



Original Research Article

Angiogenic potential of *Bambusa vulgaris* leaves: Results of an *in-vitro* study with chicken embryo chorioallantoic membrane (CAM) model



Vijaykumar Gupta ^{a, *}, Vivek Nalgirkar ^a, Anant Patil ^b, Priyanka Mirdha ^a,
Geetanjali Gajanan Ranade ^c

^a Department of Physiology, Dr. DY Patil Medical College, Navi Mumbai, Maharashtra, India

^b Department of Pharmacology, DY Patil University, School of Medicine, Navi Mumbai, India

^c Quintessence Fragrances, Mumbai, Maharashtra, India

ARTICLE INFO

Article history:

Received 4 February 2021

Received in revised form

13 June 2022

Accepted 13 July 2022

Available online xxx

Keywords:

Angiogenesis

Plant extract

Wound healing

CAM model

Medicinal plants

Chorioallantoic membrane

ABSTRACT

Background: Herbal compounds have an important place in the management of wounds. However, very few compounds have been tested for their proangiogenic potential.

Objective: The objective of study was to evaluate angiogenic potential of *Bambusa vulgaris* Schrad. plant extract.

Material and methods: Chicken embryo chorioallantoic membrane model was used for estimation of angiogenic potential of *B. vulgaris* leaves extract with different concentrations. Angiogenic potential was estimated using focal application method and AbGel™ sponge application method. The test samples were loaded on eight embryonic development day of embryonic development of chick embryo and angiogenesis was observed on eleventh embryonic development day. Counting of blood vessels and photographic evaluation was done for estimation of angiogenic potential. The sponge specimen was examined for histological changes. Angiogenic potential of *B. vulgaris* leaves extract was compared against Plermin®.

Results: All tested concentrations (85 mcg, 170 mcg, 255 mcg, 340 mcg and 425 mcg/disc) of *B. vulgaris* showed angiogenic potential as indicated by increase in the number of blood vessels. Maximum growth in blood vessels was seen at the concentration of 255 mcg. Photographic evaluation showed changes in angiogenesis with *B. vulgaris* leaves extract. Angiogenic potential was also confirmed on histological examination. Plermin control groups also showed the growth of blood vessels measured by counting the number of blood vessels in photographic evaluation. The growth of blood vessels with Plermin 40 mcg was similar to *B. vulgaris* 255 mcg.

Conclusion: The results of current study suggest angiogenic potential of *B. vulgaris* Schrad leaves as confirmed by visual observation and histological examination.

© 2022 The Authors. Published by Elsevier B.V. on behalf of Institute of Transdisciplinary Health Sciences and Technology and World Ayurveda Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Angiogenesis is defined as formation of new blood vessels from already existing blood vessels. Angiogenesis has a role in growth as well metastasis of tumour [1]. Acute as well as chronic wounds represent an important health burden. Acute wound, if not treated and/or healed spontaneously, can become chronic [2]. Several

options are available for treatment of wounds including moist dressings, autologous split-thickness skin graft, surgical debridement, and use of topical growth factors [2]. Each of these methods have its own advantages and limitations.

Herbal medicines or chemicals extracted from the plant may influence the angiogenesis process [3]. They may have anti-angiogenic properties [4–6] or promote angiogenesis [7]. Identification of these activities are important before they are used for specific purpose. Angiogenesis is one of the important beneficial mechanisms involved in the process of wound healing. Angiogenic activity of plant extracts helps in the formation of blood vessels. Envisaging this action, many of the herbal preparations are used

* Corresponding author.

E-mail address: vijaygupta15@gmail.com (V. Gupta).

Peer review under responsibility of Transdisciplinary University, Bangalore.

empirically for wound healing. Quality, safety and efficacy of alternative and complementary medicine should be tested before they are used for wider population [8].

Recently, we evaluated angiogenic potential of *Moringa oleifera* lam [7]. *Bambusa vulgaris* has several properties including Phytoestrogenic, anti-oxidative, anti-inflammatory, and abortifacient potential [9,10]. An animal study has demonstrated its potential for the treatment of osteoporosis [9]. It also has potential for external use in skin conditions [11]. It may be of valuable use in healing of the wounds if its angiogenic potential is confirmed and proved in the human research. Currently, there is a paucity of literature related to proangiogenic effect of *Bambusa vulgaris*. To establish the benefits and to add more literature, the following is taken up to determine the angiogenic potential of *Bambusa vulgaris* Schrad. leaves extract using chicken embryo chorioallantoic membrane (CAM) model. The objective of study was to evaluate angiogenic potential of *B. vulgaris* Schrad., plant extract.

2. Material and methods

Leaves of *B. vulgaris* were procured and shed dried. Ash contents of dried leaves were studied (Table 1) [23]. Hot methanolic extract of leaves was prepared using Soxhlet extraction method [12] for 36 h by packing 100 gm dry leaf powder and the yield of extract was determined (Table 2). The phytochemical properties of dried extract were established using infrared spectrophotometry (Fig. 1), ultraviolet spectrophotometry (Fig. 2) and thin layer chromatography (Table 4 and Fig. 3). The components of *B. vulgaris* were also identified by using mass spectrophotometric study (Table 3). Results were analysed to check the stability of plant extract (Figs. 1 and 2) from time to time during the period of research. Chicken embryo chorioallantoic membrane (CAM) model was selected for estimation of angiogenic potential due to its property of high vascularization and easy accessibility [13]. The experiments on Chicken embryo does not require approval of institutional animal ethics committee [24].

