In vitro evidences of the traditional use of *Rhus coriaria* L. fruits against skin inflammatory conditions

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1. Introduction

Keratinocytes are the most abundant cells of the outer epidermis layer of the skin which play a key role in the induction and maintenance of inflammation in this organ (Colombo et al., 2017). Although playing a key role in innate immunity response, inflammation contributes to the aetiopathogenesis of many diseases including psoriasis, atopic dermatitis (AD), rheumatoid arthritis, coronary heart disorders and cancer (Chandel, 2000; Hänel, 2013). Inflammatory skin diseases are the most common dermatologic conditions which are influenced by genetic and environmental factors. Chronic inflammation results from the dysregulation and abnormal expression of inflammatory mediators or their receptors in keratinocytes (Chandel et al., 2000). Epidermal keratinocytes play a critical role in skin inflammation through their production of inflammatory chemokines (Albanesi, 2010). Therefore, chemokines are considered as pivotal mediators in the progress of inflammatory

skin diseases. Several studies reported that transcription factors, including the signal transducer(s) and activator(s) of transcription 1 and nuclear factor kappa-light-chain enhancer of activated B cell (NF-κB), act as main regulators of chemokine release mediated by tumor necrosis factor-alpha (TNF-α) and/or interferon-gamma (IFN-γ) in keratinocytes (Han et al., 2011; Jeong et al., 2015). The inflammatory process involves an intricate networking and interactions between immune cells and pro-inflammatory mediators including interleukins (i.e. IL-1, IL-6, TNF-α) and NF-κB (Balkwill, 2004).

Pathophysiological skin chronic inflammatory conditions, such as psoriasis, involve hyperproliferation of keratinocytes in combination with irregular differentiation of the epidermis layer. TNF-α is one of the most critical molecules involved in the initiation and development of psoriasis and stimulates production of pro-inflammatory molecules such as IL-6, IL-8, vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and intercellular adhesion molecule 1 (ICAM-1), which lead to the recruitment of inflammatory cells in the dermal and epidermal layers of psoriatic skin (Nickoloff et al., 2007; Udommethaporn et al., 2016). Consequently, the downregulation of keratinocytes inflammatory chemokine production and the inhibition of their interaction with immune cells may be an effective target in the treatment of inflammatory skin diseases (Kwon et al., 2012; Yang et al., 2015).

Anti-TNF-α antibodies have been used efficiently for the management of immune-mediated inflammatory diseases including rheumatoid arthritis, Crohn's disease, ulcerative colitis and psoriasis (Jung, 2014 #441). Although blockage of TNF-α, by anti-TNF-α agents, such as Infliximab, Etanercept and Adalimumab, which bind TNF-α with high affinity, has been shown to successfully treat psoriasis, they can cause serious side effects (Chan et al., 2008; Ganguly, 2009; Makol and Grover, 2008; Silva et al., 2010). Thus, there is a need for

development of novel anti-TNF-α agents aiming to minimize and attenuate the adverse side effects.

Since ancient times, natural products have been used successfully in Europe and Asia for the treatment of skin diseases (Bedi and Shenefelt, 2002). The worldwide use of approximately one-third of all traditional herbal remedies in management of dermatologic conditions has been reported (Mantle et al., 2001). *Rhus coriaria* L. (*R. coriaria*) is a medicinal herb, growing to 1-3 m high as a small tree, with pinnately compound leaves and greenish-white flowers (Mozaffarian, 2013; Peter, 2006; Shabbir, 2012). Well-known as sumac, *R. coriaria* belongs to the *Anacardiaceae* family and genus *Rhus* which covers over 200 species throughout the world (Morshedloo et al., 2018; Peter, 2006). The brown to red fruits of *R. coriaria*, are used as a very popular spice in food production with a sour lemon taste. The red fruits have an extensive range of applications in Persian traditional medicine such as treatment of diarrhea, hemorrhoids, gout and decreasing effect on cholesterol, uric acid and blood sugar levels (Abu-Reidah et al., 2014; Candan, 2003; El Hasasna et al., 2016; Kosar, 2007; Mohammad Moazeni, 2012; Mozaffarian, 2013; Pourahmad et al., 2010; Rayne and Mazza, 2007; Shabbir, 2012; Sierra Rayne 2007).

The aim of the present study was to investigate the anti-inflammatory properties of *R. coriaria* extracts, prepared using different extraction methods, in human keratinocytes (HaCaT cells). The *in vitro* model used for this screening involves treatment of cells with TNF-α prior to incubation with reference compounds or extracts. The most active extracts were selected based on their effectiveness on the inhibition of IL-8 release, and pathways involved in the mechanism of action (i.e. NF-κB signaling) were investigated. Moreover, the effect of *R. coriaria* extracts on proinflammatory mediators with key role in skin diseases, including ICAM-1, VEGF, and MMP-9, was assessed as well.

2. Materials and methods

2.1. Collection and authentication of the herb

Our studies did not involve any endangered or protected species. *R. coriaria* sample was purchased from a local market in the Taleghan region located 120 km northwest of Teheran, Iran. The plant material was authenticated by the Herbarium Unit, School of Biological Sciences, University of Science Malaysia, where a voucher specimen was deposited (reference# 11526).

2.2. Preparation of R. coriaria extracts

To prepare the extracts, two different methods were used: cold extraction or maceration. In both cases the dried fruits were ground with an electric grinder and 5 g of dried fruit powder were used. Cold extraction was performed with three different solvent or mixtures (ethanol-water 50:50, EWRC; ethanol, ERC; water, WRC) (50 mL each) using a double extraction method (6 h plus overnight incubation on a shaker at room temperature). After cooling, the insoluble material was removed by filtration (Whatman filter paper), the extraction solvent evaporated under reduced pressure (RE121 Buchi, Switzerland) and the dry residues freeze-dried (Labconco™, USA). Maceration process was performed by 48 h incubation with ethanol (Mercati, #454). In brief, the mixture of herbal material (5 g) and solvent (50 mL) was placed in a shaker for 48 h at room temperature and mixed gently using a magnetic stirrer. Then, the mixture was filtered, the extract was taken to dryness under reduced pressure and freeze dried. The extracts were kept in sterile glasses at 2˚C until use.

