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Anti-inflammatory activity and phenolic composition of a traditional medicinal plant from Turkey: *Potentilla recta* L.

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Potentilla recta L. (Rosaceae) is traditionally used for its antibacterial, anti-inflammatory effects and as a tonic in Turkey. In this study, phenolic composition and anti-inflammatory activities of aerial part extract of *P. recta* from Turkey was examined. The chemical composition was analyzed *via* spectrophotometric and chromatographic (LC-MS/MS) techniques. The anti-inflammatory activity was determined by measuring the inhibitory effects on nitric oxide (NO) production and determining the levels of tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10) in lipopolysaccharide (LPS) stimulated RAW 264.7 cells. *P. recta* extract decreased the TNF-α level significantly compared to LPS group (2045.18±476.12 pg/mL). Potentillin was identified in the extract and the extract was found to be rich in phenolic compounds. These findings support the traditional use of *P. recta* with its strong anti-inflammatory activities.

Keywords: *Potentilla recta*, Potentillin, LC-MS/MS, Anti-inflammatory activity

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The genus *Potentilla* is a member of the Rosaceae family, subfamily of Rosoidae, which is distributed in the temperate, arctic and Alpine regions, mostly in the northern hemisphere. The genus *Potentilla* contains about 500 species of longevity, rare biennials and annual grasses and small shrubs with rhizome. The therapeutic properties of this species have been known since ancient times[1]. In *Ayurvedic*, *Unani*, *Siddha*, Chinese and Tibetan systems of medicine more than three hundred species of genus *Potentilla* L. are used for various disorders as an ailment because of their high content of polyphenols present in their aerial and underground parts[2].

P. recta (sulfur cinquefoil) is used as an astringent, styptic, stomachic, anti-inflammatory, cleansing, and antipyretic and tonic agent, traditionally. Also it is used for the treatment of bleeding, diarrhea, internal and external inflammations; against gonorrhea, for lung and throat cleansing, for wound and ulcer rinsing in the North Serbia[3,4]. In Turkey aerial parts of the *Potentilla recta* are traditionally used for constipation, anti-pyretic, tonic, in various skin diseases, gastrointestinal disorders, neurological disorders, respiratory diseases and also dental and oral healthcare[5,6]. Owing to its traditional usage, several biological activities like antioxidant, cytotoxic, enzyme inhibitory and antimicrobial activities were studied up to date[3,7-9]. Antioxidant effect of the *P. recta* attributed to its ellagitannin: agrimoniin. Chemical profile studies of the *P. recta* have identified a neolignan glycoside and nine flavonoids: kaempferol, quercetin, quercetin 3-*O*-*β*-glucopyranoside, quercetin 3-*O*-*β*-galactopyranoside, quercetin 3-*O*-*β*-glucuronide, kaempferol 3-O-rutinoside, quercetin 3-*O*-α-rhamnopyranosyl–(1→6)-*β*-glucopyranoside, kaempferol 3-*O*-vicianoside, quercetin 3-*O*-vicianoside. Also 4-hydroxybenzoic acid, vanillic acid, syringic acid, chlorogenic acid, *p*- coumaric acid, ferulic acid, *p*-anisic acid, salicilic acid, cinnamic acid, methoxy cinnamic acid and 2-pyrone-4,6 dicarboxylic acid were identified in previous reports[10,11].

The aim of this study was to evaluate whether the anti-inflammatory activities can support the reported traditional uses of these plant. Despite the popular uses of this plant in traditional medicine, there is lack

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of information on its anti-inflammatory potentials. For this purpose we investigated the the effect of *P. recta* in RAW 264.7 cells induced by LPS, on the pro-inflammatory and anti-inflammatory cytokines, and nitric oxide levels. The chemical composition responsible for the anti-inflammatory effect is also elucidated by LC-MS/MS.

