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Morinda citrifolia Linn. Ethanolic-extract Improve Inflammation Condition in Acetic Acid-induced Colitis Ulcerative Mice Models

Tri Fitri Yana Utami[1,2], Muhammad Novrizal Abdi Sahid[1,3*], Ika Puspita Sari[4], Ediati Sasmito[1]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281

[2] Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy and Technology, Al-Irsyad University, Cilacap, Central Java, Indonesia, 55584

[3] Curcumin Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia, 555281

[4] Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281

ABSTRACT

Ulcerative Colitis (UC) is a chronic inflammatory disorder affecting the colon, characterized by recurrent episodes and involving multifaceted etiological factors in its pathogenesis. This study aims to explore the pharmacological effect of *Morinda citrifolia* Linn. fruit ethanolic-extract (MFE) in UC. The UC mice model was induced using 2% acetic acid administered per-rectal. Subsequently, mice were categorized into six groups, comprising a normal control, negative control, positive control, and three MFE treatment groups (100mg/kgBW; 200mg/kgBW; 400mg/kgBW). Colitis severity was assessed by scoring system to obtain the Disease Activity Index (DAI) and Hematoxylin-Eosin (HE) staining. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed to determine the free radical scavenging activity. MFE exhibited notable antioxidant activity, with an IC_{50} value of 0.553 mg/ml. Notably, MFE administration led to a discernible reduction in DAI scores, with MFE treatment demonstrating improved inflammation condition compared to the negative control from day 6 through day 11. Furthermore, immune cell infiltration within colonic tissue was markedly attenuated in the MFE-treated group compared to the negative control group. Evaluation of colon tissue damage revealed scores of 5.7, 4.2, and 3 for MFE doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, whereas the positive control group exhibited a score of 3, indicating superior tissue preservation. Notably, these scores contrasted starkly with the negative control group, which registered a damage score of 11.8. These findings exhibit the MFE potential as a therapeutic intervention for UC owing to its pharmacological properties, underscoring its potential as a natural remedy in UC management.

Keywords: Acetic acid-induced colitis; antioxidant; colon disease; inflammation; *Morinda citrifolia*

INTRODUCTION

Ulcerative colitis (UC) is a chronic condition that exerts a significant emotional and social toll on individuals affected by it. It characterized by chronic inflammation and multifactorial etiopathogenesis affecting the gastrointestinal tract (Caviglia et al., 2023). According to the data from 2009–2010, approximately 1.2% of the United States population was reported a prior diagnosis of IBD, with 1.0% indicating a history of UC, and 0.3% diagnosed with Crohn's disease (CD) (Weisman et al., 2023). This prevalence trend is also on the rise in newly industrialized nations across Africa, Asia, and South America (Kaplan & Ng, 2016).

Current therapeutic approaches for UC typically involve the administration of corticosteroids, glucocorticosteroids, aminosalicylates, and immunosuppressive agents. However, these treatments are associated with significant adverse effects, and UC often recurs despite treatment (da Silva et al., 2021). In contrast, herbal medicines offer a promising alternative due to their potential for fewer toxic side effects. Consequently, they are garnering increasing attention from researchers as viable candidates for managing these diseases. Notably, investigations into the therapeutic potential of plant-based remedies have been conducted. Previous studies have demonstrated the anti-colitis activity of *Apium graveolens*, *Curcuma xanthorrhiza* and *Eleutherine palmifolia* (L.) Merr. (Dewangga et al., 2024; Saputra et al., 2022; Wijayanti & Hasyati, 2018; Young Cho et al., 2017).