Standardization of CAM model to laboratory conditions was done by continuous monitoring of incubation temperature, humidity in incubator to 96%, rotation of eggs every 2 h for first eight days. The successful results confirmed by hatching of chick embryo at the end of 21st embryonic development day [14]. Extract of *B. vulgaris* was studied for its angiogenic potential using two methods: Whatman filter paper disc method (focal application

Table 1
Ash content of leaves of *Bambusa vulgaris*.

Name of plant	Acid Soluble ash %	Acid Insoluble ash %	Total Ash Content %
<i>Bambusa vulgaris</i>	5.12	7.44	12.56

Table 2
Yield of hot methanolic extract of leaves of *Bambusa vulgaris*.

Name of plant	Wt. of dried leaves (gm)	Total Vol. of extract (ml)	Wt. of extract in 25 ml	Yield of extract in %
<i>Bambusa vulgaris</i>	100	280	1.062	11.87

Table 3
Gas Chromatography- Mass spectroscopy of *Bambusa vulgaris*.

Name of the Compound	Molecular Formula	RT in Min.
2-Furancarboxyaldehyde,5- hydroxymethyl	C ₆ H ₆ O ₃	9.6
2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	11.7
Desaspidinol	C ₁₁ H ₁₄ O ₄	23.6

method) and AbGel™ method. Overall protocol of study is shown in Fig. 4.

Angiogenic potential was studied in dose dependent manner with both methods.

3. Focal application method

The eggs were cleaned using 70% ethanol, and a window was created on broad end of the egg on third embryo development day (EDD) and the window on the egg was covered with micropore scotch tape. The incubator was humidified and maintained at 37 °C throughout the procedure. All the procedures were performed in an aseptic condition. Whatman filter paper discs of 2-mm diameter were autoclaved and were loaded with the 20ul sample and were allowed dried on Perspex base.

This filter paper disc containing leaf extract was kept on the chorioallantoic membrane on eighth EDD by puncturing a small hole on chorioallantoic membrane with stainless steel dental needle size G24 on an area devoid of blood vessels. The areas were photographed using digital camera at the time of application of the sample. The eggs were left undisturbed for three days and observed for any mortality and dead eggs were discarded.

At the end of eleventh EDD that is after three days of focal application, the chorioallantoic membrane was observed for angiogenesis and photographs was taken for evaluation. Angiogenic potential of *B. vulgaris* leaves extract was tested at concentrations of 85, 170, 255, 340 and 425 µg/disc. The angiogenic effect was established by counting the mean number of new blood vessels around the disc [14,15]. Classical angiogenic effect was defined by spoke wheel appearance of blood vessel around the disc.

4. AbGel™ sponge application method

Sterilized small blocks of two mm³ of AbGel™ were cut from its large block. These sponge blocks were dipped into the extract and were soaked for 4 h which were then placed on the vascularized area of membrane on eighth EDD. On eleventh EDD, the section of membrane along with sponge was isolated and preserved in 10% formaldehyde solution. These sections were then processed for histological study and evaluated for angiogenesis. The number of blood vessels per focused area was counted under simple microscope. The angiogenic potential was tested at 2125 mcg/ml, 4250 mcg/ml, 6375 mcg/ml, 8500 mcg/ml and 10,625 mcg/ml.

Two eggs were used for blank disc, disc plus methanol and blank control each. Three eggs were taken as standard control with Plermin©. Angiogenic effect of *B. vulgaris* leaves extract was studied on a total of 68 eggs (Disc method n = 34; AbGel method n = 34). Additional one egg was kept as incubator control. The distribution of eggs for both methods is described in Table 5.

5. Results

The CAM model showed increase in the mean number of blood vessels after application of *B. vulgaris* leaves extract. Table 6 shows number of blood vessels on the membrane with focal application method in three groups control group, paper disc containing *B. vulgaris* leaves extract, disc containing Plermin gel which is positive control for angiogenesis, Blank discs did not show any growth in blood vessels. All discs with different concentrations (85 mcg, 170 mcg, 255 mcg, 340 mcg and 425 mcg) of *B. vulgaris* extract showed increase in the number of blood vessels. Maximum growth in blood vessels was seen at the concentration of 255 mcg. Similarly, Plermin control groups also showed increase in the growth of blood vessels. The growth of blood vessels with Plermin 40 mcg was same as *B. vulgaris* 255 mcg (Table 6).

IR Spectra Bamboo

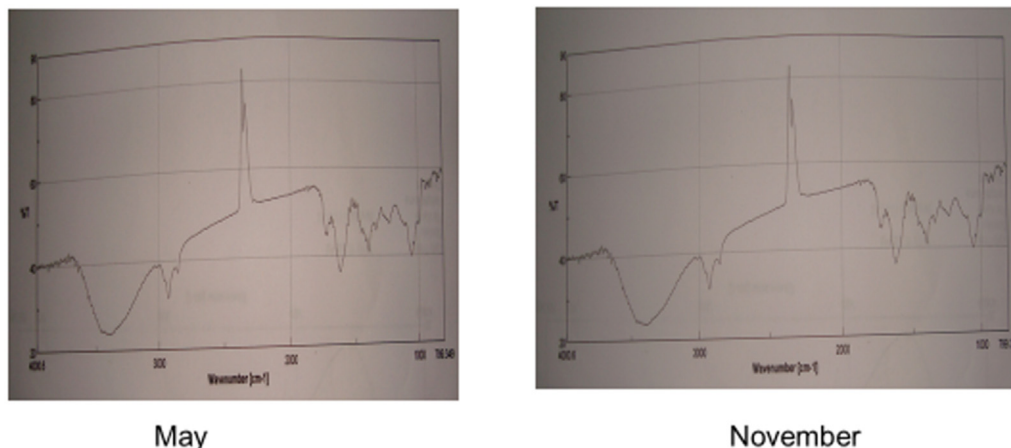


Fig. 1. Infrared spectrophotometric study of *B. vulgaris*.

