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Scope

The Journal of Ethnopharmacology is dedicated to the exchange of information and understandings about people's use of plants, fungi, animals, microorganisms and minerals and their biological and pharmacological effects based on the principles established through international conventions. Early people confronted with illness and disease, discovered a wealth of useful therapeutic agents in the plant and animal kingdoms. The empirical knowledge of these medicinal substances and their toxic potential was passed on by oral tradition and sometimes recorded in herbas and other texts on materia medica. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine) came into use through the study of indigenous remedies. Chemists continue to use plant-derived drugs (e.g., morphine, taxol, physostigmine, quinidine, emetine) as prototypes in their attempts to develop more effective and less toxic medicinals.
Histomorphometric study of ethanolic extract of *Graptophyllum pictum* (L.) Griff. leaves on croton oil-induced hemorrhoid mice: A Javanese traditional anti-hemorrhoid herb

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1. Introduction

Hemorrhoid is a common disease from which humans have suffered through the ages. Chronic progression of anorectal ailment is caused by various factors that require intensive medical intervention and may cause socio-economical loss. Until now, patients continue to seek better healing treatment, both surgical and non-surgical methods to balance patient satisfaction, postoperative complications, pain, and relapse rate (Cuk et al., 2015).

One of the features of hemorrhoids is swollen veins in the rectum or anus. Internal hemorrhoid that involves the veins far inside of rectum do not hurt because of a lack of pain-sensing nerves but often causes bleeding and the urge to defecate. The recurrence of internal hemorrhoid increases the size and swells out from the rectum, which induces itch and pain. External hemorrhoids involving a vein outside of the anus, where sufficient pain-sensing nerves exist, cause itch and severe pain (Mounsey et al., 2011; Sun and Migaly, 2016; Zaman et al., 2015).

Hemorrhoids are caused by the disintegration or alteration in the anal cushion support tissue such as abnormal venous dilatation, vascular thrombosis, degeneration of collagen fibers and fibroelastic tissue,
distortion, and rupture of the rectal sub-epithelial muscle. It is also found severe inflammation in blood vessel walls and connective tissue (Loder et al., 1994; Lohsiriwat, 2012; Sardinias et al., 2016; Sun and Migaly, 2016).

Various nutritional therapies and plant extracts for hemorrhoids had been carried out and showed significant results even though scientific evidence was still lacking (Mackay, 2001). Some plant extracts containing anti-inflammatory and antioxidative constituents have the potential for hemorrhoidal therapy and have also been shown to have increased vascular tone, capillary flow, strengthening connective tissue, and perivascular microcirculation. These plant extracts are used in various forms, both orally and topically, but only a few plants have been studied scientifically (Yildirim et al., 2017).

A variety of hemorrhoid therapy using both oral and topical medications has been known until now in traditional or modern ways. In general, these drugs are intended to relieve hemorrhoid symptoms, but unfortunately, they are unsuccessful in many cases. The combination of oral and topical medications aims to increase the effectiveness of hemorrhoid treatment, avoiding invasive surgery (Misra and Imitlemu, 2005).

_Gnaphalium pictum_ (L.) Grif. is a species of Acanthaceae family known as “handeuleum” in West Java and “Daun Ungu” in Indonesia (Levang and Foresta, 1991; Ramdhani et al., 2015). _G. pictum_ leaves (GPL) have historically been used to treat hemorrhoids (Heyne, 1987; Ministry of Health, 2010). The determination of analgesic and anti-inflammatory capabilities (Ozaki et al., 1989), the analysis of phagocytosis behavior, and immunoglobulin formation (Kusumawati et al., 2002) and the activity on the classical pathway of complement and chemoattractant activity (Kusumawati et al., 1997) are just some of the pharmacological activities relevant to hemorrhoid. However, further scientific evidence is still required to prove its effectiveness for future development as an anti-hemorrhoid remedy.

