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Antiangiogenic Effect of Salvia officinalis

Omar Hussein Ahmed[1]

ABSTRACT

Angiogenesis plays a critical role in disease evolvement and exacerbations. This article has been prepared to evaluate the Antiangiogenic Effect of salvia officinalis that have been studied for their antiangiogenic activity. The plant sources of Iraq are likely to produce effective antiangiogenic substances. The phytochemical exploration of this plant has conferred to the discovery of new treatments for many disorders. Last year owing to the fear of adverse effects people favor the use of natural plant products for disease cure. This article also helps to summarize the different methodologies and various ways to evaluate the potential natural compounds having antiangiogenic action. The information collected here will take part in an essential role to develop new drug formulation for treatment angiogenesis-related diseases and further research should be done on the above-mentioned plants.

Keywords : *Medicinal Plants, Phytochemicals, Natural Compounds, Antiangiogenic Activity*.

I. INTRODUCTION

Traditional drugs are well-advised an important part of mankind's civilized system. The knowledge acquired via slow progressive ability gained while handling plants by humans has to lead to the ongoing of new science or in other words a new segment of therapy to deal with diseases and complications associated with anatomy and physiology. Traditional medicine though is used in different propriety and is dependent on the place to place, cultures followed, and local acceptance, all over the world, but the significance of using medicinal plants into treating practices is a common assumption. The main roadblocks for the widespread popularity of the traditional system have been mainly a dearth of a proper restraining body to balance the practice in the deep areas of our society. [1]

Angiogenesis is the process involving the maturing of new blood vessels from pre-existing vessels [2]. Physiological angiogenesis takes place mainly during wound healing and menstrual cycle of the female [3], whereas, pathological angiogenesis occurs in diseases such as cancer, rheumatoid arthritis, endometriosis, and diabetic retinopathy. An abnormal or high calm of angiogenesis also contributes to vascular malformation, obesity, chronic inflammation, on the other hand, insufficient angiogenesis is related to Alzheimer's disease, coronary artery disease, stroke, myocardial infarction and ulcer formation [4]. Various challenges in treating cancers are linked to tumor progression and metastasis [5]. The growth of solid malignancies and their metastasis

[1] Department of Pharmacognosy and Medicinal Plants, College of Pharmacy University of Tikrit, Tikrit, Iraq , Email: pharmacognosy88@gmail.com , Mobile:+9647722604040.

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in addition to many other disorders depends closely on adequate oxygen and nutrient supply, which ensure the formation of new blood vessels (angiogenesis) within the tissue that is important for disease development [6]. Therefore, anti-angiogenic agents can contribute to suppressing cancer growth by preventing nutrient and oxygen supply to the tumor tissue. Plants have long been used as an important source of therapeutic agents against several diseases including cancer [7]. While several natural products are widely used in cancer treatment, the use of other plant products is limited to the support of the immune system and/or the increase in the anticancer effects of other anticancer drugs [8]. The detection of plant extracts with an anti-angiogenic response and limited toxicity can increase the effects of presently used anticancer drugs without increasing their side effects. like other countries in Iraq, there is a lot of research that has been done and some of them are still going on as it is to be mentioned that till now the prime source of antiangiogenic agents is a medicinal plant [9].

Salvia officinalis L .is a plant in the mint family Lamiaceae, subfamily Nepetoideae, tribe Mentheae, and genus Salvia [10]. Salvia is the largest genus of the Lamiaceae family, composed of around 1000 species [11], and can be found in Europe around the Mediterranean, in Southeast Asia, and Central and South America [12].

S. officinalis grows in the form of an outcrossing, perennial subshrub up to 60 cm high. The leaves are opposite and simple with white hairs on the lower leaf surface and greenish or greenish-grey on the upper surface. Stems are erect or procumbent with abundant hairy dark green branches. Leaves are elongated and petiolate with serrate margin, rugose surface, and sometimes with basal lobes. The flowers are 2–4 mm long from the pedicel, and they are in pseudo verticillasters with 5–10 violet-blue color flowers that form spurious, composed spikes. They bloom from March to July depending on habitat and climatic conditions [13,14]. Historically, sage is known as the “Salvation Plant”, originating from the old Latin word “salvage”, which means save or cure. It has been used to reduce perspiration, as a gargle for sore throat, to improve the regularity of a menstrual cycle and to break hot flashes in menopause, to fight gastroenteritis and other infections, to improve lipid status and liver function in general, to improve appetite and digestion, and to advance mental capacity [15].

