



Anti-angiogenic effect of phenolic acid fraction from *Thespesia populnea* (stem bark) in the chick embryo chorioallantoic membrane model

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ABSTRACT

Angiogenesis, which plays a significant role in a variety of physiological processes, such as embryonic growth and wound healing, is strictly delimited and finely tuned by a balance of pro-angiogenic and anti-angiogenic factors. As such, the purpose of this study was to investigate the anti-angiogenic effect of phenolic fraction of *Thespesia populnea* using chorioallantoic membrane (CAM) assay. In this study, the anti-angiogenic effect of phenolic fraction of *T. populnea* concentration of 0.7 mg/disc was applied to the incubated fertilized eggs. For the purpose of determining the anti-angiogenic effect of the drug, blood vessels of the chorioallantoic membranes were evaluated using a stereoscopic microscope. Phenolic fraction of *T. populnea* exposure in the CAM was able to reduce the formation of blood vessels in chick embryos. The present data suggest that phenolic fraction of *T. populnea* may serve as potential anti-angiogenesis agents.

KEYWORDS

Anti-angiogenic, *Thespesia populnea*, Chorioallantoic membrane, Phenolic acid, CAM assay

INTRODUCTION:

Angiogenesis is the phenomenon of formation of new blood capillaries from existing small blood vessels. It usually accounts for normal physiological and pathological changes involved in normal functioning of body, like formation of new blood vessels occurs during wound healing, organ regeneration and in the female reproductive system during ovulation, menstruation, and the formation of the placenta^[1]. It also plays a role in the pathophysiology of multiple disease processes, including tumour neovascularization, ischaemic recovery, and wound healing. In addition, angiogenesis and cardiovascular diseases are strongly related to providing the distal perfusion^[2]. The investigation was focused on ways to obtain therapeutic benefits in low-perfusion situations with neo-angiogenesis. As such, research on therapeutic angiogenesis and anti-angiogenic has become more frequent in recent studies^[3].

Thespesia populnea Soland ex. Correa, a plant of the Malvaceae family, is used in folk-medicine in India for the treatment of liver diseases and dermatitis. The decoction of the bark is used by Ayurvedic physicians for the treatment of skin and liver diseases^[4]. The bark and flower of *T. populnea* possess pharmacological properties such as hepato-protection, antioxidant, anti-inflammation, memory enhancement property and hypo-cholesterolemic activity^[5-11]. *T. populnea* fruit have anthelmintic activity and anti-diarrheal activity^[12,13]. The plant bark has not been explored for its anti-angiogenesis activity so far. The present study was therefore aimed at investigating the anti-angiogenesis activity of the stem bark phenolic acid fraction with a view to justifying the use of the plant bark in the treatment of cancer.

MATERIALS AND METHODS

Plant materials, preparation of extract and fractions: *T. populnea* stem bark was collected from Thodupuzha, Kerala, India in the month of March. The specimens were identified and voucher specimens were deposited in the Herbarium (TTP-876) of Nagarjuna Herbal Concentrates Ltd, Kerala, India.

Preparation of the extract

The dried and coarsely powdered stem bark of *T. populnea* was macerated with ethanol for 4 h by continuous stirring at room temperature and then filtered and evaporated the filtrate to dryness under reduced pressure. The yield of ethanol extract was 23% of the stem barks powder. The dried ethanol extract was then suspended in distilled water (1:50, w/v) and fractionated through

successive extractions (twice each) with chloroform (1:1, v:v), ethyl acetate (1:1, v:v), n-butanol (1:1, v:v) saturated with water. Ethyl acetate fraction was concentrated to dryness under reduced pressure and below 45 C on a rotary vacuum evaporator. The yield of ethyl acetate fraction of ethanol extract was 14%.

Fractionation of ethyl acetate fraction and isolation of phenolic acid fraction: The ethyl acetate fraction was subjected to silica gel 60-120 mesh size (Merck, Mumbai) column chromatography (column 15X400 mm, 20 g silica gel and 1g sample) and eluted with Hexane : Ethyl acetate (100:0), (90:10), (80:20), (70:30), (60:40), (50:50), (40:60), (30:70), (20:80) and (10:90), successively; each fraction was extracted in a volume of 10 ml of the solvent mixture. Eluents were combined according to TLC (NP-Silica Gel 60 F₂₅₄ plates) behaviour using solvent system: Hexane-Ethyl acetate-Acetic acid (5:5:0.1, v:v:v). The developed plates were allowed to air dry and chromatograms were observed under visible and UV light (k = 254 nm).

