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Anti-hemorrhoidal activity of *Cyperus rotundus* underground stems

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Abstract
Hemorrhoids are a common anorectal disorder characterized by inflammation, pain, and bleeding due to varicosity of rectal veins. The present study investigates the anti-hemorrhoidal activity of hydroalcoholic and aqueous extracts of *Cyperus rotundus* underground stems. Anti-inflammatory assays (protein denaturation inhibition), rectoanal coefficient studies, and histopathological examinations were conducted. The hydroalcoholic extract showed significant inhibition of protein denaturation (63%) compared to the aqueous extract (53%) and was comparable to Aspirin (76%). *In vivo* studies revealed dose-dependent protection, with 400 mg/kg extract showing activity similar to standard Pilex ointment. Histological examination confirmed reduced mucosal damage and inflammation. The findings suggest that *Cyperus rotundus* exhibits promising anti-hemorrhoidal potential, likely due to its anti-inflammatory phytoconstituents.

Keywords: *Cyperus Rotundus*, hemorrhoids, anti-inflammatory, rectoanal coefficient, herbal medicine

Introduction

Hemorrhoids, also known as piles, are swollen and inflamed vascular structures in the anorectal region that are normally present as part of the human anatomy and play a vital role in maintaining continence. When these vascular cushions become enlarged or symptomatic, they are referred to as pathological hemorrhoids. Hemorrhoids are broadly categorized based on their location relative to the dentate (pectinate) line into internal and external hemorrhoids. Internal hemorrhoids are located within the rectum above the dentate line and are covered by columnar epithelium, which is innervated by visceral sensory nerves, making them typically painless even when engorged or inflamed. The internal hemorrhoidal plexus lies in the submucosal layer and consists of arteriovenous channels supported by connective tissue and smooth muscle, forming structures known as anal cushions. These cushions, found at the classical anatomical positions of 3, 7, and 11 o'clock, are essential for anal continence by maintaining resting anal pressure and aiding in sealing the anal canal. (Skandalakis *et al*.,1993) Pathological internal hemorrhoids arise when these cushions undergo enlargement and distal displacement due to a multifactorial pathophysiology involving mechanical, vascular, genetic, and lifestyle factors. Key contributors include prolonged sitting or standing, increased intra-abdominal pressure from straining, constipation, pregnancy, or obesity, chronic diarrhea, and genetic predispositions that weaken connective tissue. These factors disturb normal venous outflow, leading to venous engorgement, inflammation, and loss of elasticity in the supporting structures, culminating in prolapse. Internal hemorrhoids are graded based on the degree of prolapse: Grade I remain within the anal canal; Grade II prolapses during defecation but reduces spontaneously; Grade III requires manual reduction; and Grade IV is permanently prolapsed and may lead to complications like thrombosis and strangulation. (Thomson, 1975) Clinical symptoms vary depending on the grade and include painless bright red rectal bleeding, mucus discharge, prolapse, pruritus ani, and rarely pain—unless complicated. Diagnosis involves thorough history taking focused on bleeding characteristics, prolapse behavior, and associated symptoms, followed by physical examination, including digital rectal examination and visualization with anoscopy or proctoscopy. Colonoscopy may be indicated to exclude other causes of rectal bleeding, especially in older patients or those with red flag symptoms. Treatment is guided by symptom severity and hemorrhoid grade. Early-stage hemorrhoids (Grades I and II) are often managed conservatively with lifestyle and dietary modifications such as a high-fiber diet, adequate hydration, avoiding straining, and maintaining regular bowel habits. Medications like topical corticosteroids, anesthetics, and oral venotonics (e.g., diosmin and hesperidin) are used to control symptoms. Minimally invasive procedures, including rubber band ligation, sclerotherapy, and infrared coagulation, are effective for Grades I to III hemorrhoids and are typically performed in outpatient settings. Surgical management, such as hemorrhoidectomy or stapled hemorrhoidopexy, is reserved for Grade III and IV cases or for patients who do not respond to less invasive treatments. If left untreated, internal hemorrhoids may lead to complications like chronic anemia from persistent bleeding, thrombosis, strangulation, or infection. Prognosis is generally good with appropriate treatment, though recurrence is common without addressing underlying causes. Prevention focuses on a high-fiber diet, proper hydration, regular physical activity, good toilet habits, avoiding prolonged sitting or straining, and maintaining a healthy weight. In contrast, external hemorrhoids are located beneath the skin around the anus, distal to the dentate line, and are covered by squamous epithelium richly innervated by somatic sensory nerves, which makes them more likely to cause pain, swelling, and discomfort compared to internal hemorrhoids. This study confirms the significant anti-hemorrhoidal and anti-inflammatory activity of *Cyperus rotundus*, specifically its underground stems, with the hydroalcoholic extract at a dose of 400 mg/kg showing effects comparable to the standard drug Pilex ointment. Selected based on literature and consultation with medical practitioners in Indore (M.P.), the underground stem—comprising corms and rhizomes—was collected in July 2024, authenticated (Voucher specimen No. ISO/Bot/03), and analyzed for morphological and physicochemical properties. (Sardinha TC *et al*., 2002) [1]

