The potency of *Polylathia longifolia* from Indonesia and the Philippines as therapeutic agents on inflammatory bowel disease (IBD) in Rats (*Rattus norvegicus*) induced by Indomethacin

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**Abstract.** Herbal medicines have the potential to be used as therapeutic agents. *Polynalthia longifolia* is widely grown in both Indonesia and the Philippines, but it has not commonly used for its therapeutic purposes. Some studies reported that *P. longifolia* leaves extract has anti-inflammatory activity. In this study, the potential of *Polylathia longifolia* leaves extracts for inflammatory bowel disease (IBD) in Indomethacin-induced rats was tested. Four groups of rats were used for this research, were control (T1), rats with IBD with 10 mg/kg BW of sulfasalazine therapy (T2), rats with IBD treated with 300 mg/kg BW of Indonesia *P. longifolia* leaves extract therapy (T3) and rats IBD treated with 300mg/kg BW of The Philippines *P. longifolia* leaves extract therapy (T4). Histopathology of gastric, duodenum, jejunum, and colon were analyzed, with protein profile and pro-inflammatory cytokines expressions. The results showed that *P. longifolia* leaves extract origin from Indonesia and the Philippines were potent as anti-inflammatory agents comparable to commercially available drugs against IBD. This works proposed the use of *P. longifolia* leaves as IBD therapy.

1. Introduction
Herbal medicines are a prominent part of healthcare whole world [1]. The use of herbal medicine has enhanced rapidly over the past few decades. Therapy with herbal medicine is the primary healthcare for 80% population in the world [2], especially in developing countries, as well as the increasing use of herbal medicine in developed countries [3]. Therapy with herbal medicine has fewer side effects when compared with synthetic drugs. Natural herbs contain phytochemicals that can use to treat various diseases such as inflammatory bowel disease (IBD). One of the plants that apply for this treatment is *Polylathia longifolia*. This plant belongs to the *Annonaceae* family which commonly found in tropical countries [4] such as Indonesia and the Philippines. This plant is widely used as an ornamental plant, reducing sound pollution and reducing fever and tonic. This plant was used to treat fever, diabetes,
hypertension. Some studies indicated that this plant has various classes of compounds terpenoids, alkaloids, and flavonoids. The content of alkaloids and flavonoids has anti-inflammatory activities, including inhibiting infiltration, reducing inflammatory mediators, and reducing oxidative stress [5,6]. Based on the therapeutic effect of the active compounds P. longifolia on the inflammatory response, it has potential in inflammatory regulation of intestinal inflammation. Inflammatory bowel disease is a gastrointestinal inflammation that has developed into a global condition with increasing incidents in developed countries and industries in Asia. Long-term treatment with synthetic drugs such as sulfasalazine will cause resistance, aggravate bleeding, male infertility, pulmonary toxicity, and nephrotoxicity [7]. Therefore, it was necessary to expand the knowledge of the use of herbal medicines such as P. longifolia leaves as IBD medicine. This study evaluates P. longifolia leaf extract used as an IBD drug induced by indomethacin and compared with sulfasalazine as gold standard.

2. Material and Methods

2.1. Plant Material
Leaves of Polyalthia longifolia were obtained from Indonesia and the Philippines. Indonesia leaves of P. longifolia were obtained from Malang City, while samples from the Philippines from Camiling, Tarlac and were brought to Biosains Institute, Brawijaya University. The taxonomy for plants was identified and authenticated in plant taxonomy laboratory, Brawijaya University.

2.2. Animals
Male Wistar rats aged two months and weighed around 150-200g each. Rats were maintained under standard laboratory conditions of temperature, humidity and 12 h light and dark. Animals have free access to feed and water (ad libitum). Before treatments, rats were acclimatized for a week. The experimental protocol was approved by the Ethic Commission of Brawijaya University (No. 1035-KEP-UB).

2.3. Experimental Design
The animals were in four groups: (T1) negative control, (T2) IBD with Sulfasalazine, (T3) IBD that is treated using P. longifolia Indonesian leaves extract, and (T4) treated P. longifolia Philippines leaves extract. Inflammatory bowel disease in rats were induced with indomethacin, with a dose of 15mg/kg orally (p.o). Treatment 2 was given 10 mg/kg BW of sulfasalazine as gold standard of IBD. T3 and T4 were given 300mg/kg BW of ethanolic extract of P. longifolia leaves from Indonesia and The Philippines, respectively. The therapies were given for 7 days orally. After therapy, rats were sacrificed and gastrointestinal contents from the gastric, duodenum, jejunum, and colon were collected, and organs were preserving in 10% neutral-buffer formalin and others in phosphate buffer saline for further use.

