Effect of Non-Volatile Constituents of *Elsholtzia ciliata* (Thunb.) Hyl. from Southern Vietnam on Reactive Oxygen Species and Nitric Oxide Release in Macrophages

Dieu T. X. Nguyen, a, b Hung Tran, a Stefan Schwaiger, *b* Hermann Stuppner, b and Stefania Marzocco c

a Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Dinh Tien Hoang 41–43, 700000, Ho Chi Minh City Vietnam

b Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 80–82, 6020 Innsbruck Austria, e-mail: stefan.schwaiger@uibk.ac.at; hermann.stuppner@uibk.ac.at

c Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, SA 84084 Fisciano Italy

© 2020 The Authors. Chemistry & Biodiversity published by Wiley-VHCA AG. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

The extract of *Elsholtzia ciliata* aerial parts was subjected to bio-guided isolation using the intercellular ROS reduction in J774A.1 macrophages to monitor the anti-oxidative activity. Fifteen compounds were isolated from the active fractions including eleven flavonoids (vitexin, pedalin, luteolin-7-O-β-D-glucopyranoside, apigenin-5-O-β-D-glucopyranoside, chrysoeriol-7-O-β-D-glucopyranoside, 7,3'-dimethoxyluteolin-6-O-β-D-glucopyranoside, luteolin, 5,6,4'-trihydroxy-7,3'-dimethoxyflavone, 5-hydroxy-6,7-dimethoxyflavone (compound 13), 5-hydroxy-7,8-dimethoxyflavone); three hydroxycinnamic acid derivatives (caffeic acid, 4-((E))-caffeoyl-L-threonic acid, 4-((E))-p-coumaroyl-L-threonic acid) and one fatty acid (α-linolenic acid). The biological evaluation of these compounds (10 – 2.5 μM) indicated that all of them exerted good antioxidant and anti-inflammatory activities, in particular compound 13.

Keywords: antioxidants, *Elsholtzia ciliata*, inflammation, oxidative stress, phenolics, Vietnamese balm.

Introduction

Reactive oxygen species (ROS), inevitable side products of normal cellular metabolism, are necessary for signaling and regulation of biological functions at physiological levels.\[1,2\] Especially they are generated in response to pathogens or foreign invasion, playing an imperative role in immune system.\[3\] However, uncontrolled ROS levels, not adequately balanced by endogenous and exogenous antioxidant defenses, can lead to an oxidative stress status giving rise to cell damage and various diseases.\[4\] Oxidative stress is known to hasten the aging process, significantly contribute to pathophysiology of a broad variety of disorders or diseases such as cardiovascular diseases, inflammatory bowel disease, rheumatoid arthritis, and others.\[5\] Moreover, a considerable body of evidence has shown that ROS trigger NF-κB which activates and/or further amplifies the pro-inflammatory response regulating pro-inflammatory mediators, such as nitric oxide (NO).\[6,7\] Therefore, regulation of redox balance is crucial for healthy living organisms. Besides natural defense mechanisms based on endogenous antioxidants such as antioxidant enzymes (e.g., glutathione peroxidase, catalase and superoxide dismutase), metal-binding proteins (like ferritin, transferrin, metallothionein, lactoferrin, ceruloplasmin) or non-enzymatic scavengers (e.g., uric acid, bilirubin),\[8\] recent studies proved that exogenous antioxidants originating from a diet rich in spices, culinary herbs, fruits and vegetables providing compounds such as vitamin C, E, carotenoids and phenolics also play an important role.
protective role against oxidative stress and inflammation.\textsuperscript{[9–11]}

The \textit{Elsholtzia} genus comprises at least 33 species and belongs to the subfamily Nepetoideae, representing the largest subfamily within the Lamiaceae.\textsuperscript{[12,13]} Many species of the genus \textit{Elsholtzia} are used as culinary herbs and remedies since ancient time, and typically contain various secondary plant metabolites like flavonoids, phenylpropanoids, as well as terpenoids. Among these compound classes, flavonoids are the most predominant substances in the genus \textit{Elsholtzia}.

\textit{Elsholtzia ciliata} (THUNB.) HYL., commonly known as Vietnamese balm (or ‘Kinh gioi’ in Vietnamese), is a spicy, lemon-scented culinary herb in Asian cuisine, especially in Vietnam, where it is typically consumed raw as an indispensable ingredient of various dishes such as Bun rieu cua (crab rice noodles soup), Bun dau mam tom (rice noodles with tofu and shrimp paste), Bun cha (grilled pork and noodles) or frequently presents in the plate of mixed garnish vegetables served in almost every Vietnamese daily meals.\textsuperscript{[14]} It has been also used in folk medicine as a detoxicant, carminative, stomachic, astringent, diuretics, antipyretic for treatment of numerous ailments such as cold, fever, headache, epistaxis, asthma, hialotosis, digestive complaints (nausea, vomiting, abdominal pain, diarrhea, cholera, dysentery, acute gastroenteritis, stomachache), heatstroke, mild renal ailments.\textsuperscript{[13,15]} The species is native to Asia and is an annual herb, reaching 40–60 cm in height with pilose, green, square stems; crenate-serrate, ovate to elliptic-lanceolate leaves and purplish flowers, located on spikes, blooming from July to October.\textsuperscript{[16]} Phytochemical investigations revealed the composition of its essential oil,\textsuperscript{[17–27]} but also the presence of flavonoids,\textsuperscript{[18,26,28]} phenolic acids,\textsuperscript{[18]} steroids and triterpenes,\textsuperscript{[15,29]} of which phenolics are the most abundant non-volatile compound class.\textsuperscript{[18]} The pharmacological data mentioned in literature, including anti-acetylcholinesterase activity,\textsuperscript{[28]} anti-oxidative properties,\textsuperscript{[30–32]} antibacterial,\textsuperscript{[22,30]} vasorelaxant,\textsuperscript{[18]} anti-leishmanial,\textsuperscript{[27]} cytotoxic,\textsuperscript{[19]} anti-inflammatory,\textsuperscript{[32–34]} and insecticidal activities\textsuperscript{[21]} mainly refer to total extracts\textsuperscript{[30,31,33,34]} and/or the essential oil.\textsuperscript{[18,19,22,27]} Interestingly, the essential oil showed a promising activity also \textit{in vivo} in a rat paw swelling assay induced by serotonin or carrageenan, and in a model for chronic arthritis induced by formaldehyde.\textsuperscript{[35]}