Indonesia has a diverse plant species, among which *M. citrifolia* stands out for its potential in mitigating colitis. Research indicates

***Corresponding author : Muhammad Novrizal Abdi Sahid**
Email : m.novrizal.a@ugm.ac.id

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that the extract of *M. citrifolia* holds promise in ameliorating the inflammatory response associated with ulcerative colitis (UC), positioning it as a viable candidate for alternative UC treatment modalities (Utami et al., 2023). Notably, *M. citrifolia* contains phytochemicals, including iridoids, flavonoids, anthraquinones, coumarins, phenolics, and triterpenoids (Singh & Sharma, 2020). These compounds revealing a broad spectrum of pharmacological activities, such as anti-inflammatory, antioxidant, ulcer-protective, anticancer, analgesic, and immunomodulatory effects (Dussossoy et al., 2011; Jin et al., 2019; Kwon et al., 2021; Rezeki & Vidirachmilla, 2017; Sasmito et al., 2015). *M. citrifolia* has demonstrated its capacity to inhibit inflammation effectively. *M. citrifolia* promotes the expression of zonula occludens-1 (ZO-1) and occludin proteins, along with enhancing mucus secretion, thereby mitigating damage to the colonic mucosa induced by dextran sulfate sodium (DSS) (Jin et al., 2019). In addition, it reduces tissue damage and modulate mRNA expression of inflammatory cytokines, including reducing IL-6 and IFN-γ mRNA expression while enhancing IL-10 mRNA expression (Kwon et al., 2021). The utilization of *M. citrifolia* fruit juice presents a compelling therapeutic option for UC owing to its immunomodulatory effects and reinforcement of intestinal lumen (Coutinho de Sousa et al., 2017). Polysaccharides extracted from *M. citrifolia* fruit juice have demonstrated anti-inflammatory properties in experimental models of acetic acid-induced UC by mitigating neutrophil infiltration, oxidative stress, pro-inflammatory cytokine activity, and expression of COX-2 and iNOS in colon cells (Batista et al., 2020).

In this study, we investigated the protective effects of MFE on acetic acid (AA) 2%-induced colitis through both macroscopic scoring and histological analysis. In addition, we sought to understand the underlying mechanisms by which MFE mitigates inflammatory injury in experimental colitis. This research is important to conduct regarding the anti-inflammatory effects of MFE as a therapeutic candidate for UC. However, research is about activity there are not many specific anti-inflammatory of MFE for UC disease done, one of the standard indexes for assessing UC prognosis is DAI. To achieve this, we evaluated various parameters including DAI, which encompasses criteria such as weight loss, stool consistency, and the presence or absence of blood in the stool.

MATERIALS AND METHODS

Materials

The *M. citrifolia* fruit utilized in this study was purchased from Cilacap, Central Java, Indonesia, in May 2022. Chemicals and reagents employed in the experimental procedures included acetic acid (Merck), aquades (PT. Brataco), 0.25% Na-CMC (Sigma), NaCl solution (PT Widatra Bhakti, No. 410101), aqua pro injection (PT. Ikapharmindo Putramas), sulfasalazine (Sulfitis®, PT. Pratapa Nirmala), ethanol (Merck), HCl (Merck), DPPH (Aldrich), toluene (Sigma), ethyl acetate (Merck), silica gel 60 GF254 (Merck), ascorbic acid (Merck), Dragendorff's reagent (Merck), p.a. grade H_2SO_4 (Merck), chloroform (Merck), $FeCl_3$ (Merck), and p.a. grade anisaldehyde (Merck).

Methods

M. citrifolia fruit collections and determination

The *M. citrifolia* fruit used in this study was purchased from Cilacap, Central Java, Indonesia, in May 2022. Subsequently, the fresh fruits underwent taxonomic identification at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada. The fruit sample was officially confirmed to be *M. citrifolia* fruit, based on determination letter number 29.6.6/UNI/FFA.2/BF/PT/2022.

M. citrifolia fruit extraction

In this study, one kilogram of dried *M. citrifolia* fruit powder underwent extraction using the maceration technique, employing a 70% ethanol solution as the extraction solvent, with a ratio of 1:5 between the dried powder and the extraction solution. The maceration process was conducted for a duration of three days and repeated twice using fresh 70% ethanol solution each time. Subsequently, the *M. citrifolia* extract solution was subjected to concentration using a vacuum rotary evaporator at 40°C overnight to obtain a concentrated extract. The resulting extract was then weighed and designated as *M. citrifolia* fruit ethanolic-extract (MFE) throughout the entirety of the manuscript.

