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 neo-vascularization, 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-inflammatory, memory enhancement property and hypocholesterolemic activity [5, 6, 7]. T. populnea fruit have anthelmintic activity and antidiarrheal activity [8]. 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, Mumba) 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 Rf value of 0.8. The plates were sprayed with Folin – Ciocalteu’s phenol reagent and fumigated ammonia vapour, the plate developed dark blue colour band with Folin – Ciocalteu’s phenol reagent and fumigated ammonia vapour, the plate developed dark blue colour band and showed a single band with another band on TLC with an Rf value of 0.5. 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 [10].

High performance liquid chromatographic (HPLC) analysis of phenolic fraction: The active component, isolated by column chromatography was subjected to HPLC analysis [11].

Chick embryo chorioallantoic membrane (CAM) assay
CAM assay was performed as described previously [12] 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.

ABSTRACT

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

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

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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 disturbed 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: (1) 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. Therefore, novel natural herbs, such as grape seed extract and dihydroartemisinin (DHA), which have been proven to be safe for humans, are recognized as sources of effective antitumor agents.

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