INTRODUCTION

Inflammation is a host response to harmful stimuli. This biological response is a protective mechanism of organisms for defence against injurious stimuli.[1] Acute inflammation occurs with typical several processes including increased blood flow, increased permeability and migration of neutrophils and eosinophils. These migrated immune cells able to neutralize and eliminate potentially injurious stimuli.[2] If acute inflammation is not resolved, the inflammation may pass to a longer term chronic phase. In chronic inflammation, accumulation of white blood cells also continues, but the composition of the cells changes. The primary cells of chronic inflammation are lymphocytes and macrophages.[3] Macrophages play an important role in the initiation and propagation of inflammatory responses by managing inflammation related signalling pathways such as mitogen activated protein kinases (MAPKs) signalling cascade and NF-κB signalling[4] with over-production of pro-inflammatory cytokines and other inflammatory mediators (prostaglandins and nitric oxide), generated by activated COX-2 and iNOS.[5-7] A number of inflammatory stimuli such as pro-inflammatory cytokines[8] and bacterial LPS[9] activate macrophages to up-regulate such inflammatory states. The involvement of macrophages in chronic inflammatory conditions has been the subject of considerable experimental investigation in recent years for developing new anti-inflammatory agents and exploring the molecular anti-inflammatory mechanisms of potential drugs.[10] Over-expression of inflammation-producing enzymes and their inflammatory mediators in macrophage is involved in many inflammation related disease such as atherosclerosis[11], rheumatoid arthritis[12] and cancers.[13] Additionally, RAW 264.7 murine mouse macrophage cells can be stimulated to inflammatory state by lipopolysaccharide treatment has been used as an *in vitro* inflammatory cellular model to investigate the effects of anti-inflammatory drugs, herb derived compounds and plant extracts.

Pelargonium species; members of Geraniaceae family, comprises about 750 species and approximately 80% of the genus is indigenous to South Africa A native South African medicinal plant called *Pelargonium sidoides* DC. has been traditionally used to treat cough, sore throat, congestion and other respiratory ailments.[14] Pharmacological studies have demonstrated antibacterial, antituberculosis, antiviral and immune-modulatory activities of *P. sidoides.*[15-17] Following the number of clinical studies the medicine with the international name of Umkaloabo, which comes from

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the plant's local name, has been manufactured using the roots of *P. sidoides.*[18,19] In Turkey, *Pelargoniums* are represented by two species: *Pelargonium endlicherianum* Fenzl. and *Pelargonium quercetorum* Agnew. *P. endlicherianum* is known with the common name "solucanotu" (tansy) and *P. quercetorum* "tolik" in Turkey. The extracts prepared from these species roots and the fresh flowers are used for the treatment of intestinal parasites.[20]

According to the literature, no scientific study has been reported on the anti-inflammatory activities of *P. endlicherianum* and *P. quercetorum.* Therefore the aim of this study is to investigate the anti-inflammatory effects of *Pelargonium* species growing in Turkey by measuring MAPK activation, NF-κB nuclear translocation, pro-inflammatory cytokines and other inflammatory mediators (prostaglandins and nitric oxide), generated by activated COX-2 and iNOS on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.

MATERIALS AND METHODS

Chemicals

Chromatographic standards, lipopolysaccharide and all remaining reagents were of the highest purity available and obtained from the Sigma Chemical Company (St. Louis, MO, USA). Cell culture medium and medium supplements were purchased from GIBCO (Invitrogen, USA).

Plant materials and preparation of the extracts

P. endlicherianum was collected from Eskişehir, Dağküplü village at August 2013 and *P. quercetorum* was collected from Hakkari at May 2014. A voucher specimen of *P. endlicherianum* has been deposited at the herbarium of the Anadolu University Faculty of Pharmacy, Eskişehir, Turkey (ESSE 14453) and a voucher specimen of *P. quercetorum* has been deposited at the herbarium of the Hacettepe University Ankara, Turkey (HUB 30648). The dried *P. endlicherianum* and *P. quercetorum* root was powdered and extracted with sufficient amount of 70% methanol and 11% ethanol for 24 h at 40 °C in a water bath with shaking. This procedure was repeated three times using the same batch of starting material and the resultant filtrates were combined and the solvent was removed in vacuum (40 °C). All extracts lyophilized and stored at -20 °C until the analysis time.

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Determination of the total flavonoid and phenolic contents

The total flavonoid content was estimated as catechin (CA) equivalents using aluminium chloride colorimetric assay.[21] The total phenolic content of the extract was determined by the Folin–Ciocalteu method[22] and estimated as gallic acid equivalents (GAE), per gram of extract.

High-performance liquid chromatography determination

The liquid chromatographic equipment (Agilent Technologies 1200 Series) with photodiode array detector. Separations were performed on a 250 x 4.6 mm i.d., 5 µm particle size, reverse-phase Mediterrenean-C18 analytical column operating at room temperature (22 °C) at a flow rate of 1 mL min-1. Detection was carried out between the wavelengths of 200 and 550 nm. Elution was carried out using a ternary non-linear gradient of the solvent mixture $MeOH/H_2O/CH_3COOH$ (10:88:2, v/v/v) (solvent A), $MeOH/H_2O/CH_3COOH$ (90:8:2, v/v/v) (solvent B) and MeOH (solvent C). Components were identified by comparison of their retention times to those of standards.

