IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Evaluation of Angiogenic potential of different medicinal herbs using CAM Assay

Dr. Aparna Deshmukh 1, Rafat Moosa 2, Bhumika Mishra 3

Department of Biotechnology,

Thakur College of Science and Commerce, Mumbai, Maharashtra, India

1 Guiding Author, 2 Corresponding Author, 3 Student

Abstract:-

Angiogenesis is a complex process that involves the growth of new blood vessels from existing ones, and defects in the control of angiogenesis have been implicated in several pathological processes, including diabetic and retinopathy.[1] To investigate the potential vasculogenic activity of plants, this study evaluated the angiogenic potential of three herbaceous plants, namely: *Centella asiatica*, *Cissus quadrangularis*, and *Madhuka longifolia* by chick chorioallantoic membrane (CAM) assay, a high-density microvasculature extra-embryonic membrane that supports embryonic development in avian species.[2] CAM assay is a well-established tool for investigating in vivo mechanisms of angiogenesis regulation.[3] These plants have been used in various traditional medicinal practices, but their therapeutic efficacy is not fully understood. The CAM from chicken embryos was treated with various concentrations of these three herbs/plants extracted to examine their angiogenic activity.[5] The results showed that all the plant extracts have the potential to induce angiogenesis in the CAM assay. The observed angiogenic activity was measured using various parameters, including increased vascularization, higher branching complexity, and increased capillary density. The induced angiogenic activity by the plant extracts was found to be concentration-dependent and showed a dose-response relationship.

The angiogenic activity of the plant extracts has significant potential for therapeutic use in promoting tissue growth and repair, as well as in the treatment of various vascular disorders.[7] A detailed understanding of the mechanism of action, optimization of dosage and suitable formulation of these herbal extracts would enable us to consider a practical option and alternative therapies in the treatment of vascular disorders.

key words: angiogenesis, CAM Assay, blood vessels, dosage, *Centella asiatica*, *Cissus quadrangularis*, *Madhuca longifolia*, vasculature, optimization, angiogenic activity.

Introduction:

The search for novel therapeutic agents to regulate angiogenesis effectively has motivated researchers to explore the fields of botany and traditional medicine [1]. *Centella asiatica, Cissus quadrangularis, and Madhuca longifolia* are medicinal plants renowned for their pharmacological properties, including wound healing, anti-inflammatory, and antioxidant effects. This study aims to extend understanding regarding the angiogenic potential of these plants and their potential application in tissue engineering and regenerative medicine. Angiogenesis involves the coordinated activation of several angiogenic growth factors and their receptors, proteolytic enzymes, and endothelial cells.[2] The balance between various pro- and anti-angiogenic factors ultimately determines the rate and extent of vessel growth, maintenance, and regression. [3]

This research paper presents an experimental approach employing the CAM assay, a robust, versatile, and cost-effective tool to assess angiogenic properties. The CAM model allows visualization and quantification of blood vessels following treatment with different herbal extracts.[4] The CAM assay offers a simplified in vivo environment that simulates different aspects of angiogenesis, thereby facilitating the evaluation of pro-angiogenic factors.

The significance of this study lies in the identification of plant extracts that promote angiogenesis, which may pave the way for the development of novel treatments for conditions characterized by impaired vascularization. Generated data from this research have the potential to contribute significantly to the field of angiogenesis research and lead to promising therapeutic interventions that harness the natural potential of these herbal extracts.

Experimental design:

To conduct the experiment, the following steps will be followed:

1. **Preparation of Samples**: Leaves of *Madhuca longifolia, Cissus quadrangularis, and Centella asiatica* will be obtained, (FROM LOCAL NURSERY OF MEDICINAL HERBS) dried, ground into a fine powder, and dissolved in 50ml of distilled water, making it concentrated by boiling it on high flame and continue stirring in aseptic environment. and filtered using sterile cotton or filter paper. Three different concentrations of each extract (highly concentrated, 1:10 diluted, and 1:100 diluted) will be prepared.

2. Preparation of CAMs: Fertilized eggs will be cleaned with alcohol and incubated at 37°C with 75% humidity. On the 8th day, a small window will be cut at the apex of the eggshell. The prepared samples will be injected in equal amounts using a micropipette. A coverslip coated with candle wax or parafilm will be placed on the CAM to maintain proper ventilation and protect the embryo.

