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Preliminary Phytochemical Analysis and *In Vitro* Evaluation of Ethanolic Extract of *Bougainvillea glabra* Choisy Bracts

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ABSTRACT

Background: Wounds disrupt the skin or tissue integrity and may result from injuries, surgeries, or infections. Chronic wounds, such as those seen in diabetic patients, require effective treatments to address complications like impaired healing, infections, and high costs of care. Traditional medicine has employed herbs for centuries, with *Bougainvillea glabra* Choisy recognized for its medicinal properties. **Aim of the Study:** This study investigates the Ethanolic Extract of *Bougainvillea glabra* Choisy bracts (EEBGCB) as an alternative treatment for diabetic wounds. The goal is to explore its potential as a natural, cost-effective remedy with fewer side effects compared to conventional treatments. **Methods:** Phytochemical analysis of EEBGCB was conducted to identify bioactive compounds, including flavonoids, phenols, and terpenoids. The angiogenic potential of EEBGCB was evaluated using the *in vitro* Chick Chorioallantoic Membrane (CAM) assay. This method quantified its ability to promote blood vessel formation, an essential process for wound healing. The results were compared with a standard treatment (Regen D gel).**Results:** The Phytochemical analysis revealed the presence of antioxidants EEBGCB. The CAM assay demonstrated that EEBGCB significantly increased angiogenesis (28 blood vessels) compared to the control group (20 vessels) and approached the efficacy of Regen D gel (36 blood vessels). This angiogenic activity supports cell migration and proliferation, accelerating wound healing. **Conclusion:** EEBGCB exhibits significant proangiogenic activity, suggesting its potential as a natural alternative for wound care, particularly for diabetic wounds. These findings emphasize the need for further *in vivo* studies to validate its efficacy and expand its therapeutic applications.

Keywords: Wound, Angiogenesis, Ethanolic Extract, *Bougainvillea glabra* Choisy, Chick Chorioallantoic Membrane (CAM), Regen D Gel.

INTRODUCTION

Diabetic wounds are a type of chronic, non-healing ulceration that results from the synergistic effects of impaired peripheral circulation, neuropathic dysfunction, and compromised immune function associated with diabetes mellitus. The resultant impairment of the wound healing cascade leads to persistent cellular dysfunction, tissue hypoxia, and delayed recovery.[1] Wounds are categorized based on their depth, cleanliness, and cause, with surgical wounds further classified into clean, clean-contaminated, contaminated, and infected types.[2] The healing process consists of four phases: hemostasis to control bleeding, inflammation to eliminate debris and pathogens, proliferation to generate granulation tissue and collagen, and maturation to fortify scar tissue.[3] Factors such as inadequate blood flow, infections, aging, and conditions like diabetes can slow healing. In diabetic wounds, reduced immunity, poor circulation, high blood sugar levels, and nerve damage exacerbate these challenges, often leading to complications like foot ulcers and amputations.[4]

Current treatments involve surgical cleaning, specialized dressings, antibiotics, and topical agents like silver-based products, while emerging methods like stem cell therapy and nanotechnology hold potential.[5] Despite these options, issues such as antibiotic resistance, high costs, and limited access to care underscore the need for more effective and innovative therapies.[6]

Traditional medicine employs natural remedies to prevent and treat illness, with 80% of people in certain regions relying on it for primary healthcare. Antibiotic resistance has prompted exploration of alternative treatments, including plant-based remedies, to combat multi-resistant pathogens and accelerate wound healing.[7]

Bougainvillea glabra Choisy, a plant belongs to the family Nyctinaginaceae. The major constituents have been identified in this plant include flavonoids, tannins, and betacyanins. It has been used in traditional medicine and exhibits potential in enhancing wound healing.[8] This study investigates the wound healing properties of *Bougainvillea glabra* Choisy bracts, aiming to contribute to the development of alternative wound care solutions.

MATERIALS AND METHODS

PROCUREMENT OF PLANT AND EXTRACTION PROCESS

The bracts of *Bougainvillea glabra* Choisy were collected. The bracts were authenticated by Dr. K.N. Sunil Kumar, Research Officer and H.O.D., Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai – 600106. The Ethanolic Extract of *Bougainvillea glabra* Choisy Bracts (EEBGCB) was prepared by Soxhlet extraction using ethanol as a solvent.[9] The percentage yield of EEBGCB extract was calculated using the following formula.

