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In vitro anti-inflammatory activities of *Tanacetum parthenium* L. extract and its major metabolite parthenolide

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ABSTRACT

Tanacetum parthenium L. (Feverfew) is daisy-like Asteraceae plant carrying sesquiterpene lactones; used for the treatment of migraine and anti-inflammatory effect. This study aims to evaluate *T. parthenium* extract and major metabolite parthenolide for *in vitro* COX-1/COX-2, LOX inhibitory activity. The extract analyzed by HPTLC. To evaluate COX-1/COX-2 inhibition assays, studied with commercial kits (20µg/mL concentration for extract, 5 µg/mL for parthenolide). The major component of extract characterized as parthenolide. IC_{50} values for COX-1/COX-2 inhibition of extract were 10.45 and 9.81µg/mL, for parthenolide; 4.86 and 1.90µg/mL. SI values of *T. parthenium* extract and parthenolide were 0.93 and 0.39. Extract showed selective COX-2 inhibitory activity. The inhibition value of extract on LOX was 80% and inhibition value of parthenolide was 41.13%. The results suggested that *T. parthenium* extract showed selective potential for COX-2 enzyme inhibition. To the best of our knowledge, the extract was tested with COX-1, COX-2, and LOX enzymes for the first time.

Keywords: COX, LOX, anti-inflammatory, *Tanacetum parthenium*, feverfew

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INTRODUCTION

Tanacetum parthenium L., (Syn: *Matricaria parthenium* L.) also known as 'Feverfew' is an Asteraceae family plant and it has daisy-like flowers. *Tanacetum* sp. grows in a wide range of area, native to the Balkan Peninsula. It easily grows along roadsides, woods and fields in North Africa, Europe, Australia, Turkiye, China and Japan. Feverfew was mentioned in Materia Medica by Dioscorides and used as a traditional remedy for inflammation and fever[1,2].

T. parthenium extracts and preparations were used as a prophylaxis against migraine, also against fever, psoriasis and arthritis ethnobotanically. Feverfew was called as 'medieval aspirin' in 1770's[1]. The plant was used since ancient times for headache, menstrual difficulties and any kind of inflammation. Another usage of feverfew is using the fresh flowering heads as insect repellent[2]. Feverfew tincture was also useful for swellings caused by insect bites. Feverfew also was a popular herb for menstrual cycle and hormones. It was known as "general strenghtener of the wombs". Traditionally, feverfew was used for asthma, pain, stomachache, threathened misscariage, spasm, tinnitus and vertigo[3].

According to previous *in vivo* and *in vitro* studies, aerial parts of feverfew has anti-inflammatory[4], antinociceptive[5], antileishmanial[6-8], antioxidant[9], antiprotozoal[10,11], α-glucosidase inhibitory[12], anti-HSV-1[13], anti-migraine[14] and insecticide[2] activities. These activities are mainly based on the sesquiterpene lactone content of the plant. Some of these compounds are germacrane type sesquiterpene lactone; parthenolide[9], guaianolide (11,13-dehydrocompressanolide)[7] and α-methylenebutyrolactone[15].

Inflammation is a kind of defence mechanism caused by the action of inflammatory agents in the body[16]. Nowadays, anti-inflammatory drugs are widely used in order to relief the symptoms of inflammation. Regular usage of anti-inflammatory drugs can cause gastrointestinal side effects, caused by the cyclooxygenase (COX) enzyme mechanism in the body. Lypoxygenase (LOX) enzyme is another important factor for inflammation cascade. Excessive accumulation of reactive oxygene species can cause release of cytokines and activation of LOX[17].

The aim of this present study was to evaluate *in vitro* anti-inflammatory activity of *T. parthenium* extract obtained by the aerial parts of the plant and parthenolide. Standardization of *T. parthenium* extract was searched according to European Pharmacopoeia by High Performance Thin Layer Chromatography (HPTLC). Anti-inflammatory activity was evaluated by the COX-1, COX-2 and LOX assays, respectively.

METHODOLOGY

Materials

Lypooxygenase (1.13.11.12, type I-B, Soybean), linoleic acid and test materials were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). COX-1 and COX-2 enzyme assay kits were purchased from Cayman Chemical Company (Ann Arbor, Michigan). All used chemicals were of analytical grade or higher if not otherwise stated.

Plant material and extraction

Dried aerial parts of *T. parthenium* as certified Pharmacopoeial quality were obtained from Caelo. Dried plant material was grounded to fine powder. 100 g of the powdered material were weighed and extraction was carried out with methanol for 1 hour with shaking. At the end of 1 hour, the extract was filtered through Whatman no:1 filter paper and concentrated with the help of a rotary evaporator. The process was repeated 3 times in total.

