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A STUDY ON ANTI HEMORRHOIDAL ACTIVITY IN CALOTROPIS GIGANTEA R.BR.

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Abstract

Hemorrhoids, a common anorectal disorder, are characterized by inflammation, swelling, and bleeding of the rectal veins. Current pharmacological treatments often provide limited relief and may be associated with adverse effects, highlighting the need for alternative therapies. Calotropis gigantea, a medicinal plant traditionally used in folk medicine for various ailments, has been reported to exhibit anti-inflammatory, analgesic, and wound-healing properties. This study investigates the potential antihemorrhoidal activity of Calotropis gigantea through phytochemical analysis and experimental evaluation using established in vitro and in vivo models. Extracts from leaves of the plant were tested for their efficacy in reducing inflammation, pain, and bleeding associated with hemorrhoidal conditions. The results demonstrated significant antihemorrhoidal effects, particularly with the ethanolic leaf extract, likely due to the presence of flavonoids, alkaloids, and terpenoids. These findings suggest that Calotropis gigantea may serve as a promising natural alternative for the management of hemorrhoids, warranting further pharmacological and clinical investigations.

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Introduction

Hemorrhoids are one of the most common anorectal diseases affecting more than 50% of adult population. The word hemorrhoid originated from the Greek term 'Haema' and 'rhoos' refers to the Flow of blood [1]. This term was first used by hippocrates in his treaties. The word pile is derived from latin word 'pila'means a ball or mass and this word was first introduced by John Arderne. It is an anorectal disease that shows the symptoms of growth and distal eviction of typical rectoanal cushion [2].

Anal cushions comprise connective tissue housing blood filled vascular structure commonly found within anal passage. These anal cushion support anal continence and safeguard the Sphincters when perfused during defecation. Anal cushion that are distally displaced and swollen are symptom of hemorrhoid It causes Bleeding, Pain and itching [3].

Regarding the suffering age in piles it has a record by Dr Rose and charless have stated that internal hemorrhoids are exceedingly common in young man about 20 years of age and up to middle age but in elderly , many conditions like enlarged prostate, carcinoma of rectum favours their development . some others like Dr.cambell have a reported that piles can occur in infant age to child and lead to older age also, With regard to sex, the male to female ratio is 5:4 and the piles are common in males [4].

Pathophysiology [5]

It is a natural component of anal canals structure found inside the submucosal area is cushions of densely vascularised tissue. There are three primary rectal padding or supporting tissue positioned in a) Right anterior, b) Right posterior, c) Left lateral in Anal canal [6]. Blood veins, elastic tissue, smooth muscle and supportive tissue are all in these haemorrhoidal cushion.

Enlargement of cushion, loosening of supporting connective tissue through anal Canal is due to unsteady bowel moment, low fiber diet, prolonged straining, elevated intra peritoneal pressure, genetics, aging, lack of valves within piles vessels. This raise the pressure inside the cushion [7].

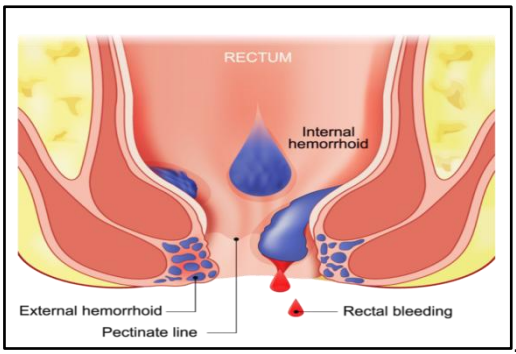

Fig 01: Types of Hemorrhoids

Depending upon connection with dentate line is divided into internal and external hemorrhoid as mentioned in Fig 01.

Internal hemorrhoid originates from inferior hemorrhoidal venous plexure above the dentate line and is covered by mucosa. While the external hemorrhoids are dilated venules of this plexure located below the dentate lines covered with the squamous epithelium. In simple word the dilation of the internal rectal plexus constitutes the internal hemorrhoid covered by the mucous membrane. The external hemorrhoids plexus also forms the external hemorrhoid covered by the skin which are placed beneath the dentate line and around the perianal region. The union of the internal and external hemorrhoid is called as intero external haemorrhoid [8].

