

# Investigating the anti-inflammatory potential of *Elaeagnus angustifolia* L. through enzyme inhibition assays

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**ABSTRACT:** Elaeagnaceae family, which is common in the steppe regions of Asia, Europe, and North America, contains about 45 species, including three genus: *Hippophae*, *Shepherdia*, and *Elaeagnus*. There are two species in the genus *Hippophae rhamnoides* and *Elaeagnus angustifolia*. The species of the Elaeagnaceae family is widely used in public and traditional medicine due to its widespread prevalence in the world and in Turkey. Ethnobotanical field studies have revealed that the fruits of *E. angustifolia* L. are used in Anatolia as an anti-inflammatory, antipyretic, urolithiatic, antidiarrheal, and anti-inflammatory agent in eye infections. Furthermore, the fruit of this plant is consumed as an appetizer and a source of nutrition. The study aims to assess the *in vitro* anti-inflammatory activity of extracts in different polarities from the leaves, branches, and fruits of *E. angustifolia* and to found the content of polyphenolic compounds (flavones and polyphenolic carboxylic acids) in the ethyl acetate extract branches using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). In this context, the anti-inflammatory effects of *n*-hexane, ethyl acetate, and methanol extracts prepared from the plant have been evaluated through *in vitro* cyclooxygenase (COX-1 and COX-2) enzyme inhibition, inhibition of protein denaturation and proteinase enzyme, and anti-lipoxygenase activity tests. The results showed the highest activity of the ethyl acetate extract from branches. The HPLC-MS analysis shown that the ethyl acetate extract prepared from the branches at a concentration of 100 mg/g contains p-coumaric acid (123.91 mg%), ferulic acid (62.07 mg%), kaempferol (43.11 mg%), sinapic acid (9.82 mg%), and quercetol (12.46 mg%). It was concluded

that the data obtained in this context supported the use of the plant in folk medicine.

**Keywords:** *Elaeagnus angustifolia*; Elaeagnaceae; anti-inflammatory; enzyme inhibition; phytonutrients

## 1. Introduction

The species of the Elaeagnaceae family is widely used in traditional medicine due to its widespread prevalence in the world and in Turkey. Distributed in Asia, Europe and North America, the family contains 3 genus and 45 species, including *Hippophae*, *Shepherdia* and *Elaeagnus* (Karamanoğlu, 1972; Bekker and Glushenkova, 2001). In Turkey, the species *Hippophae rhamnoides* L. and *Elaeagnus angustifolia* L. are found (Davis, 1984; Türk, 2017).

The *Elaeagnus* species is widely grown in Turkey, Iran and Greece due to its edible fruit. The taste of fruit is derived from the presence of basic sugars (fructose and galactose) and phenolic components (4-hydroxybenzoic, caffeic, ferulic, benzoic acid, protocatechinic, vanillic and 4-hydroxysynanic). The fruit is also used in the manufacture of alcoholic beverages (Lesica and Miles, 2001; Stannard et al., 2002). *Elaeagnus angustifolia* L., commonly referred to as oleaster, silver berry, wild olive, or Russian olive, is an excellent source of food ingredients or herbal remedies due its apparent therapeutic benefits. Oleaster's great adaptability has been shown due to its large geographic distribution, reaching from the Mediterranean to northern Russia and a diverse range of climatic circumstances. Sources published on the use of *E. angustifolia* is used in many countries around the worldwide for the treatment of gastrointestinal and urinary disorders, diarrhoea, nausea, vomiting, asthma and rheumatism (Hamidpour et al., 2017; Tuzlacı and Aymaz, 2001; Natanzi et al., 2012), as an anti-ulcerogenic (Gürbüz et al., 2003), muscle relaxant (Hosseinzadeh et al., 2003), antipyretic (Farahbakhsh et al, 2011), antinociceptive and anti-inflammatory (Ahmadiani et al., 2000; Farahbakhsh et al.; 2011; Ramezani et al., 2011), hemostatic (Hosseinsadeh and Daneshand, 2011), and wound healer (Hamidpour et al., 2017; Farahbakhsh et al., 2011; Rasekhi et al., 1999). In ethnobotanical field studies conducted in Turkey, the fruit of *E. angustifolia* has been used as a potent antipyretic, urolithiatic, antidiarrhoeal and anti-inflammatory agent in eye infections (Gürbüz et al., 2003; Bulut and Tuzlacı, 2008), while a decoction prepared from the leaves has been utilised in the treatment of kidney stones. Additionally, a decoction