Phytochemical Screening

For the determination of active constituents, the crude extract of the investigated plant underwent preliminary phytochemical screening following established protocols outlined in previous studies (Kancherla et al., 2019; Tiwari et al., 2011). A qualitative analysis of the MFE involved the detection of alkaloids, flavonoids,

saponins, terpenoids, and steroids. The phytochemical screening of the extract was carried out utilizing specific protocols, reagents, and chemicals as described below:

Alkaloids: Alkaloids were identified using the Dragendorff's test. In brief, 2 mg of MFE was mixed with 0.2 ml of dilute HCl (2 N) in a test tube. Following this, 1 ml of Dragendorff's reagent (Merck) was added, and the formation of an orange-brown precipitate indicated the presence of alkaloids.

Flavonoids: Flavonoids were identified by adding a few drops of diluted HCl 2 N to a small quantity of the extract solution. The immediate appearance of a red coloration confirmed the presence of flavonoids.

Tannins: Tannins were identified using the $FeCl_3$ test. Half g of MFE was dissolved in 10 ml of distilled water and filtered with filter paper. Subsequently, a few drops of 5% $FeCl_3$ solution were added to the filtrate. The formation of a greenish-black precipitate indicated the presence of tannins.

Saponins: Saponins were identified by diluting 1 ml of MFE with 20 ml of distilled water and shaking the mixture in a graduated cylinder for 15 minutes. The formation of foam layer (approximately 1 cm) indicated the presence of saponins.

Terpenoids: Terpenoids were detected using the Salkowski test. In brief, 5 ml of MFE was mixed with 2 ml of chloroform, followed by the careful addition of 3 ml of H_2SO_4 98%. The development of a reddish-brown coloration at the interface confirmed the presence of terpenoids.

Thin layer chromatography of flavonoid identification

MFE underwent Thin Layer Chromatography (TLC) analysis, with a silica gel 60 F254 (4 cm x 10 cm with elution distance 8 cm) plate utilized as the stationary phase. A mobile phase comprising a mixture of toluene:ethyl acetate (10:10 % v/v) was employed. Following the TLC process, staining was achieved using a spray reagent consisting of sulfuric acid-anisaldehyde. Subsequently, several stains were observed, and the relative migration (Rf) value of each stain was calculated.

$$Rf\ (relative\ migration) = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}} \quad (1)$$

Antioxidant activity (DPPH Radical Scavenging Assay)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed to determine the free radical scavenging activity, following the protocol described by (Johari & Khong, 2019). Initially, 4 mg of DPPH was dissolved in 100 mL of ethanol to obtain a DPPH solution with a concentration of 4 mg/mL. Subsequently, 1 mL of the 4 mg/mL DPPH solution was added to 3 mL of various concentrations of the MFE solution (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, and 1 mg/mL). For the blank control, the same volume of ethanol was added instead of MFE. The mixtures were thoroughly shaken and allowed to stand for 30 minutes at room temperature. The absorbance of the solutions was measured at 517 nm. The percentage of inhibition (I%) of the DPPH radical was calculated using the following formula:

$$I\% = \left[\left(\frac{A_0 - A_S}{A_0}\right)\right] x\ 100\% \quad (2)$$

$I\%$: percentage of inhibition; A_0 : Absorbance of standard; A_S: Absorbance of sample

A graph depicting the inhibition percentages (I%) against concentrations of the sample was plotted. From the graph, the concentration at which 50% inhibition (IC_{50} value) occurred was determined for each sample. To enhance accuracy, all experiments were conducted in triplicate to minimize precision errors. The mean IC_{50} value, along with the standard error of the mean (SEM) of the triplicate measurements, was reported.

Anti-colitis activity

Experimental Animals

Male Balb/c mice were obtained from Integrated Research and Testing Laboratory at Universitas Gadjah Mada (LPPT UGM). The animal experiment performed in this study was approved by the Ethical Committee of LPPT UGM with the reference number 00036/04/LPPT/X1/2022. The animal was acclimatized to the cages maintained at a temperature of 23 ± 2°C and a humidity range of 50–70%, with access to standard rodent food for a period of 7 days. For the experimental phase, mice aged between 12 to 14 weeks and weighing between 30 to 40 g were selected. A total of 30 mice were utilized and divided into 6 groups, with each group comprising 5 mice in one cage. The groupings were as follows: (1) Normal group: Animal receiving 0.25% of sodium carboxy-methyl-cellulose (Na-CMC); (2) Negative group: Animal receiving 2% of acetic acid (Colitis group); (3) Positive group: Animal receiving 2% of acetic acid and 30 mg/kg body weight (BW) sulfasalazine; (4) MFE 100 mg/kg BW group: Animal receiving 2% of acetic acid and 100 mg/kg BW of MFE; (5) MFE 200 mg/kg BW group: Animal receiving 2% of acetic acid and 200 mg/kg BW of MFE; (6) MFE 400 mg/kg BW group: Animal