2.3. Cell culture

HaCaT cells, spontaneously immortalized human keratinocyte line (Cell Line Service GmbH, Eppelheim, Germany) [15], were cultured in 5% CO_2 at 37°C in DMEM F12 (Gibco-Invitrogen). The media was supplemented with 100 units penicillin/mL, 100 mg streptomycin/mL, 2 mM L-glutamine and 10% heat-inactivated fetal calf serum (FCS) (Euroclone S.P.A., Milan, Italy). For all experiments, cells were seeded at a density of 5.7×10^3 cells⁄cm^2.

2.4. Measurement of IL-8 levels

For measurement of IL-8 secretion, HaCaT cells were grown in 24-well plates (6×10^5 cells/well) for 48 h; then, cells were treated with the pro-inflammatory stimulus (TNF-α at 10 ng/ml) and extracts/compound under study or vehicle alone (<0.2% DMSO). The cytotoxicity of extracts and reference compounds were evaluated by MTT (3,4,5-dimethylthiazol-2-yl-2-5-diphenyltetrazolium bromide) assay, as previously described. After 4 h treatment, the supernatants were removed and stored at −80°C until the assay. IL-8 was quantified by an enzyme-linked immunosorbent assay Kit (Peprotech, Rocky Hill, NJ, USA) following the manufacturer's instructions. The fold increase of IL-8 secretion reached the maximum at 6 h and this time was selected for the following experiments to test the effect of the extracts (1–50 μg/ml); quercetin (10 μM) was used as a reference compound.

2.5. NF-κB driven transcription

To evaluate NF-κB driven transcription, cells were plated in 24-well plates (6×10^5 cells/well). After 48 h cells were transiently transfected by lipofectamine with a reporter plasmid (NF-κB-luc, 250 ng/well); the plasmid contained the luciferase gene under control of a NF-κB responsive promoter (E-selectin promoter): NF-κB-luc. The plasmid NF-κB-luc was

a gift of Dr. N. Marx (Department of Internal Medicine-Cardiology, University of Ulm, Germany). After 16 h, cells were placed in a medium deprived of FCS, and stimulated with TNF-α at 10 ng/ml. mERC and EWRC were tested at 10, 25 and 50 μg/mL whereas the reference compound at 20μM. After 6 h the luciferase assay was performed using Britelite Plus reagent (PerkinElmer Inc. Massachusetts, USA) according to manufacturer's instructions; signal was read with Victor X3 (Perkin Elmer, Walthman MA, USA). Data were expressed considering 100% the luciferase activity related to the cytokine-induced NF-κB driven transcription. EGCG (20 μM) was used as a reference compound.

2.6. NF-κB nuclear translocation assay

To assess the effect of the extracts and individual compounds on the NF-κB (p65) nuclear translocation, HaCaT cells were plated at the density of 1.5×10^5 cells/mL in 100 mm plates. After 48 h, cells were treated for 1 h with the pro-inflammatory mediators and the extracts/reference compound under study. Nuclear extracts were prepared using Nuclear Extraction Kit from Cayman Chemical Company (Michigan, USA) and stored at −20 °C until assayed. The same amount of total nuclear proteins (10 μg/well), measured by the method of Bradford (Bio-Rad), was used to assess the NF-κB nuclear translocation using the NF-κB (p65) transcription factor assay kit (Cayman) followed by spectroscopy at 450 nm, 0.1s (VictorX3, Perkin Elmer, Walthman MA, USA). Data were expressed considering 100% the absorbance related to the TNF-α-induced NF-κB nuclear translocation. EGCG (20 μM) was used as a reference compound.

2.7. Assessment of ICAM-1 and VEGF release

For measurement of ICAM-1 and VEGF release, HaCaT cells were grown in 24-well plates (6×10^5 cells/well) for 48 h; then, cells were treated with pro-inflammatory stimuli (TNF-

α at 10 ng/ml) and extracts/reference compound under study. After 24 h treatment the supernatants were removed and stored at −80°C until the assay. Then the cytotoxicity of extracts and reference compounds were assessed by MTT test as previously described (Sangiovanni et al., 2015). The release of ICAM-1 and VEGF from HaCaT cells was quantified using two different high sensitivity human ELISA sets (Peprotech, Rocky Hill, NJ, USA) following the method described below. Briefly, Corning 96-well EIA/RIA plates from Sigma-Aldrich (Milan, Italy) were coated with the antibodies provided, overnight at 4°C. In all cases, 300 µl of samples were transferred in duplicate into wells at room temperature for 2 h. The results were detected by spectroscopy (signal read 450 nm, 0.1 s, by VictorTM X3) using biotinylated and streptavidin-HRP conjugate antibodies, evaluating 3,5,3,59-tetramethylbenzidine (TMB) substrate reaction. The quantification of analytes was done using an optimized standard curve supplied with the ELISA sets. Results were expressed as pg/10^6 cells. Curcumin (20 µM) and EGCG (20 µM) were used as reference inhibitors of ICAM-1 and VEGF release, respectively.

2.8 Evaluation of MMP-9 secretion

Firstly, the HaCaT cells were grown in 24-well plates (6×10^5 cells/well) for 48 h. Then the cells were treated with pro-inflammatory stimuli (TNF-α at 10 ng/ml) and extracts under study or the reference compound. After 24 h treatment the supernatans were removed and stored at −80 °C until the assay. MMP-9 secretion from HaCaT cells was evaluated by ELISA set (RayBio® Human MMP-9 ELISA kit, Norcross, GA) using a precoated 96-well plate. 300 µl of samples were transferred in duplicate into wells at room temperature for 2.5 h. MMP-9 was detected by spectroscopy (signal read 450 nm, 0.1 s, by VictorTM X3) using biotinylated and streptavidin-HRP conjugate antibodies, evaluating TMB substrate reaction. The quantification of analytes was done using an optimized standard curve supplied with the ELISA

set. Resveratrol (50 μM) was used as a reference compound. The data are expressed as pg/10^6 cells.