prepared from the branches has been used in the treatment of diabetes (Tuzlacı and Aymaz, 2001). Phytochemical studies have shown that *Elaeagnus* species are rich in vitamin C, carotenoids (licopen), linoleic acid, tanins, flavonoids, catechins, phytosterols, phenolic acids, hydroxyacids, epoxyacides, free carbohydrates, and water-soluble polysaccharides (Patel, 2015). In biological activity studies on *E. angustifolia*, antimicrobial (Bucur et al., 2006; Okmen and Turkcan, 2013; Patel, 2015), antioxidant (Farzaei et al., 2014 and 2015; Okmen and Turkcan, 2014; Yalcin and Sogut, 2014; Caliskan et al., 2010), insecticide (Khan et al., 2016), analgesic, antispasmodic (Bekker and Glushenkova, 2001; Ramezani et al, 2011; Ahmadiani et al, 2000; Hosseinzadeh et al, 2003], antimutagenic, anticarcinogenic (Okmen and Turkcan, 2014; Wang et al., 2013; Bucur et al., 2008); antiulcerogenic (Mohammed et al., 2006; Gürbüz et al, 2003; Eliassi et al., 2009; Malihezaman et al., 2007), wound healing (Natanzi et al, 2012), and anti-arthritic (Panahi et al., 2016; Nikniaz et al., 2014) effects have been reported.

Although there are many studies on the genus *Elaeagnus* in the literature, there is no study evaluating the *in vitro* anti-inflammatory activity of *E. angustifolia*. In this study, it was aimed to investigate the *in vitro* anti-inflammatory effects of extracts of different polarity prepared from various parts of *E. angustifolia*.

## 2. Materials and Methods

### 2.1. Plant Material

*Elaeagnus angustifolia* leaves, branches and fruits were collected from Konya-Selçuklu district, Türkiye, in the first week of October 2018, dried in the shade and then powdered and used. Herbarium specimens were identified by Prof. Dr. Hayri DUMAN, who is from Gazi University, Faculty of Science, Department of Biology, and the voucher sample was deposited at the Herbarium of the Faculty of Pharmacy of Gazi University, Ankara, Türkiye.

### 2.2. Preparation of plant extracts

In order to carry out chemical and activity studies, *E. angustifolia* branches, leaves and fruits

were subjected to gradual extraction. The dried and powdered *E. angustifolia* fruit (283.11 g), branch (534.89 g), and leaf (751.26 g) were extracted separately with 5 L *n*-hexane at room temperature with mechanical stirrer for 2 times with 3 days intervals. *n*-Hexane phases were combined and condensed in rotavapor at 40°C under reduced pressure. Extracts were dried and weighed. Fruit dry extract weight: 0.55 g; Branch dry extract weight: 2,67 g; Leaf dry extract weight: 15.02 g. After extraction with *n*-hexane, the remaining plant parts were extracted with 5 L ethyl acetate 2 times with 3 days intervals by stirring with a mechanical mixer at room temperature. The ethyl acetate phases were combined and condensed in rotavapor at 40°C under low pressure. Extracts were dried and weighed. Fruit dry extract weight: 0,50 g (yield: %); Branch dry extract weight: 12,44 g; Leaf dry extract weight: 13,65 g.

After extraction with ethyl acetate, the remaining plant parts were extracted with 5 L methanol at room temperature for 2 times with 3 days intervals by stirring with a mechanical mixer. The methanol phases were combined and concentrated in rotavapor at 40°C under low pressure. Extracts were dried and weighed [Fruit dry extract weight: 2.61 g (yield: %); Branch dry extract weight: 83,57g; Leaf dry extract weight: 70,93 g.

### 2.3. HPLC-MS analysis

High performance liquid chromatography coupled with mass spectrometry (HPLC-MS) was employed to determine if the Ethyl acetate extract of *E. angustifolia* branches contained polyphenolic chemicals (flavones and polyphenolic carboxylic acids).