2.4. Histopathological Observations
The gastric, duodenum, jejunum and colon were collected and fixed in 10% neutral-buffered formalin, embedded in paraffin wax and were cut into sections of 3-5µm thickness. The sections were stained using haematoxylin and eosin for histopathological observation. The effect on the different treatments on inflammation were observed especially the infiltration of the inflammatory cells, desquamation, erosion, and damage of tissue structures microscopically.

2.5. Immunohistochemistry
The paraffin section of the organs also was deparaffinized with xylol and multi-concentrations of alcohol for 15 min. Furthermore, slides were left overnight at 4°C and were washed with distilled water and PBS solution. Slides were incubated with 3% hydrogen peroxide for 40 min then washed for 5 min with PBS solution. Blocking stages were conducted with 1%BSA in PBS for overnight then washed again with PBS. Primary antibody for COX-2 and SMAD3 (Santa Cruz Biotechnology with ratio 1:50000) were added, left overnight and washed with PBS. After this, the secondary antibodies (anti-rabbit biotin conjugated) were added for 1h and washed again with PBS. Drops of Strepta Avidin-Horseradish Peroxide (SA-HRP) were made and left for 40 min and washed again with PBS. Drops of DAB was done and left for 10 min and washed with distilled water. Mayer hematoxylin was dropped on the slides, washed with distilled water, dried and then mounted. Slides were observed microscopically.
2.6. **Protein Profile Using SDS-PAGE**

0.1g of duodenum and colon were crushed and PMSF + PBS-T were added to the solution. The solution was homogenated and sonicated for 10 min and was centrifuged at 6000 rpm at 4 °C for 15 min. The supernatant was collected, and cold ethanol was added to the solution (1:1), stored at 4 °C for 12 h. Samples were centrifuged at a 6000 rpm at 4 °C for 15 min. The pellets were dried until ethanol was lost. Subsequently, Tris-HCl pH 6.8 (1:1) was added, and stored at -20 °C.

3. **Result and Discussion**

3.1. **Histopathology**

Histopathological analysis of the small intestine was conducted by the Haematoxylin-Eosin (HE) method on all treated groups. Based on previous research, induction of indomethacin caused necrosis in the small intestine, so that the goblet cell and mucus layer were lost, and villi damage (desquamations and rupture) were observed, which was also confirmed in this study. Observation showed the desquamation of epithelia, erosion, congestion, and infiltration of inflammatory cells and villi damages were seen.

3.1.1. **Gastric histopathology.** The therapy of sulfasalazine as a gold standard has maintained IBD remission [10]. It was observed that the leaf whether from Indonesia and the Philippines have potency as anti-inflammatory [11]. It also reduced the inflammatory symptoms by inhibiting the product in PGE2. As earlier studied [12,13], leaves extract from P. longifolia also had antioxidant activity. One of the compounds was liriodenine [14]. Liriodenine has anti-inflammatory and antioxidant activities that can reduce inflammation and repair tissue damage, as seen in the histopathological picture.

![Figure 1](image-url). Histopathology of gastric mucosa. (A). Gold Standard group; (B). *P. longifolia* extract from Indonesia therapy; (C). *P. longifolia* extract from The Philippines, and (D) IBD condition.

Legend:

- **M**: Mucosa
- **MM**: Muscularis mucosa
- **SM**: Submucosa
- **ME**: Muscularis external
- **D**: Desquamation of the epithelia
- **E**: Erosion
- **: Congestion**
- **: Infiltration**
- **: Parietal cells**
- **: Chief cells**
3.1.2. **Duodenum histopathology.** Indomethacin induction in rat caused intestine tissue necrosis so the duodenal villi were eroded, and goblet cells disappeared caused by the villi damages. During inflammatory conditions, indomethacin caused villi desquamation resulted in the loss of goblet cells [15]. Based on Figure 2, the histopathology of the duodenum, group treated with sulfasalazine showed an improvement in the intestinal epithelial structure after treatment, as well as no erosion in the epithelial layer, without the infiltration of inflammatory cells and measured villous forms. The use of leaves extracts from Indonesia group (T3) showed that villous improvement, evidenced by the visible arrangement of columnar epithelial cells arranged by the regular villi and the appearance of goblet cells as mucus secretor for the protection of the villi layer in the duodenum. Histopathological features of the duodenum of rats treated with the Philippine extract showed the repairment of duodenal villi, which was followed by the appearance of goblet cells and the epithelial constituents of the villi.