Methodology

Plant material and reagents

Aerial plant part of *Potentilla recta* L., was collected from the Aşağıbeyçayır Village in Pınarbaşı District of Kayseri (Turkey), in June 2013. A voucher specimen is stored in the Herbarium of Faculty of Arts and Sciences Biology Department of Erciyes University, in Kayseri, Turkey (BAAB 1001). All chemical substances used in the experiments were of analytical quality and obtained from the Sigma Chemical Company (St. Louis, MO).

Preparation of the extracts

Air-dried *P. recta* herb material (150 g) was powdered and extracted 3 times for 24 h using 70 % methanol as solvent in a shaking water bath. Thereafter, the extracts were filtered and evaporated to dryness under vacuum (37°C). Then it was lyophilized and stored at -18°C until analyses.

Total phenolics, flavonoids and flavonols

Total phenols were estimated as gallic acid equivalents (GAE), per gram of extract (Singleton *et al.*, 1999)[12]. The total flavonoid content was estimated as catechin (CA) equivalents using aluminum chloride colorimetric assay (Zhishen, *et al.* 1999)[13]. Total flavonols were estimated as rutin equivalents (RE), expressed as mg rutin eq/g extract (Miliauskas, *et al.* 2004)[14]. All determinations were carried out in triplicate and the mean values were calculated[12-14].

Analysis of phenolic compounds by liquid chromatography mass spectrometry (LC-MS/MS)

Experiments were performed using Shimadzu Prominence LC-20A and Triple Quadropolar Hybrid Linear Ion Trap Mass Spectrometer System. Shimadzu LC/MS-8040 LC-MS/MS system was used as the detector. The negative Electro Spray Ionisation (ESI) method is used in the analyses. 1 % Acetic acid in water (solvent A) and 1 % acetic acid in methanol (solvent B) were used as the mobile phase. The mobile phase consisted of 50 % acetic acid-water and 50 % acetic acid-methanol solution at a flow rate of 0.2 mL/min. Sample was eluted with aqueous methanol and injected in 1 µL volume. The resulting molecular ion peaks and fundamental peaks were evaluated.

Cell culture and cell viability: Sulforhodamine B (SRB) colorimetric assay

The murine macrophage RAW 264.7 cell line was purchased from American Type Culture Collection (ATCC TIB-71, Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100 µg/L streptomycin, and 100 IU/mL penicillin at 37 °C in a 5% CO_2 atmosphere.

The principle of the experiment is the binding of SRB dye to the protein compounds of the cell which are fixed to the cell culture plate by trichloroacetic acid. The SRB procedure consisted in the technique described by Houghton *et al*., (2007)[15]. Extracts were added at a final concentration of 7.81-1000 µg/ mL and plates were incubated at standard conditions for 24 h. The bound SRB was solubilized by adding 100 µL of 10mM unbuffered Tris-Base, and the absorbance was measured at 540 nm[15].

Measurement of NO and cytokines IL1 β, IL-6, IL-10, TNF-α

In the first experimental group (pre-treatment group), RAW 264.7 cells inoculated into the 6 well plates at a number $1x10^6$ and 24 h after plating, plates were incubated with extracts for 6 h at concentrations of 62.5 µg/mL and 125 µg/mL. After incubation, to induce inflammatory response LPS (1 µg/mL) was added to wells and incubated for 24 h.

In the second experimental group (co-treatment group), RAW 264.7 cells inoculated into the 6 well plates at a number $1x10^6$ and 24 h after plating, 62.5 µg / mL and 125 µg / mL concentrated extracts and 1 µg /mL concentrated LPS added to the wells at the same time.

In the third experimental group (post-treatment group), RAW 264.7 cells inoculated into the 6 well plates at a number $1x10^6$, after 24 h plating, 1 µg /mL concentrated LPS added to the wells. After 6 h 62.5 µg / mL and 125 µg / mL concentrated extracts were added to the wells. Also cells growing in the culture medium were included as (untreated cells) or negative control in both experimental groups. The supernatant of the cells in each well were centrifuged 10 min at 2500 rpm at 4 °C to remove the cellular debris.