The procedure was modified from an HPLC approach that has been described in the literature (Fodorea et al., 2003; Suciú et al., 2004). The extract of ethyl acetate was hydrolyzed for 40 minutes in an 80°C water bath using 2N HCl. An LC system comprising a UV detector, degasser, and quaternary pump from Varian Modular Analytical HPLC Systems was used to complete the analysis. A mass detector called the Agilent 1100 MSD Ion Trap VL was connected to the HPLC system. Using an HP Agilent 1100 autosampler, samples were injected at 45°C into a Zorbax SB-C18 analytical column (100 x 3.0 mm i.d., 3.5 µm). Agilent ChemStation was used to control the apparatus and analyze the data. Peak area measurements were used to achieve external standardization on all computations related to

quantitative analysis. With a mobile phase made up of 0.1% (v/v) methanol and acetic acid, the elution process started with a linear gradient that proceeded from 5% to 42% methanol for the first 35 minutes. This was followed by an isocratic elution that continued for the next three minutes at 42% methanol. The injection volume was 5 µL, and the flow rate was 1 mL/min. At 330 and 370 nm, the detection was carried out. A Turbo-Ionspray (ESI - electrospray ionization) interface in negative ion mode was fitted to the MS. The ESI was set up with the following parameters: negative ionization, 360°C ion source temperature, nitrogen gas, 12 L/min flow rate, nitrogen nebulizer at 70 psi pressure, and 3000 V capillary voltage. Single ion monitoring (SIM) and multiple reaction monitoring (MRM) were the analysis modes used.

### 2.3. *In vitro* activity studies

#### 2.3.1. Cyclooxygenase (COX-1 and COX-2) enzyme inhibition test

Using the same basic methods for both COX-1 and COX-2 enzyme kits, the *in vitro* anti-inflammatory activity of the test samples was examined.

The COX-1 enzyme inhibition test was carried out using the Jager et al. (1996) method. The COX-1 enzyme (Sigma-Aldrich) underwent a 5-minute pre-incubation on ice after being started with co-factor solution. After mixing 60 µL of the enzyme/co-factor solution with 20 µL of the test solution (2.5 µL extract and 17.5 µL water), the mixture was allowed to sit at room temperature for five minutes. The test sample was mixed with 20 µL of arachidonic acid substrate and incubated for 10 minutes at 37 °C. Following the incubation period, 10 µL of 2 N HCl was added to stop the reaction. 4 µL of an unlabeled prostaglandin carrier solution containing 0.2 mg/mL was added. Prostaglandins were extracted using silica gel column chromatography from unmetabolized arachidonic acid. Samples were transferred into Pasteur pipettes (0.063-0.200 mm, Merck) that were filled. with silica gel. 1mL at a time, 4 mL of an *n*-hexane:dioxane:acetic acid (350:150:1 v/v) combination were added to the column in order to extract arachidonic acid. After that, prostaglandins were separated by adding 3 mL of a 425:75 v/v ethyl acetate:methanol combination, and they were then placed into separate scintillation bottles. A Beckman LS 6000 LL scintillation apparatus was used to measure radioactivity after 4 mL of scintillation solution

was introduced to each vial containing prostaglandins.

The COX-2 enzyme assay was performed using the method described by Noreen et al. (1998) with modifications (Zschocke and Van Staden, 2000). We employed human recombinant COX-2, which was obtained from the Baculovirus expression system in S21 cells and contains six histidine sequences close to the N-terminus (Sigma-Aldrich). The methodology employed was the same as that of the COX-1 enzyme assay. Four controls were performed for every experiment. In each experiment, the standard utilized was indomethacin (5  $\mu$ M for the COX-1 enzyme assay and 200  $\mu$ M for the COX-2 enzyme assay).

By comparing the quantity of radioactivity in the sample with the amount in the solvent blind, the percentage enzyme inhibition of the test samples was computed (Eldeen and Staden, 2008).

