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# MONITORING ANGIOGENIC PROPERTY OF AQUEOUS EXTRACT FROM *BLUMEA BALSAMIFERA* LEAVES USING *IN VITRO* SHELL-LESS CHICK EMBRYO CULTURE SYSTEM

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## ABSTRACT

*In the present study angiogenic property of aqueous extract from Blumea balsamifera leaves has been examined on in vitro shell less chick (Gallus gallus) embryo cultures. Three days old incubated embryos were treated with different concentrations (0µg/mg, 50µg/mg, 100µg/mg, 150µg/mg, and 200µg/mg) of Blumea balsamifera leaf aqueous extract and monitored at an interval of 3 hour constantly for 6 hours. A positive correlation was seen between the number of capillary sprouts developed and increase in the concentrations. Formation of capillary sprouts correlated positively with exposure time as well. These indicate that Blumea balsamifera leaves can be utilized for wound healing.*

## KEY WORDS

*Blumea balsamifera, Angiogenesis, Chicken embryo, Shell-less culture*

## INTRODUCTION

Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels. This is distinct from vasculogenesis, which is the *de novo* formation of endothelial cells from mesoderm cell precursors [1]. Angiogenesis is the term used for spontaneous blood-vessel formation, and formation of new blood vessels by the splitting of existing ones. Angiogenesis is a normal and vital process in growth and development, wound healing and in granulation tissue as well. [2]

Angiogenesis may be a target for combating diseases characterized by either poor vascularisation or abnormal vasculature. Several diseases, such as ischemic chronic wounds, are the result of failure or insufficient blood vessels formation which may be treated by a local expansion of blood vessels, thus bringing new nutrients to the site, facilitating repair [3]. In adult tissues endothelial cells are quiescent but rapid proliferation occurs for a limited period of time during menstruation, ovulation, reproduction, implantation, mammary gland changes during lactation, and wound healing [4, 5]. Other diseases, such as age-related macular degeneration, may be created by a local expansion of blood vessels, interfering with normal physiological processes. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer [6]. Under pathological conditions, insufficient angiogenesis occurs in diseases such as coronary artery disease, stroke, and chronic wounds. In chronic inflammation and tumor growth, there is an imbalance between endogenous stimulator and inhibitor levels, which turns on “pro-angiogenesis switch”. Mostly, human body losses control over the balance between angiogenesis stimulators and angiogenesis inhibitors, which lead to excessive angiogenesis. When diseased cells, such as inflammatory cells and tumor cells, produce abnormal amounts of angiogenic stimulators, overwhelming the effects of endogenous angiogenesis inhibitors excessive angiogenesis occurs [7 - 9].

*Blumea balsamifera* (Kalar) belongs to family Asteraceae or Compositae. It is half woody, strongly aromatic herb. Leaves are simple, alternate, broadly elongated (7-20 cm long) with toothed margin and appendage or with divided base. Loose yellow flower head is scatter along much-branched leafy panicles.

*Blumea balsamifera* leaves are used as 'a tea' and 'a cure' for rheumatism and hypertension. Its leaves

have attracted attention as a part of the plant with various physiological activities, including plasmid inhibitors, antifungal, and liver protective effects [10]**.** It is considered as a carminative, stomachic and antispasmodic agent and has been used to treat conditions like flatulence, dyspepsia, diarrhea, intestinal colic and dysentery. A poultice of fresh pounded leaves is applied locally to treat haemorrhoids [11]. It has anti-inflammatory, anti-catarrhal and expectorant properties which renders it useful in the treatment of both upper and lower respiratory tracts like sinusitis, influenza, asthmatic bronchitis. In Vietnam a decoction of the fresh leaves is used to treat influenza and cough either by drinking the decoction or by inhalation of the vapour from the boiling of the leaves. In the case of sinusitis the Thais make cigarettes of the chopped dried leaves and smoke it. Its diaphoretic and sudorific properties have rendered it advantageous in the treatment of influenza and a type of fever known as 'Ahwah' in Bengal. In fever, a handful of the leaves is boiled and when lukewarm infects, it is used as a sponge bath. A decoction of the roots is sometimes given to treat fever [12, 13]. To treat purulent eye discharge the juice of fresh leaves is squeezed into the eyes. It has been advocated in the treatment of aphthous ulcers, angina pectoris, diabetes, hypercholesterolaemia and wounds, cut and infected ulcers [10].

