, respectively, by soaking 50 gm in each of the ten conical flasks with 200ml of the first solvent (chloroform) with continuous shaking by using the shaker water bath for eight hours at 40°C. The crude extracts were filtered using filter paper Whatman number 1 filter (20 cm), then filtrate was kept for concentration with rotary evaporator (40°C), while each time before employing the solvent of higher polarity the residue was dried and extracted by the same procedure mentioned above with the two other solvents (Methanol and then Water). The three extracts were examined for their biological activity; the extract with highest activity was depended in the other tests[11-15].

Experimental Animals

The Male Sprague Dawly rats with 12-14 weeks of age were used in the experiments. All the animals were allowed to free access to food and tap water, kept at 28-30 °C. The animals were obtained from the Animal House Facility in the Iraqi Center for Cancer and Medical Genetics Research, University of Mustansiriyah, Baghdad, Iraq. The experiments were approved by the Animal Ethical Committee in College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Rat Aorta Ring Antiangiogenesis Assay

The angiogenesis assay used in this method is according to that developed by Brown and coworkers with slight modification. Freshly excised thoracic tissues were rinsed with Hanks Balanced Salt Solution containing 2.5 µg/ml amphotericin B. The tissue specimens were then cleaned of peri adventitial fibro, adipose material and residual blood clots. This was then cut into 1mm thick aortic ring segments under a dissecting microscope. The assay was performed in a 48-well tissue culture plate, 500µl of 3mg/ml fibrinogen in serum free M199 growth medium was added to each well with 5mg/ml of aprotinin to prevent fibrinolysis of the vessel fragments. Each tissue section was placed in the center of the well and 15 µl of thrombin (50NIH U/ml) in 0.15 M NaCl. Immediately after embedding the vessel fragment in the fibrin gels, 0.5 ml of medium M 199 supplemented with 20% HIFCS, 0.1% ε-aminocaproic acid, 1% L-Glutamine, 1% amphotericin, 0.6% gentamicin were added to each well. 100 µg/ml of the test substance was added to the complete growth medium, and each treatment was performed in six replicates.

Control cultures received medium without the test substances. The sample extract was dissolved in dimethyl sulfoxide (DMSO), and diluted in M199 growth medium to make the final DMSO concentration 1%. Vessels were cultured at 37˚C in 5% CO_2 in a humidified incubator for five days. Fresh medium was added on day four of the experiment. Suramin, a well recognized antiangiogenic was used as a positive control. The blood vessel growth was quantified under 40X magnification using an inverted microscope on day five of the procedure with the aid of a camera and software packages. The magnitude of blood vessel growth inhibition was determined according to the technique developed by Nicosia and coworkers. The length of the tiny blood vessel outgrowths from the primary ex-plant was measured. The data is represented as Mean ± Standard deviation (SD) and experiment was repeated three times using six replicate per sample for validation. The percentage of blood vessels inhibition was determined according to the following formulae:

$$\text{Blood vessels inhibition} = 1 - (A_0/A) \times 100$$

A_0 = distance of blood vessels growth in µm.

A = distance of blood vessels growth in the control in µm[16-18].

Rat aorta assay dose response study of the methanol extract of *Commiphora molmol*

Serial dilution from the methanolic extract of *C.molmol* were prepared in the following concentrations. 200, 100, 75, 50, 25, 12.5 and 6.25µg/ml of the samples were dissolved in DMSO, and diluted in the M199 growth medium to make the final DMSO concentration 1%. Wells with no samples treatment were received medium with 1% DMSO used as the negative control. The data was represented as mean ± SD. 100µg/ml suramin was used as a positive control. The IC_{50}, which is the concentration that inhibit the blood vessels growth by 50%, was calculated by using the linear regression equation for the extract. Where Y=the percentage of inhibition, and X=concentration[19].