During our screening campaign to search for new antioxidant and anti-inflammatory agents, the methanolic extract of \textit{E. ciliata} was found to demonstrate antioxidant effects in both oxidative stress and inflammatory conditions in J774A.1 murine macrophages. In order to elucidate the contribution of the non-volatile constituents to the observed activities, we performed an activity-guided isolation and evaluated the antioxidant and anti-inflammatory activities of subfractions and pure compounds on J774A.1 macrophages.

**Results and Discussion**

**Activity Guided Isolation**

Bioactive compounds present in food could play a role in physical dysfunction through their effects on inflammatory mediators and pathways, barrier integrity, and/or intestinal microbiota composition. Evaluation of the antioxidant potential was performed in both inflammatory and oxidative stress conditions in J774A.1 macrophages. The impact on ROS production induced by LPS was of special interest due to its similarity to a microbial induced intestinal inflammation with an interaction of diet derived secondary plant metabolites. In order to assess the activity of the constituents of \textit{E. ciliata}, the air-dried aerial plant parts were extracted with methanol. A part of the obtained dry extract was dissolved in water and fractionated by liquid-liquid extraction with organic solvents of different polarity to yield a petroleum ether, diethyl ether, ethyl acetate, \textit{n}-butanol and the respective water subfraction. In the performed assays, all the tested extract/subfractions exerted antioxidant effects at concentrations \( \geq 5 \mu \text{g/mL} (P < 0.001 \text{ vs. } \text{LPS or } \text{H}_2\text{O}_2) \); Table 1). At lower tested concentration, only the petroleum ether, diethyl ether and water fractions were active in inhibiting ROS release during oxidative stress conditions in macrophages (2.5 \( \mu \text{g/mL} P < 0.001 \text{ vs. } \text{H}_2\text{O}_2 \); Table 1). All the tested extracts and subfractions did not exert cytotoxic activity except for the water fraction, which showed marginal anti-proliferative effects (20.67 \( \pm 1.2 \) vs. untreated macrophages) at the highest tested concentration.

HPLC Analysis of the methanolic extract and its subfractions (Figure 1) showed a good enrichment of individual compounds in the different subfractions e.g. compound \textbf{13}, \textbf{14}, and \textbf{15} in the petroleum ether subfraction. On the other hand, the analysis revealed that the activity of the extract and its subfractions could not be explained by the presence of a single active principal but due to the presence of several compounds. Aiming at the identification of those, the three most potent extract fractions (petroleum ether,
Table 1. Effect of the investigated extract and subfractions on ROS release evaluated on J774A.1 macrophages in oxidative stress conditions induced by H$_2$O$_2$ (left column) and during inflammatory conditions, induced by LPS (right column)$^{[a]}$

| Studies on Elsholtzia ciliata and subfractions in J774A.1 | % Inhibition of ROS release vs. H$_2$O$_2$ (mean ± SEM) | % Inhibition of ROS release vs. LPS (mean ± SEM) |
|----------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------|
| methanolic extract, 10 µg/mL                             | 45.7 ± 5.2$^{[b]}$                                    | 47.7 ± 0.3$^{[b]}$                                |
| methanolic extract, 5 µg/mL                              | 37.0 ± 0.6$^{[b]}$                                    | 33.3 ± 4.1$^{[b]}$                                |
| methanolic extract, 2.5 µg/mL                            | 5.0 ± 5.5                                             | 0.0 ± 0.0                                         |
| petroleum ether fraction, 10 µg/mL                       | 57.7 ± 4.1$^{[b]}$                                    | 50.7 ± 0.9$^{[b]}$                                |
| petroleum ether fraction, 5 µg/mL                        | 44.3 ± 3.0$^{[b]}$                                    | 28.0 ± 3.8$^{[c]}$                                |
| petroleum ether fraction, 2.5 µg/mL                      | 37.0 ± 1.7$^{[b]}$                                    | 0.0 ± 0.0                                         |
| diethyl ether fraction, 10 µg/mL                         | 58.0 ± 1.7$^{[b]}$                                    | 52.0 ± 5.2$^{[b]}$                                |
| diethyl ether fraction, 5 µg/mL                          | 51.7 ± 8.3$^{[b]}$                                    | 21.7 ± 8.3                                        |
| diethyl ether fraction, 2.5 µg/mL                        | 39.3 ± 2.9$^{[b]}$                                    | 0.0 ± 0.0                                         |
| ethyl acetate fraction, 10 µg/mL                         | 41.0 ± 1.5$^{[b]}$                                    | 57.7 ± 0.9$^{[b]}$                                |
| ethyl acetate fraction, 5 µg/mL                          | 27.5 ± 2.4$^{[b]}$                                    | 43.3 ± 2.2$^{[b]}$                                |
| ethyl acetate fraction, 2.5 µg/mL                        | 16.5 ± 2.5                                            | 15.7 ± 3.4                                        |
| n-butanol fraction, 10 µg/mL                             | 34.0 ± 3.5$^{[b]}$                                    | 47.7 ± 0.9$^{[b]}$                                |
| n-butanol fraction, 5 µg/mL                              | 25.3 ± 1.3$^{[b]}$                                    | 36.3 ± 4.3$^{[b]}$                                |
| n-butanol fraction, 2.5 µg/mL                            | 14.3 ± 0.9                                            | 14.0 ± 1.7                                        |
| water fraction, 10 µg/mL                                 | 46.0 ± 4.2$^{[b]}$                                    | 40.7 ± 0.9$^{[b]}$                                |
| water fraction, 5 µg/mL                                  | 32.3 ± 10.3$^{[b]}$                                   | 20.7 ± 2.6                                        |
| water fraction, 2.5 µg/mL                                | 27.7 ± 2.6$^{[b]}$                                    | 0.0 ± 0.0                                         |
| NAC, 10 µm (positive control)                            | 78.3 ± 1.3$^{[b]}$                                    | 40.0 ± 5.0$^{[b]}$                                |

