



**PHYTOCHEMICAL ANALYSIS AND EVALUATION OF WOUND HEALING AND  
TISSUE REGENERATION POTENTIAL OF SHOOT EXTRACTS OF BAMBUSA  
VULGARIS**

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**ABSTRACT**

Bambusa vulgaris shoot extracts rich in flavonoids, phenolics, alkaloids, and tannins have been demonstrated in other species to support wound healing by reducing oxidative stress, accelerating cell migration, and stimulating tissue regeneration. *Bambusa vulgaris* (bamboo) is a widely distributed species in the gramineae family, valued in traditional medicine for its therapeutic properties. Its shoots contain a range of secondary metabolites that contribute to antioxidant, anti-inflammatory and regenerative biological effects. Antioxidant and anti-inflammatory effects of bamboo shoot will contribute to wound healing. In these views, the present work is undertaken based on its traditional claims to study Pharmacognostical, Phytochemical and Pharmacological screening for shoot extract of *Bambusa vulgaris* (gramineae).

**KEYWORDS:** Bambusa, wound healing, flavonoids, phenolics, Antioxidant and anti-Inflammatory.

**INTRODUCTION**

Our skin is the key to our survival, sensing the environment, maintaining physicochemical and thermal homeostasis, acting as a reservoir of essential nutrients, providing passive and active defense, and responding to trauma and injury. Maintaining these critical functions requires robust and effective mechanisms to protect it from trauma and insult and to repair and replace critical skin functions when damaged or lost. Wound is defined as the disruption of the anatomic and cellular continuity of tissue caused by chemical, physical, thermal, microbial, or immunological injury to the tissue. Wound healing processes consist of integrated cellular and biochemical cascades leading to reestablishment of structural and functional integrity of the damaged tissue. A repaired injured tissue occurs as a sequence of events, which includes inflammation, proliferation, and migration of different cell types. The inflammatory stage begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammatory mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process.

The remodeling stage is characterized by reformulations and improvement in the components of the collagen fiber that increases the tensile strength. Factors that contribute to causation and perpetuation of the chronicity of wounds include repeated trauma, poor perfusion or oxygenation, and excessive inflammation. Imbalance in free radical generations and antioxidants has been observed to induce oxidative stress and tissue damage that delays wound healing. Therefore, elimination of ROS could be an important strategy in healing chronic wounds.<sup>[1,2]</sup>

Fibroblasts regulate the synthesis of collagen and other proteins throughout the process of wound repairing. Numerous cytokines, such as transforming growth factor beta (TGF- $\beta$ ), platelet-derived growth factors (PDGF) and epidermal growth factor (EGF), are responsible for fibroblast stimulation for collagen synthesis. TGF- $\beta$ 1 plays an important role as an inflammatory mediator in the initiation of wound healing by activating and stimulating macrophages to secrete cytokines that further act as fibroblast growth factors (PDGF, TNF- $\alpha$  and interleukin-1). In the

proliferative phase, macrophages secrete TGF- $\beta$ 1, T lymphocytes, and platelets. TGF- $\beta$ 1 is considered to be a major control signal that regulates fibroblast functions. Wound may be defined as a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection and is produced due to any accident or cut with sharp edged things. It may also be produced due to physical, chemical, thermal, microbial or immunological exploitation to the tissues as a result of the presence of an underlying medical or physiological condition. Based on the nature of the repair process, wounds can be classified as acute or chronic wounds.<sup>[3,4]</sup>

## METHODOLOGY

### Estimation of total phenolic content in the prepared shoot extract

The prepared shoot extract was estimated for its total phenolic content by the folin-ciocalteu method. A calibration curve was prepared by mixing 0.5 ml methanolic solution of gallic acid (10- 130  $\mu$ g/ml) with 1 ml of 10-fold diluted folin-ciocalteu reagent and 2 ml of 2% sodium carbonate. After an incubation of 30 min, the absorbance of the reaction mixtures was measured against blank at 730 nm using UV-Visible spectrophotometer. A calibration curve for standard gallic acid was plotted between concentration and absorbance. Similarly, bamboo shoot extracts (100  $\mu$ g/ml) was mixed with the reagents and absorbance was measured. The total plant phenolic contents were measured using the calibration curve plotted for gallic acid, and it was reported as gallic acid equivalents GAE/g of dried plant material.<sup>[5,6]</sup>