Methods and materials

1. Selection of plant materials

Cyperus Rotundus underground stems were selected for the present study, based on their utility as anti-inflammatory agent. There are no scientific reports available depicting their efficacy in hemorrhoids containing these plants parts.

2. Collection and authentication of plant material

Plants parts were collected in the month of Jul 2024 from local market of Indore (M.P.). All these plants parts were identified and authenticated by. Voucher specimens of the plant parts were submitted in Govt. Holkar (Model, Autonomous) Science College, Indore (M.P.) with voucher specimen No. ISO/Bot/03; for reference purpose.

3. Extraction of underground stems with various solvents

The underground stems have been dried and powdered separately. Underground stems were subjected to hot extraction process has been done. 50 gms of dried shade powder was exhaustively extracted with chloroform, ethyl acetate, ethanol and water using soxhlet extraction apparatus. The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts. (Shahid-Ud-Duaula AFM *et al*., 2009,) The percentage yield of each extract was calculated by using the formula: [8, 10]

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

4. Preliminary phytochemical screening of plants extracts

The powdered plant extracts underwent standard phytochemical screening to identify bioactive compounds. Terpenes, flavonoids, saponins, steroids, glycosides, proteins, reducing sugars, carbohydrates, tannins, phenols, and alkaloids were detected using specific colorimetric and precipitation tests. These analyses confirmed the rich phytochemical profile of *Cyperus rotundus* extract. (Obianime AW *et al*., 2008,)

5. Acute Toxicity Study

All these plants parts were identified and authenticated by. Voucher specimens of the plant parts were submitted in Govt. Holkar (Model, Autonomous) Science College, Indore (M.P.) with voucher specimen No. ISO/Bot/03; for reference purpose. Acute toxicity studies on *Cyperus rotundus* have generally demonstrated that the plant extracts are safe and well-tolerated at therapeutic doses. For example, a study published in Toxicology Reports (2017) evaluated the acute oral toxicity of *Cyperus rotundus* ethanolic extract in rodents and found no mortality or significant adverse effects even at high doses (up to 2000 mg/kg body weight). The animals showed no signs of toxicity, behavioral changes, or organ damage during the observation period, suggesting a wide safety margin for traditional and medicinal use. These findings support the safe application of *Cyperus rotundus* in herbal formulations, although further studies are recommended to confirm long-term safety and effects. *(*Toxicology Reports*,* 2017). All *in-vivo* and *in-vitro* experiments creatures were given by the Swami Vivekanand college of pharmacy Indore (1839/PO/Re/S/2015/CCSEA). The Institutional Animal Ethical Committee (Protocol No. IAEC/SVCP/2024/July/03) endorsed the system. All creature observed CPSEA guidelines.

6. *In-vitro* anti-inflammatory activity

The *in vitro* anti-inflammatory activity of the plant extract was evaluated using the albumin denaturation method with slight modifications (Ruiz-Ruiz *et al*., 2017) [31]. Extracts and aspirin (50–600 µg/mL in DMSO) were mixed with 1% bovine albumin, pH adjusted to 6.3, and incubated at 37°C for 20 min, then heated at 51°C for 30 min. After cooling, absorbance was measured at 660 nm, and the percentage inhibition of protein denaturation was calculated. The findings were presented as IC50 value of the hydroalcoholic extract of *Cyperus rotundus* was found to be 118.4 µg/mL, where aspirin was referred to as the positive control and DMSO as the negative control.

$$\text{Percentage inhibition (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

where A control is the absorbance of negative control (i.e., DMSO) and A sample is the absorbance of the Plant extract **(**Ruiz-Ruiz JC *et al*., 2017) [31].