The chemical content of GPL has been briefly reported in several studies. GPL contains essential oils such as phytol (75.7%), n-nonacosane (6.5%) and hexahydrafarnesyl acetone (2.6%) (Jiangseubchatveera et al., 2015) and other chemical substances such as myricetin and kaempferol (Kusumawati et al., 2002), alkaloid, glycoside, steroid, saponin, tannin, calcium oxalate (Ministry of Health, 2010). Ozaki suggests that the flavonoid compounds in GPL play a role in anti-inflammatory activity (Ozaki et al., 1989). Flavonoids from Ginkgo biloba have been shown to increase venous tone and lymphatic drainage, decreasing capillary hyperpermeability from inflammatory processes, and have been successfully used in clinical trials in hemorrhoidal patients (Misra and Imitlemu, 2005; Zaman et al., 2015).

The purpose of this study is to determine the histological activity of ethanol extract of GPL (GPLE) given orally, topically, and combination (orally-topically) in croton oil-induced hemorrhoid mice. Hemorrhoid symptoms are bleeding, itching, and pain due to hard stools (Yamana, 2017), so the astringency properties and hemostasis-associated activity of GPLE were also discussed.

## 2. Materials and methods

### 2.1. Drugs and chemical

Folin-Ciocalteu and DPPH reagents were obtained from Sigma Co. chemicals. Betamethasone was purchased from PT. Kimia Farma, Indonesia. All other chemicals are the highest purity and analytical grade.

### 2.2. Plant material and extract preparation

The GPL was obtained from a farm in Lawang tea plantation, Malang, East Java, Indonesia, in October 2017. A voucher specimen (RM GP102017) was identified and deposited in the Herbarium of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University. The dried GPL was grounded into a powder. The powder (100 g) was extracted using 70% ethanol (plant: solvent, 1:10, w/v), in a microwave (30% generator power), for 1 min. The extracts were dried by evaporating the solvent under reduced pressure then freeze-dried.

### 2.3. Determination of total flavonoid content

According to Christ and Müller’s method (Christ and Müller, 1960), the aglycone of flavonoid was released by acid hydrolysis of the GPLE, and then reacted with AlCl₃ in a methanol-ethyl acetate-acetic acid solvent to form a complex. The specific absorbance of the complex was measured using a spectrophotometer at 425 nm. The experiments were conducted in quintuplicate, and the amount was expressed as hyperoside equivalent (HE, mg/100 g samples) (Jafari et al., 2010; Nan et al., 2012).

### 2.4. Determination of total phenolic content

The measurement of total phenolic contents in GPLE was performed using a spectrophotometer as gallic acid equivalents using the Folin-Ciocalteu reagent according to the standard method (Singleton and Rossi, 1965). Gallic acid (10–500 mg/L) was used for a standard calibration. Folin-Ciocalteu solution (1:10 v/v in water) and sodium bicarbonate solution (7.5% w/v) were used as reagents. Each sample and standard (40 μL) was mixed with 1.8 mL of Folin-Ciocalteu reagent for 5 min at room temperature, and then added 1.2 mL of sodium bicarbonate. After 60 min, the absorbances were measured at 765 nm. The results were expressed as gallic acid equivalent (GAE, mg/100 g samples) (Odeh et al., 2014).

### 2.5. DPPH radical scavenging activity

The antioxidant activity was evaluated based on DPPH radical scavenging activity assay (Kusumawati et al., 2018; Kusumawati and Indrayanto, 2013). The GPLE solution was mixed with 100 μL freshly prepared DPPH methanolic solution (250 mM) on a 96-well microplate in triplicate. After incubation in the dark for 30 min, the remaining DPPH radical was evaluated from the absorbance at 515 nm, using a Multiscan Go Thermo Scientific microplate reader. DMSO was used as a negative control and Trolox as a positive control. The IC₅₀ of the inhibition ratio was determined by linear regression.

### 2.6. Measurement of astringent properties

Astringent activity-induced vasoconstriction was an essential factor in hemostasis (Nabavizadeh et al., 2016). Astringency activity was determined using the milk precipitation method (Dandjesso et al., 2012; Kloot et al., 2012). Briefly, 1 mL of GPLE solution (5%) was put in the tube, added with 100 μL milk and homogenized, allowed to stand for 3 min, and centrifuged for 1 min at 3000 rpm. The formation of a pellet was observed as the astringent activity.