The aim of this study is in vitro estimation of Anti-Angiogenic potential of the methanolic extracts in *Salvia officinalis* L.

II. Materials and Methods

Plant identification and Phytochemical analysis

The plant was saved in AL Jadria herbal store according to the document and authentication of classification of Dr. Sukaina Abbas Ulaiwii from the university of Baghdad – college of science – department of biology Approval Number 8 in 12-4-2017.

Preliminary qualitative phytochemical analysis

Chemical tests were done using the ethanolic extracts from plants and standard methods to identify the identity of active constituents.

Test for flavonoids:

(i) Lead acetate chemical test: One ml of lead acetate 10% solution added to 5ml of ethanolic extracts, the color of a yellowish-white precipitate indicates a positive test for flavonoids.

(ii) NaOH chemical test: Five ml of the extract was added to aqueous NaOH and HCl, and the yellow-orange color is a positive indicator.

Test for terpenoids:

Two ml of an extract of alcohol was dissolved in (2ml) of chloroform and evaporated to dryness. Then (2ml) of concentrated sulphuric acid was added and heat used for about 2 min. An indication for the terpenoids is the grayish color.

Test for alkaloids

Alcoholic extract (10 ml) was stirred with 5 ml of 1% HCL on a steam bath. Mayer's (1.35gm mercuric chloride in 60ml water + 5gm potassium iodide in 10ml water)and Wagner's reagents (1.27g of iodine and 2g of potassium iodide in 100ml of water) were added, white and reddish-brown color precipitate respectively were taken as evidence for the presence of alkaloids.

Tests for steroids

Liebermann-Burchard test: Extract (3ml) was treated with chloroform, acetic anhydride, and drops of sulphuric acid were added. The formation of dark pink or red color indicates the presence of steroids.

Test for saponins:

Froth test:

About (2 ml) of plant extract was boiled in (20 ml) of distilled water in a water bath and filtered. (10 ml) of the filtrate was mixed with (10 ml) of distilled water in a test tube and shaken vigorously. And observed the formation of froth that persists for 15 minutes that indicates the presence of saponins.

Extraction and fractionation of different active constituents

Five hundred grams of plant coarse powder were macerated in hexane for 24 hours to exclude the fatty materials and then dried at room temperature. The defatted plant materials were extracted with ethanol 80% in the soxhlet apparatus. The ethanolic extract is evaporated using a rotary evaporator at a temperature not exceeding 40 C^{o} to give a dark greenish-yellow residue color fraction. This Crude fraction was acidified with the addition of hydrochloric acid (5%) to reach pH 2 and then an equal volume of ethyl acetate is added to get two separated layers (aqueous acidic and ethyl acetate layer). The aqueous acidic layer is neglected because it contains the alkaloids and water-soluble components. The ethyl acetate layer was evaporated to dryness using a rotary evaporator under reduced pressure and then basified with 300ml of sodium Hydroxide 5% to reach pH 10 and extracted with chloroform in the separator funnel to get two separated layers, the aqueous basic layer, and chloroform layer. The aqueous basic layer was separated, evaporated to dryness, and then acidified with hydrochloric acid 5%to reach pH 2 and finally extracted with ethyl acetate to get flavonoids fraction. The chloroform neutral layer was separated and also evaporated to dryness under reduced pressure then partitioned with methanol 80% and petroleum ether to get two separated layers, methanol layer which contains terpenes and steroids fractions and petroleum ether layer which contain neglected waxes and fats fractions.

HPLC: using hyper clone ODCC C18 V-25cm column and a mixture of ethyl acetate: water (7:30 ratio) as a mobile phase with a flow rate of 0.5ml/min, and detected at 280 nm.