% Yield = Weight of the Dry Extract/ Weight of the Dry Plant * 100

PHYTOCHEMICAL ANALYSIS [10]

Test for Carbohydrate

a) Molisch's Test: To 0.5 ml of the sample, a few drops of alcoholic α-naphthol were added, followed by the slow addition of 0.2 ml of concentrated sulfuric acid along the inner wall of the test tube. Formation of purple to violet-colored ring at the junction indicates the presence of carbohydrate.

b) Fehling's Test: To 1ml of sample, a few drops of Fehling's solution A and B were added, heated for a few minutes. Formation of brick red precipitate indicates the presence of carbohydrate.

c) Barfoed's Test: 0.5ml of sample was treated few drops of Barfoed's reagent and heated for few minutes, Formation of red precipitate indicates the presence of carbohydrate.

Test for Glycosides

a) Borntrager Test: To 1ml of sample, 0.5ml of dilute sulphuric acid was added, boiled for few minutes and it was filtered. The filtrate was treated with ether or chloroform, and a few drops of ammonia solution were added to the organic layer. The appearance of a pink or violet color indicates the presence of glycosides.

b) Keller killani Test: To 0.5 ml of the sample, 0.4 ml of glacial acetic acid containing a trace of ferric chloride was added. The mixture was transferred to a small test tube, and 0.5 ml of concentrated sulfuric acid was carefully added along the inner wall of the tube. The appearance of a blue color in the acetic acid layer, along with a brown ring at the junction of the two layers, indicates the presence of cardiac glycosides.

Test for Alkaloids

a) Mayer's Test: To 0.5 ml of the sample, 1 ml of Mayer's reagent (potassium mercuric iodide solution) was added; formation of cream color precipitate indicates the presence of alkaloids.

b) Dragendroff's Test: To 1ml of sample, 1ml of Dragendroff's reagent [Potassium bismuth iodide solution] was added; formation of reddish brown precipitate indicates the presence of alkaloids.

c) Wagner's Test: To 0.5 ml of the sample, 0.5ml of Wagner's reagent [Solution of iodine in potassium iodide] was added; formation of reddish brown precipitate indicates the presence of alkaloids.

d) Hager's Test: To 1ml of sample, 0.5ml of Hager's reagent [Saturated solution of Picric acid] was added; formation of yellow precipitate indicates the presence of alkaloids.

Test for Phenolic Compounds

a) Ferric chloride Test: To 1ml of sample, 1ml of water was added, boiled for few minutes then it was filtered. The filtrate was mixed with a solution of ferric chloride, bluish black color was produced.

b) Lead acetate Test: To 1ml of sample, 0.5ml of solution of lead acetate was added, white precipitate was formed.

Test for Flavonoids

a) Shinoda Test: To 1ml of sample, few fragments of magnesium ribbon were added and then it was treated with few drops of concentrated hydrochloric acid. Magenta color indicates the presence of flavonoids.

b) Alkaline reagent Test: 2ml of sample was treated with 1ml of sodium hydroxide. Yellow color indicates the presence of flavonoids.

Test for Tannins

a) Ferric chloride Test: To 1ml of sample, 1ml of water was added, boiled for few minutes then it was filtered. The filtrate was mixed with a solution of ferric chloride, bluish black color was produced.

b) Lead acetate Test: To 1ml of sample, 0.5ml of solution of lead acetate was added, white precipitate was formed.

Test for Saponin

Foam froth Test: To 1ml of sample, 10ml of water was added, boiled for few minutes and filtered. The filtrate was shaken thoroughly and observed for stable froth and foam.

Test for Sterols

a) Salkowski Test: To 0.5 ml of the sample, 0.3 ml of chloroform and a few drops of concentrated sulfuric acid were added. The mixture was shaken thoroughly and allowed to stand for a while. A red color in the lower layer indicates the presence of steroids, while a yellow-colored lower layer suggests the presence of triterpenoids.

b) Libermann Burchard Test: To 0.5 mL of the sample, 0.3 mL of chloroform, a small amount of acetic anhydride, and concentrated sulfuric acid were added. The color changed from red to bluish green.

Test for Proteins and Amino Acids

a) Xanthoprotein Test: 0.5 ml of the sample was treated with few drops of concentrated nitric acid; orange color indicates the presence of proteins and amino acids.

b) Ninhydrin Test: 1 ml of the sample was treated with 0.5 ml of a 0.2% Ninhydrin solution and then boiled; appearance of violet color indicates the presence of amino acids and proteins.

