

Evaluation of the Angiogenic Potential of Acacia Nilotica Bark Extract Using the Chick Chorioallantoic Membrane Assay and Antioxidant Correlation

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Abstract

Background: Acacia nilotica has long been used in traditional medicine for wound management; however, evidence linking its antioxidant properties with angiogenesis remains limited. This study investigated the angiogenic potential of Acacia nilotica bark extract and explored its association with antioxidant activity.

Methods: The bark extract was characterized using Fourier transform infrared spectroscopy (FTIR). Antioxidant activity was evaluated by the DPPH free radical scavenging assay at concentrations ranging from 10 to 150 µg/mL. Angiogenic activity was assessed using the chick chorioallantoic membrane (CAM) assay, and vascular parameters, including vessel area, total vessel length, and vascular junctions, were quantified using NIH AngioTool64 Version 0.6a.

Results: FTIR analysis confirmed the presence of bioactive phytochemicals, including phenolic compounds, flavonoids, tannins, and polysaccharides. The extract exhibited concentration-dependent antioxidant activity, increasing from 33% at 10 µg/mL to 87% at 150 µg/mL, while ascorbic acid exhibited 100% antioxidant activity. CAM analysis demonstrated enhanced vascularization with increasing extract concentrations, reflected by greater vessel area, vascular junctions, and total vessel length. The highest angiogenic response was observed at 150 µg/mL.

Conclusion: Acacia nilotica bark extract demonstrated significant antioxidant and pro-angiogenic activities, supporting its traditional use in wound healing. The findings suggest that its antioxidant-rich phytochemical composition may contribute to enhanced angiogenesis and tissue repair. Further mechanistic, preclinical, and clinical studies are warranted to establish its therapeutic potential.

Keywords: Angiogenesis; Antioxidants; Chorioallantoic Membrane; Plant Extracts; Wound Healing.

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Introduction

Wound healing is a complex biological process and a major global health concern, involving a series of overlapping phases, including haemostasis, inflammation, proliferation, contraction, and remodelling. [1] This complex process is orchestrated through the coordinated interaction of growth factors, cytokines, matrix metalloproteinases, inflammatory cells, keratinocytes, fibroblasts, and endothelial cells. [2]

Among these processes, angiogenesis is a tightly regulated biological event that is essential for normal growth, tissue repair, and wound healing, whereas its dysregulation contributes to several pathological conditions. Dysregulated angiogenesis has been implicated in numerous pathological

conditions, including cancer, diabetic retinopathy, rheumatoid arthritis, and cardiovascular diseases. Owing to its pivotal role in tissue repair, angiogenesis is widely investigated using the chick chorioallantoic membrane (CAM), a highly vascularized extraembryonic membrane that serves as a well-established experimental model for evaluating neovascularization and vascular remodelling. [3]

Acacia nilotica (Family: Fabaceae), commonly known as babul or the gum Arabic tree, has been extensively used in traditional medicine across Africa, the Middle East, and South Asia for the management of various inflammatory and infectious disorders. The bark and other parts of the

plant are rich in bioactive phytochemicals, including tannins, flavonoids, phenolic acids, catechins, gallic acid, quercetin, and other polyphenolic compounds that exhibit diverse pharmacological properties. [4] Several studies have investigated the wound-healing potential of *A. nilotica*. [5–7] In addition, the therapeutic efficacy of *Acacia nilotica* root extract in the form of an adhesive paste has been demonstrated in patients with recurrent oral ulcers. [5] Furthermore, Kankara et al. reported that the aqueous extract of *A. nilotica* pods significantly enhanced wound healing in animal models by reducing oxidative stress and suppressing pro-inflammatory cytokines. [6] In addition to its wound-healing potential, *A. nilotica* possesses a rich phytochemical profile that contributes to a broad spectrum of biological activities, including antioxidant, antimicrobial, anti-inflammatory, antimalarial, and antileishmanial effects. [8] Among these, antioxidant activity is particularly important because maintaining redox homeostasis is essential for angiogenesis and tissue repair. [9] Previous studies have reported the antioxidant, antimicrobial, anti-inflammatory, antimalarial, and antileishmanial activities of *A. nilotica* bark extracts, suggesting the presence of biologically active compounds capable of influencing cellular signalling pathways involved in vascular development and tissue regeneration. [10] Controlled levels of reactive oxygen species (ROS) promote endothelial cell proliferation, migration, and angiogenic signalling, whereas excessive ROS production disrupts vascular growth and delays wound healing. [11] Therefore, plant-derived antioxidant phytochemicals may facilitate angiogenesis by maintaining cellular redox homeostasis and supporting endothelial cell function during tissue regeneration.