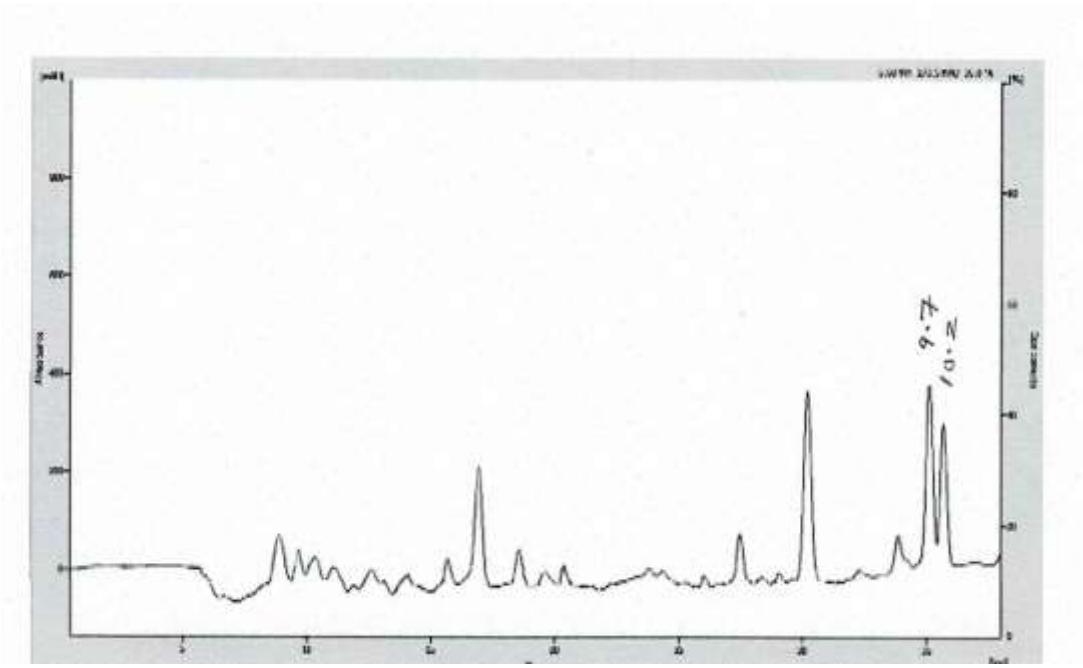


Figure 1:HPLC for flavonoid fraction

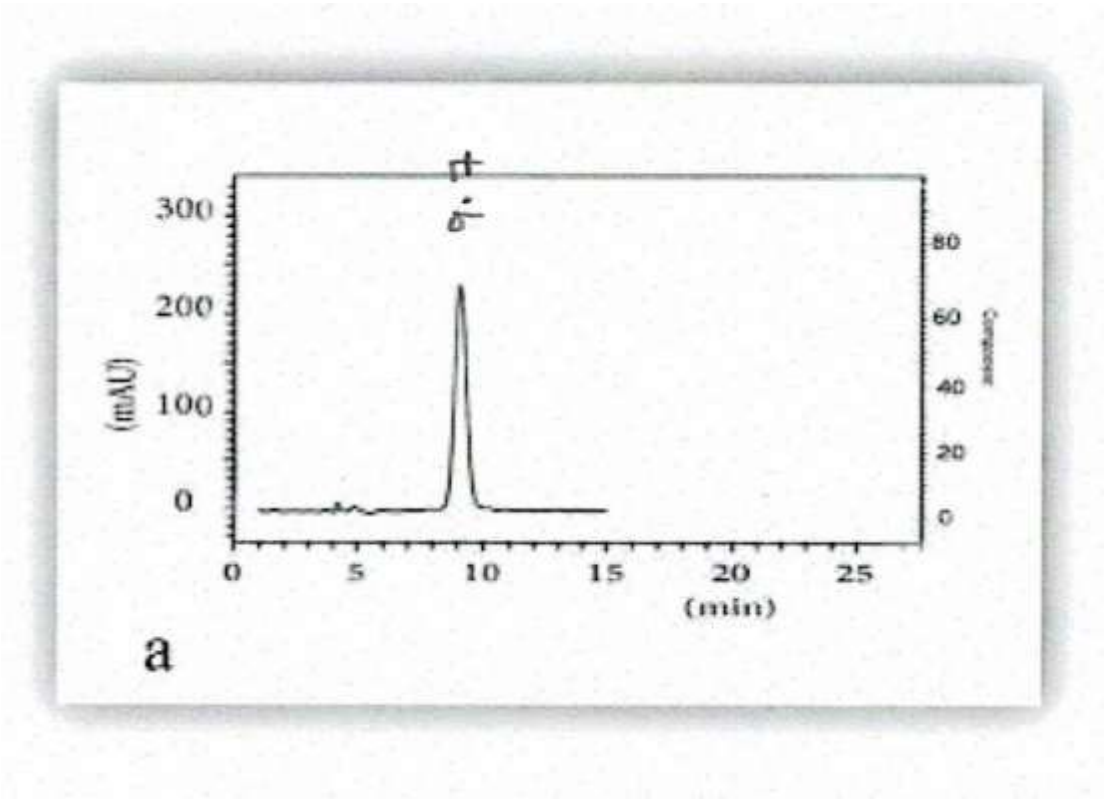


Figure 2: HPLC for S.A standard

Separation is done by PHPLC using the following condition

Isolation and purification of S.A. using (PHPLC)

PHPLC was used to separate in a pure form the major compound in flavonoids fraction (retention time 9.7) using the same condition of HPLC except :

Column: mediterranea C18 (5 µm 15 X 2.12 cm)

Flow rate: 20 ml/ min.