3. Administration of Samples: Using a micropipette, 50µl of the sample solution will be added inside the egg through the window. All samples will be prepared in duplicate to ensure method accuracy.

4. Incubation of CAMs: The eggs will be covered with parafilm and incubated until day 14 when the embryos are fully matured. On day 14, the eggshell will be cut open to observe the growth pattern of angiogenesis. Angiogenesis response of the herbal extracts will be observed visually and using a microscope.

5. Preparation of Negative Controls: A separate set of eggs will be incubated and prepared using distilled water instead of the sample solution, following the same procedure.

6. Scoring of Angiogenic Response: The angiogenic response will be scored using a modified system based on the branching pattern of blood vessels. Multiple randomized examinations will be conducted, and each blood vessel will be scored based on its branching pattern.

This experimental design aims to evaluate the angiogenic potential of the herbal extracts on the CAM model, allowing for the determination of their efficacy in promoting angiogenesis. The inclusion of negative controls will provide a basis for comparison, ensuring that any observed effects are due to the herbal extracts rather than other factors. The scoring of angiogenic response will provide a quantitative assessment, allowing for the comparison and analysis of the angiogenic capabilities of the different extracts.[19]

Results and Discussion

Aqueous extracts of Madhuca longifolia, Cissus quadrangularis, and Centalla asiatica were prepared in three concentrations (highly concentrated, 1:10 diluted, and 1:100 diluted). The angiogenic response to the extracts was studied using CAM model system [4]. Three randomized separate examinations were undertaken and branching of the blood vessels was visualized manually, images were captured as displayed in the Figure 1.

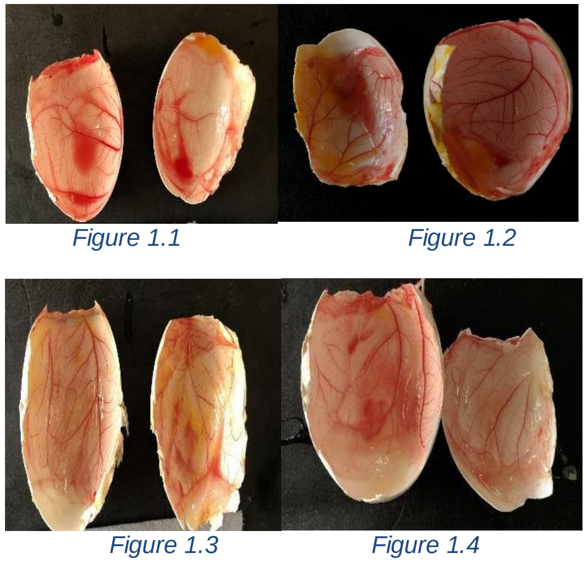

Fig.1. Angiogenesis in CAM (branches of capillaries in 1.1 standard egg, 1.2 *Centella asiatica* treated, 1.3 *Cissus quadrengularis* treated 1.4 *Madhuca longifolia* treated respectively.

Scoring each and every blood vessel was done according to their branching pattern. The primary blood vessel was given a score of 4 and the subordinating vessels were given less score according to the further branching. The average mean value of the standard chick embryos was plotted against the average mean value acquired by the different sets of herb extracts at three different doses. All three plant samples, Centella asiatica, Cissus quadrangular, and Madhuca longifolia, exhibit increased branching of blood vessels as a result of in vivo stimulation of angiogenesis, demonstrating that these plants are pro-angiogenic. These plants' angiogenic response increased in a dose-dependent manner; higher concentrations showed higher scores of capillary branches as shown in Table 1 and Figure. 2.

Concentration of the plant extract	***Cissus quadrengularis*** **(Mean±SD)**	***Centella aseatica*** **(Mean±SD)**	***Mdhuca longiflolia*** **(Mean±SD)**
concentrated	**151±6**	**153±4**	**150±9**
1:10	**142±4**	**126±5**	**103±4**
1:100	**124±7**	**128±4**	**88±8**

Table.1. Angiogenesis score of the capillary branches in medicinal herb-treated chick embryos.

