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ANTI-INFLAMMATORY AND WOUND HEALING ACTIVITY OF *PHYLLANTHUS FRATERNUS* WEBSTER

Deepika Sharma[1]*, Bhanu P. S. Sagar[2] and Amrita Singh[3]

[1]Student, IEC College of Engineering & Technology, Greater Noida, U.P.

[2]Director and Professor, IEC College of Engineering & Technology, Greater Noida,U.P.

[3]Associate Professor, IEC College of Engineering & Technology, Greater Noida, U.P.

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***Corresponding Author**
Deepika Sharma
Student, IEC College of Engineering & Technology, Greater Noida, U.P.

ABSTRACT

Herbal medicines have become widely sought-after in developing nations as a preferred choice for basic healthcare needs. *Phyllanthus fraternus* Webster, commonly referred as Gulf Leaf-Flower, is an annual herb which falls under the Euphorbiaceae family, is cultivated in tropical regions across the globe, including nations such as China, Malaysia, Indonesia, Pakistan, Sri Lanka, and various regions of India. In India, it can be found in states like Uttar Pradesh, Bihar, Gujarat, Maharashtra, Kerala, and West Bengal, and it is particularly prevalent in the entire Northeastern part of the country, especially in Assam, Arunachal Pradesh, and Manipur. So, present investigation was an attempt to investigate pharmacological potential of *Phyllanthus fraternus* Webster. Phytochemical evaluation was performed on EEPFL which indicatesthat the plant contains alkaloid, tannins, saponins, terpenoids, flavonoids and steroids. TPC and TFC estimation analysis revealed that amount of TPC and TFC found in *Phyllanthus fraternus* was 44.44 ± 6.42 mg GAE/g FW and 0.511 mg QE/g FW respectively. The plant has great antioxidant potential and showed a great anti- inflammatory agent. EEPFL 200mg and 400mg showed significant stabilization towardsRRBC. In the experimental models of carrageenan induced rat paw edema, effects of EEPFL at a dose of 200mg/kg and 400mg/kg showed a significant inhibition when compared with controls. The study results showed that EEPFL is a potent wound healer.On 10th day of post wound creation, it was observed that percentage of wound contraction was 100% in rats which were treated with EEPFL 400mg/kg.

***KEYWORDS*:** Anti-Inflammatory, Antioxidant, Total Phenolic Content, Total Flavonoid Content, Macroscopic, Microscopic, Wound Healing, Phytochemical.

INTRODUCTION

Inflammation

Inflammation constitutes the body's response towards detrimental triggers, including infectious agents, compromised tissues, harmful substances, and exposure to radiation.[1] Its function revolves around eliminating these harmful stimuli and kickstarting the recuperative processes necessary for restoration and healing.[2]

Inflammation Types (02 Types)

Acute Inflammation

A brief and immediate inflammatory reaction that the body initiates in response to injuries, illnesses, or infections.

Chronic inflammation

Persistent inflammation tends to be of longer duration and is generally associated with less intense pain compared to acute inflammation.

Inflammatory mediators

The inflammatory response is dynamically regulated by a diverse range of signaling molecules originating within the blood vessels, immune cells, and affected areas. These mediators play an active role in shaping and adjusting the immune response, ensuring a coordinated and adaptive reaction.[3]

Types of inflammatory mediator

Cell derived mediators

Cell derived mediators are released where inflammatory response occurs. Someof them are;

- Histamine
- Serotonin
- Prostaglandins
- Leukotrienes
- Platelet Activating Factor

Plasma Derived Mediators

These are predominantly synthesized in the hepatic system thereafter travel through bloodstream, circulating in the plasma. Some of them are;

- Bradykinin
- Cytokines

Wound

A wound can be described as a disturbance in the cytoplasmic and structural integrity of an organ, whether there is any pathogenic contamination, which occurs as a result of accidents or cuts with sharp objects.[4]

Classification of wounds

1. On the basis of wound creation, wounds are classified as Open wound and Closed wound.
2. On the basis of physiological process of wound healing, wounds are classified as Acute wound and Chronic wound.

Open wound

In an open wound, blood spills out from the organism and hemorrhage is clearly evident.[5]

Closed wound

In the case of closed wound, blood spills from the vascular network and stays within the organism.

Acute wound

Healthy wounds that are immediate in nature occur due to actions such as cuts, scrapes, punctures, tearing, burns, and deep cuts. These types of wounds undergo a swift and well-organized healing process, typically completing within a span of 8 -12 weeks.[6]

Chronic wound

Chronic wounds exhibit a significantly prolonged healing duration compared to sudden injuries, showing a closure rate of roughly less than one-third within a span of 12 weeks. These wounds are characterized as challenging to heal or non- responsive to conventional healing process, deviating from the typical organized and timely progression of wound recovery.[7]

Wound healing process

The initial stage is known as **hemostasis** phase, begins promptly upon injury to halt bleeding. During this stage, platelets rush to the wound site and initiate clot formation, creating a fibrin barrier. This process serves to safeguard against microbial intrusion and minimize additional blood loss by triggering vasoconstriction.[8]