Cell culture

RAW 267,4 murine mouse macrophage cells (a kind gift from Prof Asuman Sunguroglu, Department of Medical Biology, University of Ankara, Ankara, Turkey) were grown in DMEM medium containing 2 mM L-glutamine supplemented with 10% heat inactivated fetal bovine serum, 100 IU/mL penicillin,100 mg/mL streptomycin.

Cell viability assay

RAW 267.4 cell viability was evaluated using the MTT colorimetric assay as described by Janjic and Wollheim.[23] Cell monolayer with the cell density of 3×10^4 cells/well was seeded in 96- well plates. After cell attachment, 100 µL of serially diluted extract (concentrations ranging from 400 to 25 µg/mL) in DMEM with 1 % FBS was added to the wells. After incubation, 10 µL of MTT solution (5 mg/mL in dH_2O) was pipetted into each well followed by a 3-hour incubation. Violet coloured formazan crystals dissolved with 100 µL DMSO and absorbance was determined at 570 nm using a microplate reader. (Bio-Tek ELX800, BioTek Instruments Inc., Winooski, VT).

ELISA (Enzyme-linked immunosorbent assay)

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Mediums collected after pre-treatment with extracts (20 μg/mL) for 24 h followed by LPS (0,5 μg/ml) treatment (in without phenol red, serum free medium) for 6 h and assayed for cytokines (TNF-α and IL-6) and prostaglandin-E2 production using eBiosciences ELISA kits (San Diego, CA, USA) following the manufacturer's protocol.

Western blotting

RAW 267.4 cells cultured in 6 well platepre-treatment with extracts (20 μg/mL) for 24 h follo wed by LPS (0,5 μg/ml) treatment (in without phenol red, serum free medium) for 6 h. Cells were lysed in 250 μL of RIPA lysis buffer (25mM Tris (pH 7,4), 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP-40)supplemented with protease inhibitor cocktail (Complete MiniTM, Roche, Mannheim, Germany) and 1 mM Na_3VO_4. Protein concentrations were determined by using the BCA protein assay (Pierce). 30 μgprotein lysates were heated for 5 min at 94 °C in Laemmli sample buffer containing 4 % *β*-mercaptoethanol and loaded on 4–12 % Tris-glycine SDS-PAGE gels, then transferred electrophoretically to PVDF membranes. Membranes were incubated overnight at 4 °C with anti-SAPK/JNK,phospho-SAPK/JNK (Thr183/Tyr185), anti-ERK, phospho-ERK(Thr202/Tyr204), anti-P38, phospho-P38 (Thr180/Tyr182)and anti- NF-κB p65 antibodies (Cell Signalling Technology), Protein bands were detected with horseradish peroxidase-conjugated secondary antibodies (Cell Signalling Technology) and visualized by West-Pico ECL reagents (Pierce).

Quantitative Real-time PCR

RAW 267.4 cells cultured in 6 well plate pre-treatment with extracts (20 μg/mL) for 24 h followed by LPS (0,5 μg/ml) treatment (in without phenol red, serum free medium) for 6 h. Total RNA isolated using RNAzol isolation reagent (*Sigma-Aldrich*, St. Louis, MO), according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed to cDNA using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Real-time PCR was carried out using a Light Cycler Nano System (Roche Diagnostics GmbH, Mannheim, Germany). To quantify cDNA, qPCR was performed using *FastStart Essential DNA probe master mix* (Roche Diagnostics GmbH, Mannheim, Germany) and catalogue assay kit (kits consist mix of primers and probes for determination of iNOS, COX-2, β-actin). For each sample the level of target gene transcripts was normalized to β-actin.

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Nitric oxide (total nitrite-nitrate (NO_x)) measurement

Mediums collected after pre-treatment with extracts (20 µg/mL) for 24 h followed by LPS (0,5 µg/ml) treatment (in without phenol red, serum free medium) for 6 h and production of nitric oxide assayed with Griess method using nitrate-nitrite colorimetric assay kit (Cayman Chemical).

Statistical analysis

Possible associations between groups were analysed with SigmaPlot 12. statistical software using student's *t*-test. P values <0.05 were considered as statistically significant. Fold increase or decrease of mRNA levels was also calculated by REST (relative expression software tool) software developed for group-wise comparison and statistical analysis of relative expression results.

RESULTS

Total phenol and flavonoid contents of extracts were shown in (**Table 1**). The total phenolic contents in the obtained extracts ranged from 162,9 to 242,9 mg GAE/g DW. The highest concentration of phenols was measured in methanol extract of *P. quercetorum.* Total phenolic content was found to be higher in ethanol extract of *P. endlicherianum* than the ethanol extract of *P. quercetorum.*The total flavonoid contents in the obtained extracts ranged from 36,0 to 64,9 mg RE/g DW. The concentration of flavonoids in ethanol extract of *P. endlicherianum* was found to be less than the ethanol extract of *P. quercetorum.*Total phenolic and flavonoid compounds were found to be higher in methanol extracts than the ethanol extracts. High solubility of phenols and flavonoids in polar solvents provides high concentration of these compounds in the extracts dependingon the polarity of solvents used in extraction.

