Review Article

Potential Use of Flavonoid-Containing Purple Leaf Extract as an Alternative Therapy for Hemorrhoidal Disease

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Abstract

Hemorrhoids are a pathological disease that occurs in the anal cushion, marked by enlargement of vascular tissue and disposition of connective tissue, leading to the swelling of the recto-anal. This condition can cause pain, discomfort, and bleeding during bowel movements. Several risk factors are known to influence these pathological changes, such as pregnancy, constipation, the elderly, and a sedentary lifestyle. Although conventional treatments are commonly used for hemorrhoids, emerging research highlights the potential of Graptophyllum pictum (also known as purple leaf) extract as a treatment. The purple leaf extract possesses antioxidant and anti-inflammatory properties that reduce inflammatory mediators such as TNF-α, IL-6, COX-2, and MMP-9. It could minimize edema by inhibiting leukocyte extravasation to recto-anal tissue. The purple leaf extract contains flavonoids and other phytochemicals that reduce inflammation and other diseases. These findings suggest that purple leaf extract may be a promising alternative hemorrhoid treatment.
Keywords: *hemorrhoids, inflammation, flavonoid, purple leaf.*

Potensi Flavonoid dalam Ekstrak Daun Ungu sebagai Terapi Alternatif untuk Penyakit Hemoroid

Abstrak

Hemoroid merupakan kondisi patologis yang terjadi di bantalan anus, ditandai dengan pelebaran pembuluh darah dan disposisi jaringan ikat yang menyebabkan pembengkakan anorektal. Kondisi tersebut dapat menyebabkan rasa nyeri, ketidaknyamanan, dan perdarahan saat defekasi. Hemoroid dipengaruhi oleh berbagai faktor risiko seperti kehamilan, konstipasi, usia tua, dan gaya hidup sedenter. Meskipun pengobatan konvensional umumnya digunakan untuk Pengobatan hemoroid umumnya menggunakan konvensional, namun studi terbaru mengungkapkan potensi daun ungu (Graptophyllum pictum) untuk pengobatan. Ekstrak daun ungu memiliki aktivitas antioksidan dan anti-inflamasi yang mengurangi mediator inflamasi seperti TNF-α, IL-6, COX-2, dan MMP-9. Hal tersebut dapat mengurangi edema dengan menghambat ekstravasasi sel darah putih ke jaringan anorektal. Ekstrak daun ungu mengandung flavonoid dan senyawa fitokimia lain yang mengurangi inflamasi. Hal tersebut menunjukkan bahwa ekstrak daun ungu dapat menjadi alternatif pengobatan yang menjanjikan untuk hemoroid.
Kata kunci: *hemoroid, inflamasi, flavonoid, daun ungu.*

Introduction

Hemorrhoids refer to a pathological condition of anorectal marked by engorgement of anal cushions. This condition is caused by the enlargement of the vasculature of anal cushions, inflammation, and followed by the disposition of connective tissue, resulting in prolapsing of the anal cushion. Generally, hemorrhoids are asymptomatic, however under several conditions, it causes manifestations including, but not limited to, pain and bleeding during bowel movement, itching around the anal area, burning sensation, and discomfort.[1,2] It is a common pathological condition affecting approximately 4.4% of the population globally,[3] particularly those aged between 45 and 65 years old.[4]

A pathological condition of hemorrhoids is known to be influenced by several risk factors, including obesity, old age, pregnancy, prolonged straining, constipation, and a sedentary lifestyle.[5,6] Alcohol intake and smoking are recognized to increase systemic inflammation and impair the digestion system causing diarrhea; these conditions increase the risk factor of hemorrhoids.[7] Generally, hemorrhoids are classified into internal hemorrhoids and external hemorrhoids. Internal hemorrhoids are divided into four grades (I-IV) depending on the severity of prolapse and protrusion of the anal cushions.[8,9] This classification determines the appropriate treatment for hemorrhoidal patients.[10] There are various therapeutic options for hemorrhoids, encompassing non-invasive treatment, such as taking medications and increasing dietary fibers, and invasive treatment, including an operative procedure known as hemorrhoidectomy.[11]