Table 7 shows number of blood vessels on the membrane with AbGel method in three groups. Blank gel showed 5 blood vessels. All AbGel with extracts (2125 mcg/ml, 4250 mcg/ml, 6375 mcg/ml, 8500 mcg/ml, 10,625 mcg/ml) showed increased number of blood vessels in the sponge. Maximum growth was seen in AbGel with 4250 mcg/ml. Plermin control groups also showed increase in the growth of blood vessels. The growth of blood vessels with Plermin 20 mcg/ml was similar to *B. vulgaris* 2125 mcg/ml and 8500 mcg/ml and Plermin 60 mcg/ml was similar to *B. vulgaris* 10,625 mcg/ml (Table 7).

Fig. 5 shows the photographs of blank control group on CAM on day eight and day eleven. On eleventh day of EDD, no visual changes suggesting there is no angiogenesis observed in blank control group (Fig. 5).

Fig. 6 shows the photographs of *B. vulgaris* extract group on day eight and day eleven. In the *B. vulgaris* group, increase in number of blood vessels was seen on eleventh day (Fig. 6).

Fig. 7 shows the photographs of ABGel™ of blank control on day eight and day eleven of CAM. No growth of blood vessels was observed in this group on day eleven (Fig. 7).

Fig. 8 shows the photographs of ABGel™ of *B. vulgaris* extract group on day eight and day eleven of CAM. In the *B. vulgaris* group, increase in number of blood vessels was seen on eleventh day (Fig. 8).

Fig. 9 shows the photographs of ABGel™ of Plermin standard group on day eight and day eleven of CAM. In this group, increase in number of blood vessels was seen on eleventh day (Fig. 9).

UV SPECTRA BAMBOO

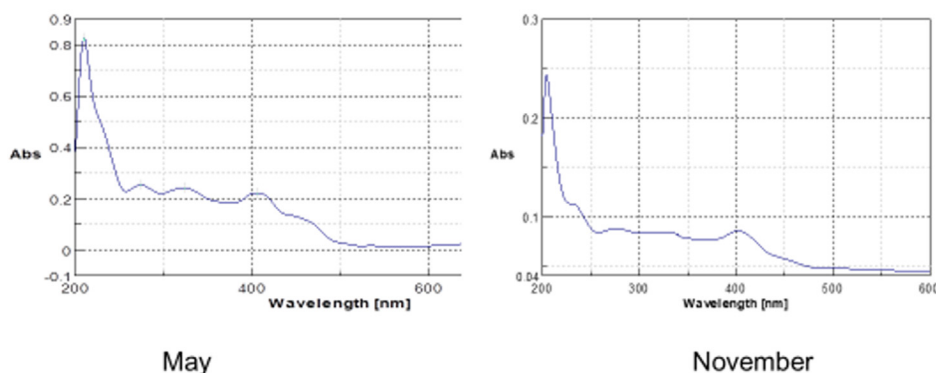


Fig. 2. Ultraviolet spectrophotometric study of *B. Vulgaris*.

Table 4
Thin layer Chromatography *Bambusa vulgaris*.

Contents	<i>Bambusa vulgaris</i>
Alkaloids	+
Anthraglycosides	-
Arbutin	-
Cardiac glycosides	-
Bitter Principles	+
Flavonoids	-
Saponins	-
Didrovaltrate	-
Valepotriates	+

Fig. 10 shows histological study of AbGel method on the membrane with haematoxylin and eosin staining (H and E Staining). New blood vessels were observed in the slide.

6. Discussion

Bamboos are a group of woody grasses in the family Poaceae and subfamily Bambusoideae [16]. This family has significant potential for human use including timber, human consumption, biomass, textile [17]. Actions and uses of Bamboo differ based on its parts.

Baboo has nutritional value. Its shoots have nutritional value with high amount of protein, moderate fibre, and less fat [16]. Bamboo derived products also has several medicinal applications and protective effects due to its antioxidant and anti-inflammatory actions [18].

Impaired wounds healing is a concern in many patients especially those with diabetes, elderly and chronically ill people. With an ageing population and increasing prevalence of chronic diseases like cancer, diabetes, wound care will certainly become an even more noteworthy issue for health systems. Delayed wound healing is signified with alteration in physical properties of collagen, flattening of the dermo-epidermal junctions, nutritional depletion and cellular immunity leading to abnormal changes in pro-inflammatory and anti-inflammatory cytokines. Several evidence have been collected to show immense potential of medicinal plants used in various traditional systems [5–8].

The leaves of *B. vulgaris* have anti-inflammatory action with potential for use in wound healing process [11,19]. India ranks second for the bamboo reserve in Asia after China [20]. The complete potential of Bamboo for its medicinal use remains to be exploited.