7. *In vivo* anti-inflammatory activity Animals

As per literature survey the wistar albino rat are suitable for the evaluation of anti-inflammatory activity, so wistar albino rat will be used as experimental model.

8. *In-vivo* activity

This study will be conducted using healthy wistar albino rat of either sex with body weight 150-250gm. The wistar albino rat will be kept in clean and dry cages and maintained in a well-ventilated, temperature controlled 22º C (±3ºC) animal house with 12 hours light and 12 hours dark cycles. The animals will be fed with standard pellet diet and water will be given as libitum. For experimental purpose, the rats will be fasted overnight but allowed access to water. (Sarvesh Cn, Jennifer Fernandes 2017).

a. Material required for above objective

Croton Solution (intra rectal), *cyperus rotundus* underground stems extract (200 and 400 mg/kg, oral), Pilex ointment (200 mg/kg intra rectal)

Table 1: Animal study Design

Group	Treatment Description	Dose / Route	No. of Animals
Group I	Control group – received saline only	Saline / Intra rectal	5
Group II	Hemorrhoid control – received croton oil preparation only	Croton oil / Intra rectal	5
Group III	Haemorrhoid induced + treated with *Cyperus rotundus* extract (CRE)	CRE 200 mg/kg / Oral	5
Group IV	Haemorrhoid induced + treated with *Cyperus rotundus* extract (CRE)	CRE 400 mg/kg / Oral	5
Group V	Haemorrhoid induced + treated with Pilex ointment	Pilex 200 mg/kg / Intra rectal	5
	Total		25

9. Experimental Method

Histological studies were conducted on rectal tissues of croton oil-induced hemorrhoidal rats to identify inflammation-related markers. The study involved 25 albino rats, randomly divided into five groups (n=5), each receiving treatment for 5 days. Hemorrhoids were induced via intra-rectal application of croton oil. Group I (normal control) received saline; Group II (positive control) received croton oil; Group III and IV received 200 mg/kg and 400 mg/kg of the test extract orally; Group V (standard) received Pilex ointment (200 mg/kg, intra-rectally). At the end of the study, all animals were sacrificed except Group I, and 20 mm recto-anal tissue samples were collected and preserved in 10% neutral buffered formalin for histopathological examination. In many experimental hemorrhoid models, researchers use weight – based ratios such as tissue weight/body weight – as a quantitative marker of inflammation severity. For instance, measuring the rectoanal coefficient in this way allows a decrease in RAC to reflect edema and swelling (Bartels *et al*., 2021; Budiono *et al*., 2021). In our study, we adapet this principle via a length-to-weight ratio:

$$RAC = \frac{\text{Length of rectoanal tissue (cm)}}{\text{Weight of rectoanal tissue (mg)}}$$

The following parameters will be measured-
Histopathological studies will be carried out on recto-anal tissues.

Recto-anal tissues (20 mm) were dissected and weighed after sacrificing the animals after anesthetizing them using thiopental sodium. After placing a small portion of the tissue in a 10% formaldehyde solution for histological examination, the rest was stored at –20°C to estimate biochemical parameters. For determining the recto-anal coefficient (RAC), the recto-anal tissues of the rats were weighed and compared with the individual body weight of the rats, and the RAC was calculated using the formula: The transverse section of recto-anal tissue was taken using a microtome and then examined for inflammatory cells, necrosis, congestion, hemorrhage, and vasodilatation **(**Azeemuddin *et al*., 2014**)** [30]

Result

1. Selection of the plant

On the ground of literature review and deep discussion with medical practitioners of the Indore (M.P.) *Cyperus rotundus* undergrounds stem was selected for evaluation of the anti-inflammatory activity.

2. Collection and authentication of plant materials:

All the plants parts were collected in the month of July 2024 from local market of Indore (M.P.). All these plants parts were identified and authenticated by Govt. Holkar (Model, Autonomous) Science College, Indore (M.P.). Voucher specimens of the plant parts were submitted in Botanical department with voucher specimen No. ISO/Bot/03; for reference purpose.