Internal hemorrhoid traditional goligher staging 4 degrees as shown in Fig 02.

- First degree - Bulging into anal passage without prolapsing.
- Second degree - Prolapse outside anal passage but spontaneously reduce.
- Third degree - Bulge outside the anal passage upon pushing and annual realignment.
- Fourth degree - Unavoidable and continuously prolapse.

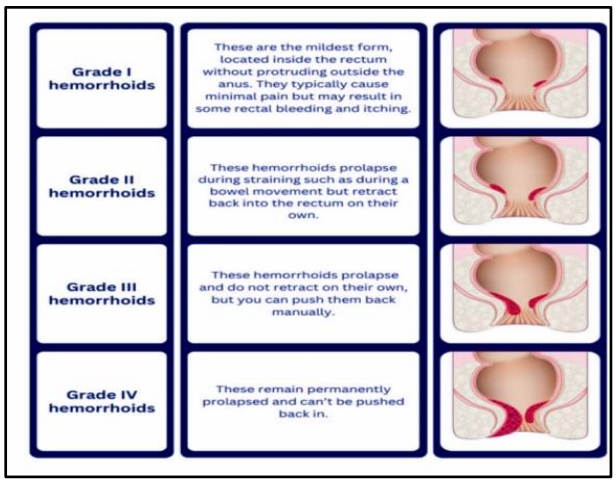

Fig 02: Stages of Internal Haemorrhoids

By physiological classification a) Venous or vascular hemorrhoids - the vessels are involved significantly to form the pile mass. b) arterial hemorrhoids - it is a haemangiomatous condition of superior rectal artery entering the pedical of internal hemorrhoid which bleed profusely . c) mucosal hemorrhoids this type of hemorrhoides are thick with mucus membrane which slide downwards such haemorrhoids along with prolonged dilated component may cause third or fourth degree hemorrhoids.

Common Clinical Characters

The general sign and symptoms of the piles are sensation of heat, itching, bleeding, prolapse, discharge, pain and anal irritation [9].

Causative factors of piles where the primary idiopathic factors include anatomical factors in in which during defecation the constriction of muscular tissue causes piles, hereditary factor, constipation, prolonged standing and sitting, occupation hazards such as heavy weight lifting, muscles straining work, due to addiction factor such as excess of alcohol intake causes liver congestion and improper diet [10]

Treatment of therapies in piles such as diet modification, common management, sclero therapy, rubber band ligation, cryosurgery, infrared coagulation ,bipolar diathermy, laser vaporization [11].

Plant Profile [12]

Calotropis is one among 250 genera And 2000 Species of Asclepiadeacea family, which are laticiferous, poisonous, Tropical shrub, Herb and climbers Which first looked like a member of apocynaceae [13]. Calotropis are widely spreaded Weed species with poisonous milky latex as shown in Fig 03. Calotropis has been derived from Greek word 'kalos' - Beautiful and 'Tropis' means the Keel of a boat, which referring to the shape of the coronal scales. This genus comprises of erect, glaborous Shrub with milky juice. The leaves are usually subsessile, Fleshy and opposite. Flowers are large and borne in umbelliform cymes. The fruits are follicles and are single thick, straight on the ventral and convex on the dorsal side, oblique and sharply incurved near the base. The seeds are comose. Generally Six pieces are found in tropical African Asia and three species grown in India of these one is found throughout India. This plant or having the rich phytochemical compound such as cardio tonic glycosides, indole, terpenoids, Alkaloids, Anthocyanins and proteolytic enzymes [14].