### 2.3.2. Protein denaturation inhibition test

Following a 15–20 minute incubation period at  $37 \pm 2^\circ\text{C}$  in a water bath, the reaction mixtures were heated to  $70^\circ\text{C}$  for 5 minutes. After that, the reaction mixture was given fifteen minutes to cool at room temperature. Colorimetric measurements of the reaction mixture's absorbance were made at 680 nm for each concentration (1000  $\mu\text{g/mL}$ , 100  $\mu\text{g/mL}$ , 10  $\mu\text{g/mL}$ , 1  $\mu\text{g/mL}$ , 0.1  $\mu\text{g/mL}$ , and 0.01  $\mu\text{g/mL}$ ) before and after denaturation. The mean absorbance was measured after each test was conducted three times.

Using the following formula, the percentage of protein inhibition was calculated on a percentage basis in relation to the control (Dharmadeva et al., 2018).

Protein denaturation inhibition (%) =  $\frac{\{\text{Abs control} - \text{Abs sample}\}}{\text{Abs control}} \times 100$

### 2.3.3. Proteinase enzyme inhibition test

The antiproteinase activity assay was carried out using the methodology established by Sakat et al. (2010) and Oyedapo and Famurew (1995). 2 mL of reaction mixture [0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH: 7.4) and 1 mL test sample at different concentrations (100– 500  $\mu\text{g/mL}$ )] was incubated at  $37^\circ\text{C}$  for 5 min and then 1 mL 0.8% (w/v) casein was added. The mixture was incubated for a further 20 min. To stop the reaction, 2 mL of 70% perchloric acid was added. After centrifuging the suspension, the supernatant's absorbance was measured at 210 nm. The experiment was carried out in three

replicates. Using the following formula, the percentage inhibition of proteinase inhibition activity was determined (Leelaprakash and Mohan Dass, 2011).

Antiproteinase activity (%) =  $\frac{\{\text{Abs control} - \text{Abs sample}\}}{\text{Abs control}} \times 100$

### 2.3.4. Anti-lipoxygenase activity determination

Linoleic acid and lipoxidase enzyme were used as substrates in an anti-lipoxygenase activity test. Following the dissolution of test samples in 0.25 mL of 2 M borate buffer (pH=9.0), 0.25 mL of lipoxidase enzyme solution (20 000 U/mL) was added, and the mixture was incubated for five minutes at  $25^\circ\text{C}$ . After adding and thoroughly mixing 1.0 mL of a 0.6 mM linoleic acid solution, the absorbance at 234 nm was determined. An indomethacin reference material was employed. The following formula was used to determine the percent inhibition.

% inhibition =  $\frac{\{\text{Abs control} - \text{Abs sample}\}}{\text{Abs control}} \times 100$

The IC<sub>50</sub> values were calculated by plotting the dose response curve. Every test and analysis was run three times and averaged (Shinde et al., 1999; Leelaprakash and Mohan Dass, 2011).

### 2.4. Statistical analysis

GraphPad Prism 6.0 (San Diego, CA, USA) software was used for statistical analyses. ANOVA test was performed for all parameters followed by Dunnett's test. Statistical significance in the experimental results compared with the control and reference group was expressed as follows: \* :  $p < 0.05$ ; \*\* :  $p < 0.01$ ; \*\*\* :  $p < 0.001$ .

## 3. Results

### 3.1. Effect of Cyclooxygenase

Cyclooxygenase (COX-1 and COX-2) enzyme activities of *n*-hexane, ethyl acetate and methanol extracts prepared from branches, leaves and fruits of *E. angustifolia* were recorded as percentage inhibition on prostaglandin biosynthesis. In this study, it was determined that the ethyl acetate extract prepared from the branches of *E. angustifolia* plant showed 61.8% COX-2 enzyme inhibition activity and gave results very close to the indomethacin used as a reference drug (Table 1).

