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# Anti-angiogenic activity of *Oroxylum indicum* L. Kurz a medicinal tree

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**Abstract :** The present investigation has been undertaken to study the angiogenic effect of different parts of medicinally valuable tree *Oroxylum indicum* L. Kurz. The chick embryo chorio allantoic membrane (CAM) assay was employed for the evaluation of anti-angiogenic property of petiole, leaf, seed, fruit wall, stem bark and root extracts of *O. indicum*. A significant inhibition of vascular endothelium growth factor (VEGF), and induced neovascularization was recorded. Results revealed that among the various parts of the plant used in this investigation, except leaf extract all the other extracts of seed, fruit wall, stem bark and petiole showed maximum anti-angiogenic effect. Thus, based on our investigation it can be concluded that various parts of O. *indicum* may be employed in the preparation of pharmaceuticals that are used in the treatment of various diseases related to angiogenesis.
**Keywords :** *Oroxylum indicum*, Plant extracts, Angiogenesis, CAM assay.

## Introduction

Medicinal plants are well known to be a rich source of bioactive constituents with wide range of medicinal properties acting against various diseases. The popularity of plant based medicine is being increased in order to reduce the risk of side effects caused by modern medicine, and hence phytotherapy is still used by a majority of the world's population. The natural products are playing a crucial role in healthcare systems of ayurveda, tribal, folk and herbal medicine[1]. Hence, it will be necessary to identify the chemical entities which are responsible for potential activities of plants against disease causing entities. Thus, they may be considered as potential nutraceuticals which may modulate angiogenic processes.

Angiogenesis is a natural process of development of new blood vessels from pre-existing vasculature necessary for growth, repair and healing of tissues occurring inside the body; whereas abnormal angiogenesis leads to cancer, rheumatoid arthritis, and diabetic retinopathy[2].

An angiogenesis inhibitor is a substance that inhibits the growth of new blood vessels that provide oxygen and nutrients to cancer cells and can be endogenous, found in the body naturally and involved in the day-to-day process of regulating blood vessel formation or exogenously influenced through pharmaceutical drugs or diet. Human diet contains significant angiogenic inhibitors.

Endogenous inhibitors are less toxic and useful in cancer therapy than exogenous inhibitors[3-5] but they are required in high dosages needed to be used for longer time to prevent tumor growth. Plants interact with stressful environments by physiological adaptation and altering the biochemical profile of plant tissues and producing a spectrum of secondary metabolites with enormous medicinal properties.

In traditional medicine many herbs are used in the treatment of angiogenic diseases such as chronic wounds and rheumatoid arthritis. Thus, it is rational to explore these medicinal plants as a source of novel

angiomodulators. A wide range of plants contain compounds with angiogenesis modulating properties. One such plant with enormous range of medicinal properties is *Oroxylum indicum* (L.) Kurz.[6]

The species *O. indicum* is an endangered medicinally important tree and known for its enormous usage in various traditional systems of medicine such as ayurveda, unani and folk. All the parts of this valuable tree are found to possess secondary metabolites [7, 8] with anti-inflammatory, anticancerous, anti-helminthic, anti-leucodermatic, anti-rheumatic, anti-anorexic, antibacterial, antioxidant, analgesic and anti-tussive activities [9-14]. Hence, we have made an attempt to study the effect of extracts of different parts of *O. indicum* on angiogenic activity using CAM assay.

## Experimental

### Collection of fertilized chicken eggs

The fertilized eggs were obtained from Venkateshwara Central Poultry breeding farm Hyderabad, Telangana State, India.

### Chorioallantoic membrane (CAM) assay

For the CAM assay fertilized chick embryos were pre-incubated for 8 days at 37.5 $^{o}$ C and 85 % humidity[15-17]. A hole was drilled over the air sac at the end of the each egg and an avascular zone was identified in the CAM. A 1 cm×1 cm window in the shell was sectioned to expose the CAM. Plant extracts were prepared by following the method[6] and have been applied to the CAM surface. Windows were sealed with clear tape and eggs were incubated for 48 hrs. Blood vessels were viewed and photographed with Nikon digital camera. The anti-angiogenic effects of plant extracts on CAMs were quantified by counting the number of blood vessel branch points.