Apart from the conventional treatments, currently, there is evidence of growing interest in herbal-based treatment in many pathological diseases, including hemorrhoids. Purple leaf (*Graptophyllum pictum*) is one of the medicinal plants that might alleviate hemorrhoids as it shows its promising potential to provide an anti-inflammatory effect and promote tissue repair in animal models due to its phytochemicals containing such as flavonoid, saponin, and tannin.[12–14] This plant belongs to the family of Acantaceae and is most likely native to Papua New Guinea. It is spreading widely in the United States, India, and most of Southeast Asia, including Indonesia.[14] The purple leaf is now locally grown in many regions in Indonesia and is commonly known as ”daun ungu”. Traditionally, the purple leaf has been used to treat diseases such as diabetes, arthritis, hemorrhoids, and open wounds.[12,14] Numerous studies have extensively documented the potential of purple leaf extract in animal models of hemorrhoids.[4,12,15,16] However, a comprehensive review of the potential of purple leaf extract towards hemorrhoids is still needed to provide a thorough understanding. In this review, we documented an overview of the pharmacological effect of the purple leaf extract on the inflammation and pathogenesis of hemorrhoids.

Inflammation and Pathogenesis of Hemorrhoids

Although the precise mechanism and pathogenesis of hemorrhoids remain incompletely understood, various risk factors might contribute to the development of hemorrhoids. The following risk factors, such as pregnancy, obesity, and constipation, are recognized to increase intra-abdominal pressure, causing venous hypertension and interfering with venous return. This condition can cause blood stagnation in the lower body, leading to localized coagulant factors in a small part of the abdominal venous.[10] Venous hypertension is associated with venous insufficiency caused by instability of venous valves and inflammation marked by infiltration of several types of activated white blood cells, including neutrophils, macrophages, and monocytes.[10,17] These activated leukocytes secrete a range of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and matrix metalloproteinase-9 (MMP-9). In particular, obesity and smoking have been found to exacerbate this condition by promoting systemic inflammation.[6] Consequently, both abdominal pressure and inflammation can inflict venous injury and abnormal dilatation, leading to pathological changes in anal cushions.[9]

MMP is an endopeptidase that plays a crucial role in many physiological functions, such as tissue remodelling and regeneration, cellular differentiation, maintaining the integrity of vascular connective tissue, cellular proliferation, and regulating inflammation.[18,19] In pathological conditions, including inflammation, deregulation of MMP expression occurs. Overexpression of MMPs leads to the production of various proinflammatory cytokines such as IL-1β and TNF-α.[20] MMP is one of the proteolytic enzymes that degrades extracellular matrix (ECM) and glycoprotein, resulting in tissue repair and morphogenesis. This activity also happens to be pathological in some diseases, such as atherosclerosis and hemorrhoids.[19,21]

According to the study,[22] there was a significant elevation in MMP-1, -2, -7, and -9 levels among patients with hemorrhoids with grade-dependent patterns. These findings align with other study[21] that stated a significant increase in serum MMPs of hemorrhoidal patients compared with the control group. An animal model study also showed croton oil-induced hemorrhoid rats exhibit a substantial increase of MMP-9 in blood serum,[4] indicating that MMP-9 plays a crucial role in both inflammation and development of hemorrhoids. In hemorrhoids, MMP, particularly MMP-9, is implicated in the degradation of different types of connective tissue, including collagen, elastin, and fibronectin.[17] Degradation of the extracellular matrix may be one of the crucial factors in the development of hemorrhoids. Upon this process, the connective tissue of anal cushions is disintegrated, causing engorgement and prolapse.[9] The occurrence of tissue damage in the hemorrhoidal plexus triggers the release of an inflammatory signal, thereby eliciting additional activated proinflammatory cells to the anorectal.[9,10,22] As connective tissue degradation persists, it continually releases inflammatory signals, resulting in the ongoing activation of immune cells. These activated immune cells produced inflammatory markers such as proinflammatory cytokine, reactive oxygen species, and other inflammatory proteins, including MMP-9, creating a positive feedback loop that exacerbates tissue damage and inflammation.