receiving 2% of acetic acid and 400 mg/kg BW of MFE.

Induction of Experimental Colitis

Prior to the induction of colitis, the animals underwent a 24-hour fasting period with free access to water. An intraperitoneal injection of ketamine at a dosage of 100 mg/kg body weight (BW) was administered to anesthetize the mice. Subsequently, 0.2 mL of acetic acid (2% v/v in 0.9% saline) was slowly administered per-rectal using a catheter inserted into the anus to a depth of approximately 1-2 cm. The mice were then positioned in a Trendelenburg posture for 30 seconds to ensure the thorough distribution of the solution within the colon. Following the induction of colitis, the degree of colon damage was assessed and graded on a scale ranging from 0 to 5, as described by Minaiyan et al., 2014. The grading scale is delineated as follows: (1) Score 0: No evidence of damage; (2) Score 1: Localized hyperemia; (3) Score 2: Presence of erosions or ulcers without inflammation; (3) Score 3: Presence of erosions or ulcers with single inflammation; (4) Score 4: $\geq$ 2 sites of ulceration and/or inflammation (5) Score 5: $\geq$ 2 major inflammation and ulceration sites or one major inflammation and ulceration site more than 1 cm along the colon`s length.

Evaluation of Colitis Conditions

In this study, colitis severity was assessed using the DAI, which encompasses evaluations of body weight, stool consistency, and the presence of blood in the stool. The mean score of these parameters was calculated to determine the overall disease activity index. In addition, the total sum of the scores was utilized to ascertain disease severity. The scoring and calculation methodology for the DAI were adopted from previous (Bang & Lichtenberger, 2016; Jeengar et al., 2017).

Statistical Analysis

The data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS version 19, employing one-way analysis of variance (ANOVA) followed by Post Hoc tests for multiple comparisons. Graphical representations were generated using Microsoft Excel 2010. Statistical significance was considered at a p-value less than 0.05, indicating significant differences between the mean values.

RESULTS

M. citrifolia-fruit ethanolic-extract investigated in this study was found to contain various classes of phytochemical compounds, including alkaloids, flavonoids, saponins, terpenoids, and steroids. Table I provides an overview of the results obtained from the analysis for the presence or absence of these phytoconstituents.

The identification of scopoletin in the MFE was conducted using Thin Layer Chromatography (TLC), with silica gel F254 serving as the stationary phase and a mixture of toluene:ethyl acetate (10:10 % v/v) as the mobile phase. The TLC test results are presented in Table II.

Table I. Phytochemical screening of *M. citrifolia*-fruit ethanolic-extract

Compound	Observation
Alkaloids	+
Flavonoids	+
Saponins	+
Terpenoids	+
Steroids	+

Antioxidant activity

The findings demonstrated significant antioxidant activity, which was quantified and expressed as IC_{50} values. The MFE exhibited the highest antioxidant activity against DPPH, with an IC_{50} value of 0.553 mg/mL (refer to Table IV), while ascorbic acid demonstrated an IC_{50} value of 0.400 mg/mL (refer to Table III).

Anti-colitis Activity

Mice body weight monitoring was conducted by recording the body weight of the mice on day 0 as the baseline weight prior to induction and from day 1 to day 11 following induction with 2% acetic acid, with concurrent administration of the test extract from day 5 to day 11 (Figure 1). The use of 2% AA served as an inducer to establish an acute ulcerative colitis (UC) mice model, as it has been demonstrated to improve DAI scores in UC mice models (Figure 2).