![Figure 2. Histopathology of duodenum. (A). Gold Standard group; (B). P. longifolia extract from Indonesia therapy; (C). P. longifolia extract from Philippines.](image)

> Legend:
> - Columnar epithelial tissue
> - Goblet cell

3.1.3. **Jejunum histopathology.** Thick walls and villi characterized the jejunum histology because they contained many blood vessels. Like duodenum, jejunum suffered damage when exposed to toxic substances such as indomethacin. Figure 3 showed the histopathological features of jejunum in the IBD group treated with sulfasalazine (Figure 3A), showed the improvements in villous shape, neat columnar epithelial cell structure and the appearance of goblet cells. However, inflammation cells were still found
coming out of the lymphatic channels and spaces in the Crypt of Lieberkühn, which indicated improvement was not yet completed. The histopathology of jejunum with IBD treated with the leaves extract from Indonesia and the Philippines showed improvement as evidenced by the reduction in inflammatory cell infiltration, neat layer of the columnar epithelial cell structure, goblet cells and no desquamation compared to the control group (Figure 3D).

![Figure 3](image)

**Figure 3.** Histopathology of jejunum; (A). Gold Standard group; (B). *P. longifolia* extract from Indonesia therapy; (C). *P. longifolia* extract from Philippines, and (D) IBD condition.

**Legend:**
- S : Serosa
- M : Mucosa
- SM : Submucosa
- CL : Crypt of Lieberkühn
  - → : Cell infiltration
  - → : Columnar epithelium tissues
  - → : Goblet cells

3.1.4. **Colon histopathology.** Histopathology analysis showed that the sulfasalazine therapy group has undergone tissue repair in the colonic mucosa marked by the appearance of goblet cells in the mucosal lining of the colonic epithelium. Goblet cells in the colon function as a barrier in the colonic mucosa by removing mucin compounds. As for the colonic histopathology of the leaves extract therapies group from both countries, indicated tissue repairs. However, inflammation cell infiltration was still found, and the surface structure of the epithelium were not yet intact.

The goblet cells in the process of repairing the digestive tract were observed due to sulfasalazine, which has the ability to suppress the production of free radicals thus accelerating the time of inflammation and increasing the work of TGF-β as an anti-inflammatory cytokine for regenerating intestinal stem cells and differentiating into several types of cells namely enterocytes, goblet cells, and cells Paneth [16]. In addition, the content of secondary metabolites in the ethanol extract of *P. longifolia* leaves has quercetin and rutin, which can inhibit the formation of free radicals, reduce pro-inflammatory mediators, improving the protective function of the epithelium in the intestine organ. Flavonoids were known to increase colonic permeability [17].
3.2. Immunohistochemistry of COX-2 and Smad3

Inflammatory bowel disease (IBD) is a chronic inflammatory condition that occurs in the gastrointestinal tract. Characterization of the inflammatory process is with the presence of COX-2 expression which increases rapidly and decreases anti-inflammatory cytokines such as Smad3. COX-2 and Smad3 expression were observed by immunohistochemistry methods and analyzed with one-way ANOVA statistics. The results showed that the treatment affected COX-2 and Smad3 expression (p <0.05).

| Groups          | COX-2        | Smad3        |
|-----------------|--------------|--------------|
| T1 (control)    | 0.6533±0.05465<sup>a</sup> | 14.8000±0.33466<sup>a</sup> |
| T2 (Sulfasalazine) | 1.2433±0.11130<sup>b</sup> | 12.6000±0.56569<sup>b</sup> |
| Indonesia       | 1.3267±0.05164<sup>b</sup> | 12.2000±0.43818<sup>b</sup> |
| Philippines     | 1.5467±0.08262<sup>c</sup> | 8.0333±0.52789<sup>c</sup> |

Note: The notations a, b and c show a significant difference between treatment groups (significance <0.05). While the same notation shows no significant difference between treatment groups.