Taxonomical Classification [15]

Kingdom - Plantea
Sub Kingdom - Tracheobionata.
Sub division - Spermatophyta
Division - Magnoliopsida
Order - Gentianales
Family - Apocynaceae
Subfamily - Asclepiadeacea
Genus - Calotropis
Species - gigantia

Fig 03: Whole plant of Calotropis gigantean

Among 2,000 species of Calotropis There are two variety of plant named Madar (i.e) Calotropis procera & Calotropis gigantia. It is an erect spreading shrub reaching a height of about 3 meters with the yellowish white furrowed bark and Stout, cylindrical branches covered with fine pubescence. Stem and leaves containing a Milky latex. Leaves are simple opposite, Oval, oblong 6 -11.5 cm, Delicious green. A White milky liquid latex exudes when broken. Flowers are regular,bisexual Stout ,Whitish purple Corolla ,Twisted ,Deep lobed and fused five petals ,Five stamen With Superior ovary, Two styles flat on top. Fruits or 8.5 -10 centimeter long and 4 - 4.5 centimeter Broad, Cylindrical. Seeds are very numerous, compressed with Long coma. It's pod resembles a mango When it ripes it breaks which is filled with white substance resembling silk where seeds are fixed[3].

Habitat or Distribution [16]

This shrub abounding in Milky juice is found chiefly in wastelands in Madras, South India, ceylon, Punjab, Assam. It occurs throughout India, Singapore, South China. Throughout India chiefly in Wastelands

Vernacular Names [17]

Synonym - Giant Milkweed, Crown Flower
English - Mudar, Gigiantic Swallow Wort
Tamil - Erukku, badabadam, yercum
Hindi - Madar, Ak
Sanskrit - Arka, alarka, mandara, surya pattra
Malyalam - Erikka
Telugu - Mandaramu, ekke
Bengali- Akanda
Propagation: Through seeds
Part used: Leaves, flowers, root, bark, inspissated juice.

Phytochemical Compounds [18]

Various principles of calotropis bark and sap are Madar alban, Madar fluavil of gutta percha, Black acid resin; Yellow Bitter resin.The root bark has high percentage of acrid and bitter resiness matter than younger plant but no Alkaloids [19]. The latex yield bitter principle such as Calotropin, Proteinase and Calosterol. It also contains Cardenolide, triterpenoids, Resins, Anthocyanin, Polysaccharides, Gigantin, Giganteol, Flavonoids, sterols, etc [20].

Therapeutic Uses [21]

It is used in leprosy, Constitutional syphilis, Dysentery, chronic rheumatism. In India it is potentially cures fever, piles, skin disease, asthma ,Epilepsy ,Indigestion ,leprosy .The leaves with rock salt roasted in vessels and Ash produced is given for Ascites ,abdominal viscera. The Milky juice is for toothache. Leaves are also used in Eczema and skin diseases [22].

Objective of This Study

1. To collect, Identify and Authentication of the plant Calotropis gigantia.
2. To prepare Aqueous and Alcoholic extract from leaves of Calotropis gigantia.
3. To perform Phytochemical screening of plant extract to identify the presence of bioactive compound.
4. To analyse the bioactive compound traces for therapeutic effects.
5. To evaluate Anti hemorrhoidal activity of leaf extract using in vitro and in vivo methods.

Duration of This Research

Researchers have taken 21 months to complete this Research work of “A study on anti-haemorrhoidal activities in Calotrophis Gigantia”. First 6 month for review of literature, 6 month of research methodology. Researchers have taken 6 month for analysis and additional 3 month of writing the reports.

Research Methodology [23]

Primary Sources

Calotropis gigantia plant parts sucha as Leaves, flowers,stems are collected by hand picking method in the month of August from Wastelands of Ambattur, Tiruvallur District Chennai. By using Natural Method of drying in room temperature the leaves are dried. For Extraction process the dried leaves are pulverized using grinder machine and seived in sieve no.80.

Secondary Sources

Database include Google scholar, Pubmed, Scopus, science direct, web of science, springer and pharma journal websites.

Plant Material

The leaves of Calotropis gigantia was collected in the month of August 2023 from the Wastelands of tiruvallur District Chennai. The collected leaves were washed in Running water several times then dried in Shaded place at room temperature for about 2-3weeks as shown Figure 4. After complete drying the leaves were grinded into fine powder using grinder machine. The powder was then sieved through sieve number 80. About 20 grams of powder was then subjected for extraction process. The 2g of powdered sample is used for Microscopical studies using Electronic microscope [24].

