Research Article

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Efficacy of *Pyrus elaeagnifolia* subsp. *elaeagnifolia* in acetic acid–induced colitis model

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Abstract: In Turkish folk medicine, the fruits of *Pyrus elaeagnifolia* subsp. *elaeagnifolia* have been used to treat diarrhea and detoxify poisonous snake bites by enlarging the wound. The aim of the study was to confirm the ethnopharmacological usage of the plant using *in vivo* and *in vitro* models. Experimental colitis was performed under anesthesia by intrarectal administration of acetic acid in rats, and the extracts were administered orally. The colonic malondialdehyde (MDA), tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and nitrite levels, in addition to the myeloperoxidase (MPO) and caspase-3 activities, were measured to determine the effects of the plant extracts. The methanol (MeOH) extract revealed a significant decrease in MPO and caspase-3 levels. The MeOH extract was found to have the highest total tannin content. It was also found to have significant antioxidant (*p* < 0.01) and anti-inflammatory activities (*p* < 0.05) in acetic acid induced colitis rat model. According to our results, the present study exhibited a decrease in MDA, nitrite, IL-6, and TNF-α levels in the colon tissue and blood in the MeOH extract treated group. The findings of this study can help in treating various disorders, such as *Clostridium difficile* infection, irritable bowel syndrome, and inflammatory bowel diseases.

Keywords: Caspase-3; colitis; myeloperoxidase; *Pyrus elaeagnifolia*; Rosaceae.

1 Introduction

The *Pyrus* L. species (Rosaceae) is the most economically important species in the Old World, from Western Europe and North Africa to Asia. Some pear species are cultivated in Asia and Europe [1]. There are twenty *Pyrus* species in the worldwide. Nine species are growing in Turkey, namely, *Pyrus boissieriana* subsp. *crenulata* Browicz, *P. hakkiarica* Browicz, *P. communis* L., *P. syriaca* Boiss., *P. amygdaliformis* Vill., *P. salicifolia* Pall., *P. anatolica* Browicz, *P. bulgarica* Kuth. & Sachok., and *P. elaeagnifolia* Pall. [2]. One of the main centers of the wild pear (*Pyrus elaeagnifolia*) is Anatolia and the plant is called as “ahlat” or “çördük” in Turkish [3,4]. Two additional subspecies are *P. elaeagnifolia* subsp. *elaeagnifolia* and subsp. *kotschyana*. The fruits of the plant are one of the most highly consumed fresh or dried fruits in the world. The fruits of this species have splendid taste and aroma [5].

Various parts of *Pyrus* species are used in folk medicine worldwide. The flowers of *P. communis* L. have been used as a remedy for treating fever, pain, and spasm, whereas leaves and bark can be used in wound healing on account of their astringent action [6,7]. In Turkish folk medicine, *P. elaeagnifolia* subsp. *elaeagnifolia* is widely consumed as preservative and occasionally pickled and dried. The fruits have also been used primarily for treating diarrhea and detoxifying poisonous snake bites. The leaves are used against swelling and wild animal bites (wolf, jackal, snake, and so forth) pounded with garlic and yogurt, to drain out the poison by enlarging the wound externally [4,8,9]. An infusion of the bark is used to treat intestinal ulcers, nausea and palpitations. A decoction is used for hemorrhoids, intestinal upsets and diarrhea and to hasten the onset of labor while a colic remedy is made from the root [10]. Various biological activities, including analgesic, anti-inflammatory, antioxidant,
antispasmodic, antimicrobial, antibacterial, and wound healing, were also reported for Pyrus species [10-14].

Phytochemical studies have revealed that Pyrus species contains phenolic compounds, including β-arbutin [15], chlorogenic acid derivatives [10,16], catechin derivatives [10,16-19], as well as flavonoids [11,20] hydroxycinnamoylmalic acids and their methyl esters [21], flavonoids [17], caffeoyl triterpenes [22] and sterol glucosides [23].

The present study aimed to evaluate the efficiency of P. elaeagnifolia subsp. elaeagnifolia fruits in treating colitis.

