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RESEARCH ARTICLE

Anti Angiogenic Activity of *Carica papaya* Leaf Extract

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Abstract

Cancer is a major cause of death worldwide and angiogenesis is critical in cancer progression. Development of new blood vessels and nutrition of tumor cells are heavily dependent on angiogenesis. Angiogenesis is the formation of new blood vessels from existing vasculature. There are several "on" and "off" switches that regulate the process. Decreasing or inhibiting angiogenesis can be therapeutic in cancer and other diseases. Thus, angiogenesis is an important process that occurs both during health and disease. This study was conducted to investigate the anti-angiogenic activity of *Carica papaya* leaf. The anti-angiogenic activity of papaya leaf was evaluated using docking behaviour of known bioactive compounds of leaf as ligands with angiogenic receptors VEGFR1 and VEGFR2 along with their putative binding sites using Swiss Dock Web server (*In silico*) and further based on docking results leaf aqueous extract was used for implantation in chorioallantoic membrane (CAM) egg yolk angiogenesis model (*in vivo*). Docking studies and binding free energy calculations revealed that among known bioactive compounds of leaf Ascorbic acid, quercetin, riboflavin and lycopene have maximum free energy as compared to other investigated ligands. Similarly, CAM assay also showed the inhibitory effect of the *Carica papaya* leaf with respect their reduction in length, size and junctions of blood capillaries compared to untreated egg yolk. The results showed that Ascorbic Acid, Quercetin, Riboflavin and, Lycopene (leaf compounds) can attenuate angiogenesis in pathological conditions and can be potent in drug discovery as well as medical science.

Keywords: *Carica papaya*, Angiogenesis, Medical Science, Cancer, Pathological, Phytochemical.

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INTRODUCTION

Angiogenesis is a multistep process. In normal physiology, angiogenesis has a role in embryogenesis, the female reproductive cycle, and wound healing and bone formation[2]. Angiogenesis is controlled by number of growth factors and inhibitors. Well known angiogenic (stimulatory) growth factors include basic Fibroblast Growth Factor (bFGF), Vascular Endothelial Growth Factor (VEGF), Granulocyte Colony-Stimulating Factor (G-CSF), Interleukin-8 (IL-8) and Transforming Growth Factors alpha and beta (TGF-α and TGF-β) [8]. When these factors are over expressed leads to diseases for example in Cancer-when solid tumor can't grow beyond a limited size but VEGF over expression leads to Metastasis of Cancer[6,15,25]. Angiogenic inhibitors include Angiostatin, Interferons (alpha, beta and gamma), Endostatin, Interleukin-12 and retinoids. Many plants have been identified and used as medicinal herbs from prehistoric times[12,19]. Treatment with medicinal plants is considered very safe as there is no or minimal side effects[18,19]. *Carica papaya* is a giant herbaceous plant-resembling a tree but not woody. It is one of the 22 accepted species in the genus *Carica* of the family *Caricaceae*[26,27]. It is native to lowland tropical areas of Central and South America, but is now grown in tropical to subtropical regions throughout the world both commercially and in kitchen gardens[19]. This evergreen tree is commonly called as papaya with broadleaf that bears papaya fruits throughout the year. The leaves are large, 50-70 cm in diameter and spirally arranged confined to the top of the trunk with palmately lobed structure. Whole plant parts fruits, seeds, bark, pulp, peel of *Carica papaya* have different medicinal properties[1,24]. Further studies can be done on these parts to identify and isolate the most active phytochemical that attributing the medicinal properties of the plant. The aim of the present study is to evaluate anti angiogenic activity of the leaves of *Carica papaya*. In this study, reported constituents of *Carica papaya* leaf aqueous extract docked with angiogenic receptors using one of the molecular docking methods. Based on preferred orientation and docking results of some compounds leaf aqueous extract was selected and used for *in vivo* CAM model assay. To analyze the mechanism underlying normal and pathological angi-ogenesis, numerous *in vivo* angiogenic assays have been stabilized employing different species of laboratory animals, including (mouse, rat and rabbit) and fish. In this study, we focussed on major models of angiogenesis in the chick embryo. In the chick embryo, the chorioallantois membrane is formed between the days 4 and 5 of development, when the outer mesodermal layer of the allantois fuses with the mesodermal lining of the chorion and a network of blood vessels is gradually formed between the two layers. The central portion of the CAM is fully developed by the day 8 to 10[21]. At this time, it becomes capable of sustaining tissue grafts, while the outskirts of the CAM are still developing and expanding until the CAM fully envelops the embryo at the day 12 of incubation[5]. The CAM consists of three germ layers, ectoderm, mesoderm and endoderm. After developing the CAM model, we assessed the potential role of *Carica papaya*(s) active ingredients as anti-angiogenic factors by which it can treat or prevent disease(s) like Cancer.