Further scientific research indicates that individuals with hemorrhoids exhibit markedly increased blood serum nitric oxide and endothelial nitric oxide synthase levels while demonstrating significantly lower levels of asymmetric dimethylarginine (ADMA).[23] Moreover, a study on experimental animals showed a significant upregulation of the inducible nitric oxide synthase (iNOS) gene in croton oil-induced rats, followed by the upregulation of cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF), and other proinflammatory gene expression.[3] Nitric oxide (NO) is recognized for its vasodilatory properties and physiologically regulates blood pressure homeostasis by promoting vascular relaxation. NO also serves a pivotal role in immune system regulation by inducing inflammation and oxidative stress, thereby triggering the activation of immune cells. Overexpression of NO can lead to tissue damage due to excessive inflammation.[24] This condition is associated with abnormal vascular dilatation observed in hemorrhoidal disease; however, additional research is required to investigate this matter further. A combination of extracellular matrix degradation, venous hypertension and insufficiency, and inflammation is thought to be the pathogenesis of prolapsing anal cushions that leads to hemorrhoids.

Hemorrhoids are classified into four degrees based on the degree of prolapsed anal cushions, according to Goligher's classification. The first grade of internal hemorrhoids is characterized by venous engorgement during bowel movement without prolapse, although bleeding may occur. The second grade is characterized by prolapsing of the anal cushions beyond the dentate line only when defecating and spontaneously reduced; this also may be accompanied by bleeding. The third grade of internal hemorrhoid shows characteristics of prolapsing anal cushion that has to be reduced manually and is accompanied by several symptoms, i.e. itching, pain, bleeding, and burning sensations. This grade is further classified into third-degree A, in which prolapsing of anal cushions only happens when defecating, and third-degree B, in which the prolapse happens almost all the time. The fourth grade is characterized by prolapsing anal cushion through the anal canal on a usual time and irreducible. Patients with fourth-degree hemorrhoids also experience chronic inflammation and ulcerations and often require surgery for treatment.[8,25,26]

Purple Leaf (*Graptophyllum pictum* L. Griff)

Purple leaf is a highly valued plant because of its pharmacological properties. It belongs to the Acanthaceae family and possesses antioxidant, anti-inflammatory, anticancer, and antibacterial properties. The plant promotes wound healing and is commonly used to treat diabetes, hemorrhoids, and other inflammatory diseases. The plant is considered indigenous to Papua New Guinea and is now widely distributed throughout Indonesia and other parts of Asia.[12,14] The purple leaf is an herbaceous plant, commonly referred to as the caricature plant, and is a species that can grow up to two meters in height. It is characterized by its striking and colorful patterned leaves, exhibiting shades of pinkish and green.[27] These hairless leaves have a glossy appearance and an uneven texture. Notably, ethanolic extracts of purple leaves are known to contain a variety of phytochemicals, including flavonoids, tannins, coumarin, anthraquinone, phenolic, and saponin.[12] However, a scientific study revealed that the hexane extract of purple leaf showed a significantly higher phytochemical concentration than the ethanolic extract.[28] It has

also shown pharmacological properties beneficial to human health, such as antibacterial, anticancer, antioxidant, and nephroprotective activity.[29]