### 3.2. Effects of heat-induced albumin denaturation

In our study, among the extracts of different polarity studied for the activity of *E. angustifolia* on protein denaturation, ethyl acetate extract

**Table 1.** Effects of test materials on COX enzyme inhibition

Plant part	Extract	COX-1 inhibition	COX-2 inhibition
Branches	<i>n</i> -Hexane	41,0±5,1	37,3±2,4
	EtOAc	<b>59,3±4,8</b>	<b>61,8±3,9</b>
	MeOH	42,7±6,3	44,2±3,5
Leaves	<i>n</i> -Hexane	14,8±2,6	11,9±1,1
	EtOAc	33,6±9,7	27,8±5,9
	MeOH	49,0±6,6	41,7±8,4
Fruits	<i>n</i> -Hexane	24,4±5,2	18,6±8,3
	EtOAc	44,6±3,1	18,1±6,2
	MeOH	9,5±1,4	2,6±0,8
<b>Indomethacin</b>		<b>80,2±4,6</b>	<b>65,1±5,7</b>

Prostaglandin inhibition values were determined using cyclooxygenase (COX-1 and COX-2) assays. The inhibition rate (%) obtained was expressed as statistical mean±S.D. EtOAc: Ethyl acetate; MeOH: Methanol.

**Table 2.** Effects of test samples on protein denaturation

Test material	Extract type	Concentration (µg/mL)	Absorbance	Inhibition of protein denaturation (%)
<b>Control</b>	-	-	0,43±0,09	-
Branches	<i>n</i> -Hexane	100	0,31±0,04	28
	<b>EtOAc</b>	<b>100</b>	<b>0,16±0,04**</b>	<b>63</b>
	MeOH	100	0,28±0,03	35
Leaves	<i>n</i> -Hexane	100	0,47±0,05	-
	<b>EtOAc</b>	<b>100</b>	<b>0,22±0,05**</b>	<b>49</b>
	MeOH	100	0,33±0,02	23
Fruits	<i>n</i> -Hexane	100	0,29±0,05	33
	EtOAc	100	0,55±0,07	-
	MeOH	100	0,46±0,09	-
<b>Acetyl salicylic acid</b>		<b>100</b>	<b>0,11±0,01***</b>	<b>74</b>

Experimental results are expressed as Statistical mean±S.D. \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001; EtOAc: Ethyl acetate; MeOH: Methanol

prepared from branches inhibited heat-induced albumin denaturation by 63%, ethyl acetate extract prepared from leaves inhibited heat-induced albumin denaturation by 49% and acetyl salicylic acid used as a reference substance inhibited heat-induced albumin denaturation by 74% (Table 2).

### 3.3. Effect of antiproteinase activity

In our study, it was found that ethyl acetate extract prepared from the branches of *E. angustifolia* showed 59% antiproteinase activity and acetyl salicylic acid, which was used as a reference substance, showed the highest activity with an inhibition rate of 67% at a concentration of 100 µg/mL (Table 3).

**Table 3.** Effects of test samples on proteinase enzyme inhibition

Test material	Extract type	Concentration (µg/mL)	Absorbance	Inhibition of proteinase enzyme (%)
<b>Control</b>	-	-	0,59±0,17	-
<b>Branches</b>	<i>n</i> -Hexane	100	0,48±0,29	19
	<b>EtOAc</b>	100	<b>0,24±0,03**</b>	<b>59</b>
	MeOH	100	0,33±0,18	44
<b>Leaves</b>	<i>n</i> -Hexane	100	0,60±0,38	-
	EtOAc	100	0,48±0,16	19
	MeOH	100	0,31±0,14	47
<b>Fruits</b>	<i>n</i> -Hexane	100	0,46±0,19	22
	EtOAc	100	0,50±0,15	15
	MeOH	100	0,52±0,11	12
<b>Acetyl salicylic acid</b>		<b>100</b>	<b>0,19±0,06***</b>	<b>67</b>

Experimental results are expressed as Statistical mean±S.D.

\*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001; EtOAc: Ethyl acetate; MeOH: Methanol.

### 3.4. Effect on lipoxygenase activity

In this study, it was determined that ethyl acetate extract prepared from the branches of *E. angustifolia* had an inhibitory effect of 63% and

56% at a concentration of 100 µg/mL, while indomethacin used as a reference substance had an inhibitory effect of 89% (Table 4).