Fig 04: Dried and Powdered Drug

Extraction [25-27]

The extraction was done by successive extraction method using soxhlet apparatus. The choice of solvent are distilled water and alcohol. The property of the menstrum such as water is highly polar which is used in Extraction of Polar compound and also it can be dissolved a wide range of substance. It is cheap, non-toxic, non-flammable. Alcohol is a polar in nature, miscible with water and could extract the polar secondary metabolites. It is self-preservative, non-toxic at low temp concentration and small amount of heat is required for concentrating the extract.

About 10 gram of powdered material was subjected for each successive extraction with 100ml of Ethanol and distilled water .The extraction process continued until the soluble bioactive compound get soluble with menstrum. After each extraction the solvent was distilled off and the extract was concentrated by vacuum dryer at a temperature below 45 degree celsius. Finally the percentage yield of Ethanol and distilled water extract where recorded. Then extract were stored in refrigerator at 45 degree celsius.

To detect the presence of bioactive component in calotropic gigantia the phytochemical screening test suggest alkaloid terpenoids, saponin, Tannin, glycosides, flavonoids,etc.

Phytochemical parameters [28]

powdered leaves of C. gigantea The 1 g powdered leaves of Calotropis gigantea was weighed and kept in the muffle furnace at a temperature of 500°C for about six hours. The total ash obtained was calculated. Followed by Extractive value and moisture content also determined.

Experimental Evaluation [29]

Invitro Anti-Inflammatory Method

To access the ability of the extract to inhibit denaturation of protein which causes an inflammation is evaluated by Protein Denaturation method material requireds are bovine serum albumin, Buffer pH 6.8, Spectrophotometer. Procedure- prefers reaction mixture of 0.45 mL of 5% BSA, 0.05 mL plant extract at various concentrations. Then incubate at 37 degree Celsius for 20 minutes and heat at 70 degree celsius for 5 minutes to induce denaturation. Cool and measure the absorbance at 660 nm. Finally calculate the Percentage inhibition of denaturation.

% inhibition= (Abs control - Abs Sample / Abs control) × 100

Invitro Antioxidant Method by DPPH radical scavenging Assay:

To determine and measure the plant substance has the ability to neutralize the Free radicals by this method.

Materials - 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution 0.1 mM in methanol , Methanol ,Spectrophotometer procedure mix 1 mL of dpph solution With 1mL of extract with various concentration Incubate in the dark for 30 minutes. Measure the absorbance at 517 nm.

Calculation: % radical scavenging = (Abs control - Abs Sample / Abs control) × 100.

In Vivo Methods - Carrageenan Induced Paw Edema [30]

Carrageenan(a polysaccharide) is injected into the rat's hind paw, inducing inflammation. The increase in paw volume is measured over time to measure the sample has Anti-inflammatory agents reduce this swelling.

Procedure: Rats are divided into control, standard (e.g., indomethacin), and test groups. Drug/extract is administered (usually orally or intraperitoneally). After 1 hour, 0.1 mL of 1% carrageenan is injected subcutaneously into the hind paw. Paw volume is measured at 0, 1, 2, 3, and 4 hours using a plethysmometer. % Inhibition of edema is calculated.

Analytical Techniques [31]

Thin Layer Chromatography (TLC)

Materials: Precoated TLC plates (Silica gel 60 F254, Merck), Capillary tubes or micropipette, Saturated TLC chamber,Solvent system (Mobile Phase),UV Chamber (254 & 366 nm), Derivatizing agent (e.g., anisaldehyde-sulfuric acid reagent), Common Mobile Phase Options: (Choose one based on best separation – trial may be needed) Toluene : Ethyl acetate : Formic acid (5:4:1), Chloroform : Methanol (9:1).

Procedure: Activate the TLC plate by drying at 110°C for 10 min. Apply small spots (5–10 µL) of the extract solution 1.5 cm from the bottom using a capillary. Develop the plate in a saturated chamber using the selected mobile phase. Allow the solvent front to travel ~8 cm, then remove the plate and dry. Observe under UV light at 254 nm and 366 nm. Spray with anisaldehyde-sulfuric acid reagent and heat at 105°C for 5 minutes. Record the number of bands and their Rf values [32].

Rf Calculation: Rf=. Distance traveled by the compound / Distance travelled by the solvent.