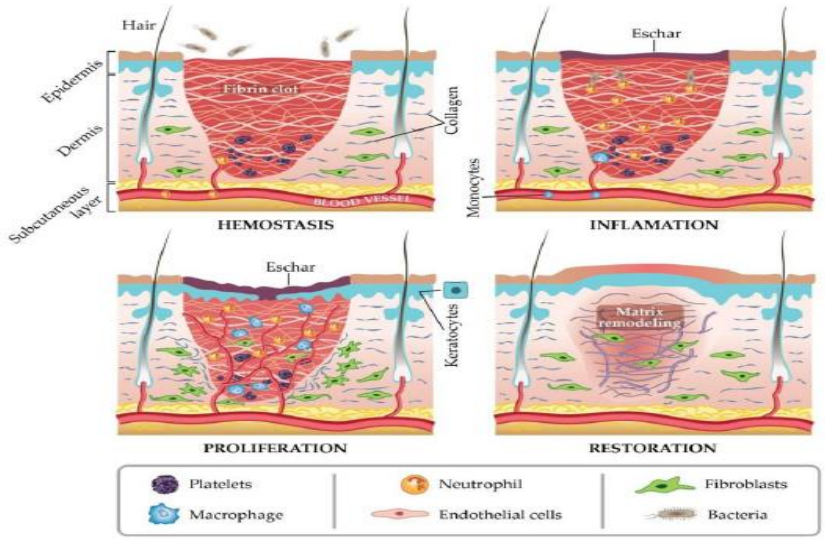


Figure 1: Wound healing stages.

Moreover, the **inflammatory** phase begins nearly concurrently with hemostasis, as activated platelets release cytokines to recruit neutrophils, monocytes/ macrophages, and lymphocytes. Neutrophils serve an essential purpose in cleansing an injury as they secrete antimicrobial peptides/ proteases and generate free radicals.[9] Neutrophils remain in the wounded area for approximately 24 hours prior to entering programmed cell death.[8]

The secreted cytokines additionally enhance multiplication of connective tissue cells, vascular and epidermal cells, initiating the successive stage of wound repair which is known as **proliferation** phase, typically lasts 2 to 3 days. It involves the development of healing bed, production of extracellular matrix (ECM) components.[10] Endotheial cells continue to move towards the wound area, promoting neovascularization growth. Meanwhile, keratinocytes migrate as well as multiply, aided by plasma which removes excess fibrin, aiding in the regeneration of the surface layer by epithelial repair components.[8]

During the last stage (**Remodelling** phase), the healing bed is substituted with an

indelible mark.[11,12]

Pathophysiology of inflammation

Chronic inflammation serves as the fundamental trigger for numerous ailments. All bodily discomfort finds its origins in inflammation and the body's reactive response to it. Various factors such as pathogens (like bacteria, viruses, or fungi), external traumas (such as cuts or injuries from foreign objects), as well as exposure to chemicals or radiation, incite the inflammatory process.

Initially, inflammation progresses through three sequential stages: the acute phase, followed by the sub-acute phase, and eventually transitioning into the chronic or proliferative phase. Inflammation involves specialized cells known as inflammatory cells, integral components of the immune response. The acute stage, lasting approximately one-three days, showcases five hallmark clinical indicators: heat, redness, swelling, pain, and impaired function. Inflammation arises when the immune system reacts to various triggers. Transitioning from brief to prolonged inflammation can disrupt immune balance, resulting in significant changes across tissues, organs, and regular cellular functions. This elevation in inflammation duration can heighten susceptibility to a range of non-communicable illnesses in people of all ages. In the presence of chronic inflammation, the body's immune reactions can gradually harm normal cells, tissues, and organs in animals. This continuous process may result in genetic material damage, tissue decay, and inner scarring over the long term. Chronic inflammatory conditions stand as the primary reason for global mortality presently, responsible for over half of all fatalities linked to inflammation-related ailments.[13,14,15]

Pathophysiology of wound

Sometimes, when you get a wound, it doesn't heal properly, especially if you're older or have conditions like diabetes. Natural wound healing is your body's way of fixing itself after an injury. It involves different stages like hemostasis, inflammation, proliferation, re-epithelialization and remodeling. When you get a cut, your blood forms a clot to stop bleeding. Then, special cells and chemicals come in to clean up the wound and start repairing tissue. This process involves a lot of interactions between cells and molecules in your body. Understanding how this works better could help improve treatments for wounds. When platelets clump together, they release clotting factors that form a clot, stopping bleeding and helping the wound to heal. At the same time, affected tissues and platelets secrete chemicals that attract immune cells like neutrophils and macrophages to start the healing process.

Inflammation usually helps protect the body, but if it gets out of control, it can actually cause more damage to tissues. A chronic wound happens when inflammation lasts a long time. As inflammation decreases, the skin starts to heal by creating regenerative cells and tissues, and the wound gets smaller. Re-epithelialization occurs when skin cells move to cover the wound. Finally, the remodeling phase begins, where collagen fibers organize to strengthen the new tissue. Inflammation is a key part of how wounds develop. Understanding the signals that cause inflammation at a basic level could help improve treatments.[16,17]

Pharmacotherapy of Anti-Inflammatory Agents

NSAIDs

- Acetylated salicylates (Like Aspirin)
- Non- acetylated salicylates (Such as diflunisal and salsalate)
- Propionic acids (Including naproxen and ibuprofen)
- Acetic acids (Like diclofenac and indomethacin)
- Enolic acids (Such as meloxicam and piroxicam)
- Anthranilic acids (Including meclofenamate and mefenamic acid)
- Naphthylalanine (Including nabumetone)
- Selective COX-2 inhibitors (Including celecoxib and etoricoxib)

Pharmacotherapy of wound healing drugs

- Surgical debriments
- Skin grafting
- Topical Medications
- Wound dressings
- Bioengineered Artificial Skin Replacements
- Nanotherapeutic approach

***Phyllanthus fraternus* Webster**

Scientific classification

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida- Dicotyledons
Subclass	:	Rosidae
Order	:	Euphorbiales