In Gujarat the leaf extracts of these plants are commonly used by local tribes for wound healing. The aim of the present study was to know whether *Blumea balsamifera* leaves could show such angiogenic property which may be involved with wound healing phenomena. Shell less culture forms an excellent *in vitro* culture system for easy and rapid visual detection of such activities. Any blood capillary sprouting after addition of leaf extract reflects the angiogenic potentials. We, therefore, analyzed the effect of this extract in *in vitro* shell less cultures.

## MATERIALS AND METHODS

*Blumea balsamifera* leaves were collected locally after proper authentication. Leaves were dried for 3-4 days at room temperature. After drying completely, it was ground crumbly in a mixture and extract was prepared by refluxing approximately 50 gm of leaf powder with double distilled water (DDW) in Soxhlet extractor for 36 hrs. The liquid extract was allowed to cool and concentrated by evaporating *in vacuo* and freeze dried. The extract was stored at low temperature (4$^{o}$C) until further use. Extracts were redissolved in sterile pyrogen free water prior to use [14]**.**

The entire embryo culture was processed under aseptic conditions. The assembly of our model for the shell less chick embryo culture consisted of a transparent glass Petri plate and its cover. Incubated eggs were allowed to cool, and then wiped with 70% alcohol to minimize contamination from the shell surface. The eggs were kept in a horizontal position to assure that the embryo was properly positioned. Thin albumen from an unfertilized egg was poured into sterile glass Petri plate. Albumen acted as a shock absorber and limited desiccation. Fertilized egg was then cracked (approximately 3-3.5 cm) with the help of scalpel from narrow end. The contents of eggs were gently released over albumen in the Petri plate, covered with a sterile lid and incubated at 37.5$^{o}$C with saturated humidity [15].

A group of five eggs was maintained for each time point and dose. 50µg/mg, 100µg/mg, 150µg/mg, and 200µg/mg of *B. balsamifera* leaf aqueous extract was administered as single dose separately to fertilized chick embryos incubated for 48 hours. The untreated embryos were kept as controls. After the treatment, each shell less culture was observed at intervals of three hours. The above experimental set up was replicated thrice and average capillary sprouts were counted as measure of angiogenesis.

## RESULTS AND DISCUSSION

The present study was initiated to see whether treatments of *Blumea balsamifera* aqueous leaf extracts to chicken embryo increase the capillary sprouts. It was seen **(Table I)** that after addition of extracts, for all concentrations, at every interval of 3 hours, the number of capillary sprouts increased. There was statistically significant ($P<0.05$) increase in total number of capillary sprouts after addition of *Blumea balsamifera* aqueous leaf extract in the final concentration of 200 µg/mg in chicken embryo as compared to control treatment (0 µg/mg).. This indicated clearly the angiogenic potentials of aqueous extracts of *Blumea balsamifera.* **Plate I** indicates capillary sprouts formations in embryos after different exposure timing and concentrations.

Numerous bioactive chemical compounds of plant origin may influence the angiogenic activity of various cell types and may thus affect the formation of blood vessels. Food and medicinal plants are well known to be a rich source of bioactive constituents and it is necessary to identify the chemical entities which are responsible for these effects on endothelial cells and