$^{[a]}$ Data are expressed as mean ± sem of % inhibition of ROS release vs. macrophages treated with H$_2$O$_2$ or LPS alone. $^{[b]}$ denotes $P < 0.001$ and $^{[c]}$ $P < 0.01$ respectively vs. H$_2$O$_2$ or LPS alone treated macrophages.

diethyl ether and ethyl acetate fraction) were selected for further phytochemical investigation.

In total fifteen compounds were isolated from aerial parts of E. ciliata, including eleven flavonoids (vitexin (4),$^{[36]}$ pedalin (5),$^{[37]}$ luteolin-7-O-β-d-glucopyranoside (6),$^{[38]}$ apigenin-5-O-β-d-glucopyranoside (7),$^{[39]}$ apigenin-7-O-β-d-glucopyranoside (8),$^{[40]}$ chrysoeriol-7-O-β-d-glucopyranoside (9),$^{[41]}$ 7,3’-dimethoxy-luteolin-6-O-β-d-glucopyranoside (10),$^{[42]}$ luteolin (11),$^{[43]}$ 5,6,4’-trihydroxy-7,3’-dimethoxyflavone (12),$^{[44]}$ 5-hydroxy-6,7-dimethoxyflavone (13),$^{[45]}$ 5-hydroxy-7,8-dimethoxyflavone (14),$^{[46]}$ three hydroxycinnamic acid derivatives (caffeic acid (1),$^{[47]}$ 4-(E)-caffeoyl-l-threonic acid (2),$^{[48]}$ 4-O-(E)-p-coumaroyl-l-threonic acid (3),$^{[49]}$ and one fatty acid (α-linolenic acid (15) $^{[50]}$). Their chemical structures, elucidated by spectral data (1D-, 2D-NMR and MS, Tables S1 to S4) interpretation in comparison with previously reported data, are depicted in Figure 2. The spectroscopic and spectrometric data of the isolated compounds are reported in the Supporting Information. Interestingly, rosmarinic acid, reported to be present in higher concentration,$^{[18]}$ could be only detected in trace by a LC/MS experiment in the investigated extract. Instead of this phenolic acid, we were able to isolate two unusual hydroxycinnamic acid derivatives 4-(E)-caffeoyl-l-threonic acid (2) and 4-O-(E)-p-coumaroyl-l-threonic acid (3), which are described here for the first time as constituents of a Lamiaceae member. Up to now, these esters were known to occur in Chelidonium majus (Papaveraceae),$^{[48]}$ Crataegus species (Rosaceae),$^{[49]}$ and Viguiera pazensis (Asteraceae).$^{[51]}$ Furthermore, 2, 3, 4, 5, 7, 9, 12 have not been reported as constituents of E. ciliata before, 4, 5, 9, 12 were found for the first time in the genus Elsholtzia.

Anti-Oxidative and Anti-Inflammatory Activity of Pure Compounds

Prior to the evaluation of the antioxidant and anti-inflammatory activities, the anti-proliferative activity of the pure compounds at all tested concentrations (10–25 µm) were analyzed. With the exception of compound 14, which showed an anti-proliferative activity (38.0 ± 9.1 vs. untreated macrophages) and was therefore excluded from the further experiments, none of the tested compounds displayed at the tested concentrations a potential cytotoxic activity. Evaluation of the antioxidant activity indicated that all tested compounds exerted significant effects on ROS release during oxidative stress conditions at the highest tested concentration (10 µm). In particular, compounds 6, 11

www.cb.wiley.com (3 of 10) e2000577 © 2020 The Authors. Chemistry & Biodiversity Published by Wiley-VHCA AG
Figure 1. HPLC chromatogram of the crude methanolic extract of *E. ciliata* (A) and its subfractions (petroleum ether B; diethyl ether C; ethyl acetate D; butanol E; water F), each 5 mg/ml. Compound assignment according to Figure 2. HPLC Parameters: Column Phenomenex Gemini 5 μ C18 110 Å (150×3 mm, 5 μm); temperature, 45 °C; injection volume, 5 μL; wavelength, 280 nm; flow rate, 1 mL/min; mobile phase, water with 0.9% FA and 0.1% AA-acetonitrile gradient (2% acetonitrile; at 30 min, 22% acetonitrile; at 40 min, 100% acetonitrile); Total time: 45 min, equilibration: 10 min.