Family	:	Euphorbiaceae
Genus	:	Phyllanthus L.
Species	:	*Phyllanthus fraternus*
Common name	:	Gulf Leaf-Flower, bhui-amla, hazarmani, bhuinanvalah, kanocha, kirunelli, nelanelli, kiizhaarnelli, chakpa-heikru, bhuiavali, mithi-sunhlu, bhumyamalaki, tamalaki, kila-nelli, nelausiri

Plants have historically served as the most ancient wellspring of bioactive compound with medicinal properties, offering humanity a wealth of valuable therapeutic substances for countless generations.[18]

Phyllanthus fraternus Webster, commonly referred to as Gulf Leaf-Flower, is an annual herb which is cultivated in tropical regions worldwide, including nations such as China, Malaysia, Indonesia, Pakistan, SriLanka, and various regions of India. In India, it can be found in states like Uttar Pradesh, Bihar, Gujarat, Maharashtra, Kerala, and West Bengal, and it is particularly prevalent in the entire northeastern part of the country, especially in Assam, Arunachal Pradesh, and Manipur.

Phyllanthus fraternus was investigated with following objectives of *Phyllanthus fraternus* Webster:

- Pharmacognostical Evaluation of *Phyllanthus fraternus* Webster
- To develop phytochemical analysis of the leaves extract of *Phyllanthus fraternus* Webster.
- To evaluate Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in *Phyllanthus fraternus* Webster.
- Estimation of *in-vitro* antioxidant property of *Phyllanthus fraternus* Webster.
- To evaluate *in-vitro* and *in-vivo* anti-inflammatory activity of *Phyllanthus fraternus* Webster.
- To perform in-vivo wound healing activity of *Phyllanthus fraternus* Webster inrats

MATERIALS AND METHODS

- Various research papers published so far will be summarized scientifically, assessedstatistically analyzed to establish scientific research protocols.
- *In-vitro* antioxidant property of EEPFL by DPPH Free Radical-Scavenging Activity and Ferric Reducing Antioxidant Power (FRAP) Assay
- *In-vivo* anti-inflammatory activity by Carrageenan Induced Rat Paw Edema inRats.

- *In-vitro* wound healing property by Rat Red Blood Stabilization (RRBC)method.
- *In-vivo* wound healing property by linear incision method.

Pharmacognostical Evaluation of *Phyllanthus Fraternus* Webster

Macroscopic Evaluation

The macroscopic characterization of medicinal plant material relies on the visible attributes of the naked eye and external characteristics observed like shape, size, color, taste, apex, surface, base, margin, texture, vascular patterning, fracture, and odor.

Leaf: simple leaves arranged alternatively and oppositely. Upper surface of the leaves is green while lower surface is pale green. They possess a bitter taste with indistinct odor. With rounded tips and bases, leaves measures 6-13mm in length and 3-6 mm in width.

Stem: Stem is pale green, angular, slender, and extending outward with 30- 60cm height and 4mm diameter. They possess a bitter taste with indistinct odor.

Root: The primary root is relatively straight, small, measuring 2.5-11.0 cm in length, gradually narrowing towards the tip. It has light brown outer surface.

Capsule: Capsule measures 2.5mm in diameter, is smooth and roundish. There are six sepals, which are enlarged, covering more than half of the capsule, typically appearing light green in color.

Microscopic evaluation

Cross-section of a leaf reveals epidermal layers on both sides consisting of thin- walled cells elongated tangentially, coated externally with a thick cuticle. The mesophyll is divided into palisade and spongy parenchyma; the palisade layer, which is single-layered, occupies approximately half of the space between the two-epidermal layers. Stomata of the anisocytic type are present on both epidermises, followed by respiratory chambers underneath. The mesophyll comprises three to five layers of loosely arranged cells, with several veins running through this area. Additionally, a few calcium oxalate cluster crystals are found within the spongy parenchyma.

Phytochemical evaluation of *phyllanthus fraternus* webster

Phytochemical screening of qualitative chemical tests of EEPFL showed thepresence of the Phytoconstituents.

Table 1: Phytochemicals present in *phyllanthus fraternus* webster.

Chemical constituent	Leaf extract of *Phyllanthus fraternus*
Alkaloid	Present
Tannins	Present
Saponins	Present
Phlobatannins	Absent
Flavanoid	Present
Terpenoid	Present
Cardiac Glycosides	Absent
Steroid	Present

Estimation of the TPC in *Phyllanthus fraternus* Webster

Extraction of *Phyllanthus fraternus*

- *P.f* leaves were pulverized and extracted with crude ethanol
- Extract fractions were pooled up, filtered and lyophilized. Yield was found as3.25%

Determination of the amount of TPC in *Phyllanthus fraternus* Webster

- Phenol reagent (Folin- Cicocalteu; 1.0 mL) was added and mixed.
- Allowed to stand for 5 min then add 10 mL of 7% Na_2CO_3 solution.
- Absorbance recorded after 90 min at 750 nm.
- Gallic acid was used as reference drug.
- 1 ml of distilled water was added to 1.0 mL of Folin- Cicocalteu reagent and was processed similarly as above and used as blank.
- TPC of the extract was estimated.

Estimation of the TFC in *Phyllanthus fraternus* Webster

TFC estimation method and Quercetin was used as standard for preparation of calibration curve.[19] In this experiment, an attempt has made to determine TFC content of *Phyllanthus fraternus* by using colorimetric assay method of Negro.[20]

Table 2: Standard values of quercetin.