MATERIALS AND METHODS

Fertilized chicken eggs taken from nearby poultry, and fresh green papaya leaves, randomly collected from Yadav Nursery, Geeta Colony, East Delhi.

Chemical Requirements

Phosphate buffer saline (7.4 pH), Ethanol, Distilled water.

Aqueous extract preparation

The leaves were washed, cut into small pieces and crushed finely into grinder. The sample was filtered, an aqueous ratio of 1:10 ml was prepared and stored into refrigerator at 4°C in an airtight container prior to use for analysis.

Docking studies

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecules[22]. Docking studies were carried out on the crystal structure of the angiogenic receptors VEGFR1 and VEGFR2 retrieved from the Protein Data Bank (PDB) using Swiss Dock web server under protein ligand interaction with known bioactive components of *Carica papaya* as ligands whose structures were determined from Zinc database. Most negative Gibb's free energy interaction pose was chosen and leaf aqueous extract was selected for implantation in CAM model assay.

Egg yolk angiogenesis (CAM) assay[5]

Fertilized leg horns chicken eggs were taken from nearby poultry. To check the viabilities of embryos, eggs were observed over a self-made lamp and kept in a humidified incubator at 37°C for 8 days. After the 8th day of incubation small window was cut in each egg and the plant extract was implanted at different concentration through the implanted disc in the experimental eggs while the control was implanted with normal saline under sterile conditions. Windows were sealed with tape and incubated for 3 days. After the period of incubation windows were reopen. Images were taken using Kodak digital camera and quantification of angiogenesis was done by observing the number of vessels and their branching. Experimental groups were compared with control groups.

RESULTS

Reported studies of *Carica papaya* leaves showed that aqueous extract contains great portion of steroids, quinones, tannins and alkaloids[3]. Compounds known in leaf aqueous extract are tocopherol, ascorbic acid, carpaine, quercetin, deoxyquercetin, lycopene, folic acid, riboflavin and cystine. Among all the compounds reported few compounds showed docking interaction with angiogenic receptors (VEGFR1 and VEGFR2).

Docking results

Negative sign signifies that binding between ligands (Ascorbic acid, Ouercetin, Riboflavin, and Lycopene) and receptors (VEGFR1 and VEGFR2) is going to occur at normal temperature and pressure (i.e. 293K and 1atm) and this reaction is thermodynamically spontaneous.

Table 1. Docking Results

Compound	Gibb's free energy with **VEGFR1** (kcal/mol)	Gibb's free energy with **VEGFR2** (kcal/mol)
Ascorbic acid	-10.56	-7.37
Quercetin	-7.36	-7.97
Riboflavin	-6.99	-7.50
Lycopene	-10.04	-8.18

It can be inferenced from the table 1, that Ascorbic acid binds most efficiently with VEGFR1 as compared to others. And, Lycopene binds with VEGFR2, there by inhibiting their functions (Fig. 1).

CAM results

On the basis of docking results compounds with most negative interaction energy i.e. ascorbic acid, quercetin, riboflavin and lycopene were studied and leaf aqueous extract was used for implantation in CAM model assay to determine the anti-angiogenic property of *Carica papaya* leaves. Anti-angiogenic behaviour of leaf extract varies depending on the concentration. In control egg there was the development of number of blood vessels as the embryonic growth took place (Fig. 2). Implantation of the experimental egg with papaya leaves extract at different concentration inhibits the formation of blood vessels at different extend. Two concentrations of papaya leaves extract 5% and 10% were used. It was observed that there was less number of blood vessels in egg with 5% concentration as compare to control and the number of vessels formation inhibits as the concentration of extract was increased (Fig. 2). On the basis of these results it can be conclude that papaya leaves may be beneficial in various health conditions where formation of blood vessels leads to disease conditions.