Histological Assessment

In this study, histopathological examinations of the colon were conducted to assess the impact of administering 2% acetic acid on the colon and to evaluate the effectiveness of treatment in improving or inhibiting colonic damage induced by 2% AA. Staining with

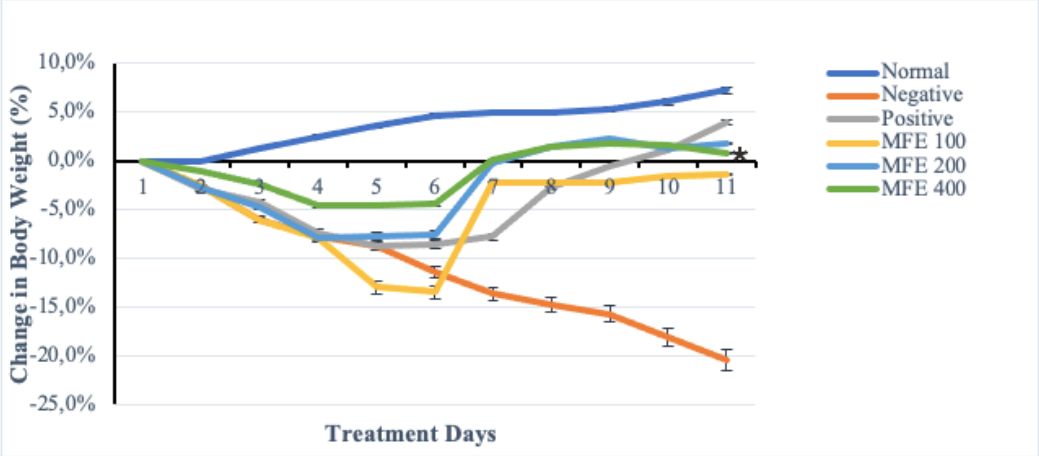

Figure 1. Effect of giving MFE on the percentage of body changes in mice induced by 2% AA. Data are shown as mean ± SEM (n=5). MFE 400 = *M. citrifolia* fruit ethanolic extract dose 400 mg/kgBB, MFE 200 = *M. citrifolia* fruit ethanolic extract 200 mg/kgBB, MFE 100 = *M. citrifolia* fruit ethanolic extract 100 mg/kgBB, Negative = Negative Control AA 2%, Normal = Na CMC 0.25%, Positive = Positive Control Sulfasalazine dose 30 mg/kgBW. p<0.05 MFE 400 vs Negative and Normal.

Table II. Result of thin layer chromatography of flavonoid identification

Sampel	Mobile Phase	Marker Compound	Detection	Spot characteristics	Rf
Scopoletin	Toluen : Ethyl Asetat (10:10 % v/v)	*Scopoletin*	UV 366	Bright blue fluorescent	0.65
			UV 254	Dark blue spot	0.65
M. citrifolia extract	Toluen : Ethyl Asetat (10:10 % v/v)	*Scopoletin*	UV 366	Bright blue fluorescent	0.65
			UV 254	Dark blue spot	0.65

Table III. Percentage of scavenging activity of Ascorbic Acid

No. Sample	IC50 value (mg/mL)	Avarage IC50 value (mg/ml) ± SEM
Ascorbic Acid 1	0.392	0.400 ± 0.005
Ascorbic Acid 2	0.401	
Ascorbic Acid 3	0.408	

Table IV. Percentage of scavenging activity of MFE

No. Sampel	IC50 value (mg/mL)	Avarage IC50 value (mg/ml ± SEM
MFE 1	0.553	0.553 ± 0.001
MFE 2	0.555	
MFE 3	0.553	

Hematoxylin and Eosin (H&E) was performed to identify the morphological alterations in the mice colon following induction with 2% AA and subsequent administration of the extract treatment. Figure 3 provides an overview of the normal colon morphology and the alterations resulting from the damage induced by 2% AA, as observed under a microscope with a magnification of 400x. The scoring of histopathological results depicting the condition of the mice colon for each treatment group are presented in Figure 4.