Despite the well-documented pharmacological properties of *A. nilotica*, limited experimental evidence is available regarding its direct pro-angiogenic activity and the relationship between its phytochemical composition, antioxidant properties, and angiogenic potential. [7]

Investigating the angiogenic activity of *A. nilotica* bark extract using the CAM model may provide valuable insights into its potential application in wound healing, tissue engineering, and regenerative medicine. Therefore, the present study aimed to investigate the dose-dependent angiogenic effects of *A. nilotica* bark extract using the chick chorioallantoic membrane model and to correlate these effects with its antioxidant properties. The findings of this study may contribute to the identification of natural angiogenic modulators with potential applications in wound healing.

Materials and Methods

Materials: *A. nilotica* bark was collected from the local area of Chengalpattu, India. Methanol (99.9%), 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH), and ascorbic acid were purchased from Sigma-Aldrich. Ethanol (99%) was used for the study. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from HiMedia. Fertilized chicken eggs (24 h old) were obtained from the Tamil Nadu University of Veterinary and Animal Sciences, Chennai, India.

Preparation of *Acacia nilotica* Bark Extract: Fresh bark of *Acacia nilotica* was collected from the vicinity of Karpaga Vinayaga Medical College Campus, Chengalpattu, India.

The bark was washed thoroughly, shade-dried, and coarsely powdered. One gram of the powdered bark was mixed with 100 mL of distilled water and heated in a water bath at 60–70°C for 6 h. The resulting mixture was filtered through Whatman filter paper and lyophilized to obtain a dried extract. The dried extract was stored in airtight containers at 4°C until further use.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis: The phytochemical profile of *Acacia nilotica* bark extract was characterized using Fourier transform infrared spectroscopy (FTIR). The FTIR spectrum was recorded over the wavenumber range of 4000–400 cm⁻¹, and the obtained spectra were analyzed to identify the functional groups corresponding to the major phytochemical constituents present in the extract. [12]

Antioxidant Activity: The antioxidant activity of *Acacia nilotica* bark extract was evaluated using the DPPH free radical scavenging assay. Different concentrations of the extract (10, 25, 50, 100, and 150 µg/mL) were prepared in methanol. A methanolic DPPH solution (1 mg/mL) was prepared, and equal volumes (1 mL each) of the extract solution and DPPH solution were mixed and incubated in the dark for 30 min.

Following incubation, absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as the positive control. Antioxidant activity was expressed as the percentage of DPPH radical scavenging. [13]

Chick Chorioallantoic Membrane (CAM) Assay: The angiogenic potential of *Acacia nilotica* bark extract was evaluated using the chick chorioallantoic membrane (CAM) assay. Freshly fertilized chicken eggs (approximately 24 h old) were cleaned with 70% ethanol and incubated at 37°C under 50–60% relative humidity.

On the third day of incubation, approximately 2–3 mL of albumin was aseptically removed using a

sterile needle to separate the developing embryo from the shell membrane and create an air chamber.

The opening was sealed with sterile adhesive tape, and the eggs were returned to the incubator. On the fifth day of incubation, a small window was created on the upper surface of the eggshell using a sterile mini-driller to expose the CAM. The membrane was treated with the control, heparin (3 $\mu\text{g/mL}$) as the positive angiogenic control, and different concentrations of *A. nilotica* bark extract. Following treatment, the windows were resealed, and the eggs were incubated for an additional 24 h. On the sixth day, the CAM was examined, and images were captured using a high-resolution camera.