Each 100 µL of culture supernatant was mixed with an equal volume of Griess reagent (0.1 % N-(1-

naphthyl)-ethylenediamine, 1 % sulfanilamide in 5 % phosphoric acid) and incubated at room temperature for 10 min, The absorbance at 550 nm was measured in a microplate absorbance reader (Bio-Rad Laboratories Inc.) and a series of known concentrations of sodium nitrite were used as the standards[16].

Measurements of interleukin-1 beta (IL-1β) (eBioscience Mouse IL-1β BMS6002), Interleukin-6 (IL-6) (Sigma-Aldrich Mouse IL-6 (RAB0308), Interleukin-10 (IL-10 (Sigma-Aldrich Mouse IL-10(RAB0245-1KT), TNF-α (eBioscience Mouse TNF-α (BMS 607/3)) cytokine levels and nitric oxide assays were performed according to the commercial kit procedure with the supernatant collected from the wells[18].

Statistical analysis

The normal distribution of the data was evaluated by histogram, q-q graphs and Shapiro-Wilk test. The variance homogeneity was tested by the Levene test. Independent two sample t tests were applied for quantitative variables in the intergroup comparisons. One-way analysis of variance was used in comparison between multiple groups. Dunnett test and Tukey test were applied for multiple comparisons. The data was evaluated using the R Studio 3.2.2 program. Significance level was accepted as $p < 0.05$.

Results

Extract yield, total phenolics, flavonoids and flavonols

Total phenol, flavonoid and flavonol amounts of the extract were calculated using the spectrophotometric method and found that the extract contained a high amount of phenolic material (185.85 ± 6.51) (Table 1).

Analysis of phenolic compounds by Liquid Chromatography Mass-Spectrometry (LC-MS/MS)

The chemical content of the extract was evaluated using the LC-MS/MS system, based on the molecular ion peaks and base peaks formed. Total ion spectra of *P. recta* extract [E (-) Q3 scan mode] is given in Fig. 1. The compounds identified in the extract are given in Table 2. The results of LC-MS/MS analyses showed the presence of quinic acid, ellagic acid, caffeic acid derivative, kaempferol glycoside, quercetin glucuronide, bis-HHDP glucose and potentillin in the 70 % methanol extract of *P. recta*.

Table 1 — Extract yield, total phenol, total flavonoid and total flavonol contents

		Spectrophotometric results[b]		
Extract	Yield[a] [%]	Total phenol[c] [$mg_{GAE}/g_{extract}$]	Total flavonoid[d] [$mg_{CA}/g_{extract}$]	Total flavonol[e] [$mg_{RE}/g_{extract}$]
Pr70	20.5	185.85 ± 6.51	43.46 ± 1.48	54.14 ± 0.88

Pr70: 70 % methanol extract

a Extract yield expressed as milligrams of extract per gram (dry weight) of material.

b Values (mg/g) are expressed as mean ± standard deviation.

c Total phenol expressed as gallic acid equivalent: milligram of gallic acid per gram (dry weight) of extract.

d Total flavonoid expressed as catechin equivalent: milligram of catechin per gram (dry weight) of extract.

e Total flavonol expressed as rutin equivalent: milligram of rutin per gram (dry weight) of extract.