**Table 4.** Effects of test samples on lipoxygenase enzyme activity

Test material	Extract type	Concentration (µg/mL)	Absorbance	Inhibition of lipoxygenase enzyme
<b>Control</b>	-	-	0,84±0,26	-
<b>Branches</b>	<i>n</i> -Hexane	100	0,42±0,14	50
	<b>EtOAc</b>	<b>100</b>	<b>0,31±0,05**</b>	<b>63</b>
	<b>MeOH</b>	<b>100</b>	<b>0,37±0,09*</b>	<b>56</b>
<b>Leaves</b>	<i>n</i> -Hexane	100	0,76±0,41	10
	EtOAc	100	0,69±0,21	18
	MeOH	100	0,66±0,39	21
<b>Fruits</b>	<i>n</i> -Hexane	100	0,70±0,48	17
	EtOAc	100	0,51±0,35	39
	MeOH	100	0,74±0,28	12
<b>Indomethacin</b>		<b>100</b>	<b>0,09±0,01***</b>	<b>89</b>

Experimental results are expressed as Statistical mean±S.D.

\*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001; EtOAc: Ethyl acetate; MeOH: Methanol

### 3.5. Effect of HPLC-MS analysis

The HPLC-MS analysis revealed that the ethyl acetate extract prepared from the branches of *E. angustifolia* at a concentration of 100 mg/g extract

contains *p*-coumaric acid (123.91 mg %), ferulic acid (62.07 mg %), kaempferol (43.11 mg %), sinapic acid (9.82 mg %), and quercetol (12.46 mg%).

## 4. Discussion

Inflammation is the typical and basic protective response to noxious stimuli such as antigen antibody reactions, infectious agents, physical, chemical, thermal agents and ischaemia and is

the body's own defence mechanism against damage, disease or destruction caused by impaired physiological functions (Goldyne et al., 1984). Physical damage is caused by a variety of stimuli, including UV exposure, microbial attack

and immune reactions. The classic appearance of inflammation is fever, redness, pain, and swelling (Punchard et al., 2004). Diseases including psoriasis, multiple sclerosis, arthritic joints, chronic asthma, and inflammatory bowel illness can all spread as a result of inflammation cascades (Brown and Mackey, 1968).

The inflammatory condition known as rheumatoid arthritis is fatal and causes cartilage, bone, and synovial membranes to deteriorate. Even though the etiology and pathogenesis of rheumatoid arthritis have not been definitively determined, it is known that pro-inflammatory cytokines have an important role in the pathogenesis of rheumatoid arthritis (Smolen et al., 2016). Various cytokines such as Interleukin-1 (IL-1) and tumour necrosis factor (TNF- $\alpha$ ) have a serious role in the pathogenic mechanisms of arthritis and are known as pro-inflammatory cytokines. The sequence of arthritic illness is linked to denaturation of proteins, suppression of inflammatory mediators, and lysosomal membrane weakening.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed worldwide due to their confirmed efficacy in reducing inflammation and pain. NSAIDs are involved in the prevention of protein denaturation and inhibition of cyclooxygenase enzyme activity, which acts as antigens and triggers autoimmune diseases (Morrow and Morrow, 2001). Ibuprofen is a non-selective NSAIDs, a derivative of propionic acid, and is prescribed as an analgesic, anti-inflammatory and antipyretic agent. Prednisolone is recognised as an alternative oral steroid, particularly for its alleviating effect in asthma, allergic conditions and infections. However, these drugs cause side effects such as bleeding, perforation and obstruction, especially gastric irritation leading to the development of gastric ulcer (Sostres et al., 2010). The increase in the prevalence of multidrug resistance has accelerated the development of new synthetic antimicrobial, antioxidant and anti-inflammatory drugs and led to the search for new antioxidant, antimicrobial, and anti-inflammatory sources from alternative sources. It is acknowledged that the primary source of novel chemicals with possible medical applications is medicinal plants. Due to their low cost and few side effects, the usage of natural products and herbal treatments has increased recently (Nostro et al., 2000).

There are two isoforms of cyclooxygenase enzymes (COX-1 and COX-2), which are produced by distinct genes on separate chromosomes. The two isoforms differ physiologically even though they exhibit about 50% homology and comparable catalytic activity. While COX-1 is often in charge of platelet aggregation and gastric cytoprotection, COX-2 is activated in inflammatory cells and is mostly expressed in response to harmful stimuli like inflammation (Pasinetti, 2001; Cronstein, 2002). Studies have shown that COX-1 enzyme inhibition causes adverse effects on gastrointestinal mucosa and increases the risk of gastric ulceration and cardiovascular diseases. However, it has been documented that the COX-2 enzyme is constitutively expressed in certain tissues, and the prostaglandins that the COX-2 enzyme produces are crucial for certain physiological and/or biological processes. (Cronstein, 2002).