Table 2: Percentage yield and extractive values of plant extract

S. No.	Solvent	% Yield	Extractive value	
			Water soluble extractive	Alcohol soluble extractive
1	Hydroalcoholic (1:1)	49.74	49	47

Table 3: Phytochemical evaluation of plants extract

S. No.	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
1	Carbohydrate	Molisch's test	+	-	+	+	+
		Fehling's test	+	+	+	+	+
2	Glycosides	Legal's test	-	+	+	+	+
		Borntrager's test	+	+	+	+	+
		Baljet test	+	+	+	+	+
3	Fixed oil and Fats	Spot test	-	+	+	+	+
		Saponification test	+	+	-	+	+
4	Proteins and Amino Acids	Biuret test	-	+	+	+	+
5	Saponins	Foam test	+	+	+	+	+
6	Phenolic Comp. and Tannins	$FeCl_3$ test	+	+	+	+	+
7	Steroids	Libermann-bucchard test	-	-	-	-	-
8	Alkaloids	Dragendorff's test	+	+	+	-	-
		Mayer's test	-	+	+	+	+
		Wagner's test	+	+	+	+	+
9	Terpines		+	+	-	+	+
10	Flavonoids	Lead acetate test	+	+	+	+	+
		Con. H_2SO_4 test	+	+	+	+	+
		FeCl3 test	+	+	+	+	+

3. *In-vitro* anti-inflammatory activity

Protein denaturation causes inflammation, and plant extracts' ability to inhibit heat-induced albumin denaturation was tested for anti-inflammatory activity. The hydroalcoholic extract showed the highest inhibition (63% at 500 µg/ml), followed by water extract (53%). Ethanolic, ethyl acetate, and chloroform extracts showed moderate to low activity. Aspirin showed 76% inhibition at 100 µg/ml. This highlights the strong anti-inflammatory potential of the hydroalcoholic extract.

4. *In-vivo* anti-inflammatory activity

From the observations, Croton oil preparation caused a significantly increased extravasation of Evans blue dye as

observed from the recto-anal tissues of the negative control as compared to the normal control. However, on treatment with plant hydroalcoholic extract (CRE 200, and CRE 400 mg/kg; oral) and Pilex ointment (200 mg/kg Intra rectal), a significant reduction in Evans blue concentration was noted as compared to the negative control. From the overall observation, extract at 400 mg/kg; p.o. was found to be most effective, which was quite comparable with the standard Pilex ointment.

Table 4: *In-vivo* anti-inflammatory activity

S.no	Hemorrhoidal Parameters	RAC (Mean ± SD) (The Recto anal Coefficient)
1	NC (negative control)	1.192 ± 0.032
2	DC (disease control)	3.84 ± 0.218
3	CRE (*Cyperus rotundus* extract) 200	1.72 ± 0.12
4	CRE (*Cyperus rotundus* extract) 400	1.12 ± 0.11
5	Pilex200	1.14 ± 0.12

The Rectoanal Coefficient (RAC) is a critical parameter for assessing the severity of hemorrhoidal conditions. The data reveals significant differences across the treatment groups. In the negative control (NC) group, the RAC was 1.192 ± 0.032, representing baseline values in the absence of induced severity. The disease control (DC) group demonstrated a markedly elevated RAC of 3.84 ± 0.218, indicating severe rectoanal damage associated with untreated hemorrhoids. Treatment with *Cyperus rotundus* extract (CRE) at doses of 200 mg/kg and 400 mg/kg reduced the RAC to 1.72 ± 0.12 and 1.12 ± 0.11, respectively, suggesting dose-dependent efficacy in mitigating rectoanal damage. Similarly, the standard treatment group, Pilex 200 mg/kg, showed a RAC of 1.14 ± 0.12, comparable to CRE400, highlighting its effectiveness as a reference treatment.

These results demonstrate the potential of *Cyperus rotundus*, particularly at higher doses, in reducing hemorrhoidal severity, comparable to standard treatment. Further investigations are warranted to explore its therapeutic mechanisms. In many experimental hemorrhoid models, researchers use weight – based ratios such as tissue weight/body weight – as a quantitative marker of inflammation severity. For instance, measuring the rectoanal coefficient in this way allows a decrease in RAC to reflect edema and swelling (Bartels *et al*., 2021; Budiono *et al*., 2021).