Chemical compositions of the extracts were determined by HPLC analyses (**Table 2**). Apocynin (1-(4-Hydroxy-3-methoxyphenyl) ethanone) identified in the *P.endlicherianum* extracts as the main compound. Based on the results of quantitative HPLC analysis, the highest content of apocynin (3,51±0,016 $\%_{extract}$) had 70% methanol extract of *P.endlicherianum*. *P.endlicherianum*%11 ethanol extract had lower(2,48±0,002 $\%_{extract}$) apocynin levels than 70% methanol extract of *P. endlicherianum.*The apocynin content of 11% ethanol and 70% methanol extracts of

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P.quercetorum was found 0,51± 0,01 and 0,049±0,01%$_{extract}$, respectively. Gallic acid was also identified both in *P. endlicherianum* and *P. quercetorum*. Highest gallic acid content was found in 11% ethanol extract of *P.endlicherianum* (1,070 ± 0,004%$_{extract}$).The gallic acid content of *P.quercetorum* was found 0,012 ± 0,001 and 0,098 ± 0,001%$_{extract}$ respectively for 11% ethanol and 70% methanol extracts.

RAW 264.7 macrophage cells were pre-treated with increasing concentration (10, 20, 25, 50, 100, 200 µg/mL) of *P. sidoides* (EPs ® 7630) and each other extracts for 24h. According to these findings (**Figure 1**), *P. sidoides* (EPs ® 7630) was found to be most cytotoxic towards RAW 264.7 macrophage cells and non-toxic (20 µg/mL) concentration of extracts were selected and used in the subsequent experiments for testing their protective effect on LPS-induced inflammatory response.

Response to LPS (0,5 µg/mL, 6 hr) treatment, release of pro-inflammatory cytokines TNF-α (**Figure 2A**) and IL-6 (**Figure 2B**) were increased approximately 2,0 and 38,2 fold, respectively. Pre-treatment with *P. endlicherianum* 11% ethanol extract and *P. quercetorum* 11% ethanol extract caused a reduction in the release of TNF-α and IL-6, but the reduction of IL-6 release after treatment with *P. quercetorum* 11% ethanol extract did not reach any statistically significance.

Treatment with LPS resulted in significant upregulation of COX-2 (**Figure 3A**) and iNOS (**Figure 3B**) mRNA levels 5,8 fold and 2,9 fold respectively. However, pre-treatment with *P. sidoides*, *P. endlicherianum* 11% ethanol and *P. endlicherianum* 70% methanol extract displayed a marked decrease in the induction of COX-2 mRNA levels. Only pre-treatment with *P. endlicherianum* 11% ethanol extract inhibited the mRNA expression levels of iNOS in LPS-activated RAW 264.7 cells. The increase of COX-2 and iNOS expression in LPS-activated murine macrophage RAW 264.7 was accompanied by the release of large amounts of their products, respectively, PGE_2 (**Figure 4A**) and NO (**Figure 4B**). *P. sidoides*, *P. endlicherianum* 11% ethanol and *P. endlicherianum* 70% methanol extract produced also a considerable decrease in the levels of the COX-2 product, PGE_2, which confirms the inhibitory effect of *Pelargonium* extracts toward the COX-2 enzyme. *P. endlicherianum* 11% ethanol extract and *P. quercetorum* 11% ethanol extract completely suppressed the NO production induced by LPS.

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In the current study an increased expression of Nuclear Factor-κB (p65)protein in cytoplasm and enhanced p65 nuclear translocation were observed upon LPS activation. All studied extracts excluding *P. sidoides* caused a significant reduction on the cytoplasmic p65 protein expression (**Figure 5A**). Moreover, *P. endlicherianum* 11% ethanol and 70% methanol extracts have an inhibitory effect on p65 nuclear translocation (**Figure 5B**).

Finally, we investigated the inhibition of MAPKs activation by Pelargonium extracts. Western blot analysis of RAW 264.7 cells exposed to LPS revealed expression levels of MAPKs, which were partly or markedly downregulated by *Pelargonium* extracts (**Figure 6**). LPS exposure to RAW 264.7 cells resulted in significant activation in MAPKs including phosphorylated extracellular signal-regulated protein kinases (p-ERK) (**Figure 7A**), phosphorylated P38 MAPK (p-P38) (**Figure 7B**) and phosphorylated c-Jun N-terminal kinases (p-JNK) (**Figure 7C**). The LPS-induced activation of ERK was prominently blocked by both *P. endlicherianum* 11% ethanol and 70% methanol extracts. The overactivation of P38 by LPS was suppressed in the presence of *P. sidoides* and *P. endlicherianum* 11% ethanol extract. Activated JNK by LPS exposure was blocked by *P. endlicherianum* 11% ethanol, 70% methanol and *P. quercetorum* 70% methanol extracts.

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DISCUSSION

Medicinal plants continue to be an important source of new chemical substances with potential therapeutic effects. Numerous natural products have been tested in various *in vitro* and *in vivo* models for the development of new anti-inflammatory agents. This study was designed to investigatethe anti-inflammatory activities of the root extract (%11 ethanol and %70 methanol) of *P. endlicherianum and P. quercetorum* and especially compare with the effects of *P. sidoides (*EPs ® 7630*)* on the induction of inflammatory signalling and production of pro/anti-inflammatory substances in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.