DISCUSSION

In recent decades, herbal drugs have experienced a resurgence in popularity for treating various human ailments (Beg et al., 2011). This resurgence can be attributed to concerns over the potential side effects and serious adverse reactions associated with many synthetic drugs commonly used to treat inflammatory disorders. Consequently, there is growing interest in exploring herbal extracts for their potential therapeutic benefits, as they are

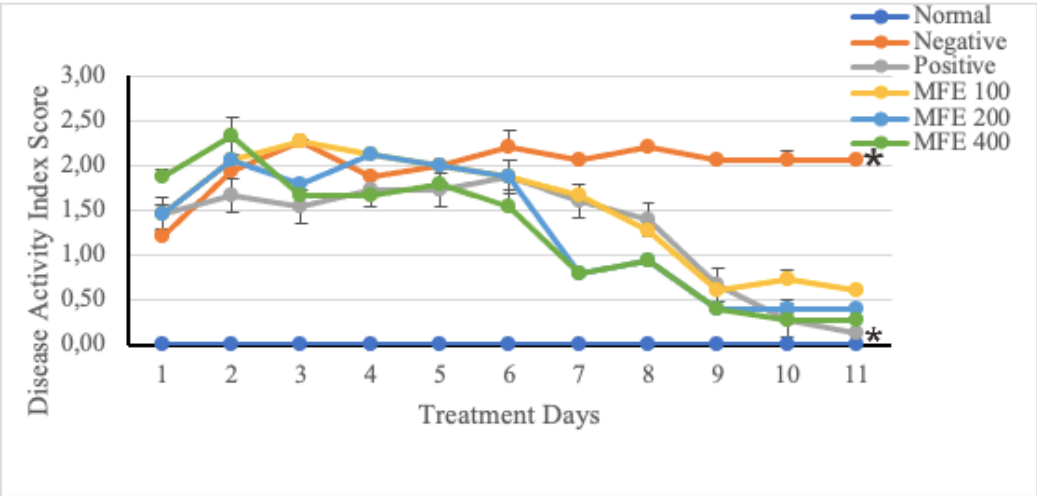

Figure 2. Effect of giving MFE on Disease Activity Index (DAI) in mice induced by 2% AA. Data are shown as mean ± SEM (n=5). MFE 400 = *M. citrifolia* fruit ethanolic extract dose 400 mg/kgBB, MFE 200 = *M. citrifolia* fruit ethanolic extract 200 mg/kgBB, MFE 100 = *M. citrifolia* fruit ethanolic extract 100 mg/kgBB, Negative = Negative Control AA 2%, Normal = Na CMC 0.25%, Positive = Positive Control Sulfasalazine dose 30 mg/kgBW. p<0.05 Positive vs Negative.