Quercetin (µg/mL)	Absorbance (510nm)
0	0
100	0.062
200	0.168
400	0.272
600	0.362
800	0.456
1000	0.548

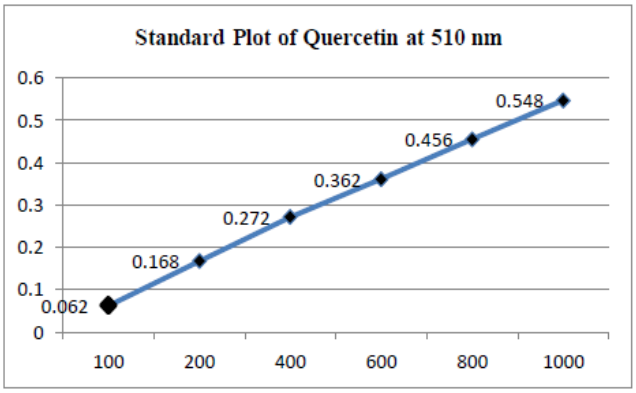


Figure 2: Standard Plot of Quercetin at 510 nm.

Estimation of *in-vitro* Antioxidant property of *Phyllanthus fraternus* Webster

1. DPPH Free Radical-Scavenging Activity
2. Ferric Reducing Antioxidant Power (FRAP) Assay

DPPH Free Radical-Scavenging activity

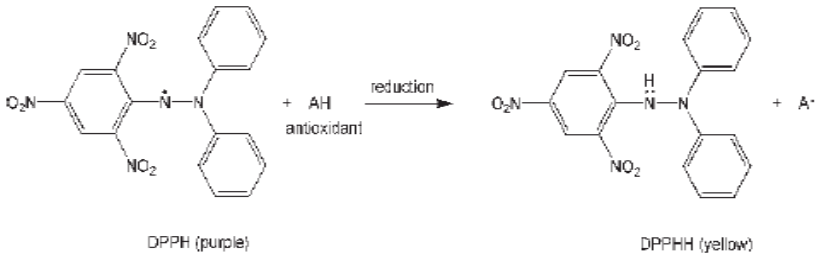


Figure 3: DPPH reduction.

Table 3: Values from DPPH Regression Curve.

S. No.	Concentration(µg/ml)	Cleared ratio (%)	
		Phyllanthus fraternus	Ascorbic Acid
1	0	0	0
2	10	7.2	14.6
3	20	14.8	24.6
4	40	22.8	35.2
5	60	31.6	44.8
6	80	38.2	55.9
7	100	44.2	68.4

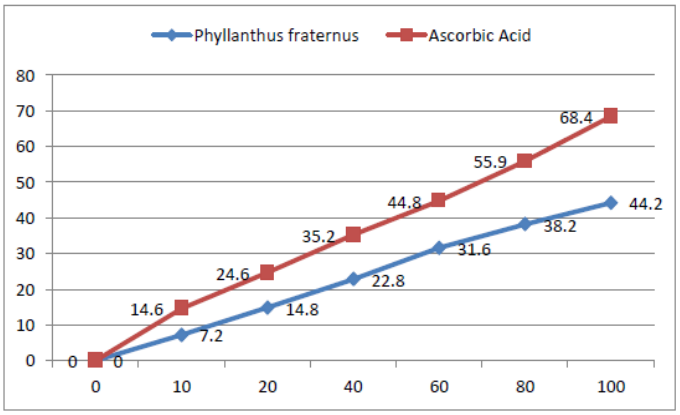


Figure 4: Regression curve plot of DPPH showing *Phyllanthus fraternus* as test sample and ascorbic acid as reference.

Ferric Reducing Antioxidant Power (FRAP) Assay

This method essentially assesses the antioxidant power of the extract based on its ability to act as a reducing agent. The antioxidant reducing power of EEPFL (100- 1000 µg/ml) was evaluated using Oyaizu's method.[21] Data was presented as concentration- absorbance curve.

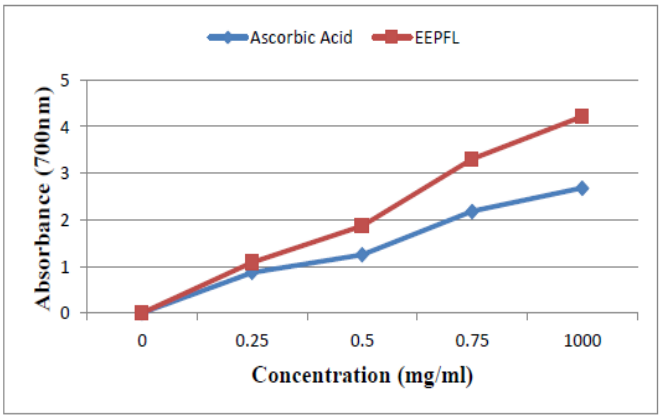


Figure 5: Ferric reducing power of EEPFL and ascorbic acid.

***In-vitro* anti-inflammatory effect of Ethanolic extract of *Phyllanthus fraternus* leaves (EEPFL)**

Rat Red Blood Stabilization (RRBC) method

Table 4: Effects of EEPFL 200mg and 400mg on RRBC.

S. No.	Percentage of Inhibition %	
	EEPFL (200mg)	EEPFL (400mg)
1	34.11 + 0.88	42.40 + 0.23
2	36.31 + 0.20	52.20 + 0.16
3	52.38 + 0.24	60.18 + 0.22
4	60.19 + 0.18	77.14 + 0.36
5	67.29 + 0.34	82.22 + 0.32

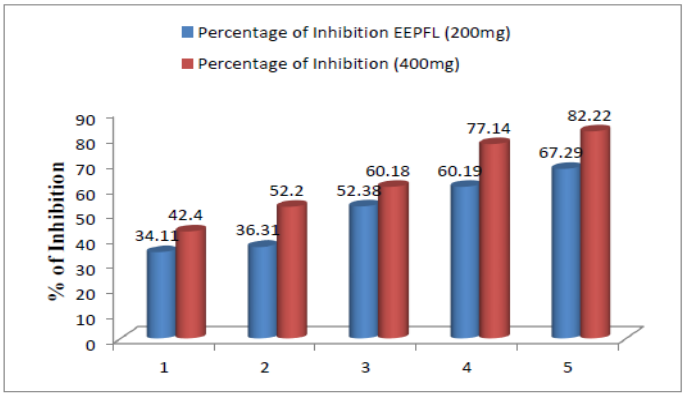


Figure 6: Effect of EEPFL 200mg and 400mg on RRBC.