Numerous scientific studies have provided evidence for the potential benefits of purple leaf extract in treating diseases. One study reported a significant decrease in blood glucose levels in mice with hyperglycemia induced by alloxan following treatment with purple leaf extract.[30] In vitro studies have also suggested a possible cytotoxic effect of the extract on human colon cancer cells, with treated cells exhibiting notable morphological changes, such as smaller size, irregular shape, and unclear inter-cell boundaries.[31] As an antioxidant, the purple leaf extract has shown its potential to inhibit reactive oxygen species (ROS) by measuring DPPH radical scavenging activity. This result may be due to the phenolic compound, particularly flavonoids, which can inhibit oxidative reactions within the cells.[29] The oral administration of both ethanolic and hexane extract of purple leaf at high doses did not lead to any observed toxicity. This was demonstrated by the absence of alteration in hematological profile or pathological changes in the liver.[28]

Purple Leaf Extract Alleviate Inflammation in Hemorrhoid

The flavonoid in purple leaf extract is known for its potent antioxidant and anti-inflammatory properties. Numerous scientific studies have reported the potential benefits of purple leaf extract in treating hemorrhoids in animal models. According to these studies, the ethanolic extract of the purple leaf has been found to reduce the serum levels of COX-2 and increase the SOD level in croton oil-induced rats. COX-2 is a potent enzyme known to induce inflammation, and its levels are significantly high in rats with hemorrhoids, whereas the SOD level is low. Croton oil originated from the seed of the *Croton tiglium* plant, which showed cytotoxicity in a particular dose and was highly irritant to the skin and mucosa. The experimental group that received purple leaf extract orally (100 mg/kgBW) showed significantly lower levels of COX-2 compared to the control group. In contrast, the serum level of SOD was found to be higher in the experimental group compared to the control group. SOD is an endogenous antioxidant that plays a crucial role in reducing the levels of ROS.[15]

Croton oil obtained from *Croton tiglium* seeds contains tigliane diterpenes compound, particularly phorbol esters. Various phorbol ester compounds, such as 12-O-tetradecanoylphorbol-13-acetate (TPA), phorbol 12-myristate 13-acetate (PMA), 12-O-tiglylphorbol-13-isobutyrate, phorbol-13-decanoate and others, have been discovered in croton oil, with a minimum of eleven compounds identified in total.[32] PMA and TPA are well-documented for their ability to elicit profound skin inflammation by stimulating histamine release from immune cells, notably mast cells. Moreover, TPA is also recognized for its capacity to induce the release of HMGB1 protein, a damage-associated molecular pattern, and a TLR4 ligand, thereby triggering a TLR4-dependent inflammation reaction.[33] A recent study observed that PMA induces the upregulation of MMP-2 and MMP-9, consequently promoting the migration of endothelial progenitor cells.[34] In conjunction with elevated levels of MMP-9, the release of DAMP from necrotic cells initiates an inflammatory response and degradation of the extracellular matrix, particularly in the anorectal region. This cascade of events establishes a detrimental cycle of inflammation, ultimately leading to increased leukocyte migration and subsequent tissue damage.[20,35]

Another recent study found that purple leaf extract may be beneficial in reducing MMP-9 levels in hemorrhoidal rats. The control positive group, induced with croton oil, had the highest level of MMP-9 when compared to the normal group and the treated groups. The group that received a dose of 100 mg/kg BW of purple leaf extract showed the lowest level of MMP-9 expression compared to the control positive group. Furthermore, at a higher dosage of 300 mg/kg BW of purple leaf extract, MMP-9 levels were significantly lower than those of the control positive group. This indicates that a 100 mg/kg BW dose of purple leaf extract may effectively reduce MMP-9 levels in hemorrhoidal rats.[4]

In hemorrhoidal rats, pro-inflammatory cytokines TNF-α and IL-6 have been identified and studied (Figure1). TNF-α has been found to stimulate the expression of adhesion molecules, leading to immune cell activation, while IL-6 can further stimulate the C-reactive protein. Significantly higher levels of these cytokines were observed in the hemorrhoidal rats compared to the normal and treated groups. Specifically, the levels of TNF-α and IL-6 in hemorrhoidal rats were significantly higher in the treated group. The administration of purple leaf extract was studied in doses of 100 mg/kg BW and 300 mg/kg BW. When given at a dose of 100 mg/kg BW, the expression levels of TNF-α and IL-6 were significantly lower compared to the hemorrhoidal group. At a 300 mg/kg BW dose, TNF-α and IL-6

levels were significantly lower than the hemorrhoidal group but not significantly different from the lower dose. These findings suggest that the purple leaf extract has the potential to modulate the expression of pro-inflammatory cytokines in hemorrhoidal rats.[36]