The angiogenic response was quantified using NIH AngioTool64 Version 0.6a by measuring vessel area, total number of junctions, and total blood vessel length to evaluate the pro-angiogenic effect of the tested samples.

Results and Discussion

FTIR Analysis: FTIR analysis of *A. nilotica* bark extract revealed several characteristic functional groups corresponding to bioactive phytochemical constituent (Fig. 1). A broad absorption peak at 3200–3500 cm^{-1} indicated O–H stretching vibrations characteristic of phenolic compounds and alcohols. The peak observed near 2900 cm^{-1} corresponded to C–H stretching vibrations of

alkanes, whereas absorption bands between 1600 and 1700 cm^{-1} suggested the presence of carbonyl (C=O) groups and aromatic C=C stretching vibrations. In addition, peaks in the region of 1000–1300 cm^{-1} were attributed to C–O stretching vibrations, indicating the presence of polysaccharides, flavonoids, and tannins.

The observed FTIR peak assignments were consistent with those reported by Ulpathakumbura et al. [12]. These observations are also consistent with the comprehensive phytochemical profile summarized by Hafez et al. [8] who reported that *A. nilotica* contains abundant flavonoids, tannins, phenolic acids, alkaloids, saponins, terpenoids, and other polyphenolic compounds that underlie its diverse pharmacological activities, including antioxidant, anti-inflammatory, and wound-healing effects.

Recent reviews have further highlighted that polyphenol-rich medicinal plants exhibit enhanced antioxidant and tissue regenerative properties, reinforcing the importance of phytochemical characterization in identifying bioactive compounds with potential wound-healing applications. [14] Overall, these findings confirm the presence of several bioactive phytochemical constituents in the bark extract that may contribute to its medicinal, antioxidant, and antimicrobial properties.

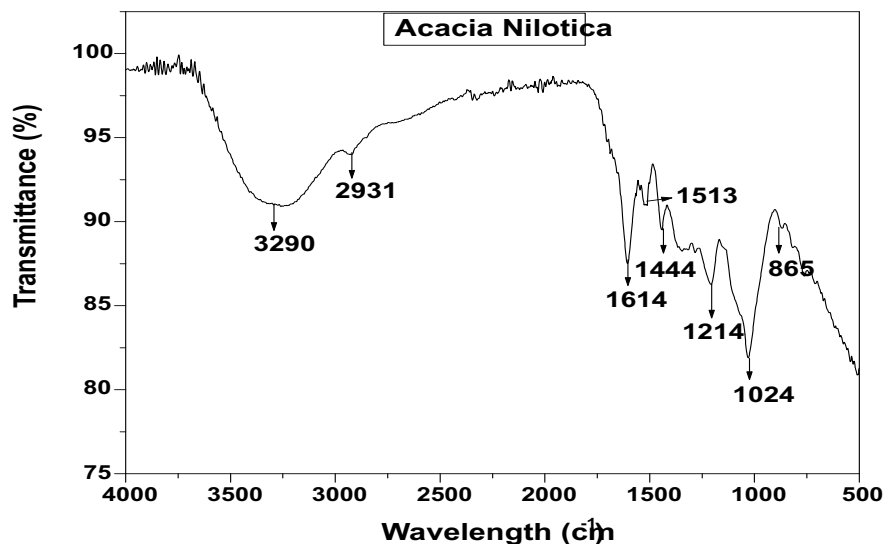


Figure 1: FTIR Spectrum of *Acacia nilotica* bark extract

Antioxidant Study: The antioxidant activity of *Acacia nilotica* bark extract was evaluated at different concentrations (10, 25, 50, 100, and 150 $\mu\text{g/mL}$) and compared with the positive control (ascorbic acid) (Fig. 2). The extract exhibited a

concentration-dependent increase in antioxidant activity.

At 10 $\mu\text{g/mL}$, the extract demonstrated 33% antioxidant activity, which increased to 45% at 25

$\mu\text{g/mL}$ and 56% at 50 $\mu\text{g/mL}$. Further increases in extract concentration resulted in antioxidant activities of 78% and 87% at 100 and 150 $\mu\text{g/mL}$, respectively. Ascorbic acid, used as the positive control, exhibited the highest antioxidant activity (100%). The concentration-dependent increase in DPPH radical scavenging activity suggests the presence of bioactive phytochemicals with potent antioxidant properties.