2 Experimental

2.1 Plant Material

The fruits of P. elaeagnifolia subsp. elaeagnifolia were collected from Kahramankazan, Ankara-Turkey in July, 2016. The voucher specimen of the plant was authenticated by Prof. Dr. Murat Ekici from Gazi University, Department of Biology, Faculty of Science and Art, Ankara and specimen of the plant (GUEF3424) was deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

2.2 Extraction procedure for the bioassays

Shade-dried and powdered fruits (1000 g) of P. elaeagnifolia subsp. elaeagnifolia were successively extracted with 1500 mL n-hexane, ethyl acetate (EtOAc), and MeOH at room temperature for 48 h. The extracts were filtered and evaporated under reduced pressure till dryness at 40°C. The yields of each extract were 7.5% for n-hexane (75 g), 9.2% for EtOAc (92 g), and 33.7% for MeOH (337 g).

2.3 Determination of total phenolic content of the extracts

Total phenolic contents of the extracts were calculated using the method of Spanos et al. [24].

2.4 Estimation of total tannin content

The total tannin content of the extracts of P. eleagnifolia subsp. eleagnifolia fruits was measured by Singleton and Rossi’s method [25].

2.5 Determination of total flavonoid content

The total flavonoid content of each plant extract was measured according to the method of Zhishen et al. [26].

2.6 Pharmacological experiments

2.6.1 Animals

Male Sprague–Dawley rats (weighing 200–250 g) purchased from Kobay Laboratory Experimental Animals were used in the experiments. The rats were held for at least 3 days under laboratory conditions, prior to beginning the experiment, to adapt them to the environment. During this adaptation period, the animals were fed standard pellet feed and water and housed at room temperature in a 12-h light/12-h dark cycle. Six rats were used in each group. The present study was performed according to the international rules of the animal experiments and biodiversity rights. All animals were hospitalized in accordance with the Guide for the Care and Use of Laboratory Animals, and the experiment was approved by the Experimental Animal Ethics Committee of Kobay (Protocol number: 234).

2.6.2 Preparation of test samples for bioassay

For the activity test models, the samples were given orally to the rats after the extracts suspended with 0.5% sodium carboxymethyl cellulose (CMC). The control group rats received 0.5% CMC. Sulfasalazine (100 mg/kg) in 0.5% CMC was used as a reference drug.

2.6.3 Acetic acid-induced colitis and treatments

The effect of the test samples on colitis in rats was determined as described previously with some modifications. Thirty rats were used in acetic acid-induced colitis rat model and they were divided into five groups including control, n-hexane extract treated, EtOAc extract treated, and MeOH extract treated. From day 1 to day 3, the extracts were given to the rats at 100 mg/kg dose using oral gavage whereas the control group received 0.5% CMC. On day 4, all the rats received 2.0 mL acetic acid (3% v/v) via the intracolonic route 2 h after administration of the extracts. After anesthetizing the rats, a polyethylene catheter was inserted 8 cm proximal to the anus. The rats were kept in the same position for a few minutes and
washed with saline. On day 6, extracts were applied to the all groups. On day 7, all the rats were sacrificed. The 7-cm distal colon was removed, opened longitudinally, cleaned of fecal content using normal saline, and examined for the presence of ulcers. The proximal part of colon (6-7 cm) was removed for biochemical estimation such as thiobarbituric acid reactive substances (TBARS), MPO, and caspase-3, and the colon part were kept in physiological buffer pH 7.4 until the samples were homogenized. A small part of the proximal colon was taken and stored in 10% formalin for histological studies [27].

The tissue homogenization was done in an ice container at a concentration of 10% (w/v) in 11.5 g/L solution of potassium chloride using a homogenizer, and the homogenized samples were centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant part was taken by micropipette and separated into aliquots for biochemical estimations [27].

2.6.4 Determination of plasma lipid peroxidation level in blood

The methodology described by Kurtel et al. (1992) [28] was used. Briefly, 1 mL plasma was mixed with 2.0 mL trichloroacetic acid (TCA; 15% w/v)–thiobarbituric acid (TBA; 0.375%)–0.25N HCl thoroughly and centrifuged at 10,000g for 5 min. The supernatant was mixed with 20 mL butylhydroxytoluene (BHT; 0.02% in 95% EtOH, w/v) to prevent further oxidation and heated for 15 min in a boiling water bath. After cooling under running water, the flocculent precipitate was removed by centrifugation at 10,000 rpm for 5 min. The absorbance of the sample was measured at 532 nm against blank that contained all the reagents except plasma. 1,1,3,3-Tetraethoxypropan was used as a standard for the curve calibration.