***In-vivo* anti-inflammatory effect of Ethanolic extract of *Phyllanthus fraternus* leaves (EEPFL)**

Carrageenan Induced Rat Paw Edema in Albino Wistar Rats

Animal study was approved with Form B: IEC/IAEC/2023/08; 02-11-2023

Table 5: Grouping of animals for anti-inflammatory activity.

Groups	Treatment	No. ofAnimals
Ⅰ: Normal Control	Water *ad libitum*	6
Ⅱ: Toxic Control	Carrageenan 100mg/kg	6
Ⅲ: DrugTreated	Carrageenan 100mg/kg + EEPFL(200mg/kg)	6
Ⅳ: DrugTreated	Carrageenan 100mg/kg + EEPFL(400mg/kg)	6
Ⅴ:Standard Drug	Carrageenan 100mg/kg + Indomethacin 5mg/kg	6

Table 6: Effect of EEPFL 200mg/kg and 400mg/kg on paw edema.

	Volume of Paw Edema (ml)				
	1 Hour	2 Hour	3 Hour	4 Hour	5 Hour
Ⅰ: Normal	-	-	-	-	-
Ⅱ : Toxic Control [Carageenan 100mg/kg]	1.76±0.22	1.81±0.06	1.88±0.06	1.97±0.09	1.89±0.057
Ⅲ: DrugTreated [EEPFL 200mg/kg)]	1.58*±0.03	1.47*±0.02	1.53*±0.08	1.43*±0.07	1.40*±0.10
Ⅳ : Drug Treated: [EEPFL 400mg/kg]	1.62±0.05	1.45*±0.07	1.51*±0.06	1.43*±0.02	1.36*±0.05
Ⅴ : Standard Drug: [Indomethacin5mg/kg]	1.50*±0.02	1.40*±0.03	1.44*±0.06	1.34*±0.07	1.28*±0.05

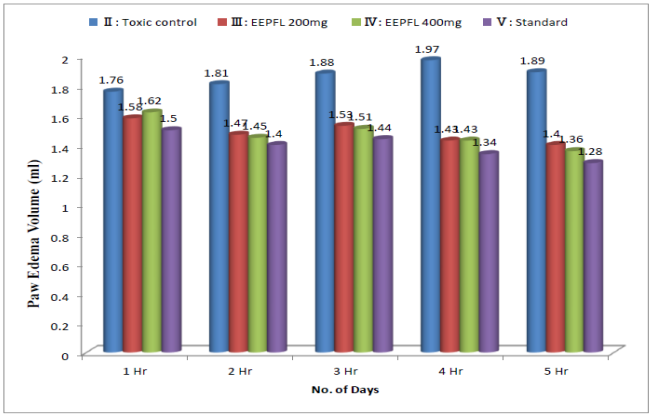


Figure 7: Effect of EEPFL 200mg/kg and 400mg/kg on paw edema (Carrageenan induced albino rats).

Table 7: % inhibition of edema in EEPFL 200mg and 400mg in albino rats.

Group	% Inhibition of edema				
	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
Group Ⅱ: Drug Treated [EEPFL (200mg/kg)]	7.46	17.08	16.10	23.04	26.72
Group Ⅲ: Drug Treated [EEPFL (400mg/kg)]	9.48	19.88	18.10	24.04	30.70
Group Ⅳ: Standard Group	13.70	21.54	20.28	28.12	34.98

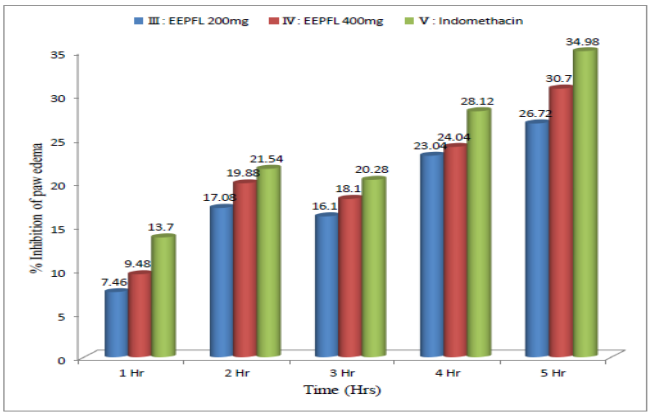


Figure 8: %inhibition of edema in EEPFL 200mg/kg and 400mg/kg in albino rats.

Stair climbing activity test

Table 8: Stair climbing activity scores.