The results of the phytochemical screening are given in the table

Table 1- Phytochemical Screening of extract

Alkaloids	Flavonoids	Steroids	Terpenoids	Saponins
+	+	+	+	-

+, - represent the presence and absence of phytoconstituents respectively.

Figure 3: TLC for separated S.A with the standard. (mobile phase) mixture use is:

Ethylacetate: formic acid: glacial acetic acid: water (100: 11: 11:27).

III. Results and Discussion

Chic Chorioallantoic Membran Assay (CAM Assay)

fertilized White Leghorn chicken eggs (6 per group) were incubated at 37∘C at constant humidity. On day 3, a square window was opened in the shell after removal of 2-3mL of albumen to detach the developing CAM from the shell. At day three of incubation, a small hole made on the fine pinpoint, and the egg left to be incubated at 36 °C for one more day. On day four the egg's sac punctured and a small window was made in the shell. The window was resealed with adhesive tape and eggs were returned to the incubator until day 10 of chick embryo growth. On day 10, the sample prepared as 500mg/ml. 20 µl placed on a round disc of filter paper left to dry and then transferred to the cam and eggs were resealed and returned to the incubator for 72 hours until day 14 (n = 6 chicken embryos per sample); the zone of inhibition was photographed and calculated [17].

Quantification and Immaging of CAM

Six CAMs were used in each control and experimental group. The responses may be graded (+ 3-up to 6 mm; ++ 6 up to 9mm; +++ > 10 mm3. the quantification of zone of inhibition was done by using image analyzer (BIOCOM Visiolab TM 2000) [18].

Calibration

Five different concentrations of Sallvolinic acid were prepared in methanol ranging between 1-100 µg/mL. Triplicate 10µL injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak areas obtained from injections were plotted against the concentrations to establish the calibration graph [19].

Chicken Embryo Chorioallantoic Membrane Assay (CAM ASSAY)

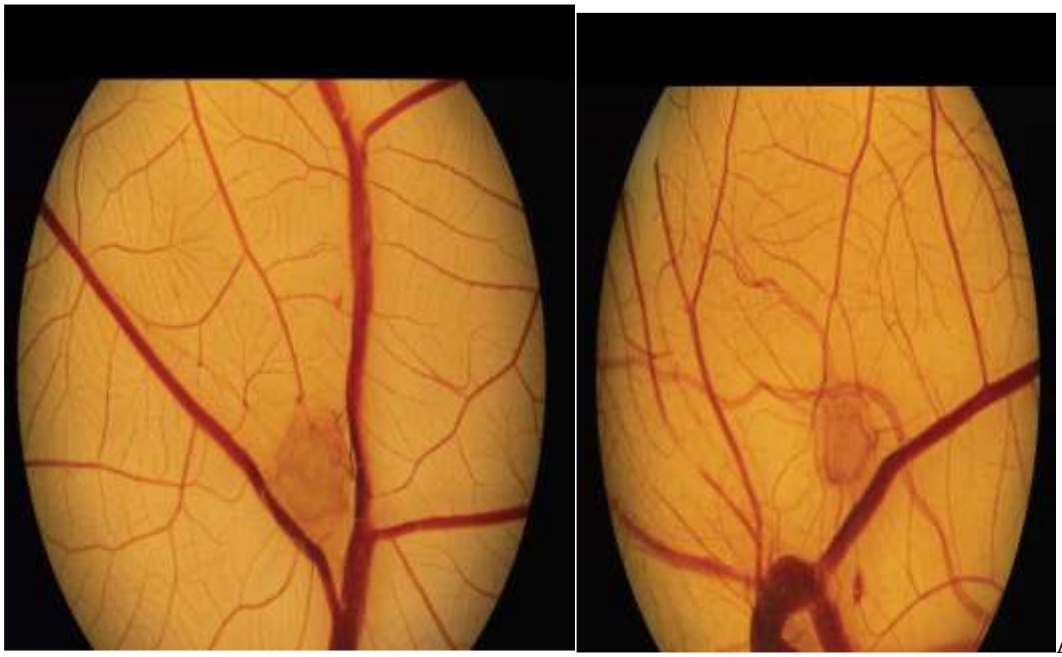
the current research has launched CAM Assay as one of the several classical *in vivo* models for studying angiogenesis [20]. Table 2