Assessment of Proliferation Inhibition of Cell Line

The (3-(4, 5-Dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) MTT assay was used as a measure of cell line proliferation according to Mosmann method[20]. All of the cells were between passages 4-7. The cells were treated with several concentrations of *C. molmol* extract. MTT was prepared by adding 5 mg/ml in PBS (phosphate buffer saline). Twenty µl of MTT was used per well and

the plates were incubated at 37°C, in 5% CO_2 for 5 hours. The plates were removed from the incubator and the supernatant was aspirated. DMSO (200 µl) was added to each well. The plates were shaken vigorously for one minute at room temperature to dissolve the dark blue crystals. The absorbance reading was taken at 570 nm and the reference at 650 nm by using micro-plate reader. The absorbance of cells cultured in control media was taken to represent 100% viability. The viability of treated cells was determined as a percentage of that for the untreated control. Each concentration was tested four times, and the experiment was repeated twice. The concentration of the cells in each well was $1x10^4$, the percentage of cell line inhibition was determined as the mean ± SD, using the following equation:

$$1-(A_0-A_1)/(A_2-A_1)$$

A_0=Absorbance of sample, A_1=Absorbance of blank, A_2=Absorbance of control

IC_{50} values were calculated by the linear and logarithmic correlation equation.

Cell Culture

Human umbilical ventricular endothelial cell line (HUVEC) was purchased from American Type Culture Collection (ATCC). The HUVEC was used to test the viability of endothelial cells against the most active antiangiogenic extract. The cells were maintained in ECM-2 (Science cell. USA). The medium was supplemented by 10% HIFCS and 1% penicillin/streptomycin. Polylysine purchased from Sigma-Aldrich was used to coat the flask that was used to culture the HUVEC cell for 48 hrs in the CO_2 incubator. Serial dilutions from *C. molmol* extract were prepared by dissolving the samples in DMSO and diluting it with the medium used for each cell line. The final DMSO concentration in the medium was 1% while the control wells received 200 µl from the medium with the final DMSO concentration; Samples were added to the well in quadruplicate and incubated in the CO_2 incubator at 37°C, with 5% CO_2 for 48hrs. MTT added on the cell and incubated for 4 hours prior to the absorbance measurements at 570nm by micro-plate reader[20].

DPPH Radical Scavenging Activity

The free radical scavenging activity of the *C. molmol* extracts were measured by 1, 1-diphenyl -2-picrylhydrazyl (DPPH) scavenging activity assay. One milliliter of 0.1 mM solution of DPPH in methanol was added to 2 ml *C. molmol* chloroform, methanol and water extracts with the following concentrations (200, 100, 50, 25, 12.5, 6.5 and 3.125µg/ml); after 30min, absorbance was measured at 517 nm. All concentrations of the three extracts were tested three times. Percentage reduction of DPPH (Q) was calculated according to the following formula[21].

$$Q = 100 \times (A_0 - A_C) / A_0$$

(A_0= Absorbance of control, A_C=Absorbance of the two samples after 30 min incubation)

Concentrations that inhibit 50% (IC_{50}) values describe the concentration of sample required to scavenge 50% of DPPH free radicals. IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The measurements were triplicates and their scavenging effect was calculated by percentage of DPPH scavenged[22,23].

Statistical Design and Analysis

The experiment design used for these studies was Rationalized Complete Block Design (RCBD). The results were presented as means ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey test comparison *t*-test (2-tailed) was used to compare between treatments groups. The differences between the means are considered significant at the 0.05 confidence level. The concentration that inhibit 50% of the blood vessels growth and cells proliferation (IC_{50}) value was analyzed by linear regression equation and logarithmic equation. The statistic analysis was carried out by using SSPS 16.0 for Windows (SPSS Inc, Chicago, IL), the level of significance at $P<0.05$.

RESULTS

Commiphora molmol oleo-gum-resin crude extract Yields

The percentage yield of the extract was determined gravimetrically using the dry weight of extracts and weight of powdered sample material, the extraction procedure yielded a highest percentage of 232 g (46.4%) of water extract and the lowest yield was for the methanol extract 25.5 g (5.1%) while the chloroform extract was 42 g (8.4%).