2.6.5 Determination of lipid peroxidation in liver tissue

The method of Ohkawa et al. (1979) [29] was used to determine lipid peroxidation in tissue samples. The rats were sacrificed using an overdose of diethyl ether. The colonic tissue of each rat was immediately excised, chilled in ice-cold 0.9% NaCl, then perfused via the portal vein with ice-cold 0.9% NaCl. After washing with 0.9% NaCl, 1.0 g of wet tissue was weighed exactly and homogenized in 9 mL of 0.25M sucrose using a Teflon homogenizer to obtain a 10% suspension. The cytosolic fraction was obtained by a two-step centrifugation first at 1000g for 10 min and then at 2000g for 30 min at 4°C. A volume of the homogenate (0.20 mL) was transferred to a vial and mixed with 0.2 mL of an 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.50 mL of a 0.8% (w/v) solution of TBA. The final volume was adjusted to 4.0 mL with distilled water. Each vial was tightly capped and heated in boiling water bath for 60 min. The vials were then cooled under running water.

Equal volumes of tissue blank or test sample and 10% TCA were transferred to a centrifuge tube and centrifuged at 1000g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm in Versamax microplate reader. The control experiment was performed using the same experimental procedure, except that the TBA solution was replaced with distilled water due to the peroxidative effect of acetic acid on the tissue.

2.6.6 Assay of colonic MPO

MPO is an enzyme presented in the intracellular granules of neutrophils. The estimation of MPO was performed using a 96-well plate, and the absorbances were measured on a microplate reader (Versamax microplate reader) at 490 nm. MPO activity was evaluated according to Kondamudi et al. [27].

2.6.7 NO measurements

One hundred microliter of the serum and colonic tissue homogenates were taken, and 100 μL of Griess reagent was mixed with the samples. The absorbances were measured at 540 nm using spectrophotometer (Beckmann Dual Spectrometer). The concentrations were calculated with the standard plot using sulfanilamide as the standard [30].

2.6.8 TNF-α and IL-6 measurements

The serum and tissue levels of TNF-α and IL-6 were measured using an enzyme-linked immunosorbent assay kit (eBioscience, Austria) according to the manufacturer’s instructions.

2.6.9 Caspase-3 measurement

The enzymatic activities of caspase-3 in the tissue samples were measured using the method of Jonges et al. [31].
2.7 Techniques for histopathological investigation

All colonic tissues from the all groups were fixed in 10% formaldehyde. The tissues were processed using a Thermo Scientific Excelsior. All tissues were embedded in paraffin blocks prepared using the Histocentre 2. The Leica RM2255 microtome was used to generate 3.5-µm sections with a marine glass. All sections were stained with hematoxylin–eosin (HE) in the Shandon Varistan machine and examined under a light microscope [27].

3 Results and Discussion

Inflammatory bowel disease (IBD) is a disorder involving both autoimmune and immune-mediated disorders [32]. In ulcerative colitis (UC), an autoantigen named human tropomyosin isolated form 5 (hTM5) is vital in activating humoral and cell-mediated responses [33]. Many plants have been used in folk medicine for treating intestinal disorders worldwide. Previous studies have reported that *P. elaeagnifolia* subsp. *elaeagnifolia* fruits are also used primarily for treating diarrhea and detoxifying poisonous snake bites by the people in the rural areas [4,8].