Table 1 shows the number of COX-2 and Smad3 expressions when Sulfasalazine, leaves extracts from Indonesia and The Philippines as compared to the adverse treatment. Results showed improvement
of IBD by decreasing COX-2 expression and decreasing Smad3 compared with the untreated group. The leaves extract from the Philippines showed significant differences (P <0.05) in treating IBD. The leaves extract from Indonesia has a comparable result as with the gold standard treatment prescribed with sulfasalazine and showed significant differences between the treated and untreated groups. The leaves extract from the Philippines was found better because of increased COX-2 expression and decreased Smad3; this result concurred with the studies of Peng et al. [5,17].

Therapy of *P. longifolia* leaves extract from the Philippines and Indonesia has the potential of accelerating healing and cell regeneration in IBD rats. Flavonoid and alkaloid compounds in the leaves of *P. longifolia* can act as antioxidants and anti-inflammatory agents. They play a role in exhibiting COX-2 so that there were limited number of inflammatory cells that migrate to the wound tissue. Furthermore, the inflammatory reaction was shortened and the proliferative ability of Smad3 was not inhibited [16]. Flavonoid and alkaloid compounds stabilize the reactive oxygen species (ROS) by reacting with the compounds from free radicals so that these compounds become inactive [18]. This was evidenced by the histopathological picture of the duodenum which looked normal and has improved.

### 3.3. Protein profile of duodenum and colon

**Table 2.** The result of protein profile of duodenum analysis based on SDS-PAGE.

| Group                          | Molecule weight (kDa) | 289.7 | 201.4 | 140.7 | 87.3 | 59.5 | 45.6 | 35.8 | 21.2 |
|-------------------------------|-----------------------|-------|-------|-------|------|------|------|------|------|
| Gold standard sulfasalazine (P1) | ✓                     | ✓     | ✓     | -     | ✓    | ✓    | -    | ✓    | ✓    |
| *Polyalthia longifolia* from Indonesia (P2) | ✓                     | ✓     | ✓     | ✓     | -    | ✓    | ✓    | ✓    | ✓    |
| *Polyalthia longifolia* from the Philippines (P3) | ✓                     | -     | -     | ✓     | ✓    | ✓    | ✓    | ✓    | ✓    |

**Figure 5.** Protein bands of duodenum (SDS PAGE 12%).

Legend: M = marker; P1 = Sulfasalazine group; P2 = EEPL from Indonesia group; P3 = EEPL from Philippines

The 40 kDa protein appeared in sulfasalazin therapy and leaves extract from the Philippines. This protein was thought as Vasoactive Intestinal Polypeptide (VIP) which was secreted by cells in the intestinal mucosa. VIP was produced by inflammatory tissue; VIP increased the permeability of
duodenal tissue stimulated the secretion of fluid and electrolytes from duodenal tissue which triggered watery diarrhea and dehydration [19].

Table 3. The result of protein profile of colon analysis based on SDS-PAGE.

| Group                                    | Molecular weight of Protein (kDa) |
|------------------------------------------|----------------------------------|
|                                          | 288.2   | 198.3  | 167.4 | 114.6 | 43.5 | 37.1 | 22.0 |
| Negative control (K-)                    | √        | √      | √     | -     | √    | √    | √    |
| Gold standard sulfasalazine (K1)         | √        | √      | √     | -     | √    | √    | √    |
| Polyalthia longifolia from Indonesia (K2)| √        | √      | √     | -     | √    | √    | √    |
| Polyalthia longifolia from the Philippines (K3) | √        | √      | √     | -     | √    | √    | √    |

Figure 6. Protein bands of colon (SDS PAGE 12%).

Legend: M = marker; K- = negative control; K1 = Sulfasalazine group; K2 = EEPL from Indonesia group; K3 = EEPL from Philippines

Protein with molecular weights 114.6 kDa were not synthesized in all treatment groups, but it was synthesized in healthy conditioned rats. A protein with molecular weight of 114 kDa is a protein that binds to glycocalyx in blood vessels, namely the β-galactosidase [20]. This β-galactosidase was enzyme that very important to convert carbohydrates into disorders in IBD conditions. This enzyme was located at the peak of the villi to hydrolyze lactose to glucose and galactose [21]

4. Conclusion

Polyalthia longifolia has been proven to improve gastrointestinal tissue in rats with infectious bowel disease-induced indomethacin. It was proven by the histopathological profiles, showed decreasing pro-inflammatory signs, enhanced of anti-inflammatory expression, and improved protein profiles.

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