In this study, we evaluated angiogenic potential of *B. vulgaris* leaves extract using CAM assay [1,4]. It is a well-established,

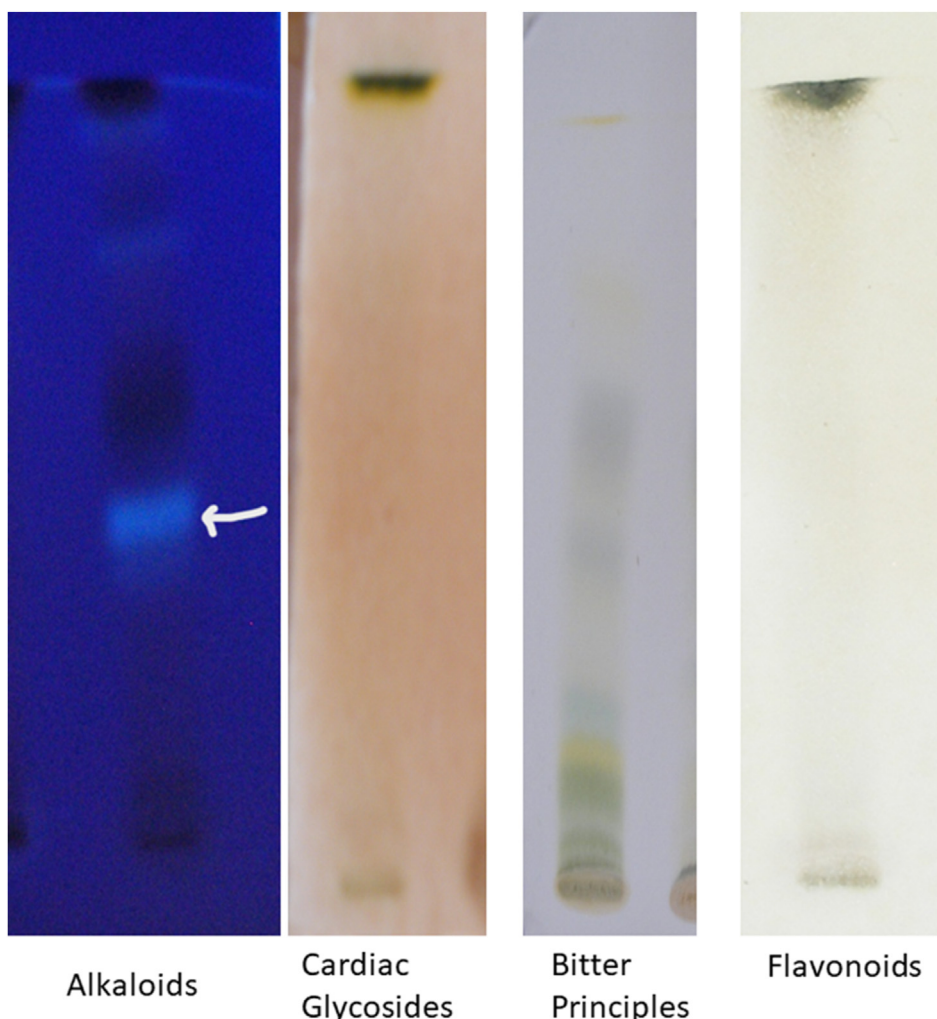


Fig. 3. Thin layer chromatography of *B. vulgaris*.

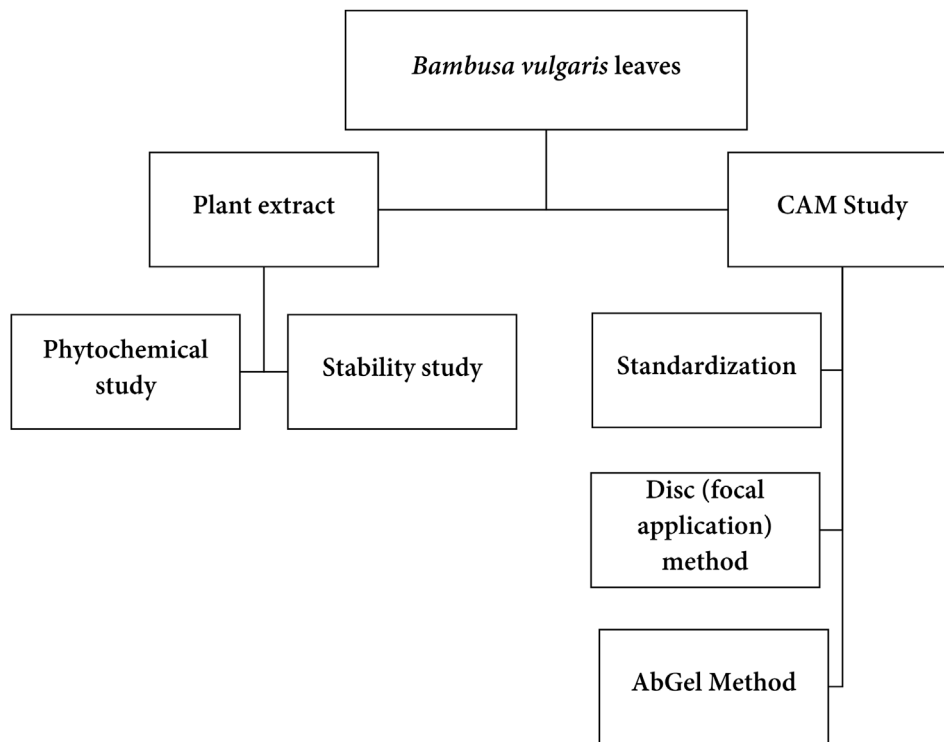


Fig. 4. Flow chart of study procedures.