In South Africa, polyphenol-rich herbal preparations made up from roots of *Pelargonium sidoides* and *Pelargonium reniforme* are traditionally used to treat respiratory and gastrointestinal infections, dysmenorrhea, and hepatic disorders. [14-16] Inspired by the healing of his tuberculosis, Charles Henry Stevens introduced this phytomedical drug to England already in 1897.[24] More than seven decades later, a special ethanol extract of *Pelargonium sidoides* roots, was finally developed [EPs® 7630] (ISO Arzneimittel, Ettlingen, Germany)]. In Germany, EPs 7630 is approved today for the therapeutic use in patients with acute bronchitis. In addition, EPs 7630 was shown to be effective in clinical trials with patients suffering from tonsillopharyngitis, rhinosinusitis, common cold or COPD.[25, 26-30]

According to a meta-analysisabout *P. sidoides*, there are several studies exploring the effects of *P. sidoides* in treating acute upper respiratory tract infections. 2,871 patients participated in these studies; an average of 261 in each and the clinical trials were performed in adults, adolescents and children.[26]

In Turkey, *Pelargoniums* are represented by two species: *P.endlicherianum* Fenzl. and *P. quercetorum* Agnew. Molecular mechanisms of *P. sidoides* have been partly identified but there is not any relevant data revealing the similar effects of *P. endlicheiranum* and *P. quercetorum* so far. Understanding the molecular effects of *P. endlicheiranum* and *P. quercetorum* is key to take advantage of their medical potential like *P. sidoides*. So far, data regarding its target cells and effects there of within the human immune system are lacking. To address this issue, we first investigated how *P. endlicherianum* and *P. quercetorum* influences the anti/pro-

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inflammatory responses of LPS-induced RAW 264.7 cells and compared the results with *P. sidoides*.

When investigating the signalling cascades induced by *P. endlicherianum* and *P. quercetorum*, we firstly activated the RAW 264.7 cells by LPS. LPS exposure to RAW 264.7 cells resulted in significant activation in MAPKs including phosphorylated extracellular signal-regulated protein kinases (p-ERK), phosphorylated P38 MAPK (p-P38) and phosphorylated c-Jun N-terminal kinases (p-JNK) whereas the LPS-induced activation of ERK was prominently blocked by both *P. endlicherianum* 11% ethanol and 70% methanol extracts. The overactivation of P38 by LPS was suppressed in the presence of *P. sidoides* and *P. endlicherianum* 11% ethanol extract. Activated JNK by LPS exposure was blocked by *P. endlicherianum* 11% ethanol, 70% methanol and *P. quercetorum* 70% methanol extracts.

Witte et al.[25] has treated the human peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors to compare the bacterial and viral stimulation of the immune system and EP7630. They found that a strong MAPK kinase pathway activation, which included phosphorylation of JNK, ERK1/2, and P38. Furthermore, EPs 7630 slightly provoked NF-κB and PI3K pathway activation. However, pharmacological blockage of only P38 resulted in a strongly decreased monocyte TNF-α production. The observation that this signalling pattern differed from that induced by TLR3 and TLR4 ligand, inflammatory cytokines, and CD3/CD28 engagement suggest that Pelargonium extract affects monocytes via receptors different from those used by the mentioned stimuli.

Differing from this study, in our experimental design the cells were stimulated by LPS and the effects of *P. sidoides, P. endlicherianum, P. quercetorum* has been compared on the inflammatory response. Pre-treatment with the extracts have decreased the effects of inflammation. Furthermore, stimulation of the RAW 264.7 cells with LPS (0,5μg/ml, 6 hrs treatment) caused an elevated production of pro-inflammatory cytokines (TNF-α and IL-6), increased mRNA expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) with release of prostaglandin E2 (PGE-2) and nitric oxide (NO), activated mitogen activated protein kinase (phosphorylation of JNK, ERK, P38) signalling pathway and nuclear translocation of Nuclear Factor-κB (p65). Which were markedly inhibited by the pre-

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treatment with %11 ethanol and %70 methanol root extracts of *P. endlicherianum* without causing any cytotoxic effects. *P. quercetorum* root extract decreased only TNF-α production and *P. sidoides* root extract alleviated P38/MAPK activation and COX-2 mRNA expression with PGE2 production.

In a study designed to investigate the effects of *P. sidoides* on inflammatory responses was conducted on Leishmania major-infected murine macrophages. In that study, EPs 7630 increased cellular nitric oxide production and mRNA levels of iNOS and several cytokines (IL-1β, IL-10, IL-12, IL-18, TNF-α, IFN-α, IFN-γ).[31] However, our knowledge regarding the influence of Pelargonium extract on human immune cells, in particular on their cytokine production, is still highly restricted. To gain insight into this matter we comprehensively studied the immune-regulatory effects of *P. endlicherianum* and *P. quercetorum* and compared them with the effects of *P. sidoides* on murine macrophage cells. According to our study, TNF-α, IL-6, COX-2, PGE2, iNOS expressions were inhibited by *P. endlicherianum.*