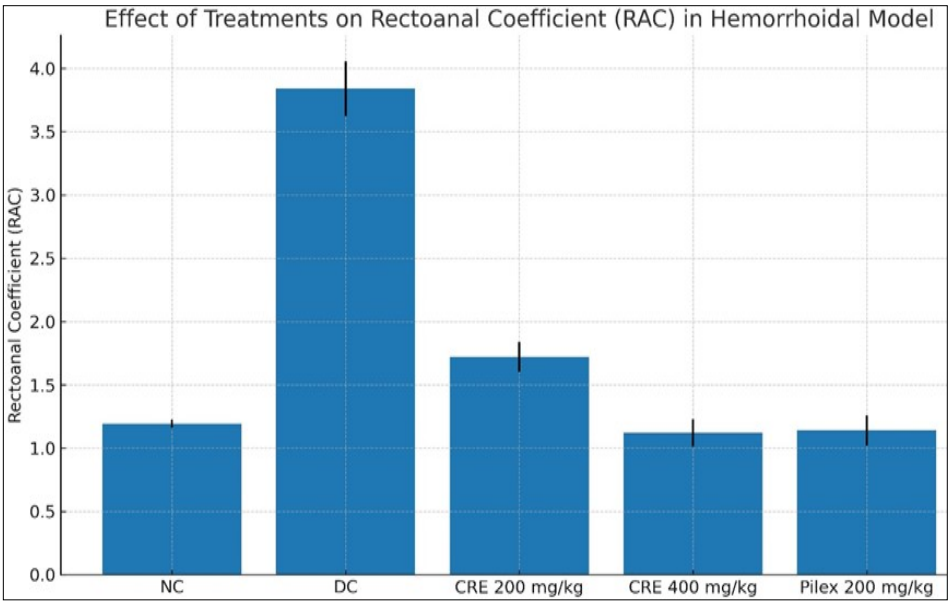

Fig. 1: Results of treatment with (CRE) *Cyperus rotundus* hydroalcoholic extract and Pilex ointment on recto anal tissue concentration.

In the histopathological study, the recto-anal tissue of the normal control group exhibited an intact architecture, with all three layers—muscular, mucosal, and submucosal—well-preserved (Figure 6.2A). In contrast, the COP-treated rats displayed acute inflammatory reactions (Figure 6.2B), characterized by necrosis, severe edema, and degeneration of the mucosal layer. Additional abnormalities included dilated blood vessels, focal areas of congestion, and hemorrhages compared to the normal control. The mucosal layer showed thickening, along with mild to moderate fibroblast proliferation in the inflamed areas.

In treatment group III (200 mg/kg ORAL), the recto-anal tissue exhibited less severe lesions compared to the negative control group, with minimal to mild histological damage (Figure 6.2C). Groups IV and V, treated with CRE 400 mg/kg and Pilex ointment, respectively, showed moderate to marked protection against histological damage, indicating significant tissue recovery (Figures 6.2D and E).