MPO activity is a quantitative measure of disease severity and a method of evaluating drug action in animal models of intestinal inflammation [34]. MPO is an enzyme found within the granules of neutrophils. It had been demonstrated that these levels reflect the state of inflammation in the mucosa of the intestine. In the present study, MPO activity in the inflamed colon was detected. A significant decrease in the levels of MPO was observed in the MeOH and reference groups compared with the control group (Figure 1). The treatment of the MeOH extract reduced MPO concentration from 220 to 52 (p < 0.001) µg/mg of tissue. The MeOH extract could cause a decrease in MPO activity, which could be considered as a result of the anti-inflammatory activities of the samples in the acetic acid model. In the present study, the control group showed a significant increase in the lipid peroxides, which shows oxidative stress. A significant decrease in the serum levels of MDA, nitrite, TNF-α, and IL-6 was noted in the MeOH and reference groups compared with the control group. The MeOH extract reduced serum TNF-α and IL-6 levels from 9.0 ± 1.8 to 6.1 ± 1.6 (p < 0.01), from 41.1 ± 21.0 to 30.1 ± 12.1 pg/mL (p < 0.05), respectively. On the other hand, serum nitrite level was increased from 1.6 ± 0.2 to 1.8 ± 0.1 µg (p < 0.05) whereas the serum MDA level was decreased from 8.9 ± 3.4 to 4.1 ± 1.1 nmol/mL plasma (p < 0.01) with the treatment of the MeOH extract (Table 1). The MeOH extract reduced colonic TNF-α and IL-6 levels from 261.3 ± 29.2 to 120.4 ± 13.6 pg/mg (p < 0.01), respectively. On the other hand, colonic nitrite and MDA levels was decreased from 0.9 ± 0.3 to 0.5 ± 0.1 ng/µg (p < 0.05), from 304.1 ± 21.4 to 181.6 ± 11.5 nmol/g (p < 0.01) with the treatment of the MeOH extract (Table 2). The MeOH extract could combat oxidative stress by decreasing the colonic tissue contents of lipid peroxides.
The caspase-3 level in the tissue homogenate decreased in the MeOH and reference groups (Figure 2). The treatment of the MeOH extract reduced caspase-3 level from 34 to 18 pmolAMC/min/mg protein ($p < 0.001$).

In addition to those parameters, total phenol content, total flavonoid content and total tannin content of the extracts were measured. Among the extracts, the MeOH extract had the highest total phenol content, total flavonoid content and total tannin content with the value of 53.80 mg of GA/g of extract, 58.64 mg of RU/g of extract, 35.77 mg TA/g of extract, respectively (Table 3).

The colon of the reference group showed no any histopathological alterations in mucosa, submucosa, and muscularosa (Figure 3A). The disturbed architecture of colonic mucosal glands, multiple mucosal erosions such as villus flattening, invasion of some inflammatory cells in lamina propria, and some damaging crypts and goblet cells in submucosa were observed in the control group (Figure 3B). The MeOH extract group also exhibited some histopathological alterations, such as villus flattening. It revealed that the mucosal crypts showed good cellularity of moderate length compared with the control sections. In addition, the regenerating surface columnar cells were observed covering the luminal surface of the mucosa related to mild cellular infiltration in the connective tissue of the lamina propria and submucosa. Moreover, some additional alterations, such as more connective tissue cells (fibroblasts and normal intestinal gland in lamina propria), were observed in this group, too (Figure 3C). The EtOAc extract–treated group also had some histopathological alterations, such as cylindric epithelium degeneration, dilated blood vessels, and separation of the epithelium. Some other alterations such as regular lamina propria and new villus formation for intestinal absorption were also observed (Figure 3D). The n-hexane extract–treated group also had some histopathological alterations, such as...
Figure 3: The histopathological views of all groups. (A) Reference group: No any histopathological alterations in mucosa, submucosa and musculosa M: mucosa, S: submucosa, MS: musculosa. Original magnification was 4x and the scale bars represent 200µm, HE; (B) Control group: Some generation in control group. C: invasion some inflammatory cells, D: damaging crypts, V: villus flattening. Original magnification was 4x and the scale bars represent 200µm, HE; (C) MeOH extract treated group: CT: more connective tissue cells (fibroblast) DG: a little degeneration of the crypts. Original magnification was 10x and the scale bars represent 200µm, HE; (D) EtOAc extract-treated group: E: cylindric epithelium degeneration, BV: dilated blood vessels, SE: separation of the epithelium. Original magnification was 4x and the scale bars represent 200µm, HE; (E) n-hexane extract-treated group: V: villus degeneration, C: collagen fibril degeneration, BV: dilated blood vessels. Original magnification was 4x and the scale bars represent 200µm, HE.
as villus degeneration, and mononuclear cell infiltration in lamina propria. On the contrary, the colon subgroup showed no more increased collagen fibers in the lamina propria and submucosa and regeneration of the crypts in submucosa compared with the control group (Figure 3E). Therefore, it was concluded that the MeOH extract displayed a comparable activity with the other extracts and also protected the mucosa from the harmful effects of acetic acid. The present study demonstrated that acetic acid–induced colitis was associated with a significant increase in the level of MPO. In IBD, oxidative stress mediates disease progression by disrupting epithelial cell integrity. Reactive oxygen species (ROS) are crucial in the pathogenesis of UC. Moreover, the overproduction of ROS can overwhelm the protective antioxidant mechanisms resulting in oxidative damage to the cells [35]. The results of the previous study in the form of degeneration and ulcer formation were similar to those reported earlier in animal models of colitis, which focused predominantly on excess ROS and proinflammatory mediators capable of causing extensive oxidative damage to cellular structures and neutrophil infiltration [36, 37]. The colon is more responsive to oxidative damage due to low number of antioxidants in the mucosa. The accumulation of ROS could give rise to damage to specific genes by changing in antioxidant enzyme levels. In UC, oxidative stress exists with increased ROS levels and decreased antioxidant levels in the mucosa. This could lead to severe damage to macromolecules, ultimately contributing to morphological and functional damage in the cell [38-41].