Antiangiogenesis activity of *Commiphora molmol* extracts

A concentration of 100 µg/ml of each of the three extracts was added on rat aorta embedded in complete growth medium of M199. The inhibition in growth of blood vessels was presented as mean percentage ± SD as in Table 1. The screening of chloroform, methanol and water extracts significantly inhibited blood vessels growth. Among these three extracts, the methanol extract showed the highest percentage of antiangiogenic activity in comparison to the chloroform and water extracts. There was a significant difference in blood vessels inhibitions among each of the three extracts of *C. molmol* and negative control (DMSO the vehicle used to dissolve samples) ($P<0.05$), and there were significant differences between each of chloroform, methanol and water, in term of blood vessels inhibition ($P<0.05$). Also there was a significant difference between methanol extract and suramin (positive control) ($P<0.05$).

The comparisons among the three extracts in term of blood vessels growth inhibition with both negative and positive controls reviled that the methanol extract was

the most biological active. Result of the current study depend the length of the tiny blood vessel outgrowths measured from the primary ex-plant in images shown in Figure 1.

Table 1: Blood vessel growth inhibition induced by tested agents (antiangiogenesis screening).

Extract	Mean Percentage (%) ± SD
Negative control (DMSO)*	Zero
Positive control (Suramin)	100
Chloroform extract	53.89±5.3
Methanol extract	92.92±1.72
Water extract	14.68±3.94

* DMSO = Dimethyl sulfoxide

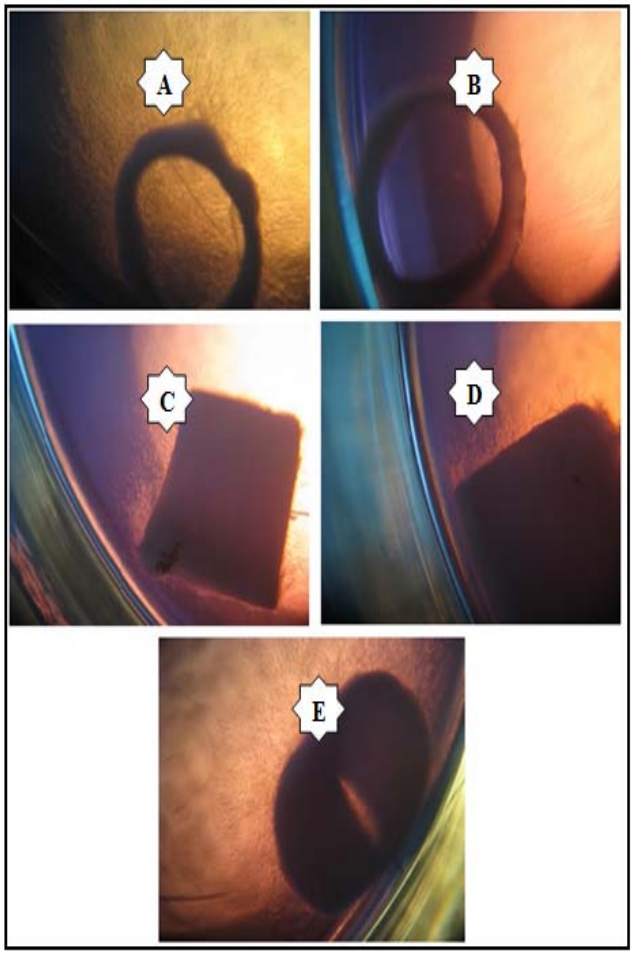

Figure 1: Images of aorta rings treated with different solvents of *C. molmol* extracts and controls, were A, B, C, D, and E represents the activity of the DMSO (negative control), Suramin (positive control), chloroform, methanol, and water respectively.

Dose response curve of *Commiphora molmol* methanol extract on rat aorta rings

The serial dilutions of the methanol extract of *C. molmol* were added to the rat aorta rings. Seven concentrations were used (3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml). These concentrations showed significant dose dependent inhibition activity (P<0.05) in comparison with negative control, except for the (3.125µg/ml) concentration which showed no significance.