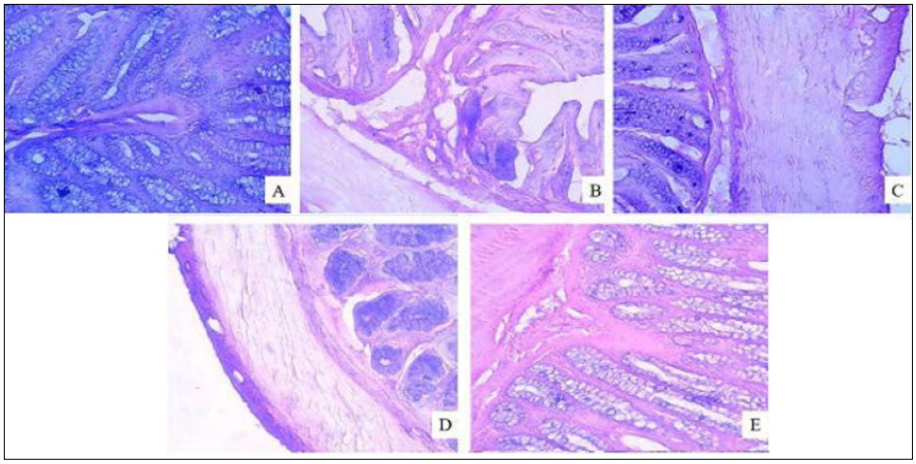

Fig. 2: Effect of CRE on Recto-Anal Tissue Histology

Microscopic images (40× magnification) of recto-anal tissue sections illustrating the impact of CRE in a rat model of COP-induced hemorrhoids:
(A) Displays the intact rectal architecture in the normal control group.
(B) Depicts severe inflammation and significant dilation of blood vessels in the disease control group.
(C) Shows moderately intense inflammation and blood vessel dilation in rats treated with CRE at 200 mg/kg orally.
(D and E) Highlight minimal inflammation and slight blood vessel dilation in rats treated with CRE at 400 mg/kg and Pilex at 200 mg/kg, respectively.

7. IC50 value of *Cyperus rotundus*

The hydroalcoholic extract of *Cyperus rotundus* showed moderate anti-hemorrhoidal activity with an IC_{50} of 118.4 µg/mL. It demonstrated dose-dependent efficacy, improving rectoanal coefficient and physiological markers. These results highlight its therapeutic potential and support its traditional use for hemorrhoid treatment. (Ruiz-Ruiz JC *et al*., 2017) [31].

8. Acute Toxicity Study of IC50 value

An acute oral toxicity study of the hydroalcoholic extract of *Cyperus rotundus* was conducted in accordance with OECD Guideline 423 for testing chemicals. The extract was administered in single doses up to 2000 mg/kg body weight in experimental animals, and no mortality or signs of toxicity were observed during a 14-day observation period. These results indicate a high safety margin for the extract at therapeutic doses. Furthermore, the extract showed moderate anti-hemorrhoidal activity with an IC_{50} value of 118.4 µg/mL, demonstrating effective pharmacological action at non-toxic concentrations (Ruiz-Ruiz JC *et al*., 2017) [31]. These findings support the traditional use of *Cyperus rotundus* and highlight its potential for safe, natural treatment of hemorrhoids.

Discussion

This study evaluated the anti-inflammatory and anti-hemorrhoidal effects of the hydroalcoholic extract of *Cyperus rotundus*, traditionally used in Ayurveda for inflammatory and gastrointestinal disorders. The underground stem was selected based on ethnomedicinal relevance, and authenticated through morphological, physicochemical, and phytochemical analyses. Phytochemical screening revealed flavonoids, tannins, saponins, terpenes, glycosides, and alkaloids, known for anti-inflammatory and venotonic properties relevant to hemorrhoid treatment. The extract inhibited heat-induced albumin denaturation *in vitro*, showing 63% inhibition at 500 µg/mL, close to aspirin's 76% at 100 µg/mL, confirming its anti-inflammatory potential. *In vivo*, the extract significantly reduced rectoanal inflammation in a croton oil-induced hemorrhoid model, with dose-dependent decreases in Rectoanal Coefficient (RAC): 1.72 (200 mg/kg) and 1.12 (400 mg/kg) versus 3.84 in controls. The 400 mg/kg dose matched the standard treatment Pilex (1.14), highlighting its therapeutic efficacy. Histopathology showed reduced tissue damage and preserved mucosa in treated groups. The extract's IC_{50} was 118.4 µg/mL, indicating moderate inhibitory activity consistent with in *vivo* results. This suggests the extract acts by stabilizing proteins and reducing inflammation and capillary permeability. Overall, the study supports the dose-dependent anti-inflammatory and anti-hemorrhoidal effects of *Cyperus rotundus* extract, attributed to its phytochemicals, validating its traditional use and potential for therapeutic development.

Conclusion

This study found that the underground stems of *Cyperus rotundus* (collected in July 2024) have strong anti-inflammatory and anti-hemorrhoidal effects, especially in a hydroalcoholic extract at 400 mg/kg, which worked as well as the standard drug Pilex. The extract was rich in useful plant compounds like flavonoids, tannins, and alkaloids. In lab tests, it showed high ability to reduce inflammation, similar to aspirin. In animal tests, it reduced tissue damage and swelling caused by hemorrhoids, with better results at higher doses. Tissue studies also showed less damage and better healing in treated groups, supporting its use as a natural and safe treatment for hemorrhoids, though more clinical research is needed.

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- **Conflicts of interest:** The authors disclose that they do not have any conflicts of interests.

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