2.9. HPLC-UV-DAD analysis

For analysis, mERC and EWRC extracts (50 mg) were solubilized in ethanol/water (10 mL) under sonication in a sonication bath at maximal power, until complete dissolution (30 min). The corresponding solutions were analysed using an LC-940 analytical/semipreparative HPLC system (Agilent, Ex Varian, Leinì, Torino, Italy) equipped with binary pumps, autosampler, fraction collector, and a with a UV-DAD detector operating in the 200-400 nm range. Separations were done using a Kinetex™ Biphenyl chromatographic column (particle size 2.6 μm, pore size 100 Å, 100 × 4.6 mm, Phenomenex, Castel Maggiore, Bologna, Italy). Gradient solvents: 0.05% aqueous formic acid (A) and 0.1% formic acid in acetonitrile (B); gradient program: 5% B for 0-3 min, from 5% to 40% B in 47 min, 40% B for 10 min. Injection volume: 10 μL. Total flow rate: 1.6 mL/min. Column temperature: 25°C.

Total polyphenols, flavonoids and anthocyanidins were measured by comparison with calibration curves built using ethanol hydroalcoholic (30:70 v/v water/ethanol) solutions standard gallic acid (observation λ=270 nm), quercetin-O-glucoside (observation λ=350 nm) and cyanidin-3-O-glucoside (observation λ=510 nm) in the concentration ranges 0.050-1.0 mg/mL, 5-50 μg/mL and 0.35-5 μg/mL respectively. Results are expressed as percentage (% w/w) of gallic acid equivalent (GAE), quercetin-O-glucoside (QE) or cyanidin-3-O-glucoside equivalent (CE) in the extracts.

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.0 via one-way ANOVA followed by Bonferroni as post-hoc test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. TNF-α

alone. Results are the mean ± s.d of at least three individual experiments performed in duplicate.