### 2.7. Measurement of plasma recalification time

Plasma recalification time (PRT) was measured to determined sample-induced effect in the clotting time of Plasma Poor Platelet (PPP) following activation of prothrombin (Factor II) by the addition of Ca²⁺ (Elahi et al., 2014). In this method, 0.1 mL PPP (defrosted and incubated at 37 °C) and 0.1 mL of the different sample solutions were combined well in test tubes (8 mm diameter). After incubation for 5 min in a 37 °C water bath, 0.1 mL CaCl₂ solution (0.025 mM) was added to each tube. The time necessary for silky fibrin formation was recorded as the PRT. Saline was used as a negative control and tranexamic acid as a positive control.
2.8. Animals and histomorphologic analysis of croton oil-induced hemorrhoid mice

Male mice (ICR), aged three months and weighing 20–25 g, were used in this study. They were obtained from the laboratory of the animal center, Faculty of Pharmacy, Airlangga University. Animal experiments were designed based on the ethical standards for animal use and were approved by the Airlangga University Ethical Committee of Animal Experimentation (protocol number 2.KE.88.05.2018). For the oral group, GPLE suspension was given to mice once daily with oral gavage at the same time without fasting. The dosage volume was set at 10 mL/kg of the body weight. For the topical group, the GPLE gel was set at 250 mg/kg and applied intrarectally on mice once daily at the same time. All samples were applied for 14 days.

2.8.1. Experimental design

Experiments were carried out using croton oil-induced hemorrhoid mice model based on the modified method described by Nishiki et al. (1988) and Azeemuddin et al. (2014). Mice were randomly divided into eight groups (8 animals each). The normal (N) group was healthy animals. For the other groups, hemorrhoid was induced by rubbing a sterile cotton swab into the anorectal for 10 s once a day for five days with a mixture of croton oil (deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in a ratio of 1: 4: 5: 10). After five days, croton oil-induced hemorrhoid mouse was randomly divided into seven groups and treated daily with different samples for 14 days as follows:

2.8.2. Histomorphological study of anorectal

On the 14th day, 1 h after the treatment, rat anorectal histology samples were obtained by fixing rat anorectal biopsies into 10% formalin solution, paraffin embedding, dissection and hematoxylin-eosin staining. All sample histology slides were observed using an Inverted system microscope, IX71-IX2 series optical microscope, the DP71 camera, and Cell D software (Olympus; Shinjuku-ku, Tokyo, Japan). Histological parameters such as the number of inflammatory cells, congestion, bleeding, vasodilation, and necrosis are observed in the histological preparations of anorectal tissue (Nishiki et al., 1988; Azeemuddin et al., 2014).

2.9. Statistical analysis

The results are expressed as means ± SD (standard deviation of the mean). Statistical differences between groups were estimated using a one-way analysis of variance (ANOVA) with Tukey’s test and were considered statistically significant at p < 0.05.

3. Result

3.1. Chemical content in GPLE

The percentage of GPLE obtained with the extraction process was 17.2% (w/w). Ozaki et al. suggest that flavonoids are responsible for the anti-inflammatory activity of the extract (Ozaki et al., 1989). Therefore, the total flavonoid and total polyphenols levels in GPLE were evaluated at first. The results indicated a presence of a significant amount of total flavonoids (16.3 ± 0.79 mg/g HE) and phenolic compounds (428.3 ± 18.01 mg/g GAE) in GPLE.

3.2. Astringent properties and PRT activity of GPLE

Plasma coagulation time was determined as the ability of the extract to stop bleeding. In the present result, the PRT of GPLE and control were...