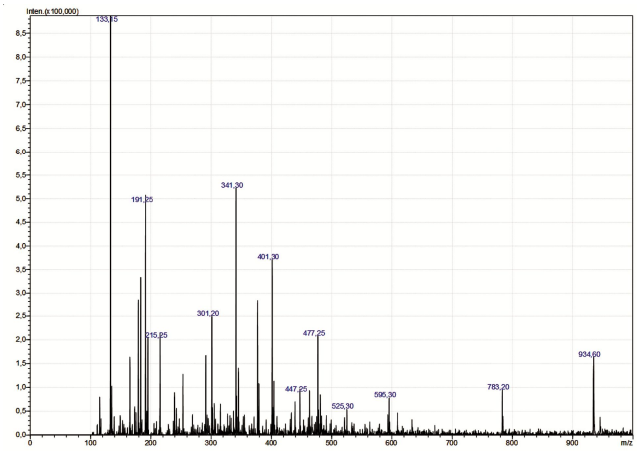

Fig. 1 — Total ion spectra of *P. recta* extract [E (-) Q3 scan mode

The compound with *m/z* 191 $[M-H]^-$ molecular ion peak seen in the spectrum was identified as the quinic acid and *m/z* 301 $[M-H]^-$ was ellagic acid[19]. It has been understood that the molecule with *m/z* 341 $[M-H]^-$ molecular ion peak is a caffeic acid derivative according to the m/z 179 [M-H] (caffeic acid) fragment seen in the spectrum[20]. The results of studies on the molecule *m/z* 447 $[M-H]^-$ molecular ion peak and *m/z* 255 $[M-H]^-$ (kaemferol) degradation were considered to be due to a sugar break in the main molecule of the resultant *m/z* 255 $[M-H]^-$. In this case, the molecule was identified as kaemferol glycoside[19,20]. The esters of glucuronic acid with flavonoids are the resultant flavanoid glucuronides, and there is a characteristic 176 amu (glucuronic acid-H_2O) difference between the molecular ion peak and the base peak in these group molecules. It was found that the investigational molecule made on the

substance having the *m/z* 477 $[M-H]^-$ molecular ion peak and the *m/z* 301 $[M-H]^-$ (quercetin) peak is the quercetin glucuronide[19-22]. It was elucidated that the molecule was a quercetin derivative of *m/z* 595 $[M-H]^-$ molecular ion peak and *m/z* 301 $[M-H]^-$ (quercetin) base peak[19-22]. The molecule with the of *m/z* 783 $[M-H]^-$ molecular ion peak and *m/z* 301 $[M-H]^-$ base peak (-HHDP glucose) was found to be bis-HHDP glucose, and *m/z* 934 $[M-H]^-$ molecular ion peak and m/z 633 $[M-H]^-$ base peak was potentillin[3,19].

The sulforhodamine B (SRB) colorimetric assay

Non-cytotoxic doses of extract from *P. recta* were determined by SRB assay using RAW 264.7 cells. The results are given in Table 3 as cell viability per cent. The extracts were tested at concentrations of 7.81 - 1000 µg / mL. At a concentration of 1000 µg / mL, cell viability was determined to be below 50 %.

Anti-inflammatory activity

NO amount

The effect of P. recta extract on NO inhibition was determined by treating the RAW 264.7 cells with LPS stimulation. Nitric oxide variants were compared between groups. Nitric oxide was found to be statistically significant in the intergroup comparisons. Results are given in Table 4. When the control group (8.43±0.00 µM) and the LPS group (70.37±0.00 µM) were compared, it was found that the LPS group was significantly higher than the control group ($p < 0.001$) and it was observed that the levels of NO amount in the extract groups (pre-treatment, co-treatment and post-treatment) were significantly lower than LPS group at both concentrations ($p < 0.001$). In the direction of these obtained results it was decided to work with pre-treatment and co-treatment groups for cytokine measurements.

Table 2 — Compounds identified by LC-MS/MS in *P. recta* extract

MW	$[M-H]^-$ m/z	Identified components	% *	References
192	191, (85)	Quinic acid	7.40	19
302	301, (199, 145)	Ellagic acid	3.75	19
342	341, (179, 119)	Caffeic acid derivative	7.86	20
448	447, (284, 255)	Kaempferol glycoside	1.37	19,20
478	477, (301, 135)	Quercetin glucuronide	3.12	19-22
596	595, (301)	Quercetin derivative	1.17	19-22
784	783, (301, 275)	Bis-HHDP glucose	1.43	3,19
935	934, (633, 301)	Potentillin	2.46	3,19

*Relative values in the total ion spectrum taken with the percentages LC-MS/MS; MW: molecular weight