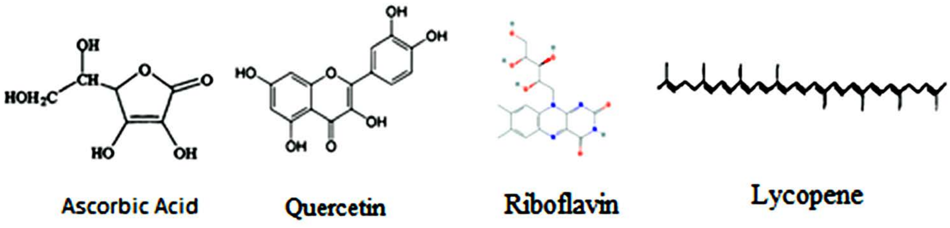

Fig. 1. Structure of compounds having most negative binding energies

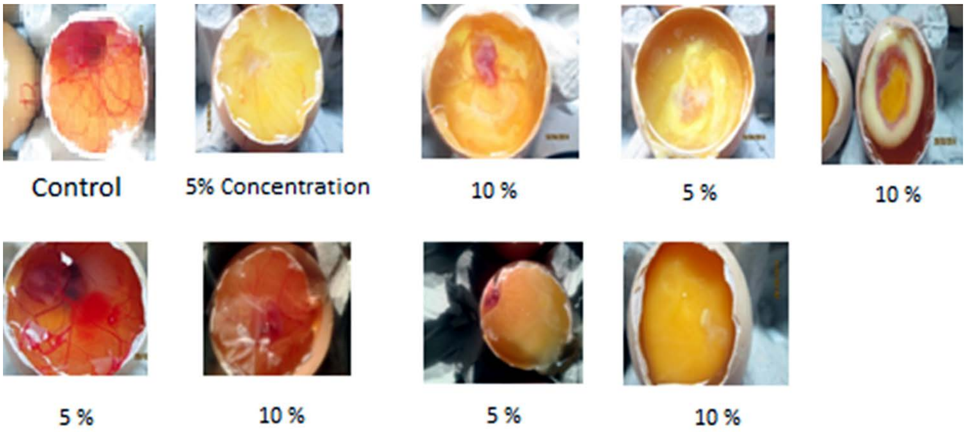

Fig. 2. Control and experimental eggs presenting formation and inhibition of blood vessels at different concentration

DISCUSSION

Papaya is not only the delicious fruit, but it is loaded with vitamins, phytochemicals and several mineral compounds which help to treat health problems. The bioactivity of plant is attributed to phytochemical constituents. Reported studies on young leaves of *Carica papaya* revealed the presence of pharma-cologically active phytocompounds, alkaloids, phenolics, flavonoids and amino acids. From this study we can concluded that *Carica papaya* leaf extract may promotes immune system that may be due to the presence of Quercetin, Ascorbic acid, Riboflavin and lycopene as these compounds showed their potent binding with angiogenic receptors during molecular docking process, also the anti-angiogenic property of leaf extract reveals that *Carica papaya* may be used as a potent cancer fighter in the field of oncology as well as pharmacy. Also, it is reported that lycopene inhibits angiogenesis at achievable concen-trations *in vivo* and it can be regarded as a promising anti-angiogenic compound[10]. It may be due to this property of lycopene that it can be used as medicinal compound in future aspects. Ascorbic acid also found to be as anti oxidant in various chronic diseases[4].

CONCLUSION

Based on reported studies and docking behaviour of some compounds we may concluded that anti angiogenic activity of *carica papaya* leaves in CAM model assay can be due to the presence of all these four compounds. As all plants *Carica papaya* leaves are rich in compounds of different properties. Further studies need to be conducted to determine anti-angiogenic pathways followed by leaf extract or juice. However, it can be concluded that administration of *Carica papaya* leaf juice is safe and does induce anti angiogenic process during disease condition like Cancer. It may play valuable role in the management of various diseases in the future.

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CONFLICT OF INTERESTS

The author declares that there are no conflict of interest.

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