Test for Fats and Fixed Oils

Stain Test: A little amount of sample was placed between two filter papers, and the appearance of a stain on the paper indicates the presence of fixed oils.

***IN VITRO* STUDY**

The *In vitro* Angiogenic activity of Ethanolic bract extract of *Bougainvillea glabra* Choisy was studied using Chick Chorioallantoic Membrane (CAM) Assay.

CHICK CHORIOALLANTOIC MEMBRANE (CAM) ASSAY [11]

The CAM consists of a multilayered epithelium, including the ectoderm at the air-facing surface, the mesoderm (or stroma), and the endoderm adjacent to the allantoic sac. Additionally, the CAM contains extracellular matrix (ECM) proteins such as fibronectin,

laminin, collagen type I, and integrin αvβ3. These ECM proteins replicate the natural environment of cancer cells, providing a physiologically relevant model.

Procedure

- Fertilized chicken eggs were obtained from a poultry supplier and incubated in a humidified environment at 37°C for 3–4 days, with the eggs gently rotated at least three times daily.
- On the seventh day, the eggs were examined under a flashlight to locate and mark the position of the embryo head.
- A small hole was made at the narrow end of each egg, and 0.5–1 mL of albumin was carefully extracted using a hypodermic needle to separate the yolk sac from the shell membrane.
- The shell around the air sac was removed using forceps, and the shell membrane at the base was peeled away.
- On the 8th day, a Whatman No. 1 filter paper saturated with 100 µg/ml of the EEBGCB sample was gently placed on the CAM surface and incubated.
- After 3 days, the CAM was removed, and the blood vessels were observed. Vessels radiating toward the center were counted under a microscope.
- A minimum of 20 eggs were used for each sample, with Regen-D Gel serving as the standard. No. of blood vessels in treated, untreated, and standard CAM samples were recorded.

RESULTS AND DISCUSSION

EXTRACTIVE YIELD

The Percentage yield of Ethanolic Extract of *Bougainvillea glabra* Choisy Bracts (EEBGCB) obtained was found to be **16.2 %w/w.**

Fig. 1 Ethanolic Extract of *B. glabra* Choisy Bract (EEBGCB)

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PRELIMINARY PHYTOCHEMICAL ANALYSIS

The Phytoconstituents found in the EEBGCB was shown in Table-1.

Table-1 Phytochemical Analysis and Results

S. No.	PHYTOCHEMICALS	EEBGCB
1.	Carbohydrates	-
2.	Glycosides	-
3.	Alkaloids	-
4.	Phenolics	+
5.	Flavonoids	+
6.	Tannins	+
7.	Saponins	-
8.	Steroids and terpenoids	+
9.	Proteins and amino acids	-
10.	Fats and fixed oil	-

The Phytochemical screening of EEBGCB demonstrated the presence of Phenols, Flavonoids, Tannins, Steroids, and Terpenoids, while Carbohydrates, Glycosides, Alkaloids, Saponins, Proteins, Amino acids, and Fats/Fixed oils were not found. On account of its high Phytochemical content, the extract was chosen for further study to investigate its potential to accelerate wound healing.

***IN VITRO* STUDY**

CHICK CHORIOALLANTOIC MEMBRANE (CAM) ASSAY

The Angiogenic activity of Ethanolic Extract of *Bougainvillea glabra* choisy Bract (EEBGCB) was studied using Chick Chorioallantoic Membrane (CAM) assay. The result of the Control, EEBGCB, and Standard Regen-D (Recombinant Human Epidermal Growth Factor) gel on angiogenesis in CAM was visualized in Figures 2 to 5. Additionally, the corresponding data is presented in Table-2.

Table-2 Chick Chorioallantoic Membrane (CAM) Assay of EEBGCB

S NO.	SAMPLE	NO. OF BLOOD VESSELS
1.	Control	20
2.	Test (100 µg/ml of EEBGCB Sample)	28
3.	Standard (100 µg/ml of Regen D Gel)	36