Table 5
Distribution of eggs used in two methods for evaluation of angiogenesis.

Eggs- Category/quantity of extract (Microgram/disc)	Number of Eggs for Disc Method	Eggs- Category/concentration of extract (Microgram/ml)	Number of Eggs for AbGel Method
Blank Disc (Only Whatman filter paper)	2	Blank Gel	2
Blank Disc + Methanol	2	Blank Gel + Methanol	2
Blank Control	2	Blank Control	2
<i>B. vulgaris</i> at 85 mcg/disc	5	<i>B. vulgaris</i> at 2125 mcg/ml	5
<i>B. vulgaris</i> at 170 mcg/disc	5	<i>B. vulgaris</i> at 4250 mcg/ml	5
<i>B. vulgaris</i> at 255 mcg/disc	5	<i>B. vulgaris</i> at 6375 mcg/ml	5
<i>B. vulgaris</i> at 340 mcg/disc	5	<i>B. vulgaris</i> at 8500 mcg/ml	5
<i>B. vulgaris</i> at 425 mcg/disc	5	<i>B. vulgaris</i> at 10,625 mcg/ml	5
Plermin control at 20, 40 & 60 mcg/disc	3	Plermin control at 40 mcg/ml	3
Total number of eggs	34	Total number of eggs	34

Table 6
Number of blood vessels on the membrane in focal application method in *Bambusa vulgaris*, Plermin and control group.

Group	Mean number of blood vessels on 8th Embryonic development day	Mean number of blood vessels on 11th Embryonic development day	Growth
Control			
Blank Disc	3	3	0.0
Blank disc + Methanol	4	5	25.0%
<i>Bambusa vulgaris</i> disc (D)			
<i>B. vulgaris</i> -85 mcg	2	4	100.0%
<i>B. vulgaris</i> -170 mcg	4	6	50.0%
<i>B. vulgaris</i> -255 mcg	3	7	133.3%
<i>B. vulgaris</i> -340 mcg	5	9	80.0%
<i>B. vulgaris</i> -425 mcg	5	10	100.0%
Plermin			
Plermin Control 1 (20 mcg)	4	9	125.0%
Plermin Control 2 (40 mcg)	3	7	133.3%
Plermin Control 3 (60 mcg)	3	8	166.6%

Table 7
 Number of blood vessels on the membrane in AbGel application method in *Bambusa vulgaris*, Plermin and control group.

Group	Mean number of blood vessels in gel on 11th EDD
Blank gel	5
Blank gel + Methanol 1	4
Blank gel + Methanol 2	4
<i>B. vulgaris</i> –2125 mcg/ml	9
<i>B. vulgaris</i> –4250 mcg/ml	12
<i>B. vulgaris</i> –6375 mcg/ml	10
<i>B. vulgaris</i> –8500 mcg/ml	9
<i>B. vulgaris</i> –10625 mcg	8
Plermin Control 1 (20 mcg/ml)	9
Plermin Control 2 (40 mcg/ml)	7
Plermin Control 3 (60 mcg/ml)	8

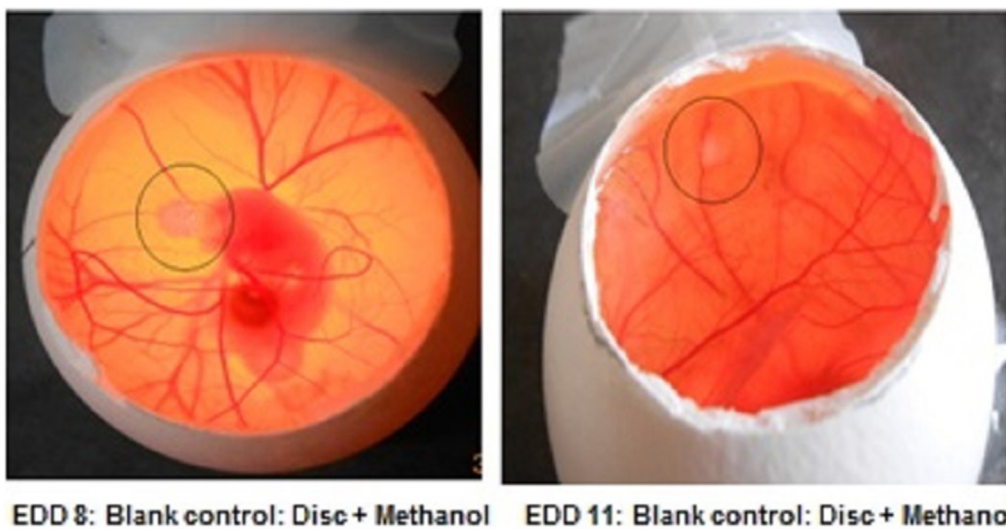


Fig. 5. Focal application method: Blank control.