HPTLC analysis

HPTLC fingerprint analysis was held with an accepted methodology determined by HPTLC Association[18]. HPTLC analyzes were performed with CAMAG HPTLC system. Glass HPTLC plates coated by silica gel was used as the stationary phase, cyclohexane:ethylacetate (50:50, v:v) was used as the mobile phase. The substances were applied to the HPTLC plate using the Linomat 5 applicator system. Samples were applied to the plates as 7-mm bands, 8 mm apart. *T. parthenium* extract was dissolved in methanol as 50mg/mL density, and applied 5µL to the plate. 0,2 mg/mL parthenolide solution was used as an analytical standart. Anisaldehyde derivatization reagent was used and spots belonged to the extract were observed under the sunlight.

Enzyme inhibition assays

LOX enzyme inhibition

The *in vitro* LOX enzyme inhibition capacity was determined by using modified spectrophotometric method described by Baylac and Racine[19].

Lypoxygenase (1.13.11.12, type I-B, Soybean), linoleic acid and test materials were purchased from Sigma (St. Louis, MO, USA). Potassium phospohate buffer solution (1,94 mL; 100 mM; pH9.0), 40 µl test solution and 20 µl lypoxygenase enzyme solution were prepared and incubated for 10 minutes in 25°C environment. 50 µl linoleic acid solution was added to the reaction and measured the absorbance change at 230 nm for 20 minutes. Test materials and

Nordihydroguairatic Acid (NDGA), which was used as a positive control were dissolved in methanol. All kinetic studies were performed using quartz cuvettes. The 50% inhibition (IC_{50}) values of the test substances were calculated.

COX enzyme inhibition

COX enzyme inhibition results were screened by using colorimetric method. For this experiment, commercial COX-1 and COX-2 enzyme kits were used and the experimental protocol was carried out under the conditions determined by the company that supplied the purchased kit[20]. The concentration used for the *T. parthenium* extract was 20μg/mL, for parthenolide was 5 μg/mL.

Statistical analysis

Statistical analysis was assessed by using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, California; Version 8.4.3). One-way analysis of variance (ANOVA) and Dunnett's or Tukey post hoc tests were used for the statistical assesment to multiple comparisons. Significance limit was stated as $P < 0.05$ and all repeated tests were in triplicate.

RESULTS and DISCUSSION

Standardization

HPTLC analysis was used for standardization of the *T. parthenium* extract and its major metabolite, parthenolide. HPTLC chromatograms of the parthenolide and *T. parthenium* extract are given in Fig. 1. As a result, parthenolide was detected between 12 spots in the *T. parthenium* extract at $R_F \approx 0.45$.

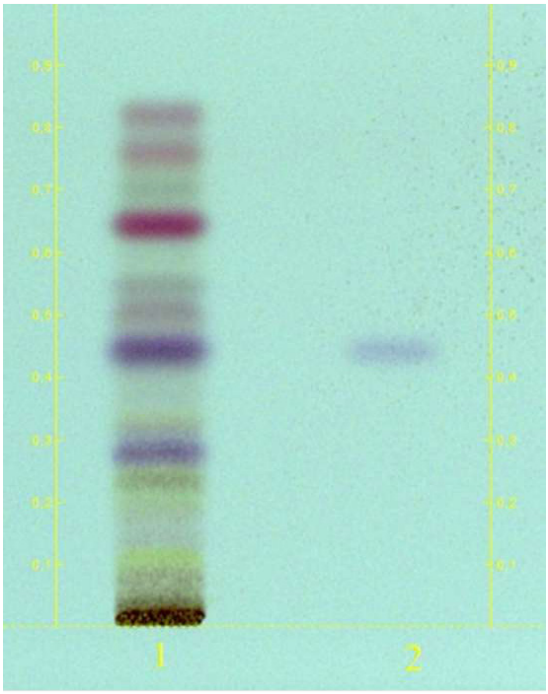

Figure 1. HPTLC chromatogram of *T. parthenium* methanolic extract (1. *T. parthenium* extract, 2. Parthenolide)

Since European Pharmacopoeia states that standardized *T. parthenium* extract should contain parthenolide, the extract was analysed and approved according to parthenolide by HPTLC system. Until now, only the essential oil of *T. parthenium* was examined with HPTLC method[21], most studies preferred to work with TLC and HPLC method[22]. This is the first study using HPTLC method for *T. parthenium* extract. In a previous study, *T. parthenium* collected from Florina was extracted by acetonitrile and examined by HPLC. Retention time was 35 min after the final purification of parthenolide [22]. Also there were studies obtaining parthenolide but using another plant instead of *T. parthenium*. *Tarchonanthus camphoratus* leaves was used and HPTLC analysis was the choice of method for parthenolide. By using BDD-run (behavior-driven development), $R_F \approx 0.15$ was detected for parthenolide[23].