IC_{50} value was calculated from the linear regression equation, Figure 2, (y=24.02ln(x)-22.84), Where: y = the percentage of inhibition and x = concentration.

The data indicates significant dose related inhibitions, which with 50% inhibition the concentration equals to 20.738µg/ml. The images of rat aorta rings showed a dose related inhibition of tiny blood vessel outgrowths from the primary ex-plant, with lowest inhibition showed in image A, and highest shown in image G, while image H represents the positive control, (Figure 3).

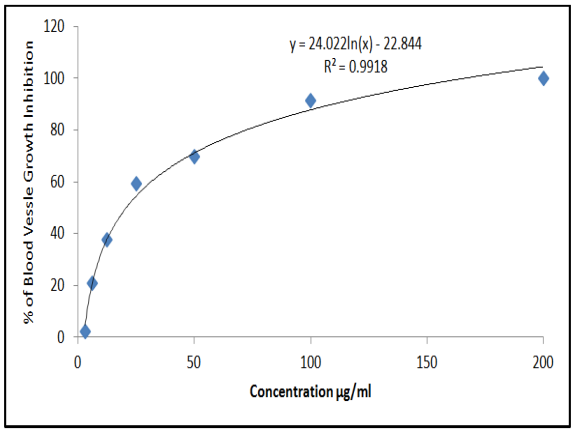

Figure 2: Dose response curve of C. molmol methanol extract on rat aorta ring assay.

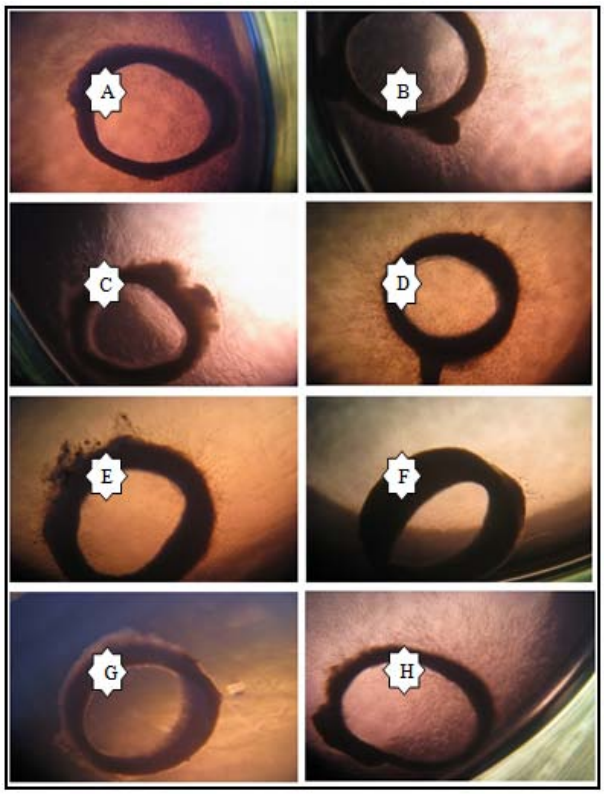

Figure 3: Dose response images of Myrrh methanol extract. A, B, C, D, E, F, G and H represented the activity of the serial concentrations (3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml) and Negative control respectively.

DPPH scavenging activity Assay

The dose response curve of chloroform, methanol and water extracts of *C. molmol* was measured using 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity test, results are expressed as mean ±SD. Result shows the percentage of DPPH scavenging activity of quercetin (positive control) and the three extracts of *C. molmol* was dose related. Serial concentrations ranged 3.125, 6.5, 12.5, 25, 50, 100 and 200 µg/ml were used. IC_{50} of DPPH scavenging activities of the tested agents were calculated by the linear regression equations; Figure (4) show the dose response curves for the extracts and the positive control. The IC_{50} of DPPH scavenging activity was calculated by the liner regression equation of each tested agent by considering Y to be 50%. The IC_{50} of DPPH for Quercetin was 1.151µg/ml. IC_{50} of DPPH for chloroform extract was 408.4 µg/ml while IC_{50} of methanol extract was 109.76 µg/ml. The IC_{50} of DPPH for water extract was 4286.6 µg/ml. The free radical scavenging activity results showed that the methanol extract had the highest activity in compared to chloroform and water extract.