showed that the zones of inhibition area are more than 10 mm, so the score is three plus. Figure 1 showed the blood vessels growth inhibition; (A) representing the control and (B) representing the treated blood vessels of CAM. The results found that CAM treated with S.A showed prominent anti-angiogenesis. A large number of vessels stopped radiated from underneath the disc which carried the 10 mg of the drug. Moreover, the vessels were sparse, disorganized, with a light yellow appearance.

Table 2. The zone of blood vessels inhibition Chick Chorioallantoic Membrane Assay (CAM Assay) assay.

Eggs	**zone of inhibition Area (mm) ±SD**	**Scoring**
1	13	+++
2	15	+++
3	6	++

4	14	+++
5	12	+++
6	7	++
Mean ± SD	12.66±2.6	+++


A

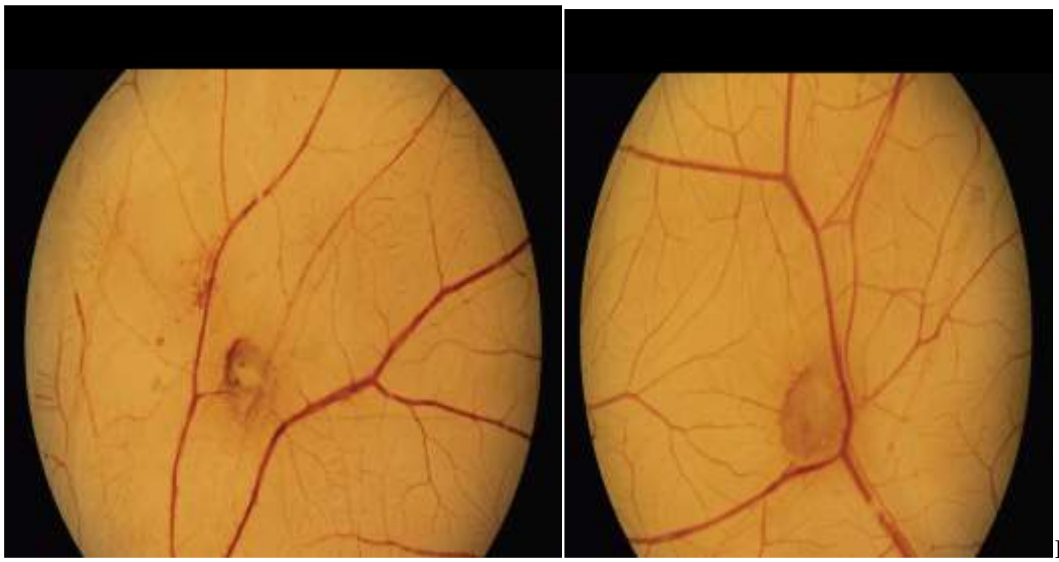

B

Figure 4:The blood vessels growth inhibition, were image (A) represents the control and (B) represent the treated blood vessels of (CAM).

IV. Conclusion

The medicinal plant's significance in amelioration of diseases has seen increasing interest among the allopathic drug industry. The lower side-effects and more effective treatment outcomes have increased the surge towards natural leads for developing drug targets for curing diseases. The present review thus puts light on the significance that Salvia officinalis holds for drug researchers to seek prospective drug molecular extractions leading to effective and efficient availability of cheap and powerful drugs. The toxicity related to drugs from natural sources has been reported in certain cases. The effective solution is to set up a standardized governing body to regularize the isolation, standardization, and dosage formation of drugs from natural sources for effective implementation of the goal of developing drugs from natural roadways.

S.A. significantly inhibited the blood vessels' growth in CAM. The mechanism may relate to regulating the expression of VEGF, matrix metalloproteinases (MMPs), EGFR, and inhibit NFκB, PI3-K/Akt, ERK1/2 signaling pathways; thereby causing strong antiangiogenic effects

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None.

Conflicts of interest

The author declares there are no conflicts of interest.

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