Witte et al.[26] revealed that *P. sidoides* (EPs 7630) strongly and dose-dependently induced the production of the pro-inflammatory cytokines TNF-α and IL-6 in human blood immune cells. Moreover, a less prominent induction of the anti-inflammatory acting IL-10 was observed. In the same line with this study *P. sidoides* root extract alleviated P38/MAPK activation and COX-2 mRNA expression with PGE2 production.Our data indicates that especially %11 ethanol root extract of *P. endlicherianum* targets the inflammatory response of macrophages via inhibition COX-2, IL-6, and TNF-α through inactivation of the NF-κB signalling pathway, supporting the pharmacological basis of *P. endlicherianum* as a traditional herbal medicine for treatment of inflammation and its associated disorders.However, *P. endlicherianum and P. quercetorum* pre-treatment has inhibited the activation of this pathway revealing that the *P. sidoides* has pro-inflammatory effects and can be used more for its protective effect but the *P. endlicherianum* can be used as a potential herbal medicine for treatment.

Furthermore, according to our results apocynin was the major component of the *P. endlicherianum* root extract which has also been identified by this study for the first time. Apocynin has shown to have strong anti-inflammatory effects in several studies.[32-34] One of the recent studies[34] conducted on the RAW 264.7 cells with

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apocynin, the effects of apocynin on the extracellular release of NO and PGE2 were examined in LPS-stimulated RAW 264.7 macrophages. Cells were incubated with apocynin for 1 h prior to LPS treatment (200 ng/mL). While LPS increased the extracellular release of NO and PGE2 production, approximately 10- and 30-fold respectively in RAW 264.7 cells, apocynin (100–500 mM) attenuated the release in a concentration-dependent manner. According to the same study, apocynin inhibited the expression of iNOS and COX-2 and reduced the TNF-α and inhibited the phosphorylation of MAP kinases JNK, ERK and P38. According to our results,*P. endlicherianum* root extracts, but not the *P. quercetorum* root extracts, were rich in apocynin and the similar strong anti-inflammatory effects that we observed by *P. endlicherianum* might be depending on the rich apocynin ingredient since the *P. quercetorum* did not show as strong anti-inflammatory effect as *P. endlicherianum*.

CONCLUSION

The present study demonstrated that *P. endlicherianum* suppressed LPS-induced inflammatory responses via the suppression of MAP kinase signalling pathways in LPS-challenged RAW 264.7 macrophages whereas the *P. sidoides* seems to be pro-inflammatory rather than being anti-inflammatory. Furthermore, it clearly showed that *P. endlicherianum* is rich in apocynin and probably exerts its anti-inflammatory effects via suppression of LPS-induced activation MAP kinase signalling pathways over apocynin. In conlusion, all these results suggest that *P. endlicherianum* might be a useful herbal medicine for dissecting inflammation-related pathologies.

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Table 1. Total phenol and flavonid content of ethanolic and methanolic extracts of *P. endlicherianum and P. quercetorum* root extracts. Each value is the average of three analysis ± standard deviation.

	Total phenols[c]	**Total flavonoids**[d]
(*P. endlicherianum*[a])	173.93±7.72	36.03±0.76
(*P. endlicherianum*[b])	201.85±6.44	41.70±0.46
(P. *quercetorum*[a])	162.90±6.95	46.63±1.93
(P. *quercetorum*[b])	242.97±5.52	64.95±2.95

[a] 11% ethanol extract
[b] 70% methanol extract
[c] mg gallic acid equivalent/g dry weight
[d] mg rutin equivalent/g dry weight

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Table 2. Phenolic compound apocynin and gallic acid content of extracts. Each value is average of three analysis ± standard deviation.

	Apocynin[c]	**Gallic acid**[c]
(*P. endlicherianum*[a])	2.486±0,002	1,070±0,004
(*P. endlicherianum*[b])	3.509±0,016	0,458±0,008
(*P. quercetorum*[a])	0,510 ±0,015	0,012±0,001
(*P. quercetorum*[b])	0,49 0± 0,012	0,098±0,001

[a] 11% ethanol extract
[b] 70% methanol extract
[c] % extract

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Figure 1. Effects of Pelargonium extracts on cell viability. n=4, *p<0,05 vs. Control.