## Results and Discussion

The anti-angiogenic potential of *O. indicum* was investigated by a chick chorio-allontoic membrane (CAM), a significant inhibition of vascular endothelium growth factor (VEGF), and induced neovascularization was recorded and presented Tables in 1-2 and shown in Fig.1. The results of investigation on angiogenic effect of different extracts of *O. indicum* revealed that except leaf extract, all the other extracts of seed, fruit wall, stem bark and petiole showed maximum anti-angiogenic activity. The score values of the different extracts were found 2.0 except the leaf extracts of *O. indicum.*

**Table-1: Standard score values for the anti- angiogenic effect on the Chorio Allontoic Membrane (CAM) assay of fertilized chicken eggs**

| Score value | Type of Effect | Effect observed |
|---|---|---|
| 0 | No effect | |
| 0.5 | very weak effect | No capillary-free area. Area with reduced capillary density around not larger than the area or area with small capillary-free area or area with significantly reduced capillary density. |
| 1 | weak-medium effect | Effect not larger than double the size of the pellet. |
| 2 | Strong effect | Capillary-free area around the pellet at least double the size of the pellet. |

**Table-2: Showing the score values of extracts of various parts of *O. indicum***

| Score value | Type of extract |
|---|---|
| 0.5 | Leaf |
| 2.0 | Stem |
| 2.0 | Petiole |
| 2.0 | Seed |
| 2.0 | Fruit wall |
| 2.0 | Stem bark |

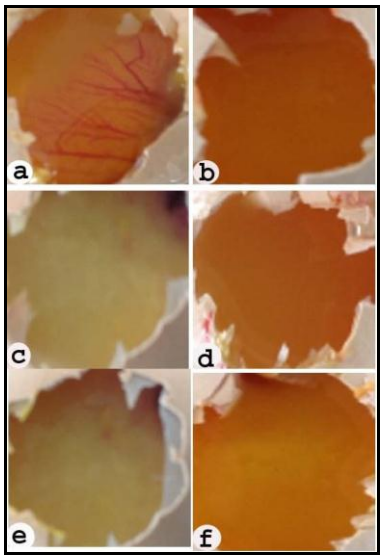


**Fig 1: Showing the anti- angiogenic effect of different extracts of *O. indicum* on chorio allantoic membrane (CAM) of fertilized chicken eggs.**
**a) Effect of leaf extract on chick CAM-(No capillary-free area and very weak effect); b-f) Effect of stem, petiole, seed, fruit wall and stem bark extracts - Capillary-free area (Strong effect)**

The CAM is a thin and extensively vascularized membrane. Due to its easy use and simplicity, the CAM assay is most commonly used to study normal angiogenesis and screen anti-angiogenic activity of new compounds [18-22]. Chorio allantoic membrane is an extra embryonic membrane, which serves as a gas exchange surface and its function, is supported by a dense capillary network, because of its extensive vascularization and easy accessibility. Our results are also found to be promising as all the extracts have shown anti-angiogenic property and may prove useful in drug discovery of anti-cancer agents.

Similarly many indian medicinal plants such as *Curcuma longa, Azadirachtha indica, Tinospora cordifolia, Calotropis procera, Andrographis paniculata*are, *Withania somnifera* and *Piper longum* were found to possess anti-angiogenic activity [23].

Thus, anti-angiogenic agents of natural origin are least toxic and may complement the necessity of chemotherapy and radiotherapy in the treatment of physiological and pathological angiogenesis. These findings suggest that the extracts of different parts of *O. indicum* possess anti-angiogenic activity and can be employed in the preparation of anti-angiogenic drugs used for anti-cancer action. Further studies are required to analyze the use of these products in the formulation of therapeutics for their confirmed use in the treatment of cancer in future.

Now a days, the interest of the scientific community has been increased in exploiting anticancer and antiangiogenic drugs. Plants, with their unique medicinal properties are known to produce diverse spectra of secondary metabolites. Thus, results obtained in the present investigation indicate the scope for utilizing the different extracts of *O. indium* that acts directly on inhibition of angiogenesis. This activity is directly related to the capacity of the plant extracts to inhibit VEGF secretion in endothelial cells.

## Conclusion

In conclusion, our present study indicates the importance of the plant extracts of *O. indicum* in inhibiting the angiogenesis, useful in the treatment of wound healing, cancer and cardiovascular diseases. Hence, these extracts can be employed in the preparation of anti-angiogenic drugs and also throws light on further investigations in this regard.

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