Researchers Suggestion to Develop Anti Hemorrhoidal Activity in Calotropis Gigantea

As a researcher I want to suggest that Calotropis gigantia is generally a toxic plant for internally by taking in high dose. In low dose, this can exhibit it's therapeutic effects and widely used to treat the common diseases such as arthritis, piles, dysentery, eczema,etc. By doing valuable effective studies with low concentrations of Calotropis gigantia will have scope in future research development.

Researchers Solution to Rectify Failures in Anti-Haemorrhoidal Effect

By performing invitro and invivo method of the Antioxidant, Anti inflammatory, Analgesic and wound healing activity we can confirm the potency of plant extract has anti hemorrhoidal activity in low dosage. By determining the correct dosage form with different concentrations of plant extract can reduce the risk level of toxicity.

Result

Pharmacognostical Studies

i) Macroscopic and Microscopic studies

The leaves of Calotropis gigantea was evaluated for its Organoleptic Characters which is mentioned in Table 01 and the characterisation was viewed as shown in Fig 05.

Table 01: Organoleptic Characters

S.No	Characteristics	Inference
1	Color	Greyish Green
2	Odour	Pungent
3	Texture	Coarse
4	Taste	Bitter
5	Shape	Oblong or obovate
6	Size	6 - 11.5 cm broad, 7-17 cm long

Fig 05: Leaves of C.gigantea

ii) Transverse sections of different plant parts show arrangements of fragments and various cell types.

Leaf anatomy

• Epidermis single-layered, with thick cuticle on both surfaces. Mesophyll differentiated into palisade and spongy parenchyma. Three rows of palisade cells under upper epidermis. Spongy parenchyma below with intercellular spaces as shown in Figure 8. Vascular bundles are bicollateral and open. Stomata type: paracytic is shown in Fig 06.

Flower anatomy: five petals (gamopetalous), five sepals, ovary with ovules.

Powder Microscopy: For C. gigantea, multicellular trichomes; epidermal cells, vessels, sclerenchyma cells, trichomes (unicellular and multicellular) as shown in Figure 7, etc.

Transverse section (young stem): cork, cortex, vascular bundles, medullary rays, xylem and phloem elements observed.

iii) Powder microscopy: The powdered leaves of Calotropis gigantea was analyzed under the microscope which showed single, capsulated, glandular and multicellular trichome. Many rosette crystals of starch grains and calcium oxalate were observed. The reddish spots that were found indicated the presence of tannin. The stomata found in the powdered leaves of C. gigantea were paracytic are confirmed.

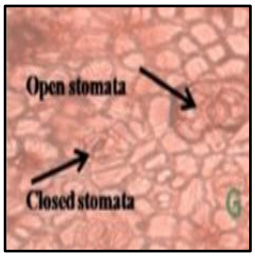

Fig 06: Stomata

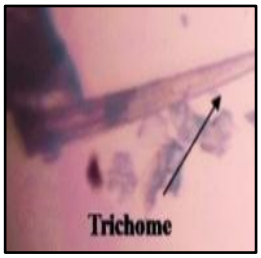

Fig 07: Trichome

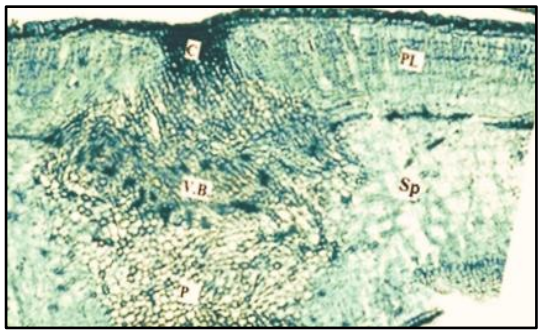

Fig 08: T.S of C.gigantea leaf

Physio chemical Evaluation

1. The ash was dissolved in water, acid, and alcohol and different phytochemical parameters were evaluated
   a. A) Ash value (%w/w). : Total ash is 12.7 ± 0.86, Acid-insoluble ash is 3.22 ± 0.09, and Water-soluble ash is 2.76 ± 0.15.
   b. B) Extractive values (%w/w): Alcohol soluble. is 5.22 ± 0.72, Water soluble is 18.45 ± 0.13
   c. C) Loss on drying (%w/w) 14.09 ± 0.46

2. *Phytochemical Screening:* In detection of the presence of bioactive component in calotropic gigantia Confirms the presence of cardiac glycoside, terpenoids, tannin, flavonoid , steroid, polyphenol and alkaloid in ethanolic extract. The Main active constituent such as calotoxin, Calotropogenin, calotropin are chief exhibit therapeutic effect in piles reduction as given in Figure 9. By screening test shown in Figure 10 confirms the presence of Phenolic compound, Flavonoids, Alkaloids, Tannins, saponnin, Glycosides and terpenoids, reducing sugars in aqueous extract of Calotropis gigantea.