Groups	Stair Climbing Activity (Mean)
Group Ⅰ: Normal	3.2 ± 0.00
Group Ⅱ: Toxic Control [Carrageenan 100mg/kg]	0.29 ±0.245
Group Ⅲ: Drug Treated [EEPFL 200mg/kg]	2.42 ± 0.36
Group Ⅳ: Drug Treated [EEPFL 400mg/kg]	2.55 ± 0.42
Group Ⅴ: Standard Drug [Indomethacin 5mg/kg]	2.9 ± 0.28

Stair Climbing Activity was observed at the time of peak inflammation (4 hoursfor carrageenan)

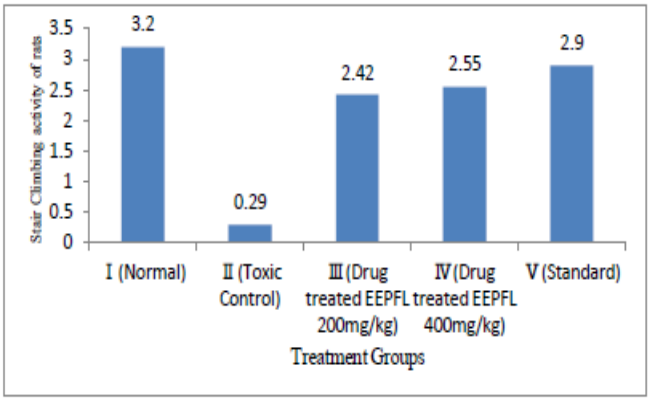


Figure 9: Effect of EEPFL 200mg/kg and 400mg/kg on impairment in stair climbing activity score associated carrageenan- induced inflammation.

Motility test

Table 9: Motility test scores.

Groups	Motility Activity (Mean)
Group Ⅰ: Normal	2.2 ± 0.00
Group Ⅱ : Toxic Control [Carrageenan 100mg/kg]	0.15 ±0.245
Group Ⅲ: Drug Treated [EEPFL (200mg/kg)]	1.34 ± 0.36
Group Ⅳ : Drug Treated [EEPFL (400mg/kg)]	1.58 ± 0.42
Group Ⅴ : Standard Drug [Indomethacin 5mg/kg]	1.95 ± 0.28
Motility Test was observed at the time of peak inflammation (4 hours for carrageenan)	

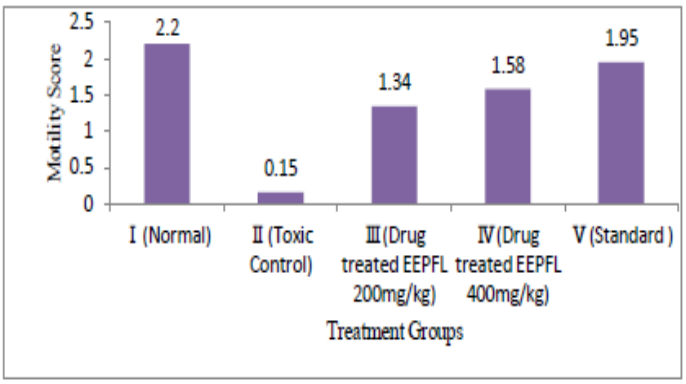


Figure 10: Effect of EEPFL 200mg/kg and 400mg/kg on impairment in motility associated carrageenan- induced inflammation.

Evaluation of the anti-wound healing effect of EEPFL in AlbinoWistar Rats

Linear Incision Wound model in Albino Wistar Rats

Animal study was approved with Form B: IEC/IAEC/2023/08; 02-11-2023

Table 10: Grouping of animals for wound healing activity.

Groups	Treatment	No. of animals
1 : Toxic Control	Distilled water	6
2 : DrugTreated	Treated with EEPFL 400mg/kg	6
3 : Standard	Treated with Soframycin Ointment	6

Figure 11: Representation of animals of Group Ⅰ, Ⅱ and Ⅲ from day 0 to Day10 of post wound creation.

Table 11: Effect of topical application of EEPFL 200mg and 400mg on wound model.

Groups	Wound Length (in cm)					
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Group Ⅰ: Toxic Control	1.01±0.016	0.96±0.03	0.87±0.03	0.59±0.06	0.35±0.04	0.11±0.04
Group Ⅱ: Drug Treated	1.01±0.013	0.93±0.008*	0.80±0.03*	0.49±0.045*	0.16±0.04*	0.00±0.00*
Group Ⅲ: Standard	1.00±0.02	0.89±0.05*	0.78±0.03*	0.30±0.075*	0.09±0.04*	0.00±0.00*

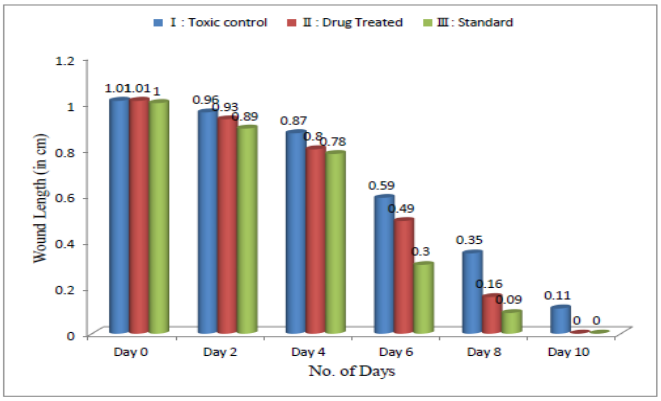


Figure 12: Effect of topical application of EEPFL 200mg/kg and 400mg/kg on wound Model.

Table 12: % of wound contraction in EEPFL 200mg/kg and 400mg/kg in albino rats.