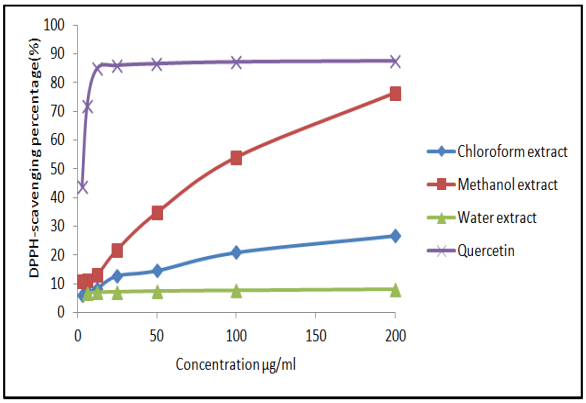

Figure 4: DPPH radical scavenging activity of quercetin (positive control), chloroform, methanol and water extracts.

The *in vitro* screening of methanol extract of *Commiphora molmol* on Human Umbilical Vein Endothelial Cell (HUVEC)

Results showed a dose related inhibition on the cell growth. The methanol extract serial dilutions of six concentrations were used (6.25, 12.5, 25, 50, 100 and 200 µg/ml) and the percentages of the HUVEC cell proliferation inhibition were 0.5±0.02, 4.7±0.04, 13.4±0.94, 42.21±0.54, 65.08±0.24, 68.14±0.04 respectively. The data indicates significant dose related inhibitions. Figure (5) show the dose response curve. Vincristine was used as a positive control, and 0.015 µg/ml of the vincristine inhibited the HUVEC growth.

The IC_{50} value of methanol extract of *C. molmol* was calculated from the following linear regression equation (y = 22.59ln(x) - 48.22), which with 50% inhibition equals to 77.3159 µg/ml.

Where: y= the percentage of inhibition and x= concentration.

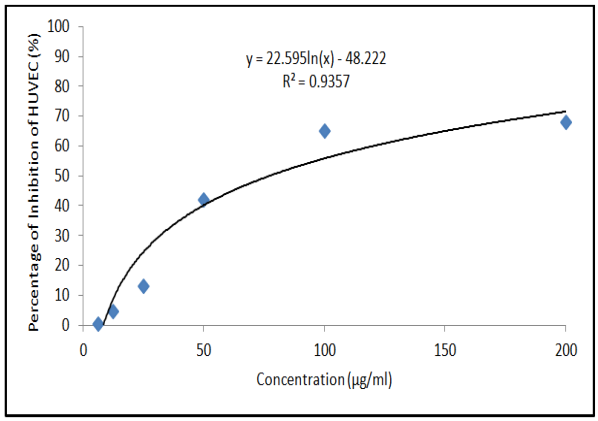

Figure 5: Cell proliferation inhibition activity of methanol extract of C. molmol on human umbilical ventricular endothelial cell (HUVEC)

DISCUSSION

Commiphora molmol (Myrrh) crude extracts Yield

In this study, the powder of oleo-gum resins of *C. molmol* was subjected to successive solvent extraction[13]. The different solvents, depending on their polarity, extracted different phytochemical groups with varying quantities of components in crude plant material that may have a pharmacological activity on the biological systems[24]. The extraction method used in this study was cold method or maceration method, this method is suitable for use in case of the thermo labile compounds as prolonged heating may lead to degradation of compounds[25].