Figure 2. Structures of compounds isolated from the aerial parts of *E. ciliata*.  

| Compound | Structure |
|----------|-----------|
| 1 (caffeic acid) | ![Structure 1](image) |
| 2 (4-O-(E)-caffeoyl-L-threonine) | ![Structure 2](image) |
| 3 (4-O-(E)-p-coumaroyl-L-threonine) | ![Structure 3](image) |
| 4 (vitexin) | ![Structure 4](image) |
| 5 (pedalin) | ![Structure 5](image) |
| 6 (luteolin-7-O-β-D-glucopyranoside) | ![Structure 6](image) |
| 7 (apigenin-5-O-β-D-glucopyranoside) | ![Structure 7](image) |
| 8 (apigenin-7-O-β-D-glucopyranoside) | ![Structure 8](image) |
| 9 (chrysosin-7-O-β-D-glucopyranoside) | ![Structure 9](image) |
| 10 (stereolenin-7,3'-dimethylether) | ![Structure 10](image) |
| 11 (luteolin) | ![Structure 11](image) |
| 12 (5,6,4'-trihydroxy-7,3'-dimethoxyflavone) | ![Structure 12](image) |
| 13 (mosloflavone/5-hydroxy-6,7-dimethoxyflavone) | ![Structure 13](image) |
| 14 (moslosoflavone/5-hydroxy-7,8-dimethoxyflavone) | ![Structure 14](image) |

© 2020 The Authors. Chemistry & Biodiversity Published by Wiley-VHCA AG
and 12 exerted significant antioxidant effects at all the tested concentrations in H$_2$O$_2$-treated macrophages ($P<0.001$ vs. H$_2$O$_2$ alone treated macrophages, Figure 3A). In LPS-induced inflammatory conditions, all tested compounds affected ROS release, of which compounds 5, 6, 13, and 15 exerted stronger, concentration-dependent activities ($P<0.05$ vs. LPS alone treated macrophages). The observed effect of linoleic acid (15) was not unexpected but helped to verify the experimental setup. More surprising was the finding that flavonoid glucosides (5 and 6) as well as the flavonoid aglycons (11 and 13) showed activities in the cell-based assay that suggests a sufficient cellular uptake of the substances or more unlikely an interaction with the extracellular LPS-signaling. In order to evaluate the contribution of the tested compounds to the inflammatory response, their effects on NO release in LPS-stimulated macrophages were evaluated. With the exception of compounds 10 and 12, all tested compounds significantly inhibited NO release in macrophages during LPS-induced inflammation ($P<0.001$ vs. LPS alone treated macrophages) at all tested concentrations (Figure 3). A possible explanation for the lack of activity of 10 and 12 might be the presence of two methoxy groups at 3’ and 7 in these compounds. The most pronounced effect could be observed for 13, which was also found to be one of the most dominant compounds according to HPLC-DAD analysis (Figure 1).

**Conclusions**

Since the essential oil of the investigated species was already shown to be active in vivo in a rat paw swelling assay induced by serotonin or carrageenan, and in a model for chronic arthritis induced by formaldehyde,$^{35}$ we were interested in the contribution of the non-volatile constituents of E. ciliata to the antioxidant effect in inflammatory and in oxidative stress conditions. The impact on ROS production induced by LPS was of special interest, since this

![Figure 3](image). Effect of the investigated pure compounds at 10, 5 and 2.5 μM on ROS release evaluated in J774A.1 macrophages during oxidative stress conditions induced by H$_2$O$_2$: A, positive control: NAC-10 μM, 78.33 ± 1.33% inhibition) and inflammatory conditions, induced by LPS (B; positive control: NAC-10 μM, 40.00 ± 5.00% inhibition) as well as on NO release induced by LPS (C; positive control: L-NAME-1 μM, 97.00 ± 0.00% inhibition). Data are expressed as mean ± SEM of % of inhibition of ROS release vs. macrophages treated with H$_2$O$_2$ or LPS alone. *** and ** denotes $P<0.001$ and $P<0.01$ vs. LPS or H$_2$O$_2$ alone treated macrophages. Numerical values are shown in Table S5.
model mimics the ROS level of a microbial induced intestinal inflammation, which is in agreement with the traditional use of *E. ciliata*.\(^{[13,15]}\)

From a phytochemical point of view, several compounds, which have not been reported as components of *E. ciliata* before, could be identified. Among those, two unusual hydroxycinnamic acid derivatives 4-\((E)\)-caffeoyl-L-threonic acid (2) and 4-O-\((E)\)-p-coumaroyl-L-threonic acid (3), were described for the first time as constituents of a member of the Lamiaceae family. In summary, the performed study supports the traditional use of *E. ciliata* against ROS/inflammation associated disorders by the found in vitro activity and identifies the aerial plant parts as a valuable source of flavonoids and phenolic acids with anti-inflammatory and anti-oxidative activities.

**Experimental Section**

**Plant Material**

Aerial parts of *E. ciliata* (‘Kinh gioi’, Vietnamese balm), botanically characterized by Assoc. Prof. Dr. Hung Tran (Pharmacognosy Department, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam), were collected in Ho Chi Minh City, Vietnam (10°48’20.6”N 106°42’08.8”E) in September 2016. The plant name is in agreement with http://www.theplantlist.org. A voucher specimen (EC-HCMC-0916) is deposited at Faculty of Pharmacy, University of Medicine and Pharmacy of Ho Chi Minh City, Vietnam.