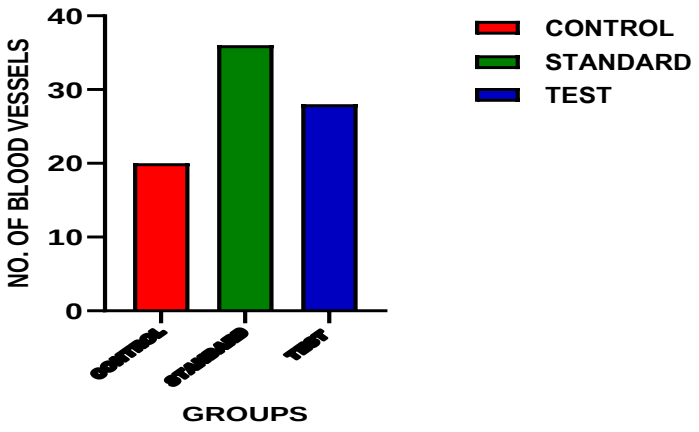

Fig.2 Comparison of blood vessels in CAM (Control, Standard and Test)

The no. of blood vessels observed in CAM were recorded. The control group showed 20 blood vessels, while the group that received 100 µg/ml of EEBGCB showed an increase to 28 blood vessels. In contrast, the group that received 100 µg/ml of standard Regen-D gel displayed the highest no. of blood vessels, with a total of 36.

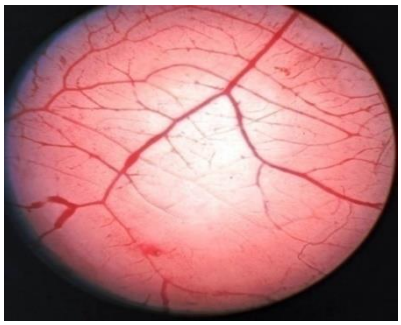

Fig.3 CAM Control

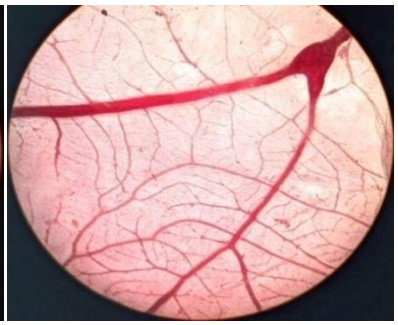

Fig.4 CAM with 100 µg/ml Sample EEBGCB

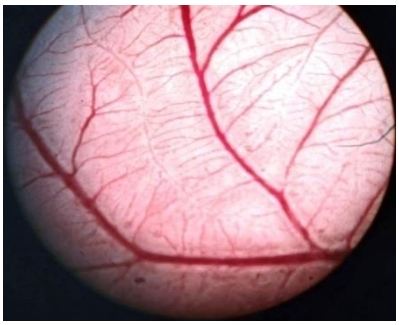

Fig.5 CAM with 100 µg/ml Standard Regen D Gel

The findings imply that EEBGCB showed a greater ability to promote the angiogenesis, suggesting that it may be a proangiogenic substance. This response exceeded the typical healing seen in untreated group and was comparable to that of the standard Regen-D gel.

SUMMARY AND CONCLUSION

Based on the traditional knowledge, Phytoconstituents and biological activities, *Bougainvillea glabra* Choisy Bract was selected for this study. The bracts were collected, authenticated and then processed by shade-drying and then coarsely powdered. Subsequently, successive extractions were performed in a Soxhlet's Extractor using ethanol as the solvent.

The Preliminary Phytochemical Analysis of Ethanolic Extract of *Bougainvillea glabra* Choisy Bracts (EEBGCB) identified various bioactive compounds, including flavonoids, phenols, tannins, steroids and terpenoids. This study confirms the wound healing properties of flavonoids and phenols, consistent with the findings of earlier studies.

In vitro Angiogenic activity was assessed by Chick Chorioallantoic Membrane (CAM) Assay. The results revealed that the Ethanolic Extract of *Bougainvillea glabra* Choisy Bracts (EEBGCB) exhibited enhanced angiogenesis, which supports the migration and proliferation of cells essential for wound closure, leading to accelerated healing outcomes. The angiogenic activity of EEBGCB was evaluated by quantifying the number of blood vessels in CAM, which was compared the results to the Standard Regen D Gel.

In conclusion, Preliminary Phytochemical Analysis identified the presence of Phytochemicals such as Phenolics, Flavonoids, Tannins, Steroids and Terpenoids. *In vitro* evaluation demonstrated significant proangiogenic activity, which is essential for wound healing. The preliminary findings suggest that the Ethanolic Extract of *Bougainvillea glabra* Choisy Bracts (EEBGCB) possesses potential wound healing activity. Further *in vivo* studies are needed to confirm and extend these findings, elucidating the wound healing property of *Bougainvillea glabra* Choisy Bracts and its potential as a herbal remedy for diabetic wound care.

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Conflict of interest statement: The authors declared no conflict of interest.

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