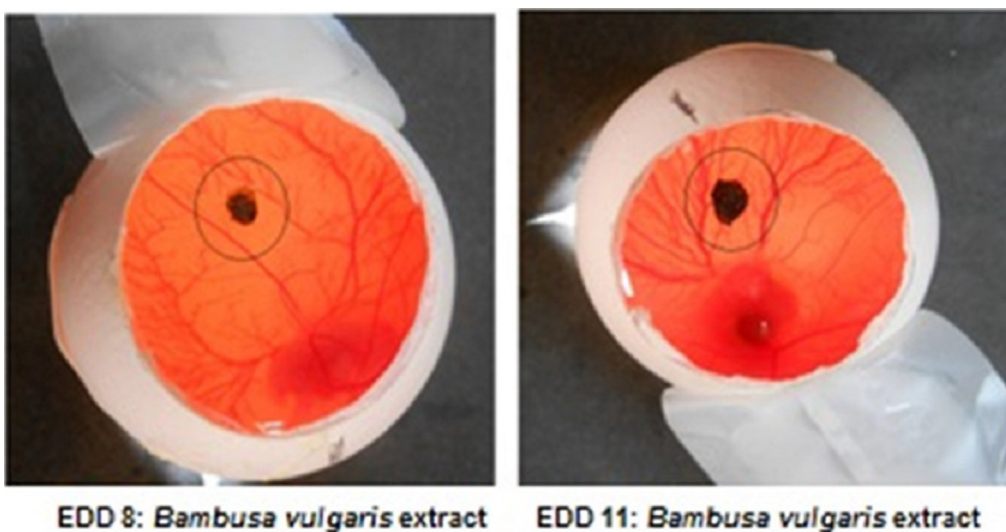


Fig. 6. Focal application: *B. vulgaris*.

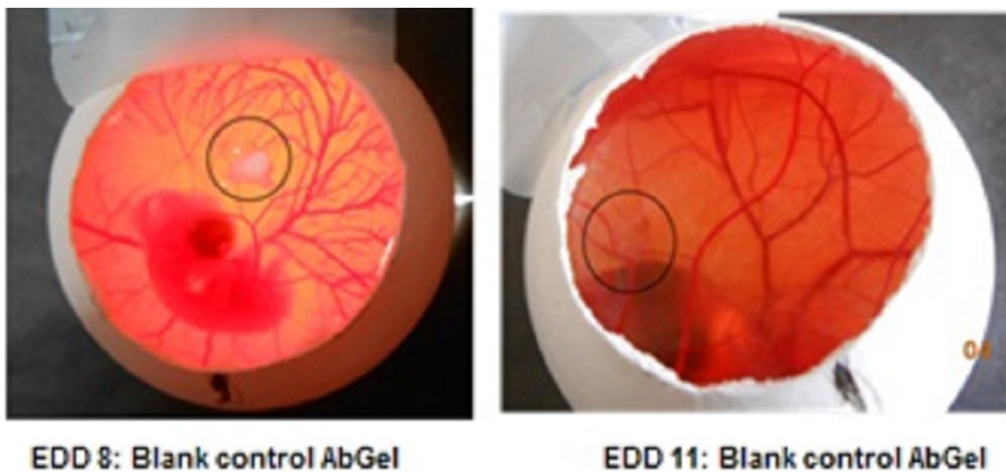


Fig. 7. Blank control: ABGel™.

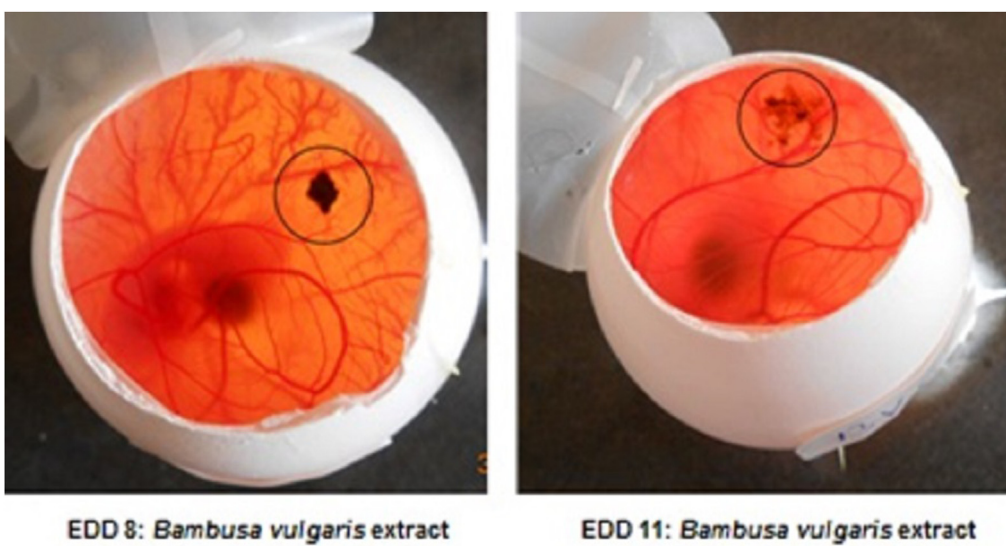


Fig. 8. ABGel™ application photograph: *B. vulgaris*.