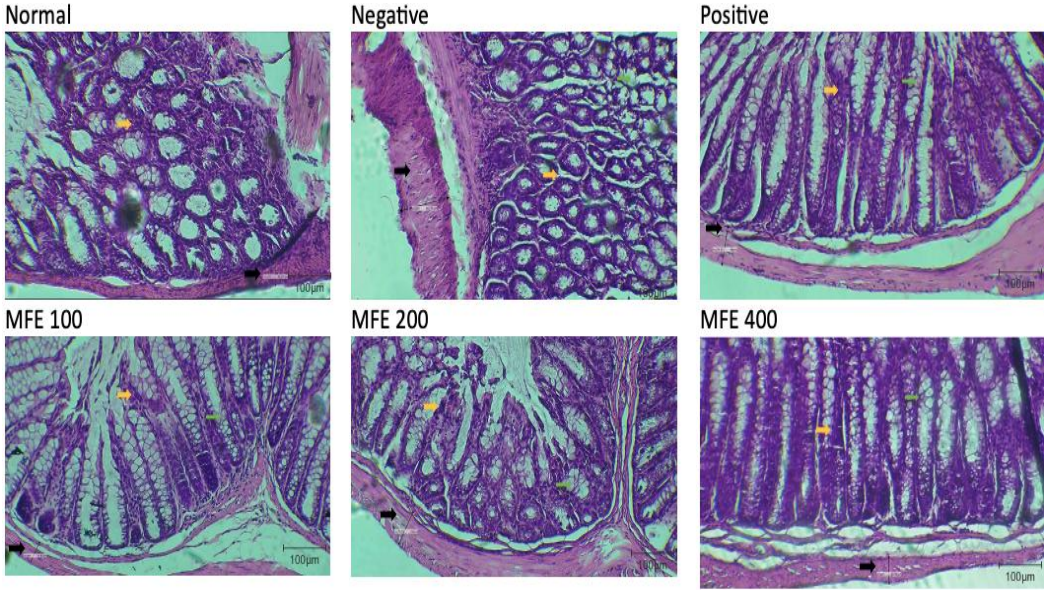

Figure 3. Normal = Na-CMC 0.25%, Negative = Negative Control Acetic Acid 2%, Positive = Positive Control Sulfasalazine dose 30 mg/kgBW, MFE 100 = EBM dose 100 mg/kgBW, MFE 200 = MFE dose 200 mg/kgBW, MFE 400 = MFE dose 400 mg/kgBW. Green arrow: architecture of the mucosa (villi and crypts), yellow arrow: cell infiltration, black arrow: muscle thickening

perceived as safer alternatives for human consumption.

The results indicated the percentage inhibition of the DPPH radical for both the MFE and the positive control at various concentrations. The findings demonstrated significant antioxidant activity, which was quantified and expressed as IC_{50} values. The MFE exhibited the highest antioxidant activity against DPPH, with an IC_{50} value of 0.075 mg/mL (refer to Table IV), while ascorbic acid demonstrated an IC_{50} value of 0.019 mg/mL (refer to Table III). It is important to note that a lower IC_{50} value indicates higher antioxidant activity, as compounds with lower IC_{50} values possess greater efficacy in scavenging free radicals. Antioxidant compounds play a crucial role in mitigating oxidative stress by impeding or preventing the oxidation process, which generates free radicals capable of damaging cells and tissues. As such, antioxidants represent a promising

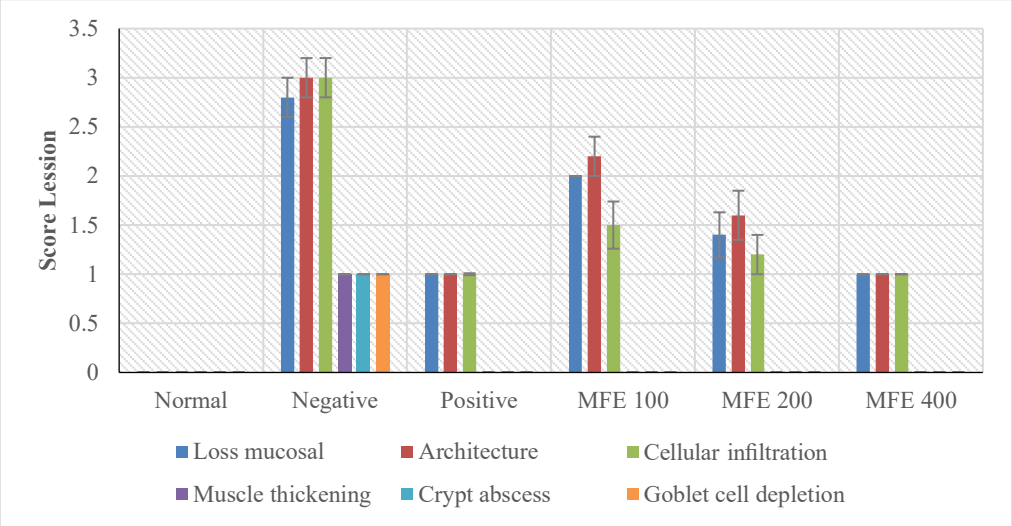

Figure 4. Histopathological score evaluation of intestinal damage in each group. Data are presented as the mean of intestinal histology damage scoring ± SEM (n=5). Normal = Na-CMC 0.25%, Negative = Negative Control Acetic Acid 2%, Positive = Positive Control Sulfasalazine dose 30 mg/kgBW, EBM 100 = EBM dose 100 mg/kgBW, EBM 200 = EBM dose 200 mg/kgBW, EBM 400 = EBM dose 400 mg/kgBW. p<0.05 vs Negative Control

therapeutic avenue for the treatment of inflammatory diseases.