COX-1, COX-2 and LOX enzyme inhibition

Anti-inflammatory activity of *T. parthenium* extract and parthenolide were examined by using the *in vitro* COX and LOX enzyme activities. COX-1 and COX-2 enzyme kits were used for measuring inhibition capacity of *T. parthenium* extract and parthenolide. IC_{50} (μg/mL) inhibition values and selective indexes are given in Table 1.

Indomethacine was used as positive control as COX-2 selective anti-inflammatory agent. The IC_{50} values of *T. parthenium* extract on COX-1 and COX-2

was 10.45 and 9.81, the IC_{50} values of parthenolide on COX-1 and COX-2 was 4.86 and 1.90, respectively. As given in Table 1, despite the inhibition power of parthenolide on COX-1 more than COX-2, *T. parthenium* extract was more powerful effect of inhibiting COX-2. The SI value of parthenolide was 0.39, hence the SI value of *T. parthenium* was 0.93. *T. parthenium* extract showed selective inhibitor effect on COX-2 compared to parthenolide.

In a previous study, parthenolide-depleted extract of feverfew inhibited the activity of pro-inflammatory enzymes 5-lipoxygenase, phosphodiesterase-3 and 4, on the other hand, the same extract was not effective on COX-1 and COX-2. In this case, the parthenolide is the responsible secondary metabolite from anti-inflammatory effect. The extract also inhibited the release of nitric oxide, PGE_2 (Prostoglandin E_2) and TNF-a from macrophages[24]. In another study, anti-inflammation activity of *T. parthenium* was studied in different pathways such as PGE_2 level, brain-derived neurotrophic factor (BDNF), interleukin-10 (IL-10), and IL-1β gene expression[4]. To the best of our knowledge, this is the first comparative study includes COX and LOX inhibiting potential of *T. parthenium* extract and parthenolide.

Regarding anti-inflammatory activity, COX-2 enzyme selectivity is important in order to avoid the side effects. COX-1 inhibition leads to classical NSAIDs side effects[25]. Since patients and health professionals avoiding from gastrointestinal side effects, selective COX-2 inhibition is very popular. The results of this study showed that ethnobotanical use of *T. parthenium* as an anti-inflammatory herb has an explanation.

In this present study, as a marker of anti-inflammatory activity, LOX inhibition capacities of *T. parthenium* extract and parthenolide was evaluated. Modified spectrophotometric method was used for determination of LOX enzyme capacity and the results support that parthenolide have a significant potential of inhibiting LOX enzyme. LOX inhibition results stated as % inhibition value which were belonged to parthenolide and *T. parthenium* extract were shown in Table 1. Parthenolide showed 41.13% and *T. parthenium* extract showed 80.12% inhibition on LOX enzyme. NDGA was used as positive control. LOX enzyme inhibition values prove that *T. parthenium* extract was a lot more powerful inhibitor compared to major component, parthenolide. This effect may be cause of synergistic mechanism of compounds beyond the standard compound, parthenolide. Isolated compounds were known as the source of biologic activity but recent studies showed that it is not true and synergistic effect usually stronger than only one molecule. A previous study stated the inhibiting capacity of *T. parthenium* extract fractions on leukocytes as strong[25].

In another research, the IC_{50} value of LOX inhibition of parthenolide-depleted extract was 11.8 ± 4.8 μg/ml[24].

Table 1. IC_{50} and SI values for COX-1 and COX-2 inhibition and % inhibition values for LOX of *T. parthenium* extract and parthenolide

Material	IC50 (μg/mL)		LOX inhibition value (%)	SI*
	COX-1	COX-2		
T. parthenium extract	10.45 ± 1.55	9.81 ± 1.07	80.12 ± 2.02	0.93
Parthenolide	4.86 ± 0.55	1.90 ± 0.14	41.13 ± 1.25	0.39
Indomethacine	1.03 ± 0.02 (μM)	7.44 ± 0.12 (μM)	NT	7.22
NDGA	NT	NT	99.8 ± 0.19	NT

*Selective Index= COX-2 IC_{50} / COX-1 IC_{50}.
*Not tested

As a conclusion, it was observed that *T. parthenium* methanol extract was effective on inflammation and it was selective on COX-2 enzyme. LOX inhibition of *T. parthenium* extract was promising. To the best of our knowledge, the extract was tested with COX-1, COX-2 and LOX enzymes for the first time. The *in vitro* biological activities may be due to sesquiterpene lactones, mainly parthenolide. Thanks to the findings of this present research, *T. parthenium* can be considered as a source of COX-2 selective anti-inflammatory compounds. These data indicated that *T. parthenium* standardized extract is a valuable plant material. Further pharmacological and clinical studies are needed for lightening the chemical reactions.

STATEMENT OF ETHICS

This study does not require any ethical approval.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Supervising: FD; Data collection and/or processing: AEK, SBK, EG; Analysis and/or interpretation: AEK, SBK, EG; Literature search and writing: RB.

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