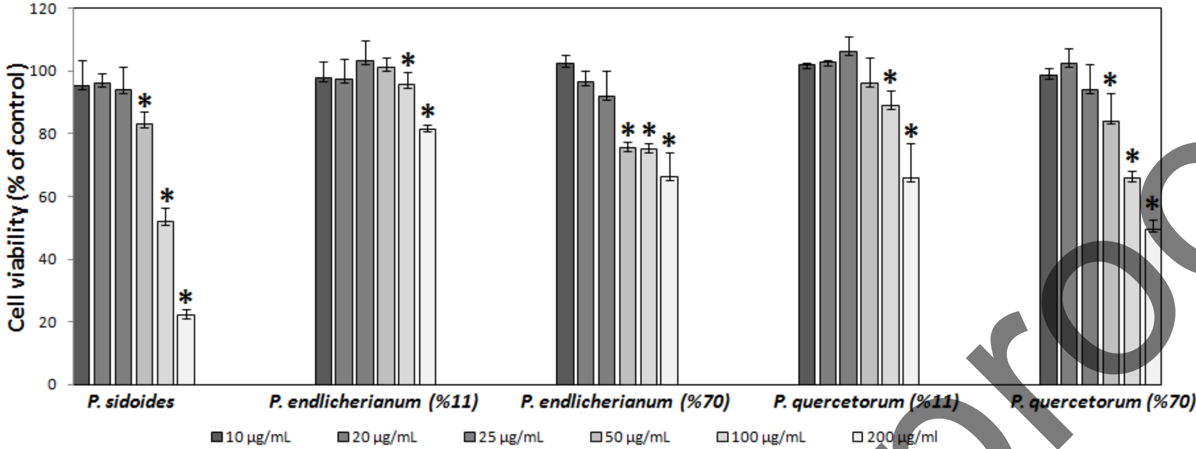

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Figure 2. Pretreatment with extracts modulate cytokine production in LPS treated macrophages. n=4,*p<0,05 vs. Control, $p<0,05 vs. LPS.

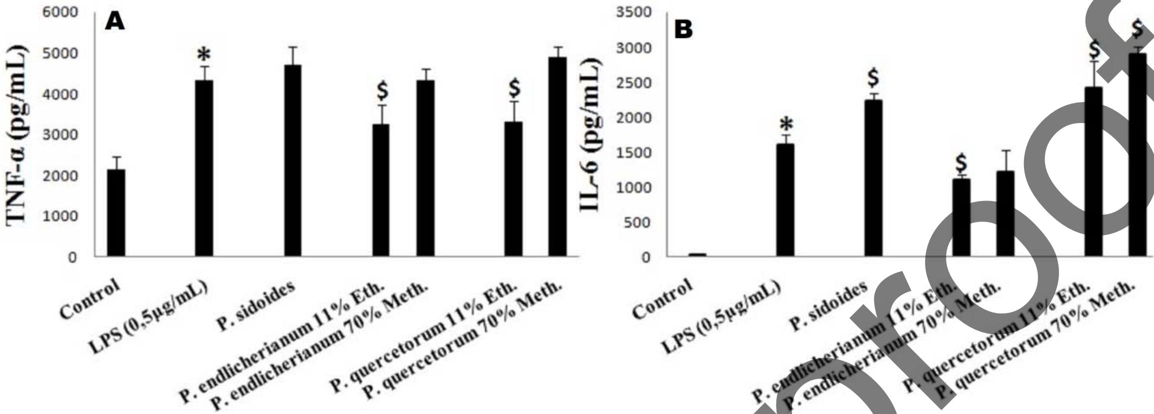

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Figure 3. Effect of extracts on inflammation-producing enzymes mRNA levels in LPS treated macrophages. n=3,*p<0,05 vs. Control, $p<0,05 vs. LPS.

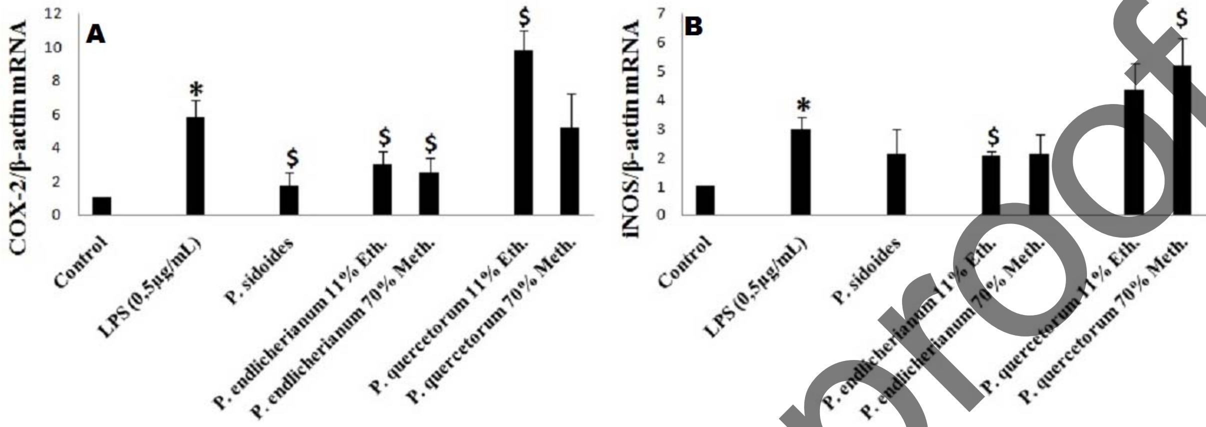

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Figure 4. Effect of extracts onthelevels of pro-inflammatory mediators; PGE2 and iNOS in LPS treated macrophages. n=3,*p<0,05 vs. Control, $^{\$}$p<0,05 vs. LPS.