UC is characterized by inflammation of the mucosa and submucosa, loss of epithelial barrier integrity, and dysregulation of the immune response (Abdallah & Ismael, 2011; Arafat et al., 2021). In severe cases, focal hemorrhage becomes evident, and the colonic mucosa becomes fragile and prone to bleeding (Lichtenstein & Rutgeerts, 2010).

In this study, administration of 2% acetic acid (AA) resulted in weight loss, soft stool consistency, and in some cases, acute diarrhea among the experimental mice. In addition, fecal blood was observed in some groups. The mice in the normal group exhibited a trend of increasing weight each day (refer to Figure 1). Furthermore, their appetite appeared good, as evidenced by the absence of leftover food daily. Conversely, the negative control group displayed a continuous decrease in weight throughout the study period, extending until day 11. In the treatment groups (100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW), weight loss was observed from the initial administration of 2% AA until the 6th day. However, significant weight gain was not observed between the treatment groups.

When assessing the Disease Activity Index (DAI), it was observed that the MFE treatment groups at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW each exhibited a decrease in DAI scores. Notably, the dose of 400 mg/kgBW demonstrated a more pronounced reduction compared to doses of 100 mg/kgBW and 200 mg/kgBW from day 7 to day 11 (refer to Figure 2). Moreover, administration of MFE at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW yielded better results than the negative control group from days 6 to 11. However, it is noteworthy that the efficacy of MFE at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW did not surpass that of the positive control group.

The histopathological analysis of the colonic organs of mice revealed discernible differences in mucosal integrity, architectural integrity, and cellular infiltration among the treatment groups. Notably, the negative control group exhibited crypt abscesses and depletion of goblet cells. As depicted in Figure 4, damage to the colonic mucosa and thickening of the muscular layer surrounding the colon were observed. This damage was characterized by the dilation of blood vessels within the glomeruli. In addition, necrosis of the epithelial cells was evident, manifesting as visible alterations in nuclear morphology, such as nuclear shrinkage, irregular borders, and a darker hue. The comprehensive structural damage to the cells was accompanied by cellular lysis and tissue inflammation. The inflammatory response was characterized by the infiltration of lymphocytes into the interstitial tissue.

In the normal group without treatment, no observable changes in colonic pathology were noted, indicating an overall intact and healthy colonic mucosa. Conversely, in the group solely induced with 2% acetic acid (AA) (negative

control), significant intestinal damage was evident. Scoring was conducted based on several criteria, including mucosal loss, architectural changes, cellular infiltration, muscle thickening, crypt abscesses, and goblet cell depletion. This underscores that intrarectal induction of 2% AA in mice can induce substantial damage to the colon organ. Upon administering MFE treatment for 5 consecutive days, the scores reflecting damage to the colon organ were as follows: MFE 100 with a score of 5.7; MFE 200 with a score of 4.2, and MFE 400 with a score of 3. In comparison, the positive control group exhibited a damage score of 3, indicating a better outcome than the negative control group, which displayed a damage score of 11.8. Notably, the scores of the MFE 100 and MFE 200 groups remained higher than those of the positive control group. This suggests that the extract treatment administered over 5 days was insufficient to fully repair or inhibit the damage induced by 2% AA induction, which was comparable to the positive control group.

The ability of MFE to repair cell damage is attributed to its antioxidant compounds. Studies indicate that *M. citrifolia* extract can significantly reduce malondialdehyde (MDA) levels and repair cells by inhibiting reactive oxygen species (ROS) and reducing the expression of inducible nitric oxide synthase (iNOS) through its antioxidant activity (Tanikawa et al., 2021; Vashti et al., 2022). Plant extracts, including those from *M. citrifolia,* are known to prevent cell damage caused by oxidative stress through their antioxidant activity. Phenolic compounds found in plants possess the ability to scavenge free radicals and inhibit lipid peroxidation (Lin et al., 2016; Shi et al., 2022).

CONCLUSION

MFE exhibited notable efficacy in reducing the Disease Activity Index (DAI) UC scores, with concentration of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW. These findings support our hypothesis that MFE holds promise as a therapeutic treatment for UC, owing to its pharmacological actions. Moreover, our results suggest that MFE may serve as a potential natural product for the treatment of UC.

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CONFLICT OF INTEREST

None

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