**Chemicals and General Procedures**

For analytical purpose, solvents were purchased from Merck (Darmstadt, Germany) and ultrapure water was produced onsite by a Sartorius Arium 611 UV water purification system (Göttingen, Germany). Solvents for NMR experiments with 0.03% tetramethylsilane (TMS) as internal standard were purchased from Euriso-Top (Saint Aubin, France). Other solvents for phytochemical investigations were purchased from WVR International (Darmstadt, Germany). For fractionation and isolation purposes, size-exclusion column chromatography on Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden), Reveleris MPLC system (Büchi, Flawil, Switzerland) or a semi-preparative HPLC from Dionex (Thermo Fisher, Waltham, USA) were used. Semi-preparative HPLC parameters: Column Phenomenex Gemini 5 μ C18 125 Å (250x10 mm, 5 μm); mobile phase: water with 0.02% TFA/acetonitrile. TLC was conducted on aluminum TLC plates, silica gel 60 coated with fluorescent indicator F\(_{254}\) (VWR, Darmstadt, Germany). HPLC analysis was performed on a HP 1100 system (Agilent, Waldbronn, Germany) connected with DAD as following parameters: Column Phenomenex Gemini 5 μ C18 110 Å (150x3 mm, 5 μm); flow rate: 1 mL/min; mobile phase: water with 0.9% FA and 0.1% AA/acetonitrile gradient (2% acetonitrile; at 30 min, 22% acetonitrile, at 40 min, 100% acetonitrile); total time: 45 min, equilibration: 10 min; temperature: 45°C; injected volume: 5 μL. NMR Spectra (1D- and 2D-experiments) were recorded on a Bruker Advance II 600 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at 600.19 MHz (\(^1\)H) and 150.93 MHz (\(^13\)C) (chemical shifts δ in ppm, coupling constants J in Hz). Mass spectrometry was performed on an Esquire 3000 ion-trap mass spectrometer (Bruker-Daltonics, Bremen, Germany) coupled to an Agilent HPLC system type HP 1100 (Santa Clara, USA) with the following MS conditions: ESI; alternating mode; spray voltage: 4.5 kV, 350°C; dry gas: 10.0 L/min; nebulizer: 30 psi; full scan mode, m/z 150–1500. HR-ESI-MS data were obtained using a Bruker mirkOTOF-Q II mass spectrometer (Bruker Daltonics, Bremen, Germany); in m/z. Optical rotations were measured on a PerkinElmer 341 polarimeter (Wellesley, MA, USA) at 20°C.

**Cell Culture and Pharmacological Assays**

**J774A.1 Murine Macrophage Cell Line**

J774A.1, a murine monocyte macrophage cell line (American Type Culture Collection, Rockville, MD), was grown adherent to Petri dishes with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS), 25 mM HEPES, 2 mM glutamine, 100 u/mL penicillin and 100 mg/mL streptomycin at 37°C in a 5% CO\(_2\) atmosphere. Macrophages J774A.1 were plated in 96 well plates (5 x 10\(^4\)) and allowed to adhere. Subsequently, the medium was replaced with fresh medium alone or containing serial dilutions of the extract, its subfractions (10–2.5 μg/mL) or the single compounds (10–2.5 μM) for 1 h and then co-exposed to a final concentration of LPS (1 μg/mL) for 24 h or to H\(_2\)O\(_2\) (1 mM) for 1h. Then ROS and NO levels were detected as described as follows.

**Anti-Proliferative Assay**

J774A.1 macrophages (5 x 10\(^4\)/well) were plated on 96-well plates and allowed to adhere. Thereafter, the medium was replaced by fresh medium alone or containing serial dilutions of the extract, extract...
subfractions (10–2.5 μg/mL) or the single compounds (10–2.5 μM) for 24 h. Cell viability was assessed through 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide (MTT) assay as previously reported. Briefly, 25 μL of MTT (5 mg/mL) was added and the cells were incubated for additional 3 h. Thereafter, cells were lysed, and the dark blue crystals solubilized with 100 μL of a solution containing 50% (mL/L) N,N-dimethylformamide, 20% (mL/L) sodium dodecyl sulfate (SDS) with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 550 nm filter used as the main absorbance and a 620 nm filter used as reference. J774A.1 macrophages viability in response to treatment was calculated as: % cellular inhibition = 100 – (OD treated/OD control) × 100.

Intracellular ROS Release Measurement

ROS intracellular production in J774A.1 macrophages was evaluated by the probe of 2',7'-dichlorofluorescein-diacetate (H$_2$DCF-DA). J774A.1 cells were seeded in 24-well plates (3 × 10$^5$ cells/well) and allowed to adhere. After the cellular treatment with the tested compounds in combination with LPS or H$_2$O$_2$ as described above, J774A.1 cells were collected, washed with PBS, and then incubated in PBS containing H$_2$DCF-DA (10 μM). Cell fluorescence was evaluated after 15 min at 37°C, using a fluorescence-activated cell sorter (FACSScan, Becton Dickinson, Franklin Lakes, NJ, USA), and was analyzed by CellQuest software version 4 (Becton Dickinson, Milan, Italy), as formerly reported.

Measurement of NO Release

NO levels were measured as nitrite NO$_2^-$, index of NO released by cells, in the culture medium of J774A.1 macrophages 24 h after LPS stimulation by Griess reaction, as previously reported. Briefly, after the cell treatment with the tested compounds and LPS, as previously described, 100 μL of cell culture medium were mixed with 100 μL of Griess reagent (equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride in water) and incubated at room temperature for 10 min. Subsequently, the absorbance was measured at 550 nm in a microplate reader Titertek (Dasit, Cornaredo, Milan, Italy). The amount of NO$_2^-$ measured in the samples is expressed in μM concentration, which was calculated via a sodium nitrite standard curve.

Data Analysis

Results of the pharmacological assays are reported as mean values in % inhibition ± standard error of the mean (SEM) referred to the LPS-stimulated control group in Table 1 and Figure 3. At least three independent experiments, each in triplicate, were performed for each extract and concentration. Statistical analysis was performed by analysis of variance test, and multiple comparisons were made by Bonferroni’s test using Prism 5 (GraphPad Software, San Diego, CA, USA). A P-value less than 0.05 was considered significant.

Isolation of Compounds

The air-dried ground plant material (50 g) was exhaustively extracted with methanol (6 × 1 L) by maceration at room temperature, and then the combined filtrate was evaporated under reduced pressure using rotavapor to yield a crude extract (12 g). A portion of the obtained crude extract (10 g), suspended in 150 mL distilled water, was sequentially fractionated with petroleum ether (6 × 0.5 L), diethyl ether (4 × 0.5 L), ethyl acetate (5 × 0.5 L) and n-butanol (5 × 0.5 L). Each of organic layers and the final aqueous layer were evaporated in vacuo to afford petroleum ether (EC_P; 1.90 g), diethyl ether (EC_D; 0.65 g), ethyl acetate (EC_E; 0.65 g), n-butanol (EC_B; 4.56 g) and water (EC_W; 2.10 g) portions.