In addition to the increasing number of incidents with oxidative damage and inflammatory processes, enhanced programmed cell death in IBD has also been exhibited in the literature [42-44]. Apoptosis was described as programmed cell death. Apoptosis is the process of programmed cell suicide because of irremediable tissue damage. Inflammatory event is related to cytokines, free oxygen radicals whereas programmed cell death is not related to cytokines, free oxygen radicals, or eicosanoid release. Excessive or inadequate apoptosis response during the inflammatory process leads to different pathological events. Although many enzymatic structures are appointed, the most important activators of apoptosis that promote DNA damage are enzymes influencing caspase-3 activity on programmed cell death. In the present study, administration of the MeOH extract for acetic acid–induced colitis in rats was associated with a significant decrease in the mean value of caspase-3 in the colon compared with the control group. In the present study, a significant increase in colonic caspase-3 activity was observed in the control group. Caspase-3, which is known to be expressed in enterocytes, has been also demonstrated to be increased in animal models of induced colitis [45,46].

**Table 2: Colonic tissue TNF-α, IL-6, NO and MDA levels in treated groups.**

| Groups           | TNF-α (pg/mg) | IL-6 (pg/mg) | Nitrite (ng/mg) | MDA (nmol/g) |
|------------------|---------------|--------------|-----------------|--------------|
| Control          | 261.3 ± 29.2  | 203.1 ± 18.9 | 0.9 ± 0.3       | 304.1 ± 21.4 |
| n-Hexane extract | 258.2 ± 27.5  | 197.4 ± 19.4 | 0.8 ± 0.1       | 291.7 ± 29.8 |
| EtOAc extract    | 251.4 ± 25.3  | 192.5 ± 17.1 | 0.9 ± 0.1       | 205.9 ± 20.1 |
| MeOH extract     | 132.6 ± 11.9**| 120.4 ± 13.6**| 0.5 ± 0.1*     | 181.6 ± 11.5**|
| Sulfasalazine    | 110.2 ± 9.8***| 100.1 ± 10.1**| 0.4 ± 0.0**    | 160.8 ± 10.4***|

* : p < 0.05; ** : p < 0.01; *** : p < 0.001; S.E.M.: Standard Error of Mean

**Table 3: Total phenolic, flavonoid, and tannin contents of the extracts.**

| Extracts        | Total Phenol Content (mg of GA/g of extract) | Total Flavonoid Content (mg of RU/g of extract) | Total Tannin Content (mg TA/g of extract) |
|-----------------|---------------------------------------------|-------------------------------------------------|------------------------------------------|
| n-Hexane extract| 22.69 ± 0.56                                | 14.52 ± 0.41                                    | 4.13 ± 0.35                              |
| EtOAc extract   | 31.92 ± 0.74                                | 47.31 ± 0.67                                    | 11.78 ± 0.28                             |
| MeOH extract    | 53.80 ± 0.93                                | 58.64 ± 0.24                                    | 35.77 ± 0.14                             |

Values are expressed as mean ± SEM of three replicates
It is generally agreed that the initiation and pathogenesis of UC are multifactorial, involving interactions among environmental, genetic, microbial, and immune factors. These factors are all involved and functionally integrated into the development of the chronic intestinal inflammatory reaction that characterizes UC [47,48].