Groups	% of Wound Contraction				
	Day 2	Day 4	Day 6	Day 8	Day 10
Ⅰ: Toxic Control	4.95	13.86	41.58	65.34	89.10
Ⅱ: Drug Treated	7.92	20.79	51.48	84.15	100
Ⅲ: Standard	11	22	70	91	100

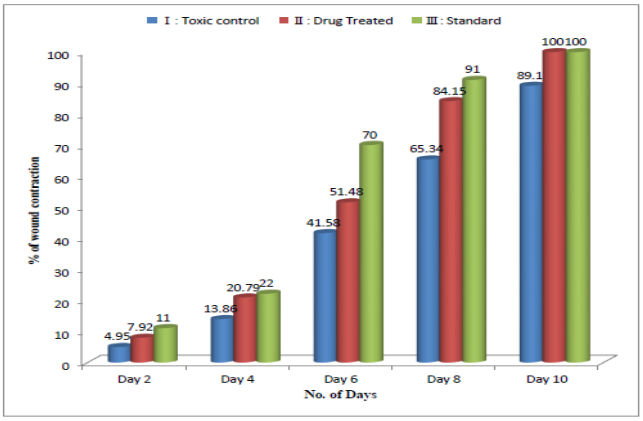
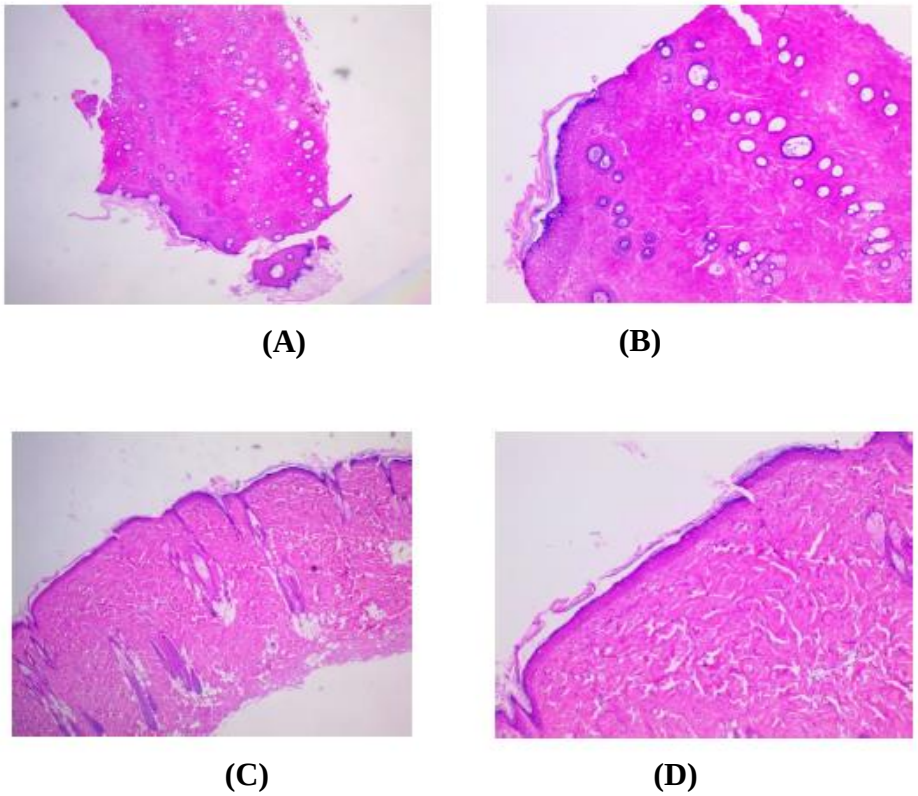


Figure 13: % of Wound Contraction in rats treated with EEPFL 400mg/k.

Histopathology



(A) **(B)**

(C) **(D)**

Figure 14: (A) and (B) are Toxic Control group; (C) and (D) are Treatment Group (EEPFL400mg/kg).

RESULTS AND DISCUSSIONS

Macroscopic and microscopic evaluation was performed to determine the visible attributes and anatomical structures at cellular level. Further, phytochemical evaluation was performed with the help of qualitative chemical tests. The analysis indicates that the plant contains alkaloid, tannins, saponins, terpenoids, flavonoids and steroids (Table 1).

The amount of TPC found in EEPFL was 44.44 ± 6.42 mg GAE/g FW. The potential of the antioxidant property was directly proportional to TPC value found in *Phyllanthus fraternus.* Standard values were prepared and TFC were estimated in *P.f.* The Total Flavonoid Content was expressed as mg quercetin equivalent /g fresh weight (mg QE/g fw) in plant extract**.** TFC value found was 0.511 mg QE/g fw. The antioxidant activity was estimated through DPPH radical scavenging activity of *Phyllanthus fraternus* Webster and was directly proportional to the concentrations (regression equations were significant at $p < 0.05$). Appearance of yellow colored DPPH2 indicates the presence of anti-oxidants present in the extract solution. Reducing potential of EEPFL was increased with increasing concentration.

This signified the consistent reduction of Fe^{3+} to Fe^{2+}. The reducing agents found in EEPFL demonstrated notable reducing capabilities in contrast to standard ascorbic acid (Figure 5).

EEPFL 200mg and 400mg showed significant stabilization towards RRBC and EEPFL 400mg showed better results than EEPFL 200mg. In *in-vivo* anti-inflammatory activity, at 5^{th} hour observation, edema inhibition in rats treated with EEPFL 400mg (30.70%) and EEPFL 200mg (26.72%) as summarized in Table 7 and Figure 8. The observation of Stair Climbing Activity and Motility Test occurred at the time of maximum inflammation (4 hours post carrageenan administration) to evaluate the effect of EEPFL 200mg/kg and 400mg/kg in the rats which were challenged with 1% Carrageenan. Stair Climbing Activity scores were found better in EEPFL 400mg/kg (2.55 ± 0.42) when compared with EEPFL 200mg/kg (2.42 ± 0.36) as presented in Table 8 and Figure 9. Motility scores were found better in EEPFL 400mg/kg (1.58 ± 0.42) when compared with EEPFL 200mg/kg (1.34 ± 0.36) as summarized in Table 9 and Figure 10.