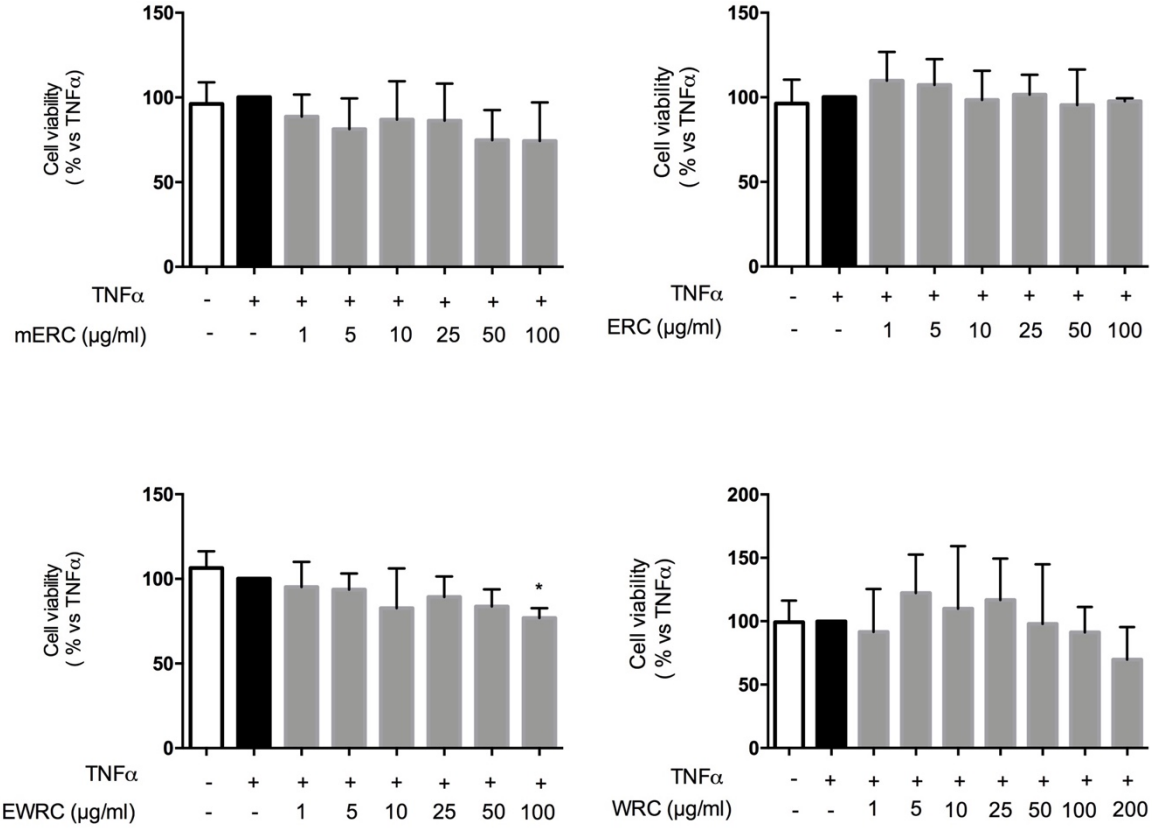
3. Results

3.1. Preparation of R. coriaria extracts and percent recovery

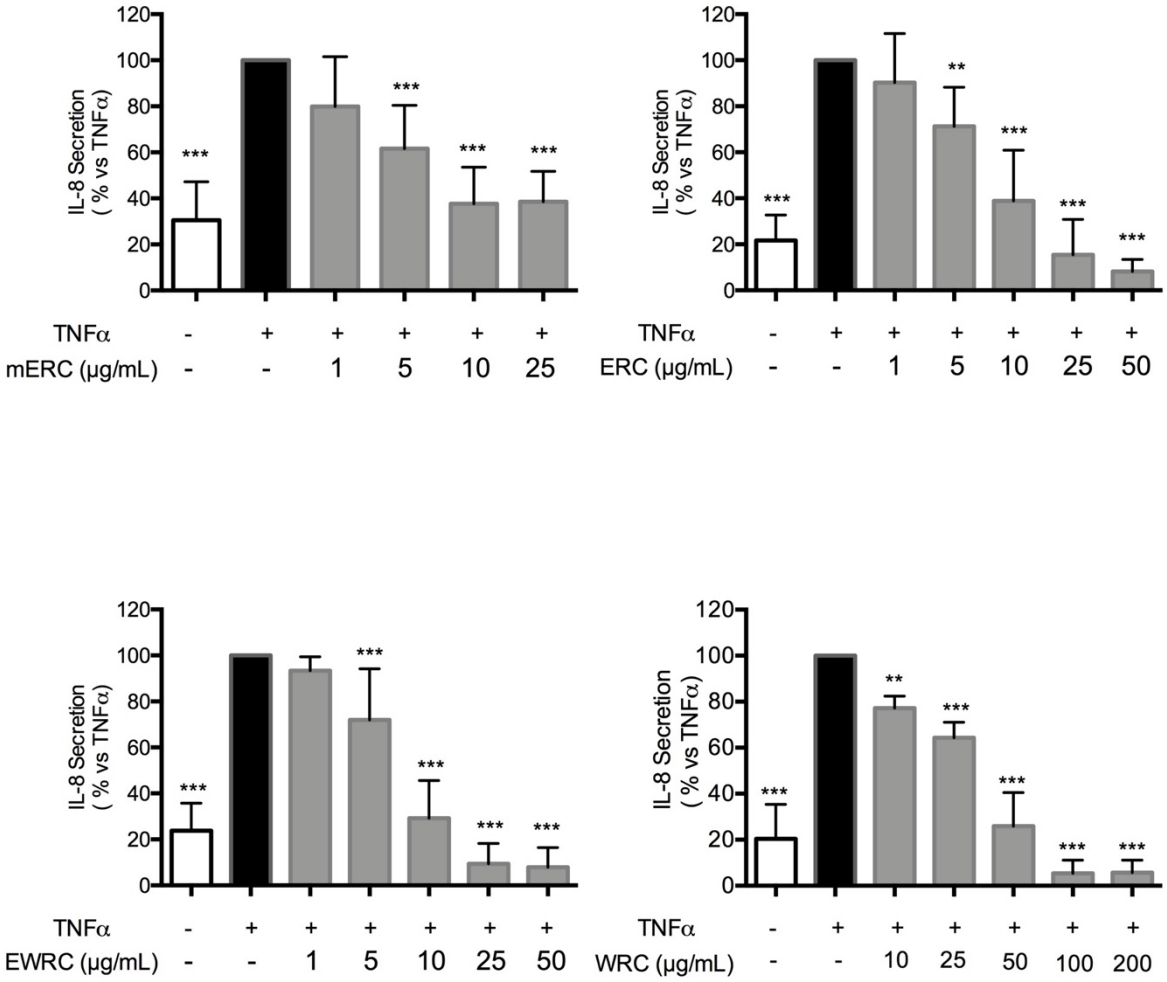
The extraction yields are reported in Table 1. The WRC extract showed the highest yield of extraction (23.2%), while EWRC, ERC and mERC extracts had lower or comparable extraction yields (14.8%, 16.2%, and 15.2%, respectively).

3.2. Screening of different extracts of R. coriaria on the TNFα-induced IL-8 release in HaCaT cells

The effect of the extracts on the viability of HaCaT cells was analysed by MTT assay. Quercetin (10 μM), a known IL-8 inhibitor, was used as reference compound. None of the extracts was found to be toxic in HaCaT cells, but slight toxicity occurred after treatment with 100 μg/mL EWRC. Therefore, this concentration was excluded from further analysis on the biological effects of this extract (Fig. A1). The preliminary screening of the extracts on the TNF-α-induced IL-8 release in human keratinocyte HaCaT cells was performed.



All the extracts differently inhibited IL-8 release, as depicted in Figure 1. mERC was the most active extract, with significant inhibition ranging between 5 and 25 µg/mL (IC_{50}=3.15±1.14 µg/mL; mean ± s.d.); EWRC was active as well, which a doubled IC_{50} value (IC_{50}=6.61±0.55 µg/mL; mean ± s.d.).