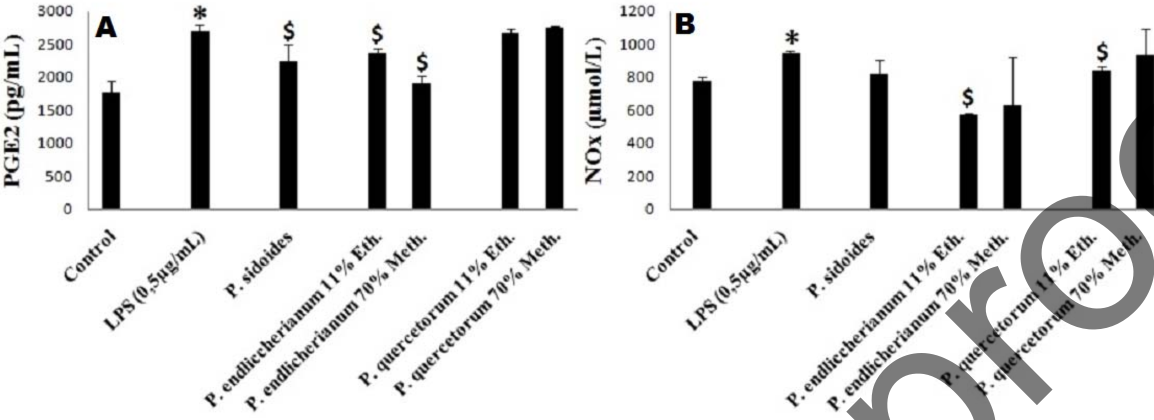

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Figure 5. Activation of NF-κBby LPS treatment in macrophage cells and the effects of extracts on cytoplasmic p65 levelsand p65 nuclear translocation. n=3,*p<0,05 vs. Control, $^{\$}$p<0,05 vs. LPS.

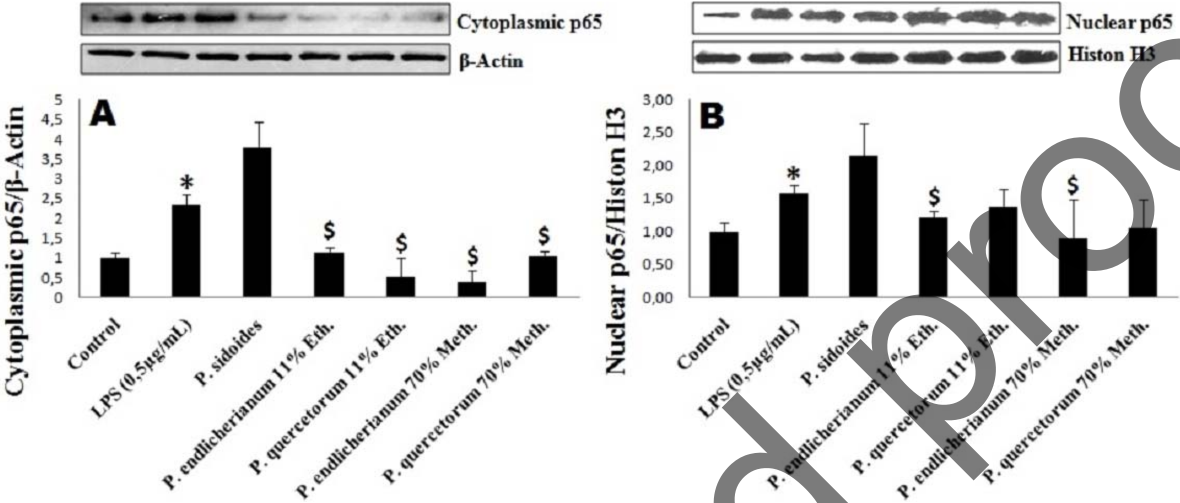

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Figure 6. Activation of MAPK pathway by LPS treatment and the inhibitory effects of extracts were shown with western blot analysis. Line1: Control, Line 2: LPS, Line 3: *P. sidoides*+LPS, Line 4: *P. endlicherianum* %11 eth.+LPS, Line 5: *P. endlicherianum* %70 meth.+LPS, Line 6: *P. quercetorum* %11 eth.+LPS, Line 7: *P. quercetorum* %70 meth.+LPS.