An aliquot of the petroleum ether residue (EC_P, 1000 mg) was subjected to flash chromatography using a Reveleris X2 system (Grace, Columbia, Maryland, USA) with the following parameters: dry sample (the homogenous mixture of 1000 mg petroleum ether fraction and 1000 mg RP18 material, which was then loaded in a 15 mL solid loader); stationary phase: Reveleris C$_{18}$ RP 24 g cartridge, 40 μm; mobile phase: methanol/water, gradient 30–100% methanol in 120 min; at 140 min, stop; flow rate: 12 mL/min; detector: ELSD, UV (205 nm, 254 nm, 350 nm); UV and ELSD sensitivity: medium; collection mode: collect all; collected volume: 10 mL/tube (peak), 20 mL/tube (non-peak). This separation led to the collection of 168 tubes, which were combined to 19 subfractions (Pa1 to Pa19). As a result, 13 (11.5 mg), 14 (7.4 mg), 15 (23.1 mg) were isolated from subfractions Pa11, Pa13 and Pa18, respectively. The diethyl ether-soluble portion (600 mg) was applied to size-exclusion chro-
matography on Sephadex LH-20 material (column Ø = 2 cm, l = 92 cm), eluted with dichloromethane-acetone (85:15, v/v) to yield 12 (6.8 mg). The ethyl acetate fraction (600 mg) was dissolved in methanol, leading to the precipitation of a fine white powder, which was then filtered and recrystallized from methanol to obtain 10 (65.1 mg). The mother liquor was separated into 13 subfractions (Ea1 to Ea13) by Sephadex LH-20 CC in methanol (column Ø = 2.5 cm, l = 85 cm). From subfraction Ea8 (39.5 mg) and Ea12 (8.0 mg), by recrystallization, 6 (11.1 mg) and 11 (4.3 mg) were isolated, respectively. Subfraction Ea6 (62.2 mg) was separated by semi-preparative HPLC (Phenomenex AQUA, flow rate: 2.5 mL/min; column temperature: 45 °C; detector: UV 280 nm; mobile phase: isocratic 13% acetonitrile in 33 min) to obtain 1 (3.8 mg, tR = 13.5 min), 2 (6.2 mg, tR = 18.0 min), 3 (6.7 mg, tR = 30.6 min). Subfraction Ea7 (56.6 mg) was fractionated into seven subfractions (Eb1 to Eb7) using size-exclusion chromatography on Sephadex LH-20 material with methanol as eluent (column Ø = 85 cm). From subfraction Eb5-7 (27.5 mg) was separated by semi-preparative HPLC (Phenomenex AQUA, flow rate: 2.5 mL/min; column temperature: 45 °C; detector: UV 325 nm; mobile phase: isocratic 19% acetonitrile in 62 min) to get 4 (3.5 mg, tR = 9.6 min), 5 (2.9 mg, tR = 16.0 min), 7 (2.3 mg, tR = 28.9 min), 8 (2.6 mg, tR = 40.9 min) and 9 (3.3 mg, tR = 60.7 min). A summary of the performed isolation steps is shown in Figure S1.

Abbreviations

| Abbreviation | Definition                          |
|--------------|------------------------------------|
| AA           | Acetic acid                         |
| DMEM         | Dulbecco’s modified Eagle’s medium  |
| FA           | Formic acid                         |
| FCS          | Fetal calf serum                    |
| H2DCF-DA     | 2’,7’-dichlorofluorescein-diacetate  |
| HEPES        | N-2-hydroxyethylpipерazine-N-ethanesulfonic acid |
| L-NAMe       | L-N-nitro-l-arginine methyl ester   |
| LPS          | Lipopolysaccharide                  |
| MTT          | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyloxazole bromide |
| NAC          | N-acetylcysteine                    |
| NO           | Nitric oxide                        |
| OD           | Optical density                     |
| PBS          | Phosphate-buffered saline           |
| ROS          | Reactive oxygen species             |
| SDS          | Sodium dodecyl sulfate              |
| TFA          | Trifluoroacetic acid                |
| TMS          | Tetramethylsilane                   |

Acknowledgements

This work was supported by MediHealth-MSCA-RISE-2015-Marie Sklodowska-Curie Research and Innovation Staff Exchange (RISE), Project number 691158 and Ernst Mach-Grants-ASEAN European Academic University Network (ASEA-UNINET). The authors thank S. Moosmang, V. Grati and S. Sturm for NMR measurements as well as Shara Francesca Rapa for the technical assistance.

Author Contribution Statement

D. T. X. Nguyen performed the phytochemical part of this study. H. Tran conducted the ethnopharmacological survey, provided and identified the plant material. S. Marzocco planned, performed, and analyzed the in vitro studies. S. Schwaiger and S. Marzocco analyzed the data and supported planning and interpretation of experiments. S. Schwaiger, S. Marzocco, and H. Stuppner contributed reagents, materials, and analysis tools. S. Schwaiger, S. Marzocco, H. Stuppner conceived and supervised the study. D. T. X. Nguyen wrote the manuscript with contributions of S. Schwaiger and S. Marzocco. All authors revised and approved the final manuscript.