In our experiment, extracts must have a minimum inhibition rate of 50% in order to be characterised as active. The ethyl acetate extract of the branches of *E. angustifolia* showed 61.8% COX-2 enzyme inhibition activity. In this context, it was concluded that the branches of *E. angustifolia* plant inhibit the formation of inflammatory prostaglandins.

Protein denaturation is one of the well-known causes of inflammation due to external factors such as strong acid or base, heat. A concentrated inorganic salt or organic solvent or causes the protein to denature, which leads to disorientation of the tertiary and secondary structure of the protein (Leelaprakash and Mohan Dass, 2011; Sen et al., 2015), preventing substrates from binding to the active site and enzymes lose their activity (Liu et al., 2013). In conditions such as cancer, diabetes, and rheumatoid arthritis, which are associated with the inflammatory process, protein denaturation leads to the production of autoantigens. Therefore, inflammatory activity can be inhibited by inhibition of protein denaturation (Sostres et al., 2010; Patel et al., 2013; Sakat et al., 2010). According to the results suggested that *E. angustifolia* branches and leaves of the plant may be effective in inflammation.

Serine proteinase, which occurs in inflammatory cells, plays an important role in various inflammatory disorders such as pulmonary and emphysema arthritis. Neutrophils are confined to a small area within lysosomes and are recognized

as a rich starting area for serine proteinase. During inflammatory reactions, leukocyte proteinase is crucial for the development of tissue damage. Deficiency in the delivery of proteinase inhibitors is the main risk factor for the growth of the inflammatory disorder (Woolf and Pfleger, 2003; Khandelwal, 2008). Proteinase inhibitors provide significant protection against inflammation (Das and Chatterjee, 1995). In our study, the results of proteinase enzyme inhibition obtained showed that the plant may have the potential to inhibit the development of inflammatory disorders due to its strong proteinase enzyme inhibitor effect.

The arachidonic acid cascades and the lipoxygenase (LOX) pathway are similar in many aspects (Gardner, 1991). As a result, lipoxygenase inhibition *in vitro* offers a useful paradigm for identifying test substances with anti-inflammatory potential. Because they are susceptible to antioxidants, LOXs primarily function by scavenging lipitoxin or lipid peroxy-radicals produced during enzyme peroxidation, which inhibits the development of lipid hydroperoxide. This could prevent the lipid hydroperoxide substrate from forming, which is necessary for the LOX catalytic cycle (Abad et al., 1995). In this context, it was concluded that *E. angustifolia* prevents the release of LOX mediators that occur in asthma, which is characterised as an inflammatory disorder through the LOX enzyme. The efficacy of the extracts prepared from *E. angustifolia* in the treatment of inflammation and pain has been demonstrated in several studies. A clinical study demonstrated that the extract from the fruit of *E. angustifolia* significantly improved pain and inflammation management of knee osteoarthritis in women. It has been demonstrated that the compound can decrease serum levels of inflammatory cytokines (MMP-1, TNF- $\alpha$ , etc.) and enhance patients' presentation of the disease (Nikniaz et al., 2014).

Two separate randomised, double-blind clinical studies were conducted to assess the efficiency of aqueous extract of *E. angustifolia* (300-600 mg/kg) which contained at minimum 0.21% kaempferol, in comparison with ibuprofen (800 mg/kg) in women with knee osteoarthritis. The *E. angustifolia* extract was found to be tolerable and safe at two doses, and was observed to be useful in reducing the symptoms of osteoarthritis (Ebrahimi et al., 2015; Panahi et al., 2016). It can be surmised that the analgesic effects of *E.*

*angustifolia* represent the most significant medical uses of this plant.