The highest antioxidant activity observed at 150 $\mu\text{g/mL}$ may be attributed to the abundant phenolic compounds, flavonoids, tannins, and other secondary metabolites present in the bark extract. These findings are consistent with those reported by Lawaly et al. [15] who demonstrated dose-

dependent DPPH radical scavenging activity in *A. nilotica* and attributed its antioxidant potential to its rich phenolic and flavonoid content.

Similar findings have been reported in recent studies demonstrating that plant-derived polyphenols effectively scavenge reactive oxygen species and protect tissues against oxidative damage, thereby supporting their therapeutic potential in wound healing and regenerative medicine. [16] Collectively, these findings further support the antioxidant potential of *A. nilotica* bark extract and provide a rationale for its subsequent evaluation in angiogenesis and wound-healing studies.

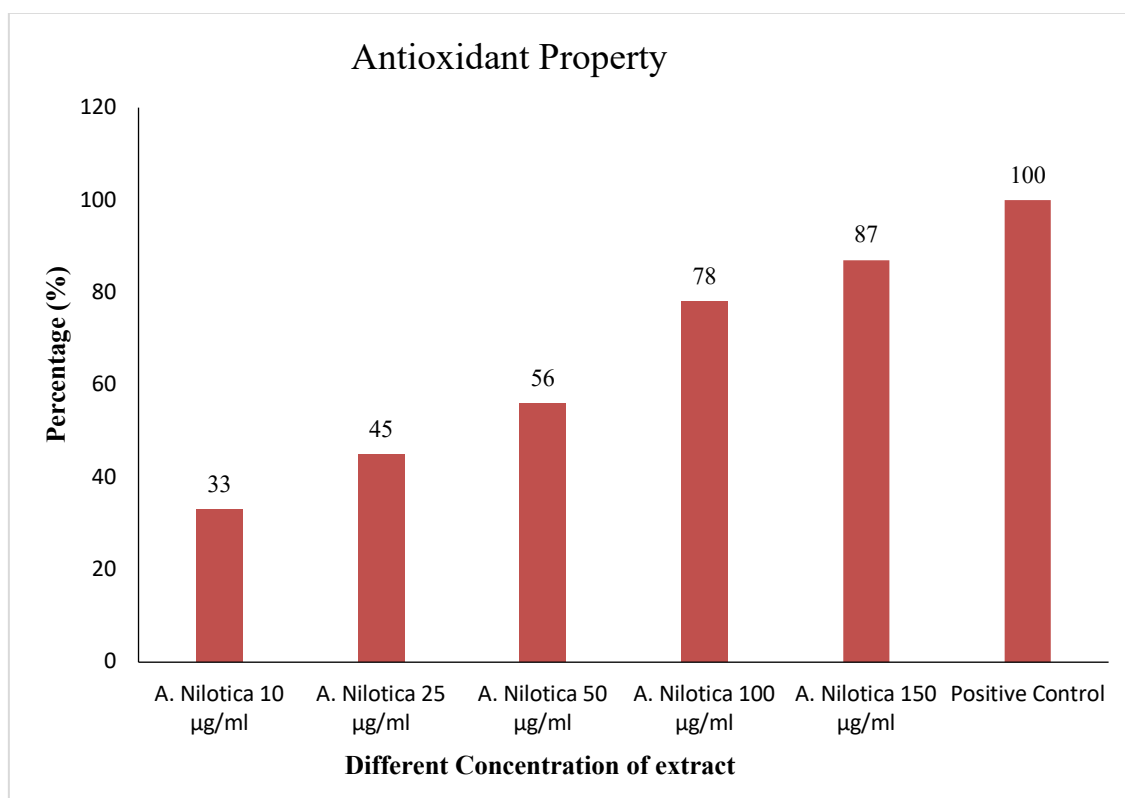


Figure 2: Antioxidant property of *A. nilotica* at different concentrations

Angiogenesis Study: The angiogenic potential of *Acacia nilotica* bark extract was evaluated using the chick chorioallantoic membrane (CAM) assay. Representative CAM images demonstrated normal vascular development with fewer vascular branches in the control group, whereas the positive control exhibited enhanced vascularisation (Fig. 3). Treatment with *A. nilotica* bark extract resulted in a concentration-dependent increase in vascular growth from AN-50 to AN-150. Quantitative analysis using NIH Angio Tool Version 0.6a further confirmed these observations by demonstrating progressive increases in vessel area, total number of vascular junctions, and total blood vessel length with increasing extract

concentrations. Among the tested concentrations, the highest angiogenic response was observed at 150 $\mu\text{g/mL}$, indicating the strongest pro-angiogenic activity under the present experimental conditions.

The enhanced angiogenic response observed at higher extract concentrations may be associated with the antioxidant phytochemicals identified by FTIR and the corresponding increase in DPPH radical scavenging activity. Recent evidence suggests that plant-derived polyphenols promote angiogenesis by enhancing endothelial cell proliferation, regulating oxidative stress, and facilitating extracellular matrix remodeling during tissue repair. [9] Plant-derived phenolic compounds, flavonoids, and tannins have been