References

[1] P. D. Ray, B. W. Huang, Y. Tsuji, ‘Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling’, Cell Signalling 2012, 24, 981–990.
[2] R. Mittler, ‘ROS Are Good’, Trends Plant Sci. 2017, 22, 11–19.
[3] Y. Yang, A. V. Bazhin, J. Werner, S. Karakhanova, ‘Reactive Oxygen Species in the Immune System’, Int. Rev. Immunol. 2013, 32, 249–270.
[4] N. Ghosh, A. Das, S. Chaffee, S. Roy, C. K. Sen, in ‘Immunity and Inflammation in Health and Disease’, Eds. S. Chatterjee, W. Jungraithmayr, D. Bagchi, Academic Press, 2018, pp. 45–55.
[5] I. Liguori, G. Russo, F. Curcio, G. Bulli, L. Aran, D. Della-Morte, G. Gargiulo, G. Testa, F. Cacciatore, D. Bonaduce, P. Abete, ‘Oxidative stress, aging, and diseases’, Clin. Interv. Aging 2018, 13, 757–772.
[6] Y. Ranneh, F. Ali, A. M. Akim, H. A. Hamid, H. Khazaai, A. Fadel, ‘Crosstalk between reactive oxygen species and pro-inflammatory markers in developing various chronic diseases: a review’, Appl. Biol. Chem. 2017, 60, 327–338.
[7] L. Flohé, R. Brigelius-Flohé, C. Saliiou, M. G. Traber, L. Packer, ‘Redox Regulation of NF-kappa B Activation’, Free Radical Biol. Med. 1997, 22, 1115 – 1126.

[8] T. A. F. Aguilar, B. C. H. Navarro, J. A. Mendoza Perez, ‘Endogenous Antioxidants: A Review of Their Role in Oxidative Stress’, in ‘A Master Regulator of Oxidative Stress - The Transcription Factor Nrf2’, Eds. J. M. Morales-Gonzalez, Á. Moralez-González, E. O. Madrigal-Santillan, IntechOpen, 2016.

[9] D. Huang, ‘Dietary Antioxidants and Health Promotion’, Antioxidants 2018, 7, 9.

[10] C. M. Andre, Y. Larondelle, D. Evers, ‘Dietary Antioxidants and Oxidative Stress from a Human and Plant Perspective: A Review’, Curr. Nutr. Food Sci. 2010, 6, 2 – 12.

[11] J. Rosado-Pérez, I. Aguiñiga-Sánchez, T. L. Arista-Ugalde, E. Santiago-Osorio, V. M. Mendoza-Núñez, ‘The Biological Significance of Oxidative Stress, Effects of Fruits as Natural Edible Antioxidants’, Curr. Pharm. Des. 2018, 24, 4807 – 4824.

[12] P. Li, Z.-C. Qi, L.-X. Liu, T. Ohi-Toma, J. Lee, T.-H. Hsieh, C.-X. Fu, K. M. Cameron, Y.-X. Qiu, ‘Molecular phylogenetics and biogeography of the mint tribe Elsholtzieae (Nepetoideae, Lamiaceae), with an emphasis on its diversification in East Asia’, Sci. Rep. 2017, 7, 2057.

[13] P. N. Ravindran, ‘The Encyclopedia of Herbs and Spices’, CABI, 2017.

[14] A. O. Tucker, T. DeBaggio, ‘The Encyclopedia of Herbs: A Comprehensive Reference to Herbs of Flavor and Fragrance’, Timber Press, 2009.

[15] B. H. Do, C. Q. Dang, C. X. Bui, D. T. Nguyen, ‘Cay thuoc va dam, by nguyen thanh hue intimidation in Vietnam’, Vol. 2, Khoa hoc ky thuat, 2004.

[16] Z. Y. Wu, P. H. Raven, ‘Flora of China’, Vol. 17 (Verbenaceae through Solanaceae), Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 1994.

[17] X. Wang, L. Gong, H. Jiang, ‘Study on the Difference between Volatile Constituents of the Different Parts from Elsholtzia ciliata by SHS-GC-MS’, Am. J. Anal. Chem. 2017, 8, 625 – 635.

[18] L. Pudziuvelyte, V. Jakštas, L. Ivanauskas, A. Laukivičienė, C. F. D. Ibe, L. Kursvietiene, J. Bernatoniene, ‘Different extraction methods for phenolic and volatile compounds recovery from Elsholtzia ciliata fresh and dried herbal materials’, Ind. Crops Prod. 2018, 120, 286 – 294.

[19] L. Pudziuvelyte, M. Stankevičius, A. Maruska, V. Petrikaite, O. Ragazinskiene, G. Draksiene, J. Bernatoniene, ‘Chemical composition and anticancer activity of Elsholtzia ciliata essential oils and extracts prepared by different methods’, Ind. Crops Prod. 2017, 107, 90 – 96.

[20] E. A. Korolyuk, W. König, A. V. Tkachev, ‘Composition of essential oils of Elsholtzia ciliata (Thunb.) Hyl. from the Novosibirsk region, Russia’, Khim. Rastit. Syr’ya 2002, 31 – 36.

[21] M. P. Zhao, X. C. Liu, D. Lai, L. Zhou, Z. L. Liu, ‘Analysis of the Essential Oil of Elsholtzia ciliata Aerial Parts and Its Insecticidal Activities against Liposcelis bostrychophila’, Helv. Chim. Acta 2016, 99, 90 – 94.

[22] G.-h. Tian, ‘Chemical Constituents in Essential Oils from Elsholtzia ciliata and Their Antimicrobial Activities’, Chin. Herbal Med. 2013, 5, 104 – 108.

[23] J.-H. Kim, D.-H. Jung, ‘Variations in volatile compounds from Elsholtzia ciliata’, J. Plant Biol. 2003, 46, 287.

[24] R. K. Thappa, S. G. Agarwal, B. K. Kapahi, T. N. Srivastava, ‘Chemosystematics of the Himalayan Elsholtzia’, J. Essent. Oil Res. 1999, 11, 97 – 103.

[25] N. X. Dung, L. V. Sac, L. H. Hai, P. A. Leclercq, ‘Composition of the Essential Oils from the Aerial Parts of Elsholtzia ciliata (Thunb.) Hyland. from Vietnam’, J. Essent. Oil Res. 1996, 8, 107 – 109.