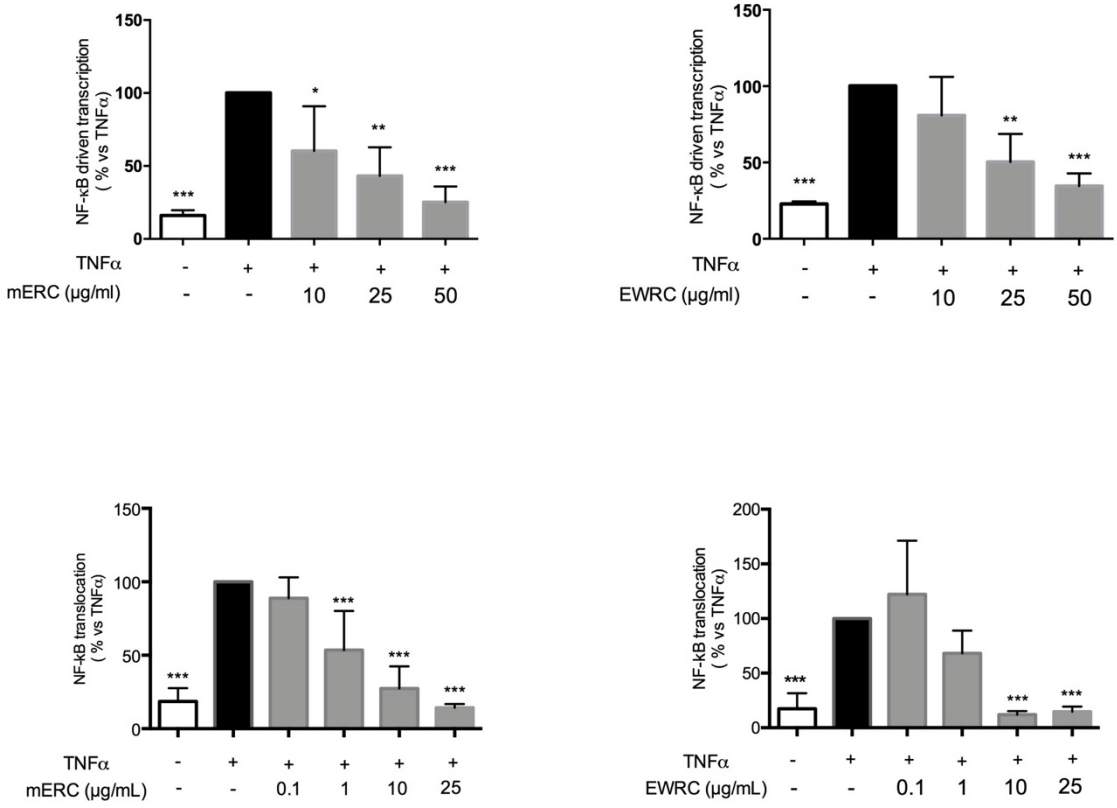


The ERC effect was similar to EWRC, while WRC showed the lowest inhibitory activity (Table 2). IC_{50s} of the extracts under study were as follows: WRC > ERC > EWRC > mERC. Quercetin 10 µM was used as reference compound (~25% inhibition). Based on these results, mERC and EWRC were selected for further studies aimed to assess the inhibitory effect on relase of pro-inflammatory mediators involved in skin diseases.

3.3 mERC and EWRC inhibit the TNF-α induced IL-8 secretion through suppression of the NF-κB signaling

To evaluate the mechanism of action by which mERC and EWRC are able to inhibit IL-8 release, and to find out the involvement of NF-κB pathways, HaCaT cells were transiently

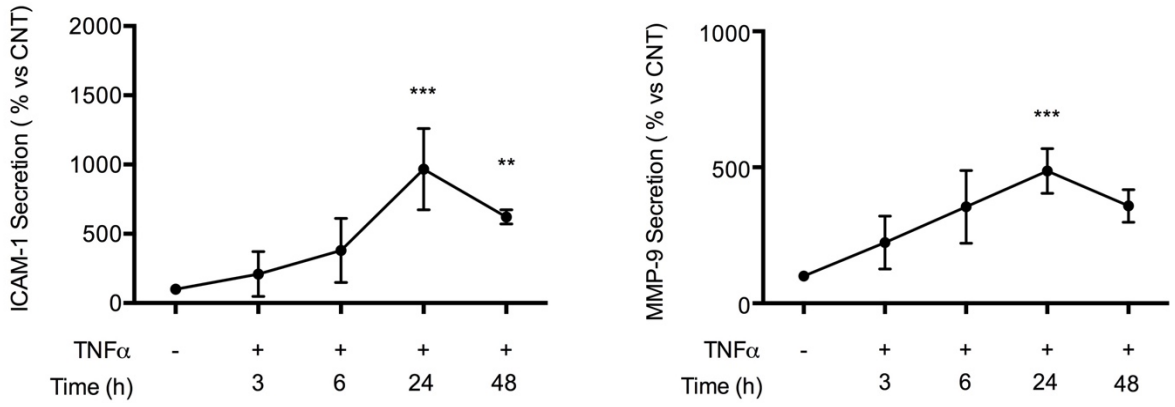
transfected with the NF-κB-luc plasmid and treated for 6 h with different concentrations of the extracts (10-50 μg/mL) or the reference compound in the presence of TNF-α (10 ng/mL). As shown in Fig. 2, both the extracts inhibited TNF-α-induced NF-κB driven transcription in a concentration dependent fashion, with comparable activity. IC_{50}s for mERC and EWRC were 11.48 ± 0.21 and 18.51 ± 0.08 μg/mL (mean ± s.d.), respectively (Table 3).



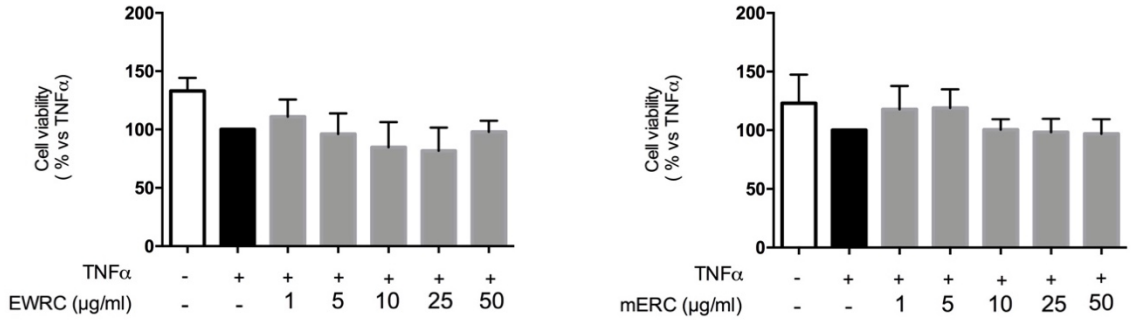
To gain further insights into the molecular mechanisms by which the mERC and EWRC exert skin anti-inflammatory activity, we tested the extracts on the NF-κB translocation. The HaCaT cells were treated for 1 h with different concentrations (0.1, 1, 10, and 25 μg/mL) of the extracts in serum free medium containing TNF-α (10 ng/mL). The supernatants were used for nuclear extraction and assessment of p65 translocation by ELISA. mERC and EWRC extracts inhibited the NF-κB translocation in a concentration dependent fashion (Fig. 2), with similar IC_{50}s (0.84 ± 0.45 and 1.03 ± 0.31 μg/mL, respectively) (Table 3).