Ex Vivo Rat aorta ring assay screening

In the current study, the main objective was to identify whether the three extracts have any antiangiogenic activity and which extract has the highest activity, it was important to screen the extracts against rat aorta *ex vivo* assay to identify the most biological active extract for further testing. The results show that all extracted samples using the cold method (maceration) successive solvent extraction, have antiangiogenic properties at the concentration of 100µg/ml. The extraction condition at low temperatures maintains the thermo labile active compounds and prevents it from volatilizing at higher temperature resulting in increasing its concentrations in the extracted sample. The results reveal that methanol extract of *C. molmol* was found to have the highest antiangiogenic activity compared to the other two extracts, chloroform and water extracts, which showed lower antiangiogenic activities. The antiangiogenic activity of chloroform and water extracts remained significant, which could be due to the presence of lower concentrations of active compounds or other compounds which may have less activity[26]. And this result agrees with previous studies that showed high concentrations of chemical groups which may have antiangiogenic power, specially the sesquiterpenes which mostly exist in the methanolic extract of oleo-gum resins of *C. molmol*[8,27]. The different solvents, depending on their polarity, extracted different phytochemical groups with varying

quantities of components in crude plant material that may have a pharmacological activity on the biological systems[28]. The antiangiogenic activity of the crude extracts was quantified by measuring the length of the micro blood vessel outgrowths from the primary tissue ex-plants with the aid of Leica Quin software package. The results are presented as mean percent inhibition[18]. And Suramin was used as positive control as it is a well-known standard agent used in angiogenesis experiments[29].

Ex Vivo Dose response curve of the Methanol Extract of *Commiphora molmol* on rat aorta rings

The results showed that methanolic extract decreased the new blood vessels formation in rat aortic ring explants, the data indicates significant dose related inhibitions with IC_{50} of 20.738 µg/ml, the result was not in the cytotoxic concentration and within the safe range, as in angiogenesis process, the boarder concentration for a herb to be considered safe is 20 µg/ml of the extract[30]. Methanol extract of *C. molmol* potently inhibited the outgrowth of microvessels in rat aortic rings in a dose dependent manner.

In Vitro Anti-proliferative activity of the Methanol Extract of *C. molmol* against Human Umbilical Vein Endothelial Cells (HUVEC)

The HUVEC experiment was as the preparatory assay to assess if the activity of methanol extract was due to its inhibitory activity. As a proem to this study, the methanol extract of *C. molmol* has been tested against the (HUVEC) cell line proliferation to measure if the antiangiogenic activity observed on the rat aorta rings was due to the cytotoxic activity of extract or to the direct and / or indirect inhibition of key angiogenesis receptors or mediators. The MTT results showed that *C. molmol* methanol extract is not cytotoxic towards the endothelial cells and the IC_{50} value was calculated, the 50% inhibition equals 77.59µg/ml. These results corroborate the results of previous experiments indicating that the diterpene resin acids compounds of *C. molmol* exhibited obvious inhibitory effects on HUVEC proliferation[31]. Because the methanol extract has IC_{50} value above 20µg/ml, this finding further reveal that methanol extract is antiangiogenic. In the present study, methanol extract of *C. molmol* exerted dose-dependent inhibitory effects on HUVEC.

The extract inhibited cell proliferation at 77.59µg/ml, but no cytotoxic activity was observed below 20µg/ml. In the crude extract, the IC_{50} value was higher than 20µg/ml, suggesting that this sample has no significant cytotoxic effect to HUVECs, and according to the National Cancer Institute (NCI) extracts with IC_{50} > 20 µg/mL are not considered cytotoxic[32]. Moreover *C. molmol* is approved by FDA for food use and was given generally recognized as "safe" status as a flavouring agent[33].

Together, the results of *ex-vivo* rat aortic ring assay and the *in vitro* anti-proliferative assay on HUVEC, both confirmed that the antiangiogenic effect observed by *C. molmol* is not due to the cytotoxic nature of the compound, but may be more related to inhibition of one or more of angiogenesis cascade. The results of the present study was supported by results of a previous study stating a new cycloartane-type triterpene named neomyrrhaol along with four known terpenes were isolated from the resin of *C. molmol* and exhibited significant inhibitory activity as shown in the MTT assay all the known compounds had inhibitory effects on HUVEC growth[34].