reported to reduce oxidative stress, modulate inflammatory responses, and support cellular processes involved in tissue repair. [17] In addition, several recent reviews have demonstrated that flavonoid-rich medicinal plants stimulate fibroblast proliferation, collagen synthesis, and vascular regeneration through coordinated antioxidant and anti-inflammatory mechanisms. [18]

Consistent with these observations, the concentration-dependent antioxidant activity demonstrated in the present study suggests that maintenance of cellular redox homeostasis may contribute to the observed angiogenic response, as oxidative stress is known to influence endothelial cell survival and vascular development. [19] These observations agree with recent reports demonstrating that maintenance of intracellular redox homeostasis is essential for endothelial cell survival and angiogenic signalling during wound repair. [20]

The present findings are further supported by previous experimental and review studies investigating the wound-healing properties of *A. nilotica*. Earlier studies have demonstrated that *A. nilotica* extracts enhance wound healing by reducing oxidative stress, suppressing inflammatory cytokines, improving re-epithelialization, and promoting tissue regeneration. These findings are supported by both

earlier and recent reviews summarizing the antioxidant, anti-inflammatory, and wound-healing properties of *A. nilotica*, which have been attributed to its rich phytochemical profile. [8,21] Recent experimental evidence has further demonstrated that polyphenol-rich extracts of *A. nilotica* accelerate wound healing through enhanced tissue regeneration, antioxidant activity, and modulation of inflammatory responses, providing additional support for its therapeutic potential. [22] Similarly, Kankara et al. [6] reported that *A. nilotica* pod extracts enhanced wound healing by reducing oxidative stress and inflammatory responses, while Nefai et al. [5] demonstrated the wound-healing activity of *A. nilotica* stem bark extract. Furthermore, the recent review by Hafez et al. [8] highlighted that *A. nilotica* promotes re-epithelialization, angiogenesis, dermal tissue regeneration, and suppression of pro-inflammatory cytokines, including TNF- α and IL-1 β , providing mechanistic support for the enhanced vessel formation observed in the present CAM assay. Unlike previous investigations that primarily focused on antioxidant activity and wound contraction, the present study demonstrates enhanced angiogenesis in the CAM model, providing additional evidence that stimulation of neovascularization may contribute to the wound-healing potential of *A. nilotica* bark extract.