3.4. Two different extracts of R. coriaria fruits inhibit TNF-α-induced ICAM-1 release in HaCaT cells

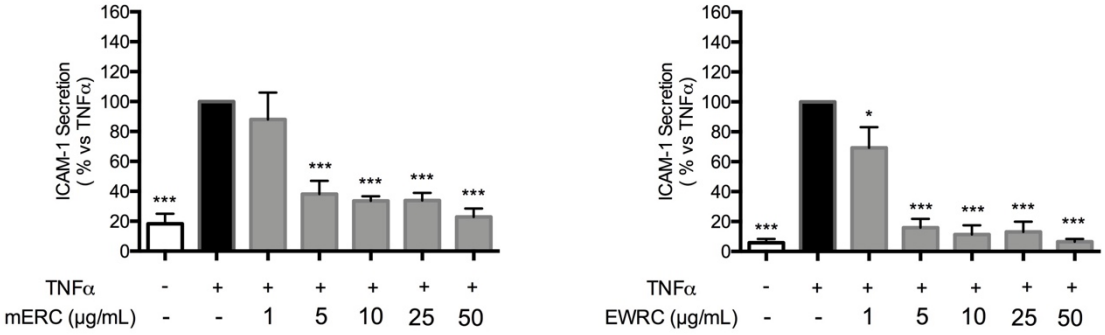
As shown in Fig. A. 2, TNF-α induced the highest ICAM-1 release at 24 h in HaCaT cells, and this time was selected for the following experiments aimed to test the effect of the extracts (1–50 μg/mL).



Viability of both the extracts was tested by MTT at 24 h and none of them was found to be toxic up to the highest concentration tested (50 μg/ml, Fig. A. 3).

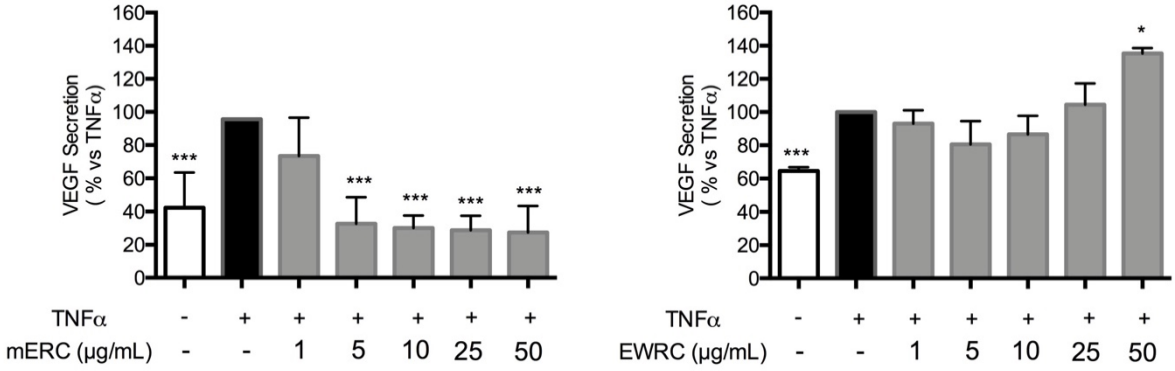


mERC and EWRC inhibited ICAM-1 release in a concentration dependent fashion; IC_{50s} were 2.59 ± 0.46 and 1.76 ± 0.24 μg/mL for mERC and EWRC, respectively (Fig. 3). The reference compound curcumin 20 μM significantly inhibited ICAM-1 release (53.55%), as expected.



3.5. Macerated ethanol and ethanol-water extracts of R. coriaria inhibited TNF-α-induced VEGF release in HaCaT cells

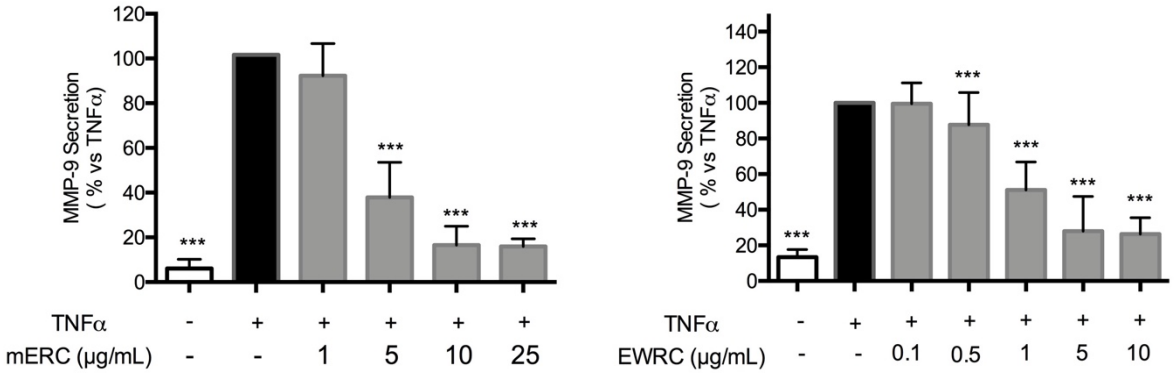
Only mERC inhibited the TNF-α-induced VEGF release at 5-50 μg/mL (Fig. 4). The IC_{50} was 1.48 ± 0.47 μg/mL; the reference compound EGCG 20 μM showed about 60% inhibitory activity. EWRC did not show any inhibitory effect on the secretion of VEGF, one of the key factors in the process of angiogenesis, thus suggesting that the extracts may have different composition.



3.6. Macerated ethanol extract of R. coriaria inhibited TNF-α induced MMP-9 release in HaCaT cells

HaCaT cells were treated with different concentration of the extracts described above, then MMP-9 production, a bioactive molecule which plays a key role in matrix remodeling and degradation, was investigated. The MMP-9 secretion reached the maximum at 24 h and

this time was selected for the experiments (Fig. A. 3). mERC showed an inhibitory effect at the concentrations of 5-25 μg/mL (Fig. 5) with the IC_{50} of 3.37 ± 0.77 μg/mL (Tab. 3), thus reflecting results obtained on IL-8 release. EWRC was also active on MMP-9 secretion with the IC_{50} of 1.24 ± 0.33 μg/mL, in comparison with the significant inhibitory activity (67.21 %). Resveratrol 50 μM inhibited the MMP-9 release (66.19 %).