The anti-inflammatory properties of purple leaf extract may be due to its numerous phytochemical compounds, particularly flavonoids. Flavonoids are active compounds that are commonly found in plants, and they have been shown to have the ability to inhibit inflammation through the NF-κB pathway. This pathway is activated by TNF-α or IL-1β, which are expressed in hemorrhoidal animal models.[13] Flavonoids also showed an inhibition activity to iNOS in RAW 264.[7] Macrophage at the mRNA level stimulated by proinflammatory cytokine.[37] Flavonoids are also known to have a similar mechanism to NSAID drugs in terms of inhibiting COX-2 as well as NOS activity. Numerous scientific studies have highlighted flavonoid compounds' potential to inhibit various inflammatory signaling mechanisms. This includes inhibiting the nuclear translocation of p65/p50 subunits and activating protein-1 (AP-1), suppressing the synthesis of proinflammatory cytokines such as IL-1, IL-6, TNF-α, and IL-18, as well as impeding MMP-9 activity and MAPK signaling through Erk/JNK/Nrf proteins, which ultimately leads to the synthesis of COX-2 and iNOS.[38] Additionally, flavonoids are recognized for inhibiting nociceptor sensitization, thereby contributing to pain relief through their analgesic effect.[39] Flavonoid compounds possess antioxidant properties by inhibiting the overproduction of ROS. While ROS can naturally form due to metabolic processes, their levels increase during inflammatory reactions, exacerbating inflammation and causing cellular and tissue damage. Exposure to TPA increases COX-2 expression, leading to elevated ROS levels. Consequently, this results in heightened activity of the IKK enzyme, which is involved in the phosphorylation of IκBα, an inhibitor of the NFκB subunit. This, in turn, leads to an increase in NFkB activity, thereby worsening inflammation. The anti-inflammatory and antioxidant effects of flavonoids play a pivotal role in inhibiting both processes.[40,41]