Chemical analysis of active component/fraction

In the column chromatography, fraction 25-50 showed a single band on TLC with an R_f value of 0.8^[15]. The plates were sprayed with Folin – Ciocalteu's phenol reagent and fumigated ammonia vapour, the plate developed dark blue colour band^[14]. The yield of active component was 53% of ethyl acetate fraction and 1.7 percentages of plant stem bark powder. This active component was used for further studies. The phytochemical identification was carried out by the methods of Trease and Evans^[15].

High performance liquid chromatographic (HPLC) analysis of phenolic fraction: The active component, isolated by column chromatography was subjected to HPLC analysis^[15].

Chick embryo chorioallantoic membrane (CAM) assay

CAM assay was performed as described previously^[16] with small modifications. Briefly, 20 fertilized chicken eggs were incubated at 37°C at constant humidity and randomly divided into 2 groups. On the 9th day of incubation, a square window (1x1 cm²) was opened in the shell. The following day, filter discs loaded with 0.7 mg/disc of phenolic fraction of *T. populnea* was placed on the top of the growing CAMs under sterile conditions. Afterwards, the window was sealed with sterilized surgical tape and the eggs were returned to the incubator. After 72 h of incubation, the CAMs were photographed using an Olympus Live View Digital SLR camera.

RESULTS

To verify the anti-angiogenic effect of phenolic fraction of *T. populnea* ex vivo, On day 9 of embryo development, fertilized chick eggs were treated with 0.7 mg/disc concentration of phenolic fraction of *T. populnea*. After 72 h of incubation, normal vascular pattern with numerous branchings was observed in the control group (Fig. B). However, phenolic fraction of *T. populnea* significantly distorted the vasculature architecture on the chorioallantoic membrane (Fig. D). However, further investigations are required to assess the in vivo, in vitro models, detailed mechanisms and to ascertain its beneficial role in the clinical setting.

DISCUSSION

Conventional treatments for cancer patients include surgery, radiotherapy and chemotherapy. Recently, some alternative treatments, such as gene therapy and targeted therapy have attracted some attention. However, these therapies are usually unaffordable for most patients and have limited efficiency and serious side-effects.

As an indispensable step for metastasis, angiogenesis is a promising target in anticancer therapy. Anti-angiogenic agents exert their effect in 2 ways^[17,18]. Direct inhibitors disrupt the proliferation, migration and differentiation of endothelial cells. On the other hand, indirect inhibitors interfere with the communication between tumor cells and endothelial cells by suppressing the expression of pro-angiogenic cytokines or blocking the binding of factors with their receptors. Although anti-angiogenic agents exhibit obvious antitumor activities, serious side-effects are often observed following treatment, such as hypertension, impaired wound healing, haemorrhaging and thrombosis^[19]. Therefore, novel natural herbs, such as grape seed extract^[20] and dihydroartemisinin (DHA)^[21], which have been proven to be safe for humans, are recognized as sources of effective antitumor agents.

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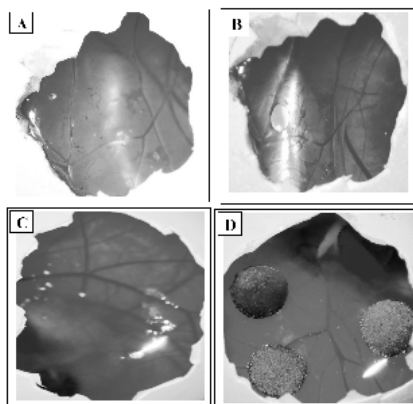


Fig. 1: Anti-angiogenic activity of *T. populnea*
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Fig. 1: Anti-angiogenic activity of *T. populnea*

A: The appearance of the control group chorioallantoic membrane (CAM) through a window on the egg shell on day 9; **B:** The appearance of the control group chorioallantoic membrane (CAM) blood capillaries increased through a window on the egg shell on day 12; **C:** The appearance of the treatment group chorioallantoic membrane (CAM) through a window on the egg shell on day 9; **D:** The appearance of the treatment group after *T. populnea* drug place on chorioallantoic membrane and inhibition of blood capillaries on day 12.

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