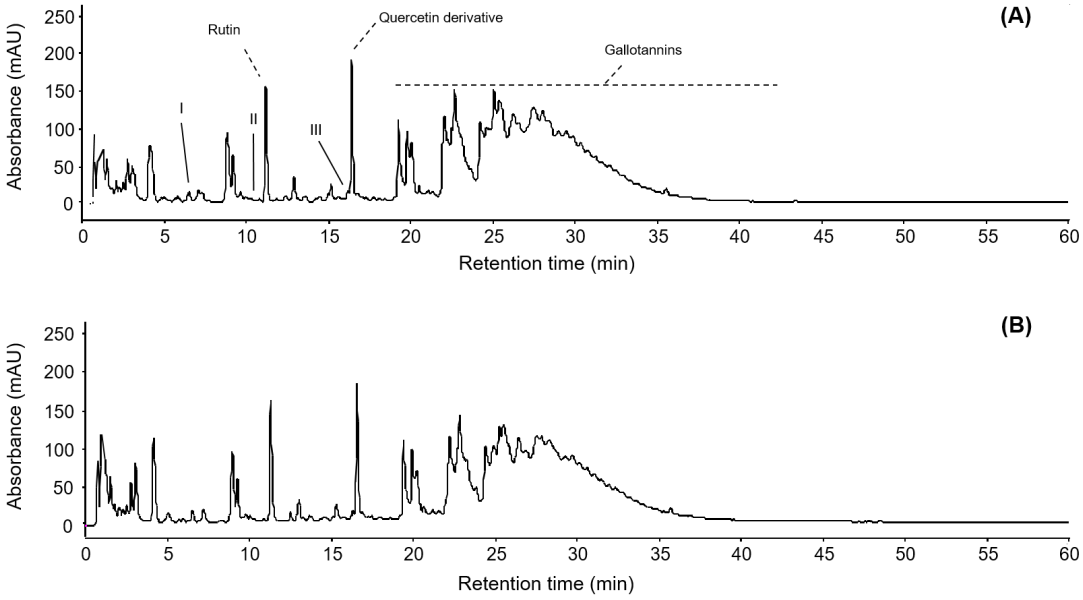


3.7. Phytochemical characterization of macerated ethanol and ethanol-water extracts of R. coriaria

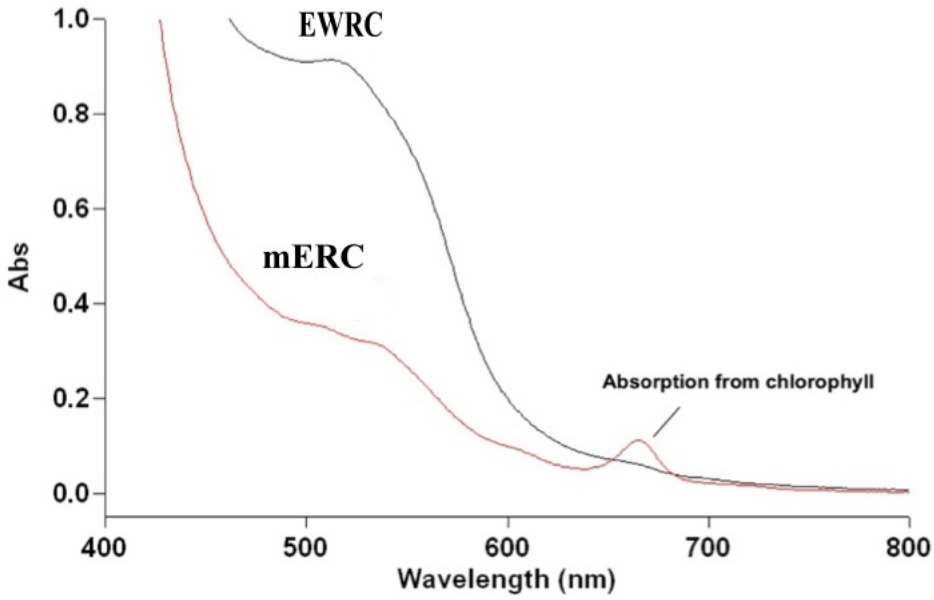
The phytochemical profile of *R. coriaria,* which includes hydrolyzable gallotannins, gallic acid derivatives and anthocyanins, have been previously reported in the literature (Abu-Reidah et al., 2015; Abu-Reidah et al., 2014; Beretta et al., 2009; Kossah et al., 2010; Shabbir, 2012).

However, to get deeper insights into the composition of macerated and ethanol-water extracts, their composition was investigated through HPLC-UV/DAD analysis. The corresponding chromatographic profiles (observation λ=270 nm) are reported in Fig. 6A and Fig. 6B respectively. The chromatograms were almost superimposable and in both cases dominated by the presence of several derivatives with UV spectra typical of the gallotannin derivatives , indicating that the employed extraction procedures and solvent compositions did not have significant impact on extraction yield and phytochemical profile of this class of compounds in the extracts.

Similarly, also the quantitative amount of the substances with UV spectral absorptions characteristic of quercetin derivatives (λ_{max}=254, 350 nm, spectra not shown) generating the peaks at RT~12.5 min and RT~20.5 min, did not change significantly. By contrast, the EWRC extract showed a comparatively higher concentration of at least three anthocyanin derivatives at RT=6.5 min (I, λ_{max}=216, 273, 350, 511 nm), RT=11.55 min (II, λ_{max}=217, 273, 350, 511 nm) and at RT= 16.5 min (III, λ_{max}=218, 275, 350, 517 nm) (see Fig. 6A, spectra not shown).



Cyanidin derivatives, consistent with those previously identified in *R. coriaria* fruits (cyanidin-3-O-(2”galloyl)-galactoside, 7-O-methyl-delphinidin-3-O-(2”galloyl)-galactoside, methyl delphinidin aglycone, 7-O-methyl-cyanidin-3-O-(2”galloyl)-galactoside), were responsible for the characteristic pink color of the hydroalcoholic extract compared to the pale brownish ethanol extract (see Fig. 7 for the spectrophometric comparison of the visible light absorbtions of the two extracts analysed by HPLC).



The quantitative analysis of these extracts is reported in Table 4. Both extracts show similar amounts of flavonoids (0.23±0.02% QE and 0.23± 0.03% Q; mERC vs. EWRC, P>0.05) and of tannins (4.33± 0.32% GAE and 4.54±0.23% GAE; mERC vs. EWRC, P>0.05). By contrast, EWRC showed a significantly higher anthocyanins content 0.207± 0.023% CE compared to mERC 0.031±0.005 % CE (P<0.05).