Table 3 — Effect of *P. recta* extract on the viability of RAW 264.7 cells

	Concentration (µg/mL)								p
	1000 (*n*=3)	500 (*n*=3)	250 (*n*=3)	125 (*n*=3)	62.5 (*n*=3)	31.25 (*n*=3)	15.62 (*n*=3)	7.81 (*n*=3)	
Viability	40.07±2.64^{a}	59.69±0.68^{b}	87.17±0.84^{c}	95.27±3.04^{cd}	101.59±12.84^{d}	93.77±3.35^{cd}	99.38±1.51^{cd}	94.47±3.23^{cd}	< 0.001

Data are expressed as mean ± standard deviation. The same letters on the same line represent similarities between groups with different letters and differences between groups.

Table 4 — Effect of *P. recta* extract on the amount of nitric oxide in RAW 264.7: Pre-treatment group, co-treatment group, post-treatment group

Concentration	Samples					p
	Pre-treatment group (*n*=6)	Co-treatment group (*n*=6)	Post-treatment group (*n*=6)	Control (*n*=3)	Control+LPS (*n*=3)	
125 mg/mL (*n*=9)	34.50±1.44^{a}	34.30±1.97^{a}	36.90±3.49^{a}	8.43±0.00^{b}	70.37±0.00^{c}	<0.001
62.5 mg/mL (*n*=9)	43.10±2.71^{a}	38.63±0.61^{b}	40.03±1.36^{ab}	8.43±0.00^{c}	70.37±0.00^{d}	<0.001
p	0.008	0.022	0.221	-	-	

Data are expressed as mean ± standard deviation. The same letters on the same line represent similarities between groups with different letters and differences between groups.

IL-1β cytokine amount

Following incubation of RAW 264.7 cells with LPS, IL-1β amount increased significantly in LPS treated group (183.03±1.60 pg/mL) compared to which LPS untreated control group (28.38±13.37 pg/mL) ($p < 0.001$). IL-1β variants were compared among the groups. IL-1β was found to be statistically significant in the intergroup comparisons. The co-treatment group was significantly lower ($p < 0.001$) than LPS group for both 125 and 62.5 µg/mL concentrations: 170.10±5.34 pg/mL and 157.17±2.43 pg/mL, respectively (Table 5).

IL-10 cytokine amount

IL-10 variants were compared between groups and the results are presented in Table 5. IL-10 was found to be statistically significant in the intergroup comparisons. LPS group (1745.83±152.75 pg/mL) was observed to be lower than the control group (2437.50±25.00 pg/mL, $p < 0.001$). In the 62.5 µg/mL ($p < 0.001$) and 125 µg/mL ($p < 0.05$) concentrated co-treatment groups were significantly higher than LPS group (Table 5). It was found that there was no significant difference ($p > 0.05$) between the LPS and pretreatment groups.

IL-6 cytokine amount

IL-6 variants were compared between groups. IL-6 was found to be statistically significant in the intergroup comparisons. LPS group (154.52±1.89 pg/mL) was significantly higher than the control group (106.33±1.39 pg/mL, $p < 0.001$) as can be seen in Table 5. In the co-treatment group it was found that the 62.5 µg/mL concentrated group (256.18±3.18 pg/mL) was significantly higher ($p < 0.001$) than LPS group whereas there was no significant difference between the LPS and 125 µg/mL concentrated group. It was observed that the high concentration of the pre-treatment group was significantly lower than the LPS ($p < 0.001$), while the lower concentration was not significantly different from the LPS.