In this case, flavonoids seem to be the primary player, while terpenoids, anthocyanins, and saponins might also be important. One of the most well-known flavonoids having analgesic properties is quercetin. It has been shown to be able to inhibit lipoxygenase and cyclooxygenase (COX) activities. It has been shown to have an inhibitory effect on immunoglobulin secretion *in vitro* (Kumar et al., 2013). The mechanism governing chronic pain is significantly influenced by bioflavonoids, which have the capacity to inhibit the release of bradykinin and arachidonic acid. Pharmacological data suggests that flavonoids can inhibit the N-methyl-D-aspartate (NMDA) receptor and lower intracellular calcium levels, which results in decreased nitric oxide and phospholipase A2-calcium-dependent protein enzymatic activity (Rang and Ritter, 2012). Flavonoids also fill the ATP binding sites of kinases (tyrosine kinases or serine/threonine). The results indicated a reduction in phospholipase A2 activation and a decrease in COX production. Additionally, certain flavonoids have been demonstrated to inhibit cAMP phosphodiesterase in platelets. Other flavonoids have been demonstrated to inhibit cAMP phosphodiesterase in platelets. The reduction of cAMP results in alterations to the cytoskeleton and the inactivation of specific protein kinases. The kinases' substrates, such as phosphoproteins activated by vasodilators, stay dormant, which eventually inhibits platelet adhesion, secretion, and aggregation. This is one of the key mechanisms in regulating inflammation (Zhao et al., 2005).

It has been proposed that the inhibition of the COX pathway, which reduces the formation of TXB<sub>2</sub>, a effective aggregating and anti-inflammatory agent, may be a viable therapeutic strategy. The anti-inflammatory effects of *p*-coumaric acid have been the subject of considerable debate. When given locally, it exhibits anti-inflammatory properties; however, when given at a dose of 200 mg/kg per day, one hour prior to carrageenan injection, it had no effect on *in vivo* on carrageenan-induced paw edema (Fernandez et al., 1998). Researchers have recently shown that pretreatment with *p*-coumaric acid (50 mg/kg) can reduce an acute intestinal inflammation produced by dextran sulphate sodium. This effect has been linked to



the inhibition of COX-2 expression and activity (Luceri et al. 2004). A later investigation showed that the decrease in TXB2 production, which is connected with the suppression of COX-1 and COX-2 activities, is coupled with the reduction in platelet aggregation caused by *p*-coumaric acid. (Luceri et al., 2007).

Pro-inflammatory cytokines, which are main in processes of inflammation, develop and include TNF- $\alpha$  and IL-6. TNF- $\alpha$  is one of these pro-inflammatory cytokines that has been receiving attention previously as an important factor in the mechanism of inflammatory diseases (Maxiaad et al., 2011). Patients with psoriatic skin lesions and joints have been found to have elevated levels of pro-inflammatory cytokines, such as TNF- $\alpha$  (Mease, 2002). TNF- $\alpha$  has the potential to be a major factor in the development of various inflammatory conditions, such as psoriatic arthritis, Crohn's disease, and juvenile rheumatoid arthritis. The reduction in IL-6 and TNF- $\alpha$  levels that was found after derivatives of ferulic acid were administered implies that they could have potential use in a range of inflammatory ailments (Ellerin et al., 2003; Kuek et al., 2007). Nile et al. examined the COX-1 and COX-2 inhibitory effect of ferulic acid-related compounds seen on isolated enzymes as a mechanism of their topical anti-inflammatory impact. Moreover, it has been shown that a variety of phenolic compounds, such as catechin, hesperetin, hesperidin, luteolin, rosmarinic acid, gallic acid, naringin, and naringenin, suppress the production of COX-2 (Raso et al., 2001; Shen

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et al., 2008). Consequently, the results suggested that the chemicals associated with ferulic acid might lower inflammatory activity by inhibiting the COX-2 enzyme, which causes pro-inflammatory activity.

## 5. Conclusions/future directions

Although there are many synthetic anti-inflammatory drugs used in the treatment of inflammatory diseases today, it is aimed to develop more effective and reliable drug molecules in the treatment because these drugs do not provide the targeted effect at the expected level and have serious side effects especially in the gastrointestinal system. The results obtained are promising preliminary studies on *E. angustifolia* and it is thought that our study may contribute to the studies carried out for the development of new anti-inflammatory drugs / drug molecules and can be considered as a source on the way from plant to drug and it may be a natural alternative to NSAIDs after further studies.

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