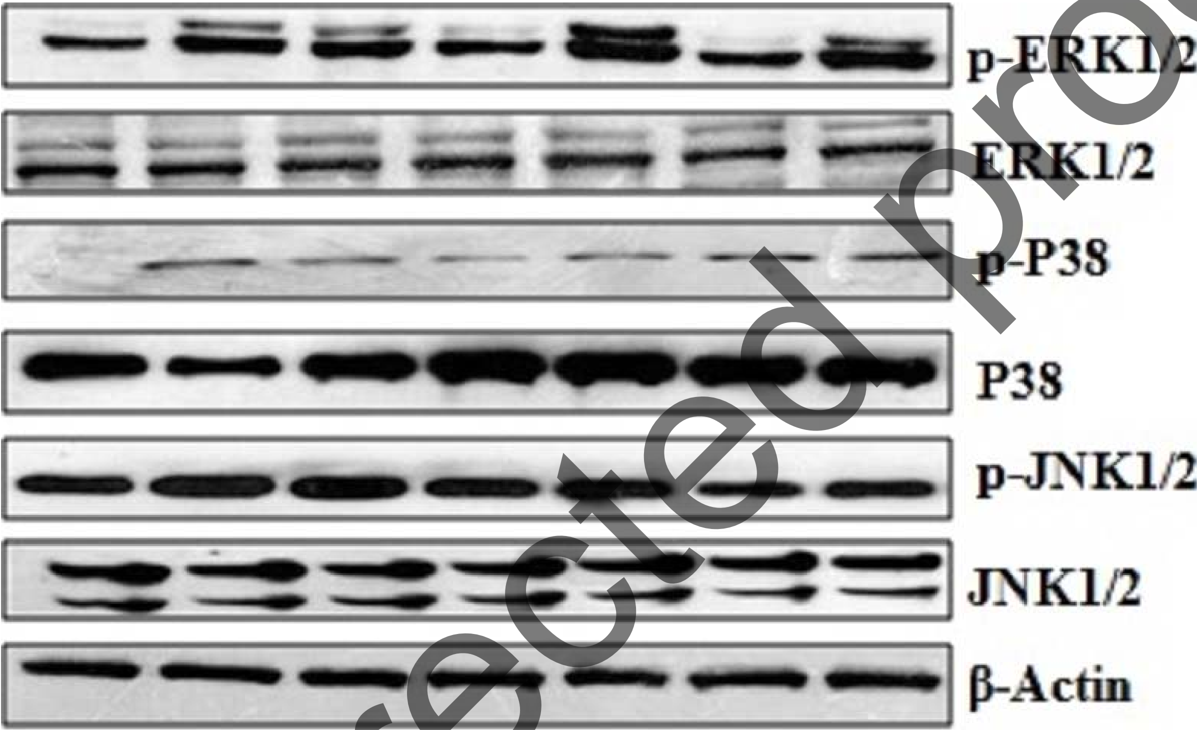

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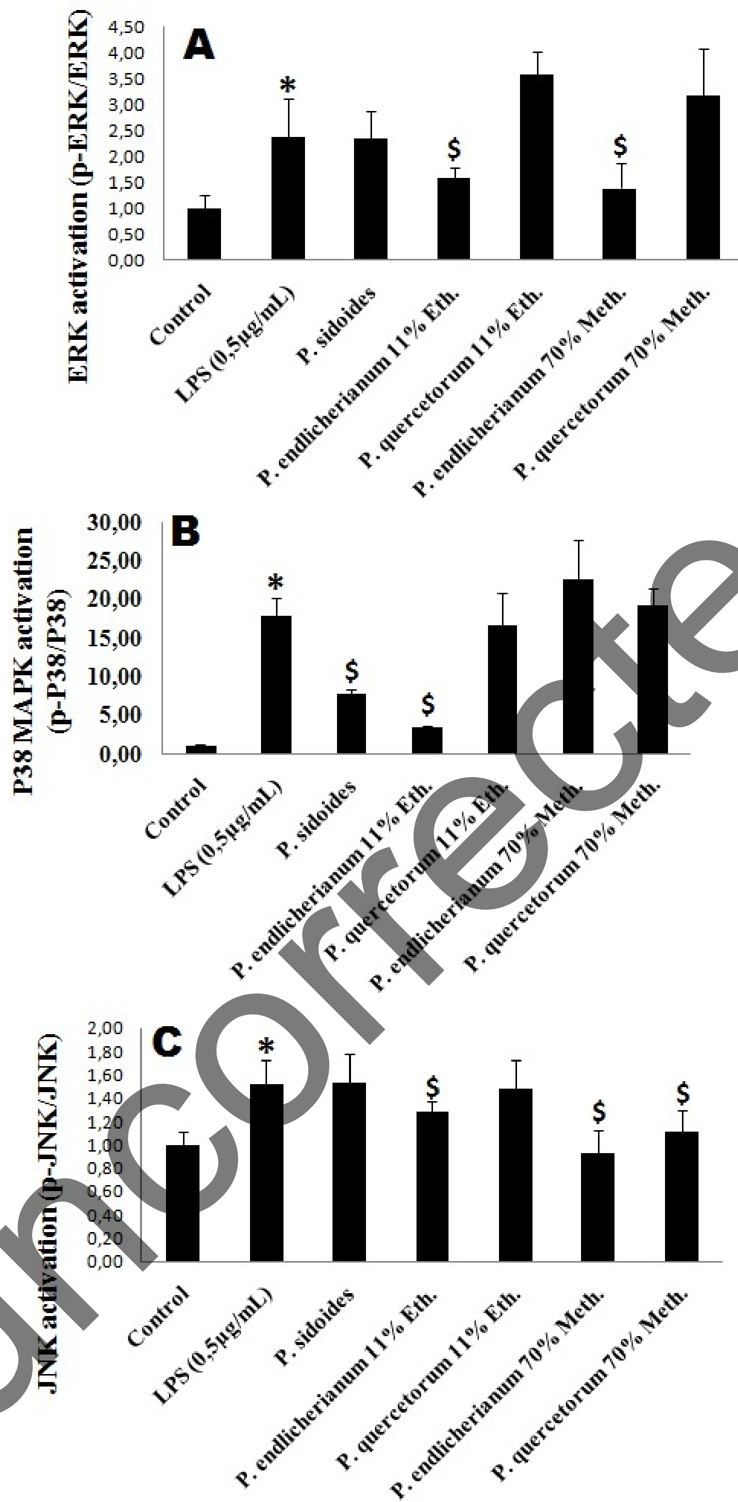
Figure 7. MAPK activation by LPS treatment and the inhibitory effects of extract. A: ERK activation, B: P38 MAPK activation, C: JNK activation. n=3,*p<0,05 vs. Control, $p<0,05 vs. LPS.



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