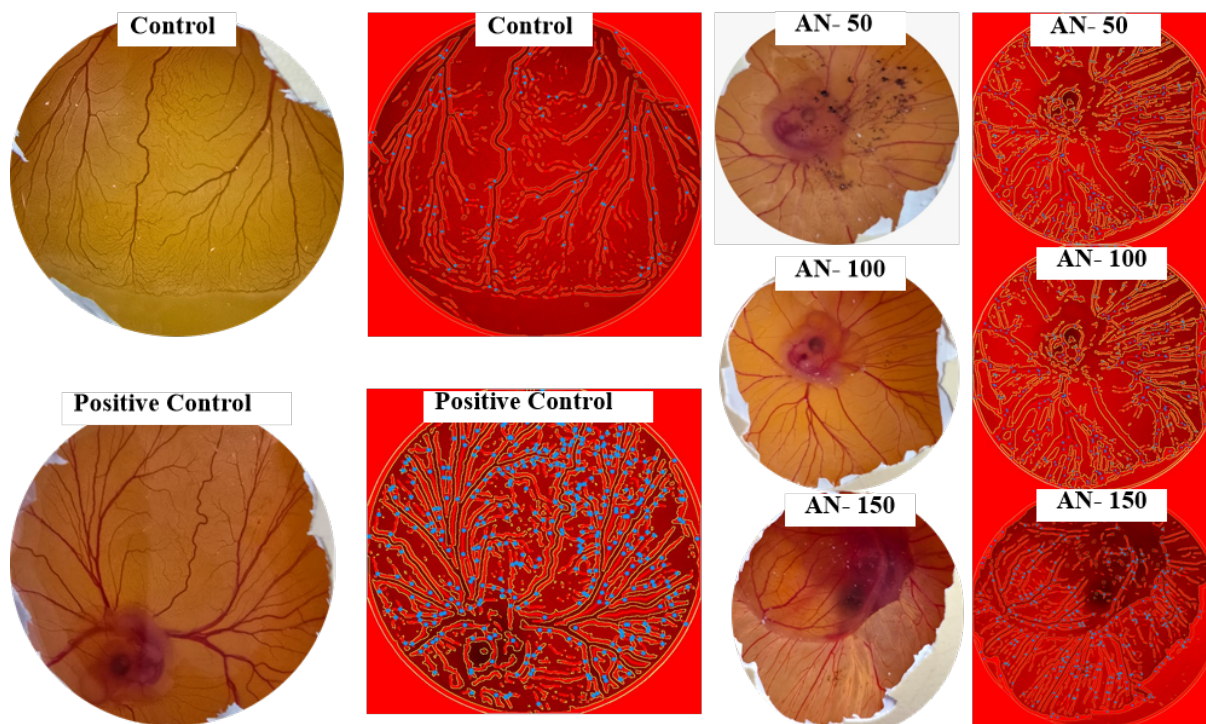


Figure 3: Dose-dependent angiogenic effect of *Acacia nilotica* bark extract using CAM assay

These findings collectively provide a plausible biological explanation for the angiogenic response observed in the present study. Angiogenesis is an

essential step in wound healing because newly formed blood vessels supply oxygen, nutrients, and growth factors to regenerating tissues [23].

Controlled levels of reactive oxygen species stimulate endothelial cell migration and proliferation, whereas excessive oxidative stress inhibits vascular growth. [19]

Therefore, the antioxidant compounds present in *A. nilotica* may create a favourable microenvironment for angiogenesis. Consistent with these observations, quantitative CAM analysis demonstrated progressive increases in vessel area, total number of junctions, and total blood vessel length with increasing concentrations of *A. nilotica* bark extract, with the greatest angiogenic response observed at 150 $\mu\text{g/mL}$. These findings suggest that the extract may promote endothelial sprouting,

branching, and vascular network formation. These observations are in agreement with the findings of Sene et al. [24], who demonstrated that *A. nilotica* pod extract enhanced wound healing in a concentration-dependent manner by promoting angiogenesis, re-epithelialization, keratinocyte migration, and collagen deposition, thereby supporting the pro-angiogenic potential observed in the present CAM model.