Regarding the microbial flora, it is widely accepted that the intestinal flora has a central role in the pathogenesis of UC. It was suggested that loss of tolerance to the gut commensal flora in cases of UC might lead to a state of chronic intestinal inflammation [49-51]. Finally, the role of immune system in triggering and maintaining the inflammatory response in UC is of paramount importance. The immunity is mediated by different cell types, including neutrophils, monocytes, and macrophages. A growing body of evidence shows that the behavior of these cells and the expression and function of their recognition receptors are altered in UC [52].

Certain pathophysiological features of human UC and CD are weight loss and diarrhea accompanied by blood and/or mucus, fever, shortened colon, crypt abnormalities, gastric dysmotility, and infiltration of inflammatory cells [53]. Most reports on animal models of colitis focused predominantly on the proinflammatory mediators that might initiate the inflammatory process. The present study included biochemical estimation of MPO and caspase-3 levels in colonic tissues and histological examination of sections stained with hematoxylin and eosin and AB/PAS for goblet cell mucus and Trichrome stain for collagen fibers as indicators of fibrosis.

Intestinal inflammation increases the formation of harmful ROS and decreases the antioxidant defense system, thus this condition triggers oxidative stress. The colon is more vulnerable to oxidative stress than other organs due to its low antioxidant protection system [52]. Phenolic compounds and flavonoids are recognized as responsible compounds for the antioxidant activity of plant extracts. Plants belonging to the genus Pyrus often contain hydroquinone and its derivatives, such as arbutin, which has high antibacterial activity. Sroka et al. [54] reported that the EtOAc extract from the leaves of P. communis and P. elaeagnifolia had antiradical and antibacterial activities. In addition, hydroquinone in extracts from these plants showed antibacterial activity for almost all investigated bacterial strains. It was found to have good protective effects evidenced by suppression of acetic acid-induced oxidative stress in the colon of rats and attenuation of the morphological changes caused by acetic acid. The positive effects of P. elaeagnifolia subsp. elaeagnifolia could be explained by many mechanisms.

It might be due to its free radical scavenging, antioxidant activity, and antibacterial effects [55].

Natural bioactive compounds such as phenols and flavonoids are the important secondary metabolites in plants having intrinsic properties that affect the appearance, taste, odor, and oxidative stability of plant-based foods. These compounds possess biological properties such as antioxidant, anti-aging, and anti-carcinogenic. They also protect from cardiovascular diseases, immune/autoimmune diseases, and brain dysfunctions [56,57]. Phenolic compounds from plants especially are able to scavenge free radicals (superoxide and hydroxyl radicals). Hence, they enhance immunity and antioxidant defense of the body [58]. Dietary supplementation of these compounds reduces the oxidative damage to cell membrane lipids, proteins, and nucleic acids due to their strong ability to quench free radicals [59]. Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight that form complexes with the proteins [60]. Various proanthocyanidins, such as epicatechin gallate, procyanidin B2, cinnamattannins B1, and aesculitannin A have been isolated from the fruit peels of Pyrus species [61-65]. It has been reported that proanthocyanidins various biological effects include antioxidative, antimicrobial, antiallergic, anticancer, anti diabetic, and antiobesity activities. Clinical studies have demonstrated the efficacy of tannins in the treatment of acute diarrhea, the anti diarrheal effects of tannin albuminate in a patient with Crohn’s disease [66-68]. Proanthocyanidin SP-303 (from sangre de drago of Croton lechleri) is a potent inhibitor of cholera toxin induced fluid accumulation and chloride secretion [69]. As a result, it was concluded that procyanidin compounds in P. elaeagnifolia subsp. elaeagnifolia were responsible from treating colitis.

4 Conclusion

The present study showed that the MeOH extract of P. elaeagnifolia subsp. elaeagnifolia had antioxidant and anti-inflammatory properties in a rat model of colitis. The highest total phenolic, tannin, and flavonoid contents were determined in the main active extract, which was the MeOH extract of P. elaeagnifolia subsp. elaeagnifolia. The findings of this study can help in treating various disorders, such as Clostridium difficile infection, irritable bowel syndrome, and inflammatory bowel diseases. In further studies, bioassay guided fractionation and isolation technique will be conducted to determine the compounds responsible for the relevant activity.
Conflicts of interest: The authors declare no conflicts of interest.

Authors’ Contributions:
MI: In vivo studies, manuscript writing
EKA: In vivo studies, study design, manuscript writing
HT: In vitro studies, histopathological analysis
FTGD: In vivo studies
IT: In vivo studies, study design, manuscript writing

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