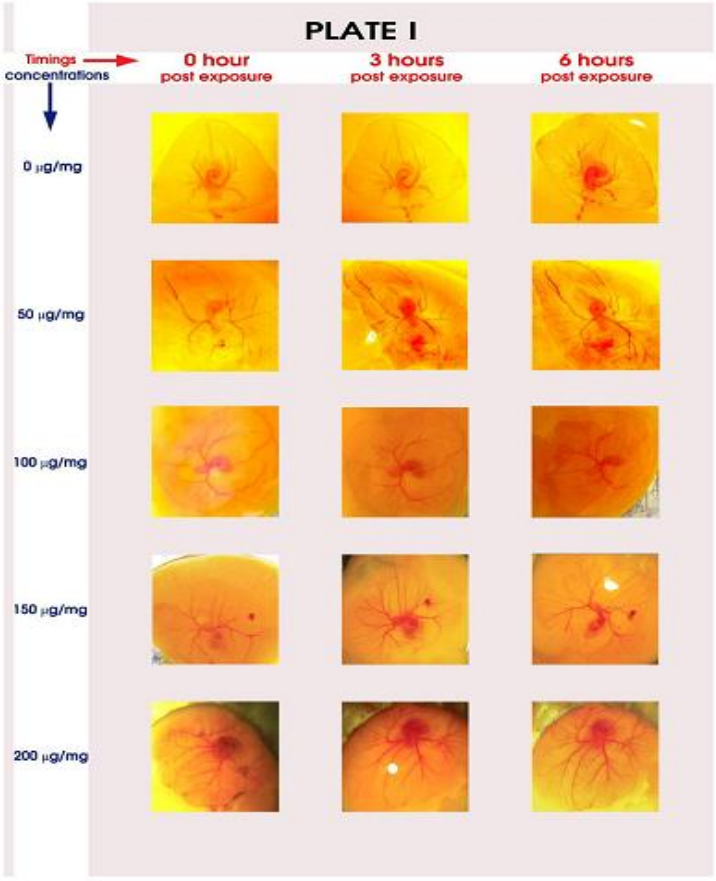
keratinocytes. Proliferation and migration of endothelial cells appears to be at least partially dependent on distinct pathways in endothelial cells.

The essential oils from *Origanum minutiflorum* have no antiangiogenic effect but, provide dilatation in the blood vessels and increase the number of new vessels [16]. In *O. minutiflorum* species, the active material was determined as carvacrol [17–22]. Similarly, it was found [23] that extract of *Thymus piperella* influenced the synthesis of Vascular Endothelial Growth Factor (VEGF), while extract from *Origanum heracleoticum* affected Hypoxia-Responsive Element (HRE) activity. The angiogenic activities as determined did not show any correlation with the contents of polyphenol compounds present in the plants. Besides affecting directly endothelial cells the compounds may act through influence on the production of angiogenic growth factors. These are not usually generated by endothelial cells, most probably due to mechanisms which prevent the unwanted autocrine stimulation of endothelial cells [24–25]. However, some endothelial cell types, namely micro-vascular cells can generate small quantities of VEGF [26 – 27] which can be responsible for angiogenic activities.

**Table I: Average numbers of capillary sprouts counted after addition of *Blumea balsamifera* leaf extract on shell less culture of 48 hour old chick (*Galus galus*) embryo**

| Timings | Concentrations | | | | |
|---|---|---|---|---|---|
| | **0 µg/mg** | **50 µg/mg** | **100 µg/mg** | **150 µg/mg** | **200 µg/mg** |
| **0 hours post exposure** | 11 | 13 | 12 | 10 | 13 |
| **3 hours post exposure** | 11 | 15 | 19 | 25 | 40 |
| **6 hours post exposure** | 13 | 18 | 24 | 31 | 48 * |

* Significantly greater than the control *Blumea balsamifera* (L.) leaf extract injected (P<0.05)




Although, plants may be considered as potential nutraceuticals which may modulate angiogenic processes, demonstration of real influence of plant based therapies on human health requires, extensive animal studies and controlled clinical investigations. This is because in any plant based system of therapy

many active compounds are present in each herb. Also the synergistic and antagonistic interactions between active compounds from different herbs are largely unknown. Further, individual active compounds are usually less potent than the total herbal extract from which they are isolated. Additionally, some compounds are pro drugs and are active only after absorption and metabolism.

The findings of current study that the aqueous extract from leaves induces capillary sprouts, should be further explored with additional studies. In addition, probable mechanisms of the possible angiogenic effects should also be studied. Once thoroughly explored, compounds from *Blumea balsamifera* can be used for wound healing and regenerative applications.

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