The standard eggs treated with saline showed an average score of 93.5± 5.6. Increased branching, indicative of more blood vessel formation is suggestive of higher angiogenic activity. The CAMs treated with the plant extracts showed enhanced vascular sprouting, elongation, and intricate vessel network formation highlighting the angiogenic potential.

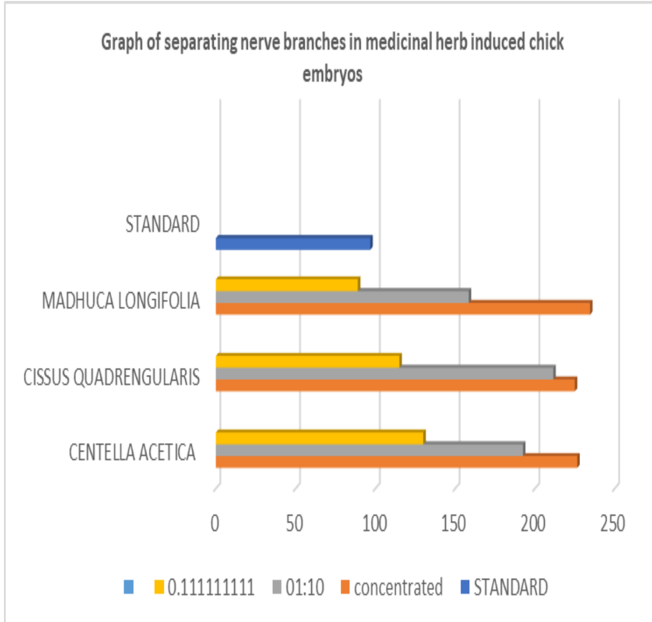

FIG.2.Graph showing separating nerve branches value induced by the medicinal herb

The exact mechanism through which these medicinal herbs induce angiogenesis is not fully understood and requires further investigation. It is believed that the active compounds present in these plants may interact with specific receptors or signalling pathways involved in angiogenesis regulation. Future studies should focus on identifying the specific bioactive compounds responsible for the angiogenic activity and elucidating their mechanisms of action. Furthermore, optimization of dosage and suit formulation of these herbal extracts is essential to ensure their safety and efficacy. Standardization of extraction methods and quality control measures are crucial in maintaining consistency and reproducibility of the angiogenic potential of these medicinal herbs.

Conclusion:

The results showed that all three plants demonstrated pro-angiogenic activity, with an increase in the branching of blood vessels indicating that they promote angiogenesis. Moreover, the concentration of the sample injected was found to be a critical factor in influencing the extent of angiogenesis. It is noted that if these plants are used as therapeutic agents in the future, it could be useful for patients suffering from diabetes and decreased healing power, people suffering from coronary heart disease, and could even be used as an anti-wrinkle agent to decrease the onset of aging. There are still studies conducted to evaluate the angiogenic potential of different plants using various assays such as cell lines and cell culture, rat aortic ring assay, in-vitro cytotoxicity assay, anti-proliferative activity, wound healing migration assay, and others.[20] However, this project represents a significant contribution, being the first to study the pro-angiogenic activity of these three specific plants using a CAM assay. Furthermore, the simplicity and accuracy of the method suggest it could be implemented successfully in studies evaluating the angiogenic potential of other agents.

The discovery of three plant samples demonstrating a clear signal as pro-angiogenic opens up avenues to further study these samples and to identify how their angiogenic qualities can be harnessed therapeutically to benefit human health.

Acknowledgement:

The authors acknowledge the support of the principal and management of Thakur College of Science and Commerce for providing infrastructure and support to conduct the research work.

Reference:

1. Naik, M., Brahma, P., & Dixit, M. (2018). A cost-effective and efficient chick ex-ovo CAM assay protocol to assess angiogenesis. *Methods and protocols*, *1*(2), 19.
2. Krupinski, J., Kaluza, J., Kumar, P., Kumar, S., & Wang, J. M. (1994). Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke*, *25*(9), 1794-1798.
3. Jonkman, J. E., Cathcart, J. A., Xu, F., Bartolini, M. E., Amon, J. E., Stevens, K. M., & Colarusso, P. (2014). An introduction to the wound healing assay using live-cell microscopy. *Cell adhesion & migration*, *8*(5), 440-451.
4. Auerbach, R., Auerbach, W., & Polakowski, I. (1991). Assays for angiogenesis: a review. *Pharmacology & therapeutics*, *51*(1), 1-11.
5. enger, D. R., & Davis, G. E. (2011). Angiogenesis. *Cold Spring Harbor perspectives in biology*, *3*(8), a005090.
6. Kerbel, R. S. (2008). Tumor angiogenesis. *New England Journal of Medicine*, *358*(19), 2039-2049.
7. Karamysheva, A. F. (2008). Mechanisms of angiogenesis. *Biochemistry (Moscow)*, *73*, 751-762.
8. Jonkman, J. E., Cathcart, J. A., Xu, F., Bartolini, M. E., Amon, J. E., Stevens, K. M., & Colarusso, P. (2014). An introduction to the wound healing assay using live-cell microscopy. *Cell adhesion & migration*, *8*(5), 440-451.
9. Naik, M., Brahma, P., & Dixit, M. (2018). A cost-effective and efficient chick ex-ovo CAM assay protocol to assess angiogenesis. *Methods and protocols*, *1*(2), 19.
10. Krupinski, J., Kaluza, J., Kumar, P., Kumar, S., & Wang, J. M. (1994). Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke*, *25*(9), 1794-1798.
11. Arribas-López, E., Zand, N., Ojo, O., Snowden, M. J., & Kochhar, T. (2022). A systematic review of the effect of Centella asiatica on wound healing. *International Journal of Environmental Research and Public Health*, *19*(6), 3266.

12. Taher, M., Mandal, U. K., Jaffri, J. M., Susanti, D., Mahmood, S., & Zakaria, Z. A. (2019). Pharmacological properties of Centella asiatica hydrogel in accelerating wound healing in rabbits. *BMC complementary and alternative medicine*, *19*(1), 1-7.
13. Kavitha, S., & Manimekalai, G. (2015). A study on properties of cissus quadrangularis plant-a review. *International Journal of Research in Applied Natural and Social Sciences*, *3*(6), 15-18.
14. Mishra, G., Srivastava, S., & Nagori, B. P. (2010). Pharmacological and therapeutic activity of Cissus quadrangularis: an overview. *International journal of pharmtech research*, *2*(2), 1298-1310.
15. Sibi, G., Zaki, S., Malathi, R., & Latha, V. (2020). A review on efficacy of Cissus quadrangularis in pharmacological mechanisms. *International Journal of Clinical Microbiology and Biochemical Technology*, *3*(1), 049-053.
16. Kamal, A. (2014). Qualitative phytochemical analysis of Madhuca longifolia. *Indian J Plant Sci*, *3*(4), 38-41.
17. Bairagi, J. H., & Ghosh, R. S. (2020). PHYTOCHEMISTRY AND ANTICANCER ACTIVITY OF MADHUCA LONGIFOLIA LEAVES.
18. Mondal, S., Bandyopadhyay, S., K Ghosh, M., Mukhopadhyay, S., Roy, S., & Mandal, C. (2012). Natural products: promising resources for cancer drug discovery. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *12*(1), 49-75.
19. Miller, W. J., Kayton, M. L., Patton, A., O'Connor, S., He, M., Vu, H., ... & Libutti, S. K. (2004). A novel technique for quantifying changes in vascular density, endothelial cell proliferation and protein expression in response to modulators of angiogenesis using the chick chorioallantoic membrane (CAM) assay. *Journal of translational medicine*, *2*(1), 1-12.
20. Kıyan, H. T., Demirci, B., Başer, K. H. C., & Demirci, F. (2014). The in vivo evaluation of anti-angiogenic effects of Hypericum essential oils using the chorioallantoic membrane assay. *Pharmaceutical biology*, *52*(1), 44-50.
21. Nia, R., Essien, E. E., Iyadi, K. C., Bassey, A. I. L., Antai, A. B., & Franz, G. (2004). Evaluation of the anti-oxidant and anti-angiogenic effects of Sphenocentrum Jollyanumpierre. *African Journal of Biomedical Research*, *7*(3).
22. Yang, E. Y., & Moses, H. L. (1990). Transforming growth factor beta 1-induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. *The Journal of cell biology*, *111*(2), 731-741.