Further, healing potential of *Phyllanthus fraternus* for the rats subjected to a straight incision of 1 cm. On 10^{th} day of post wound creation, it was observed that percentage of wound contraction was 100% in Group Ⅱ animals and Group Ⅲ animals. Meanwhile, Group Ⅰ animals had 89.10% wound contraction as shown in Table 12 and Figure 13.

Histopathology was performed for the rats treated with EEPFL and for the control and standard group as shown in Figure 14.

Table 13: Stair Climbing Activity and motility activity of rats treated withEEPFL 200mg/kgand 400mg/kg.

Groups	**Stair Climbing Activity(Mean)**	**MotilityActivity (Mean)**
Group Ⅰ: Normal	3.2 ± 0.00	2.2 ± 0.00
Group Ⅱ : Toxic ControlCarrageenan 100mg/kg]	0.29 ±0.245	0.15 ±0.245
Group Ⅲ: Drug Treated [EEPFL 200mg/kg]	2.42 ± 0.36	1.34 ± 0.36
Group Ⅳ : Drug Treated[EEPFL 400mg/kg]	2.55 ± 0.42	1.58 ± 0.42
Ⅴ : Standard Drug[Indomethacin 5mg/kg]	2.9 ± 0.28	1.95 ± 0.28

CONCLUSION

Pharmacological investigations were performed for *Phyllanthus fraternus* Webster. Phytochemical evaluation was performed on EEPFL which indicates that the plant contains alkaloid, tannins, saponins, terpenoids, flavonoids and steroids. TPC estimation analysis revealed that amount of TPC found in *Phyllanthus fraternus* was 44.44 ± 6.42 mg GAE/g FW. TFC estimation analysis revealed that amount of TFC found in *Phyllanthus fraternus* was 0.511 mg QE/g fw.

The plant has great antioxidant potential and showed a great anti-inflammatory agent. EEPFL 200mg and 400mg showed significant stabilization towards RRBC. In the experimental models of carrageenan induced rat paw edema, effects of EEPFL showed a significant inhibition when compared with controls. EEPFL, as an anti- inflammatory agent is useful in reducing the inflammation in both 200mg and 400mg dosage. The study results showed that EEPFL is a potent wound healer. On 10^{th} day of post wound creation, it was observed that percentage of wound contraction was 100% in rats which were treated with EEPFL. Taken together, the present study highlighted the positive impact of EEPFL as an anti-inflammatory agent, anti-oxidant and a potent wound healer.

REFERENCES

1. Medzhitov, R, 2010; 140: 771-776.
2. Ferrero-Miliani, L., Nielson, O., Andersen, P., Girardin, S. *Clin. Exp. Immunol,* 2007; 147: 227-235.
3. Halliwell, B., Gutteridge, J.M. *Oxford. University. Press*, 2015.
4. Lazarus, G.S., Cooper, D.M., Knighton, D.R., Margolis, D.J., Pecoraro, R.E., Rodeheave, G., Robson, M.C. *Arch. Dermatol*, 1994; 130: 489-493.
5. Strodtbeck, F. *Newborn. Infant. Nurs. Rev*, 2001; 1: 43-45.
6. Metelmann, H.R., Von Woedtke, T., Weltmann, K.D. *Springer,* 2018.
7. Boersema, G.C., Smart, H., Giaquinto-Cilliers, M.G., Mulder, M., Weir, G.R., Bruwer, F.A., Idensohn, P.J., Sander, J.E., Stavast, A., Swart, M. *World. Counc. Enteros. Ther, 2021;* 41: 21-32.
8. Thiruvoth, F.M., Mohapatra, D.P., Kumar, D., Chittoria, S.R.K., Nandhagopal, V. *Plast. Aesthetic. Res, 2015;* 2: 250-256.
9. Agrawal, P., Soni, S., Mittal. G., Bhatnagar, A. *Int. J. Low. Extrem. Wounds, 2014;* 13: 180-190.

10. Rogers, A.D., Jeschke, M.G. *Chronic. Wound. Care. Manag. Res*, 2016; 3: 59-71.
11. Diegelmann, R.F. *Methods. Mol. Med*, 2003; 78: 349-358.
12. Carlson, M.A., Longaker, M.T. *Wound. Repair. Regen*, 2004; 12(02): 134-147.
13. Furman, D., Campisi, J., *et al. Nat. Med*, 2019; 25: 1822-1832
14. Fleit, H.B. *Chronic. Inflammation. Pathobiology. of. Human. Disease*, 2014; 300-314.
15. Thorsson, V., Gibbs, D.L., Brown, S.D., Wolf, D., Bortoneet, D.S., *et al Immunity, 2019;* 51: 411-412.
16. Wang, Y., Huang, C., Cao, Han, J.S. *Life Sciences*, 2000; (67): 261-267.
17. Wilkinson, H.N., Hardman, M.J. *Open. Biol*, 2020; 10(9).
18. Pandey, A., Patil, A.A., Joshi, N.R., Kucheka, B.S. *J. Chem. Pharm. Res, 2010;* 2(3): 182- 186.
19. Kamtekar, Mishra, P.K., Pathak, N., Bande, S.S., Sriram, S. *Journ. of. Appl. Pharmaceu. Scie, 2014;* 4(09): 061-065.
20. Negro, C., Tommasi, L., Miceli, A. *Bioresource. Tech*, 2003; (87)1: 41-44.
21. Oyaizu, M. *Japan. J. Nutr*, 1986; 44: 307-315.