Table 1

Mice grouping and treatment given to each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Name of group</th>
<th>Oral</th>
<th>Drug</th>
<th>Sample</th>
<th>Topical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vehicle</td>
<td>Drug</td>
<td>Sample</td>
<td>Base</td>
</tr>
<tr>
<td>Group I</td>
<td>C group</td>
<td>0.05% of CMC-Na</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Group II</td>
<td>OB group</td>
<td>–</td>
<td>(0.065 mg/kg)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Group III</td>
<td>TB group</td>
<td>–</td>
<td>(0.065 mg/kg)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Group IV</td>
<td>OTB group (combination)</td>
<td>–</td>
<td>–</td>
<td>166.4 mg/kg</td>
<td>–</td>
</tr>
<tr>
<td>Group V</td>
<td>OG group</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>166.4 mg/kg</td>
</tr>
<tr>
<td>Group VI</td>
<td>TG group</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Group VII</td>
<td>OTG group (combination)</td>
<td>–</td>
<td>–</td>
<td>166.4 mg/kg</td>
<td>–</td>
</tr>
</tbody>
</table>

(–): not given.
0.46 and 2.12 min, respectively. GPLE showed a significant reduction of PRT to 21.7% (78.3% reduction) of the control (Table 2).

3.3. Antioxidant activity of GPLE

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical is a stable free radical with intense purple color and reacts with a hydrogen atom to form DPPH (1,1-diphenyl-2-picrylhydrazine, pale yellow). Trolox is an analog of vitamin E and is used as a reference compound. The dose-dependency of GPLE was evaluated by in vitro assay, and the result was shown in Fig. 1. Both Trolox and GPLE showed an almost linear response with the correlation coefficient value ($r$) > 0.95 (Table 3).

The IC$_{50}$ value of GPLE was 143.0 ± 1.04 μg/mL, while Trolox was 13.8 ± 0.46 μg/mL. The activity of GPLE was weaker than Trolox. However, given that the GPLE was a crude mixture, the activity appeared was promising for the efficient control of oxidative stress.
during the progression of hemorrhoids.

3.4. Analysis of anorectal histopathology of in mice hemorrhoid model

The mice pathological model of hemorrhoids was created by the application of croton oil at the anorectal region for five days. The symptoms of hemorrhoids were recorded in photographs after 14 days of treatment with each prescription (Table 1 and Fig. 2). The vehicle treatment group, after the induction of hemorrhoids, showed severe inflammation as a red swelling (C). On the other hand, both groups treated with the positive control, betamethasone (OTB, TB, and OB), or GPLE (OTG, TG, and OG) showed significant improvement. The combination of oral and topical applications (OTB and OTG) seems better than single usages such as TB, OB, TG, and OG.

Histological analysis was then carried out quantitatively. The thickness of mucosa and muscularis externa were measured from the photograph to describe the effect of the treatments. The five days croton oil treatment apparently induced inflammation and hypertrophy of goblet cells (G), mucosa (M), and muscularis externa (MES) in the control group (C) compared to the normal mouse (N) (Fig. 3).

The treatment with betamethasone (B) or GPLE (G) significantly reduced the thickness of the mucosa (Fig. 4 and Table 4) and muscularis externa (Table 5). In Fig. 4, croton oil caused an increase in the mucosal thickness of mice anorectal into 360.7 ± 39.5 μm (C), which is 3.6 times thicker than normal mice (108.0 ± 7.1 μm) (N). After oral and topical combination application of GPLE extract (OTG) for 14 days, there was a decrease of mucosal thickness to 151.0 ± 30.8 μm, whereas single topical (TG) or oral (OG) application of extract showed a decrease to 233.7 ± 35.4 μm and 237.4 ± 26.0 μm, respectively. These ameliorations in mucosal thickness by GPLE application showed significant differences compared with the control group (C). In addition, the oral and topical combination of GPLE (151.0 ± 30.8, OTG) was comparable to the clinical drug betamethasone (135.2 ± 27.6, OTB) (Table 4).