### Estimation of total flavonoid content

The prepared shoot extract was estimated for its total flavonoid content by the aluminium trichloride colorimetric method. For the study, quercetin was used as the standard. The stock solution of quercetin was prepared by dissolving 5.0 mg quercetin in 1.0 mL ethanol. The stock solution was further diluted with methanol to prepare a solution having concentrations 10-100  $\mu$ g/ml. One ml of these dilutions was mixed with 1 mL of 10 % ethanolic aluminium trichloride and incubated for 30 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 430 nm using UV- Visible spectrophotometer. Similarly extract was mixed with 10 % ethanolic aluminium trichloride solution, and absorbance was measured. The concentration of total flavonoid content in the extract was measured using the calibration plot for quercetin and reported as mg quercetin equivalent (QE)/g of dried plant material.<sup>[7,8]</sup>

### Determination of in vitro antioxidant properties DPPH (2, 2 diphenyl 2 picryl hydrazyl hydrates) radical scavenging activity

The DPPH radical scavenging activity was measured according to the method. In brief, 3ml reaction mixture containing 200  $\mu$ l of DPPH (100 $\mu$ M in methanol) and 2.8 ml of sample (at various concentrations 20-100 $\mu$ g/ml) in methanol was incubated at 37 $^{\circ}$ C for 30 minutes and

absorbance of the test mixture was read at 517nm using UV-visible Spectrophotometer. The percentage inhibition of DPPH radical was calculated.<sup>[9-12]</sup>

## Evaluation of wound healing activity

### Excision wound model

The animals were divided into a total of five groups for each of the plant containing 6 animals in each group. Group I animals was treated with the vehicle control. Group II animals was treated with Framycetin Sulphate IP as standard using topical administration. Group III, IV and V were treated with shoot extract with dose of 100 mg, 200 mg and 300 mg/kg body weight. Animals were anaesthetized with a combination of xylazine (dose rate of 13 mg/kg b.wt) and ketamin (dose rate of 87 mg/kg b.wt) by intraperitoneal route (i/p) prior to the creation of the wounds. The animals were fasted overnight without being taken out the water supplement. The rats are inflicted with excision wounds. The dorsal fur of the animals was shaved and the area of the wound to be created was marked. A full thickness of the excision wound of circular area = 500 mm<sup>2</sup> and 0.2 cm depth was created aseptically along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open. The experiment was kept for a total of 12 days. For this model, wound closure rate and epithelization periods were taken as the standard parameters to evaluate the healing ability of the respective plants. The wound closure rate was assessed by tracing the wound on days 0, 4, 8, 12 post-wounding using transparency papers and a permanent marker. The wound areas (mm<sup>2</sup>) were measured by using a sq. mm graph paper. Epithelisation period was recorded as the number of days required to falling of scar without any residual raw wound. The results were recorded as wound area (mm<sup>2</sup>) post wounding day and period of epithelialisation in day(s) and the values were expressed as Mean  $\pm$  SEM.<sup>[13-15]</sup>

### Estimation of hydroxyproline

Ten mg of dried tissue was placed in an ampoule. Two ml of 6N HCl was added and it was incubated at 110  $^{\circ}$ C for 18 hours. The ampoule was broken and a pinch of activated charcoal was added. After 30 minutes, it was filtered and neutralized with sodium carbonate solution (pH 6.5 – 7.0). One (1) ml of neutralized solution was then taken in a test tube along with blank and two (2) ml isopropyl alcohol was added to all the test tubes and mixed well. One (1) ml of Chloramin T (7%) and two ml of Ehrlich's reagent was added to all the test tubes. The sample was then incubated at 60 $^{\circ}$ C in hot water bath for 25 minutes and then allowed to cool. The optical density was measured at 560 nm in spectrophotometer and the amount of hydroxyproline was determined. The quantity of hydroxyproline was expressed in mg/g of dry tissue.<sup>[16,17]</sup>

**RESULT****Quantitative estimation of total phenolic****Table 1: Standard Gallic Acid (Concentration Vs Absorbance).**

S. No.	Conc. (µg/ml)	Absorbance (λmax= 760 nm)
		Gallic acid
1.	10	0.110
2.	20	0.176
3.	30	0.247
4.	40	0.298
5.	50	0.326

**Quantitative estimation of flavonoid****Table 2: Standard Rutin (Concentration Vs Absorbance).**

S. No.	Conc. (µg/ml)	Absorbance(λmax= 510nm)
		Rutin
1.	10	0.135
2.	20	0.151
3.	30	0.165
4.	40	0.177
5.	50	0.201

The standard curve for gallic acid using folin-ciocalteu method was found linear with regression coefficient  $R^2 = 0.9761$ . Total phenolic content in bamboo shoot extracts was found to be  $134.4 \pm 0.917$  GAE/g. The standard curve for rutin using aluminium trichloride colourimetry method was found linear with regression coefficient  $R^2 = 0.9856$ . Total flavonoid content in bamboo shoot extracts

was found to be  $67.67 \pm 3.512$  Rutin/g.