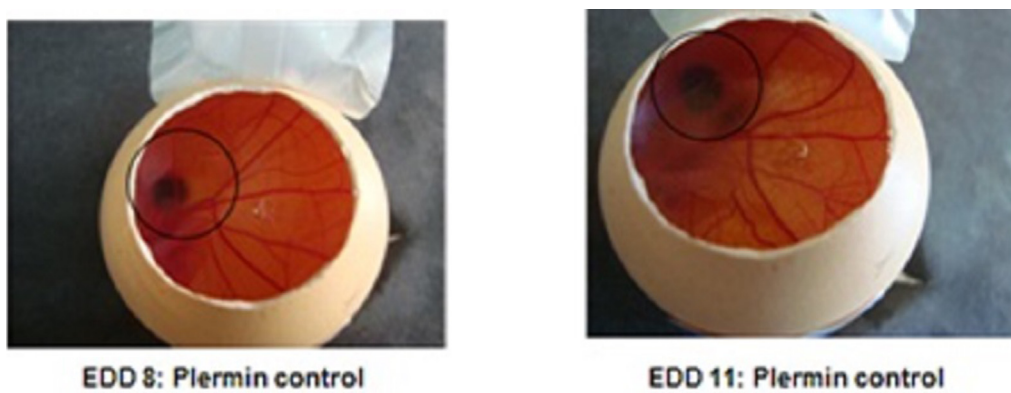


Fig. 9. ABGel™ application: Plermin standard.

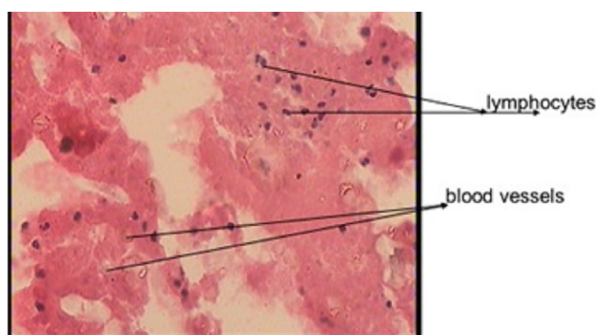


Fig. 10. Histological study of ABGel™.

specialized, and commonly used model for estimation of angiogenesis [1] because of highly vascularized tissue of the avian embryo [21].

We evaluated angiogenic potential by using CAM assay by two methods: focal application and ABGel™ method. The sponge method relies on infiltration of neovascularization in sponge block containing proangiogenic compound [14,15].

There was visible increase in blood vessels on the 11th day in both the positive control and the sample loaded with *B. vulgaris* extract. On the contrary, the CAM which is not loaded with any drug did not show improvement in angiogenesis. These observations suggests that *B. vulgaris* leaves possess angiogenic property and it has potential for studying further for its clinical effects.

The angiogenic compound in sponge causes the new blood vessels to infiltrate the sponge pores which gets stained and can be visualized very clearly from rest of sponge material under microscope.

Role of platelet derived growth factor, transforming growth factor-beta and fibroblast growth factor (FGF) in wound healing is known [22]. Overall, our observations suggest similar angiogenic potential of Bamboo leaves extract in the concentration of 255 mcg with Plermin 40 mcg.

Phytochemical study suggested that the acid insoluble ash content leaves was more than acid soluble ash this could be due high amount of silicates in the leaves of *B. vulgaris*. The hot methanolic yield of extract is 11.25% which contains maximum phytochemicals of *B. vulgaris* leaves. The infra-red and ultraviolet studies indicated specific absorbance pattern in different months of the year indicating stability of the extract. The thin layer chromatography showed the presence of alkaloids, bitter principles and valepotriates in extract which might cause the angiogenesis in synergism with each other. The mass spectroscopy identified the specific compound in extract which in the future can be isolated and its angiogenic potential can be determined.

There is paucity of literature on angiogenic potential evaluation in plants based study using CAM model. There are no head to head comparative studies between bambusa vulgaris and other plant hence, it is difficult to comment on the superiority of one plant over the other. This research is at a nascent stage. Further clinical development and human studies are required for recommending this for clinicians to use in their practise. However we have further analysed the angigenic effects of tuttha bhasma, kasis, and other ayurvedic preparations using CAM which are yet to be published.

Our results suggest that leaves extract of Bamboo has a potential for use in wound healing. Our study provides significant insights into the potential of Bamboo leaves extract for its angiogenic

potential and clinical use. The fractionated form of crude samples needs testing to establish the presence of angiogenic compound. Further studies are required to establish optimum concentration of *B. vulgaris*. Animal and human studies are also needed after estimating optimum concentration of compound showing angiogenic property on CAM model.

7. Conclusion

Visual change in the growth pattern of blood vessels as indicated by increase in number and photographs was observed with *B. vulgaris* leaves extract. Histological examination also confirmed the angiogenic potential of *B. vulgaris* leaves extract. Angiogenic potential of *B. vulgaris* extract 255 mcg was similar to Plermin 40 mg.

Financial support and sponsorship

Nil.

Source(s) of support

Nil.

Author credit statement

Vijaykumar Gupta: Conceptualization, data acquisition, data analysis, data interpretation, editing manuscript and approval of the manuscript.

Vivek Nalgirkar: Conceptualization, data interpretation, editing manuscript and approval of the manuscript.

Anant Patil: Data interpretation, preparation of first draft of manuscript, editing manuscript and approval of the manuscript.

Priyanka Mirdha: Data interpretation, editing manuscript and approval of the manuscript.

Geetanjali Gajanan Ranade: Conceptualization, data interpretation, editing manuscript and approval of the manuscript.

Declaration of competing interest

Nil.

Acknowledgement

We would like thank IIT, Mumbai for providing assistance in conducting GC–MS analysis of the extract.