Other symptoms were seen in the mucosa and muscularis externa as hemorrhage and infiltration of inflammatory cells (Figs. 5 and 6), which indicated the severity of inflammation in the mucosa and muscularis externa in the anorectal region. The area of hemorrhage and the number of inflammatory cells were then counted per 10,000 μm² for evaluating the effectiveness of GPLE application (Tables 4 and 5). All treatments showed a significant reduction in hemorrhage area and the number of inflammatory cells compared to the control group (C) (1653.0 ± 103.8 μm²/10,000 μm² and 33.6 ± 3.6 cells/10,000 μm² in the mucosa, and 369.0 ± 34.1 μm²/10,000 μm² and 7.08 ± 0.70 cells/10,000 μm² in muscularis externa, respectively) (Tables 4 and 5). The combination therapy of GPLE (OTG) dramatically decreased these symptoms both in the mucosa and muscularis externa (212.1 ± 14.0 μm²/10,000 μm² and 13.0 ± 6.0 cells/10,000 μm² in the mucosa, and 67.2 ± 7.1 μm²/10,000 μm² and 1.00 ± 0.14 cells/10,000 μm² in muscularis externa, respectively), even though the single applications of GPLE as topical or oral were also significantly effective (Tables 4 and 5). Besides, the combination therapy of GPLE (OTG) also reduced the number of necrotic cells significantly (0.52 ± 0.11 cells/10,000 μm²) compared to the control (2.88 ± 0.22 cells/10,000 μm²) (Fig. 6 and Table 5).

4. Discussion

A famous Greek physician, Hippocrates, first used the word hemorrhoids from the Greek words, haima (blood) and rheos (flow) because of the characteristic symptom of bleeding from the anus (Jeff, 1987). The prevalence of hemorrhoids is estimated from 4 to 55% of the population, with no significant difference between males and females (Yamana, 2017). Because of a kind of embarrassment, patients tend to relieve their symptoms by self-medication with over-the-counter (OTC), herbal, and ethnomedicines (Donmez 2020). The leaves of G. pictum are traditionally used to treat hemorrhoids in Indonesia (Heyne, 1987; Ministry of Health, 2010). However, sufficient scientific evidence has not been reported so far. Therefore, anti-hemorrhoidal effect of G. pictum was evaluated scientifically in this study, focusing on its antioxidant, astringent, fibrin-forming, and histomorphological amelioration in a croton oil induced hemorrhoid mouse model.

Oxidative stress by reactive oxygen species (ROS) contributes to the initiation and development of various diseases, including hemorrhoids.
Analysis confirmed the antioxidant activity of GPLE (IC₅₀ 18.01 mg/g GAE) as potential natural antioxidants. In actually, DPPH ethanol extract of G. pictum range tests.

Values were expressed as mean ± SD (n = 5); *P < 0.05 using T-tests; NA (not available).

### Table 3

Data used to calculate the IC₅₀ in DPPH assay of GPLE.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Slope</th>
<th>Intercept</th>
<th>r²</th>
<th>Range concentration</th>
<th>IC₅₀ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPLE</td>
<td>0.2807</td>
<td>9.7335</td>
<td>0.9921</td>
<td>40-200 (μg/mL)</td>
<td>143.0 ± 1.04 a</td>
</tr>
<tr>
<td>Trolox</td>
<td>2.5376</td>
<td>15.3030</td>
<td>0.9922</td>
<td>4-20 (μg/mL)</td>
<td>13.8 ± 0.46 a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 5); means in the same column followed by different letters are significantly different at P < 0.05 using Tukey’s multiple range tests.

### Table 4

Histopathology evaluation of anorectal mucosa of mice.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thickness of mucosa (μm)</th>
<th>Hemorrhage area (μm²/10,000 μm²)</th>
<th>Number of inflammatory cells/10,000 μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>108.0 ± 7.1 b</td>
<td>9.3 ± 1.4 b</td>
<td>4.0 ± 0.7 b</td>
</tr>
<tr>
<td>C</td>
<td>360.7 ± 39.5 a</td>
<td>1653.0 ± 103.8 a</td>
<td>33.6 ± 3.6 a</td>
</tr>
<tr>
<td>OTB</td>
<td>135.2 ± 27.6 b</td>
<td>134.3 ± 82.4 c</td>
<td>7.4 ± 1.5 b</td>
</tr>
<tr>
<td>OTG</td>
<td>151.0 ± 30.8 b</td>
<td>221.2 ± 14.0 a</td>
<td>13.0 ± 6.0 ad</td>
</tr>
<tr>
<td>TB</td>
<td>216.4 ± 70.5 d</td>
<td>466.1 ± 97.7 d</td>
<td>12.2 ± 2.4 d</td>
</tr>
<tr>
<td>TG</td>
<td>233.7 ± 35.4 c</td>
<td>599.2 ± 65.9 b</td>
<td>20.6 ± 5.2 d</td>
</tr>
<tr>
<td>OB</td>
<td>237.4 ± 26.0 c</td>
<td>399.2 ± 66.7 b</td>
<td>16.4 ± 2.1 da</td>
</tr>
<tr>
<td>OG</td>
<td>267.7 ± 37.5 s</td>
<td>850.1 ± 13.7 b</td>
<td>26.6 ± 3.1 f</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 5); means in the same column followed by different letters are significantly different at P < 0.05 using Tukey’s multiple range tests.