TNF-α cytokine amount

TNF-α variants were compared between the different groups. TNF-α was found to be statistically

Table 5 — Effect of *P. recta* extract on the amount of IL-1β, IL-10, IL-6, TNF-α in RAW 264.7 cells: Pre-treatment group and co-treatment group

Concentration	Pre-treatment group (*n*=6)	Co-treatment group (*n*=6)	Control (*n*=3)	Control+LPS (*n*=3)	p
IL-1β					
125 mg/mL (*n*=6)	273.28±2.72[a]	170.10±5.34[b]	28.38±13.37[c]	183.03±1.60[d]	<0.001
62.5 mg/mL (*n*=6)	259.83±0.97[a]	157.17±2.43[b]	28.38±13.37[c]	183.03±1.60b[d]	<0.001
p	0.001	0.019	-	-	
IL-10					
125 mg/mL (*n*=6)	1587.50±25.00[a]	1937.50±225.00[b]	2437.50±25.00[c]	1745.83±152.75[ab]	0,011
62.5 mg/mL (*n*=6)	1679.17±101.40[a]	2295.83±256.58[bc]	2437.50±25.00[c]	1745.83±152.75[a]	<0.001
p	0,202	0,02	-	-	
IL-6					
125 mg/mL (*n*=6)	109.06±7.35[a]	145.12±6.82[b]	106.33±1.39[a]	154.52±1.89[b]	<0.001
62.5 mg/mL (*n*=6)	145.42±7.06[a]	256.18±3.18[b]	106.33±1.39[c]	154.52±1.89[a]	<0.001
p	0.003	<0.001	-	-	
TNF-α					
125 mg/mL (*n*=6)	2045.18±476.12[a]	2338.26±112.12[a]	1152.62±10.00[b]	2800.82±1.94[c]	<0.001
62.5 mg/mL (*n*=6)	2565.18±215.54[a]	2514.92±97.36[a]	1152.62±10.00[b]	2800.82±1.94[c]	<0.001
p	0.01	0.108	-	-	

Data are expressed as mean ± standard deviation. The same letters on the same line represent similarities between groups with different letters and differences between groups.

significant in the intergroup comparisons. LPS group (2800.82±1.94 pg/mL) was significantly higher than the control group (1152.62±10.00 pg/mL, $p < 0.001$). Pre-treatment and co-treatment groups were found to be significantly lower than LPS group ($p < 0.001$) (Table 5).

Discussion

In this study, chemical composition and *in vitro* anti-nflammatory effects of *P. recta* were investigated. The yield of 70 % methanol extract prepared from the aerial parts of *P. recta* was found to be 20.5 %. The total phenol, total flavonoid and total flavonol amounts of the extract were determined spectrophotometrically. Total phenol, total flavonoid, and total flavonol contents was found to be 185.85 ± 6.51 mg $_{GAE}$ / $g_{extract}$, 43.46 ± 1.48 mg_{CA} / $g_{extract}$ and 54.14 ± 0.88 mg_{RE} / $g_{extract,}$ respectively. Comparing the here in reported results about the total phenolic and flavonoid contents are similar to the literature which Şöhretoğlu, *et al*. (2015) showed that the different extracts of *P. recta* and *P. astracanica* had a greater phenolics content, ranging from 88.60 to 277.05 mg GAE/$g_{extract}$[7].

The chemical composition of the extract was determined by LC-MS/MS analysis. As a result of these analyses, quinic acid, ellagic acid, caffeic acid derivative, kaempferol glycoside, quercetin glucuronide, quercetin derivative, bis-HHDP glucose and potentillin were determined in the extract. As our results, the ellagic acid derivatives and polyphenolic compounds such as potentillin were identified in the *Potentilla* species[23]. It has been reported in literature that the aerial parts of *Potentilla* species contains flavonoids, hydrolyzed tannins, and condensed tannin precursors, terpenoids, organic acids and phenol carboxylic acids. It has also been reported that *P. recta* contains kaempferol, quercetin, ellagic acid, *p*-coumaric acid, caffeic acid, ferulic acid, 2-pyrone-4,6 dicarboxylic acid[1]. As a chemical component in the studied *P. recta* extract, organic acid derivatives; quinic acid, phenolic acid derivatives; caffeic acid derivatives, flavonoid derivatives; kaempferol glycoside, quercetin glucuronide, quercetin derivative, tannin derivatives; ellagic acid, bis-HHDP glucose and potentillin complies with the literature[1,3].