References

- [1] Tufan AC, Satiroglu-Tufan NL. The chick embryo chorioallantoic membrane as a model system for the study of tumor angiogenesis, invasion and development of anti-angiogenic agents. *Curr Cancer Drug Targets* 2005;5: 249–66.
- [2] Langer V, Rajagopalan S. Evaluation of recombinant human platelet-derived growth factor as an agent for wound bed preparation in traumatic wounds. *Indian J Plast Surg* 2012;45:203–8.
- [3] Loboda A, Cisowski J, Zarebski A, Jazwa A, Nunez DR, Kypriotakis Z, et al. Effects of plant extracts on angiogenic activities of endothelial cells and keratinocytes. *J Physiol Pharmacol* 2005;56(1):125–37.
- [4] Hoseinkhani Z, Norooznezhad F, Rastegari-Pouyani M, Mansouri K. Medicinal plants extracts with antiangiogenic activity: where is the link? *Adv Pharmaceut Bull* 2020;10:370–8.
- [5] Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, et al. Effective medicinal plant in cancer treatment, Part 2: review study. *J Evid Based Complementary Altern Med* Oct 2017;22(4):982–95. <https://doi.org/10.1177/2156587217696927>.

- [6] Lu K, Bhat M, Basu S. Plants and their active compounds: natural molecules to target angiogenesis. *Angiogenesis* Jul 2016;19(3):287–95.
- [7] Gupta V, Nalgirkar V, Ranade GG, Kripalani Y. Study of angiogenic potential of moringa oleifera lam. On Chicken chorioallantoic membrane (cam). *Int J Sci Res* Oct 2020;9(10). <https://doi.org/10.36106/ijrs>.
- [8] Seo E-J, Kuete V, Kadioglu O, Kadioglu O, Krusche B, Schröder S, et al. Antiangiogenic activity and pharmacogenomics of medicinal plants from traditional Korean medicine. *Evid base Compl Alternative Med* 2013; 131306.
- [9] Watcho P, Kamto BGK, Deeh PBD, Nguetefack TB, Kamanyi A, Kamtchouing P. The aqueous and methanol extracts of *Bambusa vulgaris* (Poaceae) improve calcium and phosphorus levels, and bone microstructure in ovariectomized model of osteoporosis. *J Basic Clin Physiol Pharmacol* Apr 2019 5;(3):30. <https://doi.org/10.1515/jbcp-2018-0157>.
- [10] Yakubu MT, Bukoye BB. Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Contraception* Sep 2009;80(3):308–13.
- [11] Lodhi S, Jain AP, Rai G, Yadav AK. Preliminary investigation for wound healing and anti-inflammatory effects of *Bambusa vulgaris* leaves in rats. *J Ayurveda Integr Med* Mar 2016;7(1):14–22.
- [12] Shanbhag TV, Sharma C, Adiga S, Bairy LK, Shenoy S, Shenoy G. Wound healing activity of alcoholic extract of *Kaempferia Galanga* in wister rats. *Indian J Physiol Pharmacol* 2006;50(4):384–90.
- [13] Ribatti D. Chicken chorioallantoic membrane angiogenesis model. *Methods Mol Biol* 2012;843:47–57.
- [14] West DC, Thompson WD, Sells PG, Burbridge MF. In: Murray JC, editor. *Methods in molecular medicine, angiogenesis protocols*, vol. 46. Totowa, NJ: Humana Press; 2001. p. 107–29. Chapter-9.
- [15] Vargas A, Zeisser-Labouèbe M, Lange N, Gurny R, Delie F. The chick embryo and its chorioallantoic membrane (CAM) for the in vivo evaluation of drug delivery systems. *Adv Drug Deliv Rev* Sep 2007;59(11):1162–76.
- [16] Singhal P, Bak LM, Satya SSP, Naik SN. Bamboo shoots: a novel source of nutrition and medicine. *Crit Rev Food Sci Nutr* 2013;53:517–34. <https://doi.org/10.1080/10408398.2010.531488>.
- [17] Canavan S, Richardson DR, Visser V, Johannes J, Le Roux, Maria S, et al. The global distribution of bamboos: assessing correlates of introduction and invasion. *AoB Plants* Jan 2017;9(1):plw078. <https://doi.org/10.1093/aobpla/plw078>.
- [18] Panee J. Potential medicinal application and toxicity evaluation of extracts from bamboo plants. *Author Manuscript* Jun 2015;9(23):681–92.
- [19] Muniappan M, Sundararaj T. Antiinflammatory and antiulcer activities of *Bambusa arundinacea*. *J Ethnopharmacol* Oct 2003;88(2–3):161–7.
- [20] Yeasmin L, Ali MN, Gantait S, Chakaraborty S. Bamboo: an overview on its genetic diversity and characterization. *Biotech* 2015;5:1–11.
- [21] Deryugina EI, Quigley JP. Chapter 2: chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. *Methods Enzymol* 2008;444:21–41.
- [22] Fang RC, Galiano RD. A review of becaplermin gel in the treatment of diabetic neuropathic foot ulcers. *Biol Targets & Ther* 2008;2:1–12.
- [23] Li Y, Ma G, Zhou Q, Li Y, Huang Z. Nutrient contents predict the bamboo-leaf-based diet of Assamese macaques living in limestone forests of southwest Guangxi, China. *Ecol Evol* 2020 Apr 28;10(12):5570–81. <https://doi.org/10.1002/ece3.6297>. PMID: 32607175; PMCID: PMC7319238.
- [24] National Institute of Health (NHS). The Public Health Service Responds to Commonly asked Questions. *ILAR News* 1991;33(4):68–70. Office of Laboratory Animal Welfare. <https://grants.nih.gov/grants/olaw/references/ilar91.htm>.