Similar findings have recently been reported for other polyphenol-rich botanical extracts, which promote wound repair by enhancing vascularization, collagen deposition, and tissue remodeling. [25]

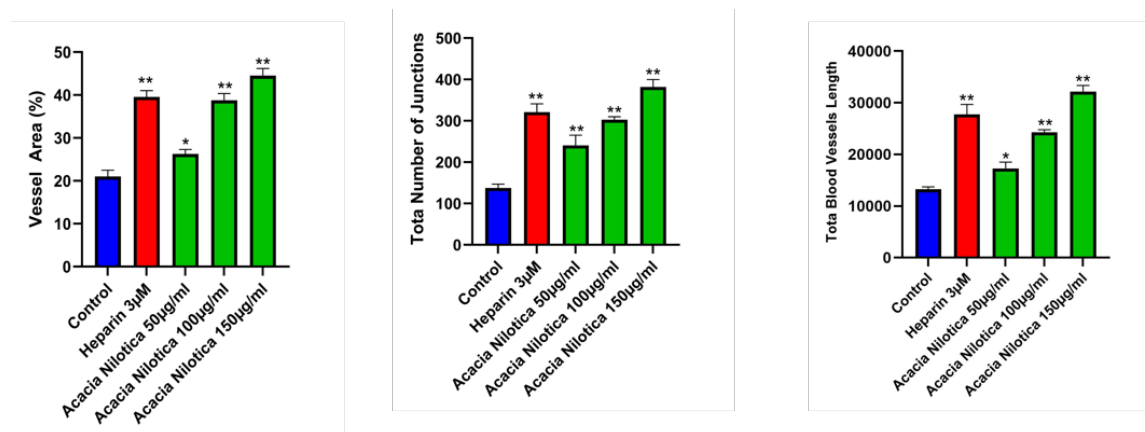


Figure 4: Dose dependent angiogenic effect of *Acacia nilotica* bark extract

Overall, the collective findings of the FTIR, antioxidant, and CAM analyses indicate that *Acacia nilotica* bark extract possesses significant pro-angiogenic activity that may be attributed to the synergistic effects of its phenolic, flavonoid, tannin, and other antioxidant phytoconstituents. The strongest effect was observed at 150 $\mu\text{g/mL}$, followed by 100 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$. A notable strength of the present study is the integrated evaluation of phytochemical characterization, antioxidant activity, and functional angiogenic assessment, providing complementary evidence supporting the wound-healing potential of the bark extract.

However, the findings should be interpreted in light of certain limitations. The angiogenic activity was evaluated only using the CAM model, and the underlying molecular mechanisms were not investigated.

Therefore, future investigations should evaluate angiogenic biomarkers, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hypoxia-inducible factor-1 α (HIF-1 α), and inflammatory cytokines, to elucidate the molecular mechanisms underlying the observed pro-angiogenic activity and validate these findings in preclinical wound-healing models.

Conclusion

The present study demonstrated that *Acacia nilotica* bark extract exhibited significant antioxidant and pro-angiogenic activities, with the greatest effects observed at 150 $\mu\text{g/mL}$.

FTIR analysis confirmed the presence of bioactive phytochemical constituents, and the observed increase in antioxidant activity together with enhanced vascularization in the chick chorioallantoic membrane model suggests that these phytochemicals may contribute to the pro-angiogenic properties of the extract.

These findings provide experimental support for the traditional use of *A. nilotica* in wound management and highlight its potential role in supporting tissue repair. From a translational perspective, *A. nilotica* bark extract may represent a promising natural candidate for the development of wound-care formulations aimed at enhancing vascularization and tissue regeneration.

Nevertheless, further mechanistic studies involving angiogenic biomarkers, followed by well-designed preclinical and clinical investigations, are required to establish its efficacy, safety, and therapeutic potential. Recent advances in phytochemical-based regenerative therapies further support the potential

application of antioxidant-rich medicinal plant extracts as natural candidates for wound management and tissue engineering.

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Author Contributions

A. Amy Evangeline: Conceptualization, methodology, investigation, data collection, formal analysis, manuscript drafting, and literature review.

B. Prathap: Study conceptualization, supervision, methodology, critical review, and final approval of the manuscript.

Vishwasrao Sunil Mhatarba: Study supervision, data interpretation, manuscript review, and critical revision of the manuscript.

Santhosh Kumar Y: Experimental design, FTIR and CAM assay support, data analysis, and manuscript review.

S. Antinate Shilpa: Experimental investigations, phytochemical and antioxidant analyses, data interpretation, and manuscript editing.

All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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