4. Discussion

Rhus coriaria L. is a plant traditionally used in some countries, including Iran and Turkey, as an anti-microbial and anti-inflammatory agent, to treat skin injuries (Behnammanesh et al., 2015; Mehrabani Natanzi et al., 2017). The traditional application of *R. coriaria* fruits to heal skin disorders including burns, wounds and eczema has been recorded (Altundag, 2011). However, the mechanism of action of *R. coriaria* is poorly understood. Recent phytochemical studies on *R. coriaria* have proved its richness in hydrolysable tannins, condensed tannins, gallic acid derivatives, anthocyanin and various organic acids such as malic and citric acids, fatty acids, vitamins, flavonoids and terpenoid derivates (Abu-Reidah et al.,

2014; Kossah et al., 2010; Shabbir, 2012). Protocatechuic acid, linolenic acid, p-OH-benzoic acid, and vanillic acid were the phenolic acids found in the leaves of this herb which contain up to 25-33% tannins. The literature related to the biological activities of *R. coriaria* highlights the antimicrobial, antifungal, antiviral, hypoglycemic, neuroprotective, hepatoprotective, and cardiovascular protective properties (Behnammanesh et al., 2015; Beretta et al., 2009; Bozan et al., 2003; Candan, 2003; Khalilpour, 2015; Khalilpour et al., 2017; Khalilpour et al., 2018; Panico et al., 2009; Peter, 2006; Pourahmad et al., 2010; Rayne and Mazza, 2007). Despite this information, the potential modulatory effect of *R. coriaria* fruit extracts on epidermal keratinocytes inflammation has not been reported so far.

Keratinocytes are active players in epidermal repair and in the skin's immune defense through the secretion of different growth factors, cytokines, and chemokines. In this study, HaCaT cells were used to investigate the release of CXCL8/IL8, VEGF, and MMP-9 in response to TNF-α as pro-inflammatory stimulus (Jeong et al., 2010; Sung et al., 2012). These factors were selected as strictly involved in the pathogenesis of inflammatory skin conditions and overexpressed during psoriasis (Chandel et al., 2000; Jeong et al., 2010; Kang et al., 2013).

In keratinocytes, TNF-α stimulation activates several intracellular signaling pathways, including those regulated by NF-κB (Sung et al., 2012). TNF-α leads to NF-κB/p65 nuclear translocation (Kang et al., 2013), which in turn is involved in the regulation of several pro-inflammatory factors in HaCaT cells (Choi et al., 2009). Since IL-8 expression is dependent on the NF-κB activation, contributing to exacerbate inflammation, we investigated this chemokine and the inhibitory effect of mERC and EWRC on TNF-α activated keratinocytes.

Our results showed that all the extract prepared from *Rhus coriaria* L. had inhibitory effect on IL-8 secretion, but mERC and EWRC revealed the highest activity. Both the extracts inhibited the expression of IL-8 by suppressing the activation of the NF-κB pathway. In

addition, mERC and EWRC reduced MMP-9 and ICAM-1 release from HaCaT cells in a concentration depenedent manner, but only mERC was active against VEGF secretion.

The two extracts were characterized by similar compositions, both in terms of gallotannins and flavonol derivatives contents, with the presence of a significantly higher proportion of anthocyanins as the only evident difference between them.

Anthocyanins are polyphenols that are responsible for the pink, red, violet, and blue coloration of many fruits and flowers (Osmani et al., 2009). These polyphenols are present as glycosides linked to 2-phenylbenzopyrylium or flavylium moiety. They have potent antioxidant and anti-inflammatory properties, and it has been reported that they stimulate the wound-induced VEGF production in keratinocytes (Nizamutdinova et al., 2009). Therefore, higher amount of anthocyanins in EWRC may be the main reason of its stimulating effect on VEGF levels in HaCaT cells.

To summarize, both the extracts affect the ability of TNF-α-stimulated HaCaT cells to produce pro-inflammatory mediators including ICAM-1, MMP-9, and IL-8 acting, at least in part, on the NF-κB pathway whereas only mERC impaired VEGF release. Our findings confirm for the first time the traditional use of *Rhus coriaria* L. as a remedy to treat skin inflammatory conditions.

Conclusion

Keratinocytes produce cytokines and chemokines involved in the development of inflammatory skin disorders, such as psoriasis and atopic dermatitis (Han et al., 2011). In this study, concentration-dependent decrease in the levels of pro-inflammatory factors were achieved in cells pretreated with mERC and EWRC, suggesting the potential use of *R. coriaria* extracts in the treatment of inflammatory skin diseases.

Both extracts were active as anti-TNF-α agents to block one of the signaling cascades which plays a central role in potentiation of the process of the keratinocytes inflammation. The main difference in their pharmacological activities appears in the lack of VEGF inhibition, probably due to the higher concentration of anthocyanins in EWRC. Inhibiting the production of the pro-inflammatory mediators, which are involved in recruitment of neutrophil, infiltration of leukocytes, degradation of matrix and formation of new blood vessels, reveals the potency of *R. coriaria* extracts as novel therapeutic opportunities. Our findings confirm for the first time the traditional use of *Rhus coriaria* L. as a remedy to treat skin inflammatory conditions.

Acknowledgements

The authors declare no conflict of interests in this work. This research was supported by grants from MIUR Progetto Eccellenza. The authors thank Professor Dr. Petra Boukamp and Dr. Norbert Fusenig from Deutsches Krebsforschungszentrum, Stiftung des öffentlichen Rechts (German Cancer Research Center), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany, for providing HaCaT cell line. The work was partially supported by Fondazione Cariplo (HyWoNNa project grant to MDA).

Authors Contribution

M.D.A. and E.S. designed the experiments. S.K., S.P., M.F., and G.B. performed the experiments. S.K., E.S., M.D.A., S.P., and M.F. and G.B. performed data analysis. S.K., E.S., G.B. and M.D.A. were involved in writing and revising the manuscript.

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