**DPPH free radical scavenging activity (% inhibition)**

The DPPH is a stable free radical with a maximum absorbance at 517 nm and can readily undergo scavenging by an antioxidant. It has been widely used to test ability of compounds as free radical scavengers by hydrogen donors and to evaluate the antioxidant activity.

**Table 3: DPPH free radical scavenging activity (% inhibition).**

Concentrations (µg/ml)	VIT C	Bamboo extract
20	$42.51 \pm 3.21$	$39.24 \pm 3.13$
40	$53.27 \pm 2.34$	$46.45 \pm 2.51$
60	$66.35 \pm 1.96$	$57.19 \pm 2.39$
80	$70.91 \pm 3.21$	$63.37 \pm 2.95$
100	$82.43 \pm 2.74$	$81.97 \pm 2.61$

Values are expressed as mean  $\pm$  sem.  $p < 0.05$ ; compared with vit C group

Dose-dependent activity was confirmed in this assay that increasing concentrations of the test sample usually show progressive inhibition of superoxide radicals. This suggests a concentration-dependent antioxidant effect. Activity is often compared with standards such as ascorbic acid, quercetin, or BHT. Comparable or higher activity indicates strong antioxidant potential. The shoot extract of plant showed comparable anti oxidant activity because of presence of flavonoid and polyphenolic compounds.

**Wound healing activity: Excision wound model****Table 4: Effect of bamboo extract on wound healing.**

Groups	Contracted wound area (mm <sup>2</sup> )					Epithelialization periods
	0 Day	4 <sup>th</sup> Day	8 <sup>th</sup> Day	12 <sup>th</sup> Day	16 <sup>th</sup> Day	
I (Control)	$384.50 \pm 1.73$	$377.20 \pm 2.80$	$359.50 \pm 3.02$	$354.50 \pm 2.94$	$344.50 \pm 2.94$	$29.67 \pm 1.41$
II Framycetin (100mg/kg)	$385.00 \pm 1.27$	$316.80 \pm 2.89$	$166.50 \pm 7.14$	$77.67 \pm 1.48$	$67.67 \pm 1.48$	$17.83 \pm 1.82$
III (100mg/kg)	$381.30 \pm 1.02$	$356.80 \pm 1.78$	$265.20 \pm 3.37$	$217.20 \pm 2.59$	$117.20 \pm 2.59$	$22.33 \pm 1.89$
IV (200 mg/kg)	$382.00 \pm 1.34$	$340.20 \pm 2.12$	$201.20 \pm 1.74$	$152.50 \pm 1.38$	$112.50 \pm 1.38$	$20.83 \pm 2.12$
V (300 mg/kg)	$385.70 \pm 1.36$	$330.00 \pm 1.65$	$183.30 \pm 2.08$	$125.50 \pm 1.61$	$77.50 \pm 1.61$	$17.17 \pm 0.60$

**Effect of Bamboo extract on hydroxyprotein of tissue****Table 5: Effect of Bamboo extract on Hydroxyprotein (mg/g tissue).**

Groups	Hydroxyprotein content
I (Control)	$42.00 \pm 1.46$
II Framycetin Sulphate IP(100mg/kg)	$94.78 \pm 1.89$
III (100 mg/kg)	$62.83 \pm 1.64$
IV (200 mg/kg)	$77.83 \pm 1.30$
V (300 mg/kg)	$92.50 \pm 0.72$

**CONCLUSION**

Wound healing is a complex and dynamic process. The physiological process of wound repair includes four overlapping phases such as the immediate hemostasis phase, inflammatory phase, proliferative phase and remodeling phase. The wound healing activity of

bamboo extract was determined using wound model which provides optimum results as wound healing agent.

Bamboo shoot extract was found to increase wound breaking strength in incision wound model study which

may indicate increased collagen content, cross-linking collagen, maturation, formation of stable intra and inter molecular cross-link, matrix deposition, and cell migration. Increase in tensile strength in the incision wound model due to antioxidant, astringent, cell proliferating property in the extract

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