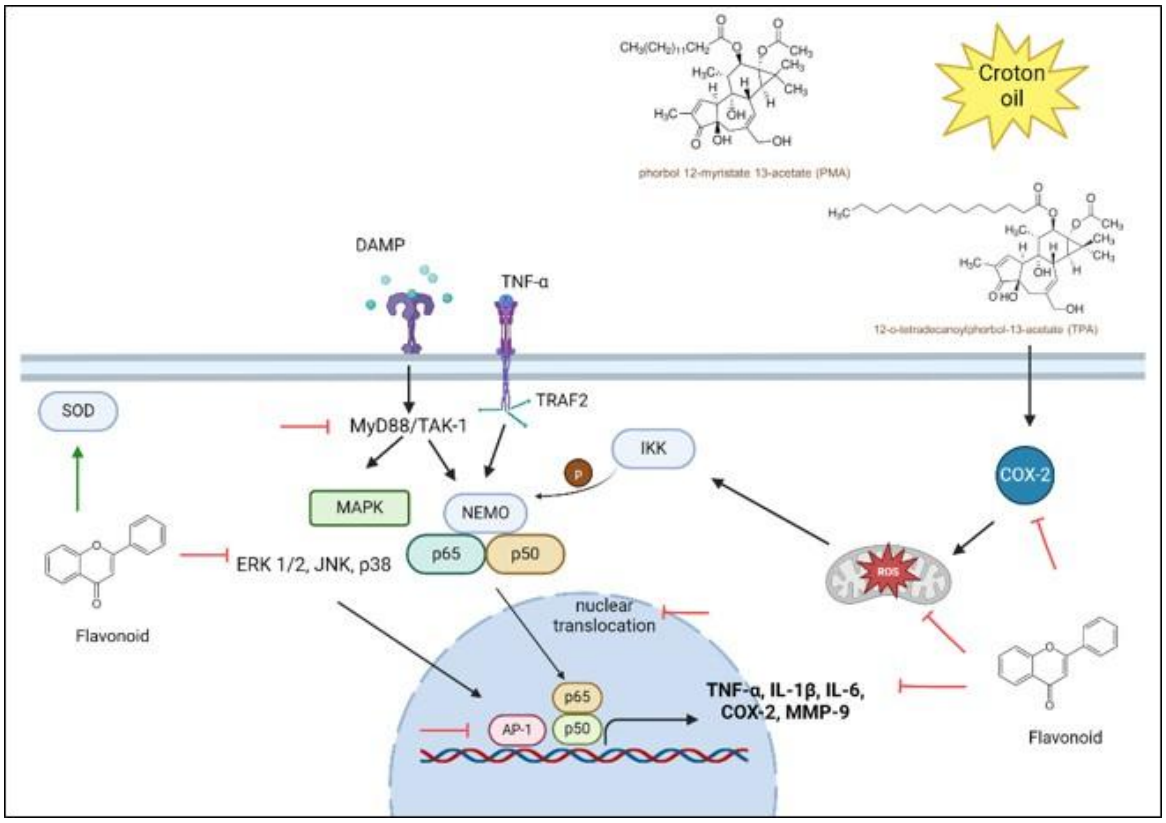

Figure 1. Molecular Mechanism of Flavonoid Inhibiting Inflammatory Process Induced by Croton Oil Made with Figure BioRender. Both TPA and PMA cause increased COX-2, leading to ROS overproduction. This leads to disruption of the regulation of IKK, which exacerbates inflammation. Flavonoid acts as an anti-inflammatory agent inhibiting several NFκB and MAPK pathways mechanisms. Furthermore, flavonoids also show antioxidant activity by inhibiting ROS production and triggering SOD levels

Based on these reports, the anti-inflammatory properties of flavonoid-containing purple leaf extract play an important role in expediting the recovery process of hemorrhoids and mitigating further tissue damage.

Purple Leaf Extract Promotes Tissue Repair

The purple leaf extract exhibits the potential to reduce the recto-anal coefficient. This coefficient is reliable for measuring edema induced by croton oil within the anorectal. The experimental rats, which received 100 mg/kg BW of purple leaf extract, showed a significantly lower anorectal coefficient compared to the control group. In addition, the number of extra vassal leukocytes in the experimental group was significantly lower than in the control group. Furthermore, administering purple leaf extract to hemorrhoid-induced rats reduced leukocyte extravasation into the anorectal.[13] Applying croton oil to the anorectal area causes severe inflammation, marked by swelling and redness. Histopathologically, hemorrhoid-induced rats showed significant thickness changes in muscularis externa of anorectal compared to the normal group. A combination of topically and oral purple leaf extract has shown a significant improvement in the histology of anorectal tissue by reducing the thickness of muscularis externa and inhibiting leukocyte extravasation.[16] This data indicates the phlebotrophic effect of purple leaf extract on hemorrhoidal animal models. Flavonoid-containing purple leaf extract showed an inhibition activity towards pro-inflammatory cytokines and adhesion molecules, which are important for recovery as they promote tissue repair.[42]

Conclusion

Hemorrhoidal disease is influenced by numerous risk factors and involves a complex process of pathological changes in anal cushions, including inflammation. Purple leaf extract is considered an alternative therapy for hemorrhoids as it is known to have a beneficial effect in treating hemorrhoids in animal models by reducing pro-inflammatory mediators and decreasing leukocyte extravasation that leads to edema as it contains numerous phytochemical compounds, including flavonoid, saponin, tannin, anthra-quinone, phenol, and coumarin.

Conflict of Interest

The authors declare there are no conflicts of interest.

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