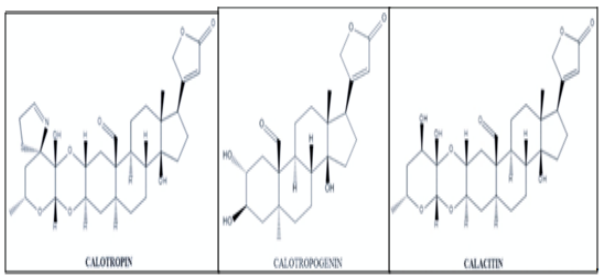

Fig 09: Chemical Structure

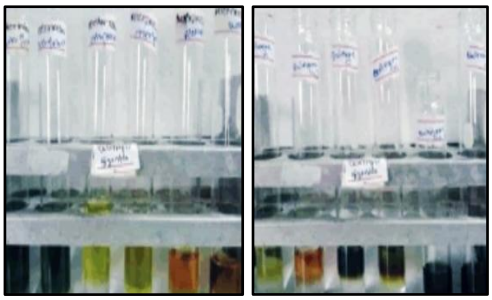

Fig 10: Chemical Test for Screening

Analytical techniques

The application of TLC techniques to the ethanolic extract of Calotropis gigantea successfully established a reproducible phytochemical fingerprint, confirming the presence of several bioactive secondary metabolites. The chromatographic profiles revealed multiple distinct spots and peaks, indicating the complexity and richness of the extract's phytochemical composition.

These findings provide valuable qualitative and semi-quantitative data that can be used for the standardization, quality control, and future pharmacological correlation of C. gigantea extracts. Moreover, the presence of compounds with known anti-inflammatory and anti cancer properties, such as flavonoids and terpenoids, supports the pharmacological relevance of the extract in the management of hemorrhoidal conditions.

Limitation of these Studies

Short Duration of Study: Haemorrhoids are a chronic condition in many cases. My study duration is short, so it may not reflect the long-term efficacy or safety of Calotropis gigantea. Lack of Comparative Analysis: The study m not compare the plant extract with standard anti-hemorrhoidal treatments (e.g., hydrocortisone, lidocaine, flavonoid-based medications), making it difficult to judge its relative effectiveness. Dosage Optimization Not Addressed: The study might not define the most effective or safest dose for treatment, which is critical for clinical application.

Conclusion

The findings of this study provide compelling evidence that Calotropis gigantea exhibits significant antihemorrhoidal activity, potentially attributable to its rich phytochemical profile, including flavonoids, terpenoids, and phenolic compounds. The plant extract demonstrated a marked reduction in 0hemorrhoidal symptoms in experimental models, likely through a multifactorial mechanism involving anti-inflammatory, analgesic, Antioxidant and wound healing effects. Histopathological evaluation further confirmed the restoration of vascular integrity and attenuation of inflammatory cell infiltration in treated groups compared to controls. These results substantiate the ethnomedicinal use of C. gigantea in the management of hemorrhoids and highlight its potential as a candidate for the development of plant-based antihemorrhoidal therapeutics. However, while the preclinical data are promising, further research is warranted to isolate and characterize the specific active constituents responsible for the observed effects. Additionally, comprehensive pharmacokinetic, toxicological, and clinical evaluations are necessary to fully establish the therapeutic potential and safety profile of C. gigantea in human subjects. In future it open a gate for researchers to find potential therapeutic effects due to presence of various secondary metabolites.

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Conflict of Interest

Authors are declared that no Conflict of Interest

Informed Consent and Ethical Statement

Not Applicable

Author Contribution

Both authors are contributed equally.

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