Another factor behind this activity could be the antioxidant properties of *C. molmol*, a previous study had reported the radical-scavenging and antioxidant properties in the DPPH radical assay for *C. molmol*, and it has been shown that antioxidants are considered as a natural angiogenesis inhibitors. In addition, presence of some components of *C. molmol* as terpenes can be extracted only at low temperature[35].

Free radical scavenging activity of *Commiphora molmol* extracts (DPPH Assay)

Anti-oxidants are well known to have potent antiangiogenic activity, amongst those that have been identified include vitamin C, vitamin D, vitamin E, vitamin A, rosmarinic acid, 3-hydroxyflavone, 3', 4'-dihydroxyflavone and 2', 3'-dihydroxyflavone[35,36]. Free radicals are considerable evidence that free radicals induce oxidative damage to bio-molecules and play an important role in cardiovascular diseases, aging, cancer, inflammatory disease and a variety of other disorders[36]. Free radical scavenging activity for the three extracts of *Commiphora molmol* (Myrrh) was studied and considered important to help understand the mechanism of action of *C. molmol* extracts. It may be due to certain chemical constituents of *C. molmol* which possess good oxygen radical scavenging and antimutagenic potential. The antioxidant and protective effects of *C. molmol* are owed to their content of antioxidant active constituents such as eugenol, cuminic aldehyde and sesquiterpenes[35]. The capability of blood vessels outgrowth inhibition in rat aorta assay screening among the three extracts may related to The presence of terpenes (specifically sesquiterpene) in *C. molmol* may explain the antiangiogenesis mechanisms as described previously, as terpenes may show their pharmacological effect through antioxidant properties. These active compounds consider very potent in inhibiting angiogenesis process[37]. This study has found that methanol extract of *C. molmol* had gave the significantly highest anti-oxidant activity in compare to the other two extracts (chloroform and water), the methanol extract also showed the highest percentage of antiangiogenic activity, as shown by the rat aortic ring assay. Its potency in inhibiting new blood vessel development could be contributed to its significant antioxidant behaviour, as shown in the DPPH scavenging assay. Results of this study showed positive correlation when comparing the *C. molmol* extracts in both

antiangiogenic activity shown by the rat aorta assay, and antioxidant power shown in the DPPH scavenging assay. This may result in a decrease in the free radicals present, which are known to activate the hypoxia responsive element gene. During the process of angiogenesis the latter gen can act as a trigger for VEGF, a key cytokine in angiogenesis activation[38]. Strong anti-oxidant properties and good anti-inflammatory responses are the two major selection criteria imposed in the good antiangiogenic agents[39]. So first antioxidants can significantly affect the angiogenesis process in a variety of ways as described previously, antioxidants can affect the physiological redox balance that will mop up reactive oxygen species (ROS) that tend to be prevalent in low oxygen tension locality such as that in the tumor[40]. Possible molecular mechanism of antioxidant rich phytochemicals is to inhibit VEGF-induced angiogenesis through the suppression of VEGF-induced ROS production[39]. The methanol extract of *C. molmol* was found to be a potent antioxidant in terms of the reducing power and with a significant capacity to scavenge DPPH. The second selection criterion is the ability of the candidate compound to confer anti-inflammatory response. The process of angiogenesis can also contribute to inflammatory response where the new blood vessels can transport inflammatory cells to the site of inflammation as well as nutrients and oxygen to the proliferating inflamed tissue. Angiogenesis plays a critical role during the pathogenesis of inflammation[41].

CONCLUSION

Angiogenesis important process in many diseases, such as in diabetic retinopathy (DR) and age-related macular degeneration (AMD), psoriasis, growing tumor and others. *Commiphora molmol* extracts showed potential inhibition activity against this process with highest effect observed by the methanolic extract, this herb may have promising activity against tumor as adjuvant with chemotherapy or in targeting angiogenesis related diseases.

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