Thus a sufficient amount of antioxidants is essential to prevent damage to anorectal tissue (Saad and Lamia, 2009; Faudjar et al., 2018). The ethanol extract of G. pictum leaves (GPLE) contained significant amounts of flavonoids (16.3 ± 0.79 mg/g HE) and phenolic compounds (428.3 ± 18.01 mg/g GAE) as potential natural antioxidants. In actually, DPPH analysis confirmed the antioxidant activity of GPLE (IC₅₀ = 143.0 ± 1.04 μg/mL). These results indicate that GPLE contains chemical components that are favorable for reducing oxidative stress in hemorrhoidal tissues.

As for bleeding control, it is thought that astringent activity is related to hemostatic properties in the aspects of vasoconstriction and blood coagulation (Nabavizadeh et al., 2016; Ebrahimi et al., 2020; Dandjesso et al., 2012; Odukoya et al., 2009). The results of the astringency assay clearly showed the positive effect of GPLE (Table 2). In addition, plasma recalcification time (PRT), which is an important parameter of blood coagulation (Abascal and Yarnell, 2005; Ohkura et al., 2015; Ream Nayal and M Yasser Abajy, 2015), has significantly reduced by the treatment with GPLE (0.46 ± 0.19 min) compared to that of control (2.12 ± 0.19 min) (Table 2). These results suggest that GPLE has beneficial features in stopping the bleeding of hemorrhoids by astringency and coagulation activities. However, the most important thing is whether or not it works in animal models, as discussed below.

Croton oil has a strong irritant property in the skin and mucosa and is generally used to induce mice hemorrhoids. In this study, we used a croton oil-induced mouse hemorrhoid model to examine in vivo activity, focusing on the following histological parameters in the anal region, such as a thickness of the mucosa and external muscle, the number of inflammatory cells, the area of bleeding, and necrotic cell number (Figs. 2–4, Tables 4 and 5). The combination of topical and oral GPLE application significantly reduced these symptoms comparable to that of the positive control, betamethasone (Figs. 2–4, Tables 4 and 5). These results strongly support the ethnomedicinal use of G. pictum as a treatment for hemorrhoids.

The release of inflammatory mediators such as prostaglandins, leukotrienes, TNF-α, nitric oxide, and bradykinin are induced by croton oil treatment. These factors regulate the activation of fibroblasts, endothelial cells, monocytes, lymphocytes, and neutrophils, which leads to severe inflammation and hemorrhoids (Azemuddin et al., 2014; Faujdar et al., 2018). Some flavonoids have been reported to have anti-inflammatory activities (Hosek and Smejkal, 2015; Kim et al., 2004). GPLE contains various compounds including flavonoids and polyphenol, which may directly or synergistically regulate the expression and function of these inflammatory mediators, but further investigation of chemical constituents and expression analysis of mRNA and proteins is needed to unveil the detailed mechanisms of this ethnomedicine.

### 5. Conclusion

The ethanol extract of Graptophyllum pictum leaves was suggested to have a therapeutic effect on hemorrhoids by its antioxidant, anti-inflammatory and hemostatic properties. The present study validates the ethnomedicinal use of this plant against hemorrhoids and suggests its therapeutic potential as a promising anti-hemorrhoid agent.

### Declaration of competing interest

There is no conflict of interest

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.114765.
PPP: Plasma Poor platelet  
PRT: Plasma recalcification time  
ROS: Reactive oxygen species  
SEM: standard error of the mean  
TB: topical betamethasone  
TG: topical Graptophyllum pictum leaves extract