The anti-inflammatory activity of *P. recta* extract was determined by the amount of anti-inflammatory and pro-inflammatory cytokines released by LPS-induced RAW 264.7 mouse macrophage cells. Macrophages are cells with functions such as phagocytosis, antigen presentation, cytokine release, and they have an important place in the fight against microorganisms[24]. Macrophage activation plays an important role in the initiation and spread of the inflammatory response by providing the production of IL-1β, TNF-α, NO and other inflammatory mediators[25]. Nitric oxide is produced in high amounts by cytokines and endotoxins in infectious cases and plays a role in the killing of parasites[26]. The Griess method was used to measure nitric oxide in our study. In the nitric oxide assay, the level of nitric oxide is higher than the LPS-administered group than in the control group, indicating that inflammation occurs. In all groups treated with extract, the decrease in nitric oxide level compared to the control group treated with LPS showed anti-inflammatory effect.

Cytokines are polypeptide constructs that are synthesized from many cells, primarily activated lymphocytes and macrophages, and play a role in regulating the function of other cells. Anti-inflammatory cytokines are molecules that suppress the activities of pro-inflammatory cytokines[27]. In this study, pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and anti-inflammatory cytokine IL-10 were investigated. Production of IL-1β by mononuclear phagocytes is induced by bacterial products such as LPS or cytokines such as TNF-α. Its functions are similar to TNF-α and co-operate with TNF-α in inflammation formation. In the LPS-administered group, there was an increase in IL-1β relative to the control group, indicating that this inflammation occurred. There was a decrease in the co-treatment group compared to the LPS group. However, in the group to which the extract was applied before (pre-treatment), an increase was observed compared to the LPS-administered group. This demonstrates that the pro-inflammatory effect occured when the extract applied before, and the anti-inflammatory effect when the extract applied together. IL-6 is secreted from endothelial cells and fibroblasts by the induction of infectious agents and cytokines such as and IL-1, TNF-α[25,28]. In the co-treatment group, it was observed that at low concentration (62.5 µg/mL), IL-6 amount was significantly higher than that of the LPS- group and there was no significant difference at the high (125 µg/mL) concentration. In the pre-treatment group, we found that the high concentration in the extract-treated group was significantly lower than the LPS group and there was no significant difference in

the low concentration. This result demonstrates that high doses of the *P. recta* act as anti-inflammatory agent when applied before LPS, and low doses of the *P. recta* act as pro-inflammatory agent when applied together with LPS. The amount of IL-10 in the control group is significantly lower than the LPS group. In the co-administered extract group IL 10 amounts were found to be significantly higher than the LPS group in both 62.5 and 125 µg/mL concentrations. In the co-administred extract group, no significant difference was found when comparing with LPS group. There was a significant increase in the level of TNF-α in the LPS group compared to the control group. In the pre and co-administered extract groups, there were decreases in TNF-α levels compared to LPS group. This significant decrease in the groups proves anti-inflammatory effect. It was observed that anti-inflammatory or pro-inflammatory characteristics may be present according to the time (before or after) and the concentration of the application of the extract. Quercetin, kaempferol, caffeic acid and ellagic acid which are present in the extract have been reported to have anti-inflammatory properties in the literature[29-31]. Also Bazylko *et al*. (2013) explained the beneficial effects of *P. recta* in treatment of skin inflammation due to its strong scavenging activity against H_2O_2, and the inhibition of the enzymatic activity of lipoxidase[3]. Several *in vitro* and *in vivo* studies demonstrated the anti-inflammatory activities of *P. reptans*, *P. erecta*, *P. anserina*, *P. chinensis* and *P. evestita* which support the traditional use of *Potentilla* species[32-36]. *P. recta* is used in the treatment of different diseases among the public and the potency and efficacy studies with *P. recta* may be helpful for reducing inflammation and it may open the door for the cure.

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