[26] U. Kobold, O. Vostrowsky, H. J. Bestmann, J. C. Bisht, A. K. Pant, A. B. Melkani, C. S. Mathela, ‘Terpenoids from Elsholtzia Species. II. Constituents of Essential Oil from a New Chemotype of Elsholtzia crista’, Planta Med. 1987, 53, 268 – 271.

[27] T. B. Le, C. Beauvay, D. T. Nghiem, M.-P. Mingeot-Leclercq, J. Quetin-Leclercq, ‘In Vitro Anti-Leishmanial Activity of Essential Oils Extracted from Vietnamese Plants’, Molecules 2017, 22, 1071.

[28] A. Nugroho, J.-H. Park, S. C. Jae, K.-S. Park, J.-P. Hong, H.-J. Park, ‘Structure determination and quantification of a new flavone glycoside with anti-acetylcholinesterase activity from the herbs of Elsholtzia ciliata’, Nat. Prod. Res. 2017, 33, 1 – 8.

[29] A.-L. Liu, S. M. Y. Lee, Y.-T. Wang, G.-H. Du, ‘Elsholtzia: review of traditional uses, chemistry and pharmacology’, J. Chin. Pharm. Sci. 2007, 16, 73 – 78.

[30] J. Ma, R.-R. Xu, Y. Lu, D.-F. Ren, J. Lu, ‘Composition, Antimicrobial and Antioxidant Activity of Supercritical Fluid Extract of Elsholtzia ciliata’, J. Essent. Oil-Bear. Plants 2018, 21, 556 – 562.

[31] X. Liu, J. Jia, L. Yang, F. Yang, H. Ge, C. Zhao, L. Zhang, Y. Ju, ‘Evaluation of Antioxidant Activities of Aqueous Extracts and Fractionation of Different Parts of Elsholtzia ciliata’, Molecules 2012, 17, 5430 – 5441.

[32] L. Pudziuvelyte, M. Liaudanskas, A. Jekabsone, I. Sadauskienė, J. Bernatoniene, ‘Elsholtzia ciliata (Thunb.) Hyl.’ Extracts from Different Plant Parts: Phenolic Composition, Antioxidant, and Anti-Inflammatory Activities’, Molecules 2020, 25, 1153.

[33] H.-H. Kim, J.-S. Yoo, H.-S. Lee, T. K. Kwon, T.-Y. Shin, S.-H. Kim, ‘Elsholtzia ciliata inhibits mast cell-mediated allergic inflammation: role of calcium, p38 mitogen-activated protein kinase and nuclear factor-kB’, Exp. Biol. Med. 2011, 236, 1070 – 1077.

[34] T.-W. Kim, Y.-J. Kim, S.-R. Park, H.-S. Lee, J.-Y. Jung, ‘Elsholtzia ciliata (Thunb.) Hyland attenuates renal inflammation and interstitial fibrosis via regulation of TGF-ss and Smad3 expression on unilateral ureteral obstruction rat model’, Phytomedicine 2016, 23, 331 – 339.

[35] Z. L. Huang, Z. M. Cui, ‘Study on pharmacology of effective constituents from Elsholtzia ciliata’, Acta Gansu Coll TCM 1991, 2, 18 – 20.

[36] U. L. B. Jayasinghe, B. A. I. S. Balasooriya, A. G. D. Bandara, Y. Fujimoto, ‘Glycosides from Grewia damine’, Nat. Prod. Res. 2002, 16, 499 – 502.

[37] V. D. S. Bolzani, A. A. L. Gunatilaka, D. G. I. Kingston, ‘Bio-active Guanidine Alkaloids from Pterogyne nitens’, J. Nat. Prod. 1995, 58, 1683 – 1688.
[38] Q. Zhang, Z. Lu, T. Ren, Y. Ge, Y. Zheng, D. Yao, X. Ye, H. Gu, Q. Shi, C. Huo, ‘Chemical Composition of Achillea alpina’, Chem. Nat. Compd. 2014, 50, 534–536.

[39] M. Veit, H. Geiger, F.-C. Czygan, K. R. Markham, ‘Malonylated flavone 5-O-glucosides in the barren sprouts of Equisetum arvense’, Phytochemistry 1990, 29, 2555–2560.

[40] Y. Chang, P. Zhang, X. Zhang, J. Chen, W.-D. Rausch, A. Gula, B. Bao, ‘Cytotoxic activities of flavonoids from a traditional Mongolian medicinal herb Clematis aethusifolia Turcz.’, Nat. Prod. Res. 2017, 31, 1223–1227.

[41] C. V. Minh, N. X. Nhiem, H. T. Yen, P. V. Kiem, B. H. Tai, H. Le Tuan Anh, T. T. T. Hien, S. Park, N. Kim, S. H. Kim, ‘Chemical constituents of Trichosanthes kirilowii and their cytotoxic activities’, Arch. Pharmacal Res. 2015, 38, 1443–1448.

[42] S. Mathuram, K. K. Purushothaman, A. Sarada, J. D. Connelly, ‘A new flavone 6-glucoside from Citharexylum subserratum’, Phytochemistry 1976, 15, 838.

[43] L. Zhu, J. Chen, J. Tan, X. Liu, B. Wang, ‘Flavonoids from Agrimonia pilosa Ledeb: Free Radical Scavenging and DNA Oxidative Damage Protection Activities and Analysis of Bioactivity-Structure Relationship Based on Molecular and Electronic Structures’, Molecules 2017, 22, 195.

[44] E. Maldonado, A. Ortega, ‘Neo-clerodane diterpenes from Salvia thyoides’, Phytochemistry 1997, 46, 129–1294.

[45] F. A. Tomas-Barberán, J. D. Msonthi, K. Hostettmann, ‘Antifungal epicuticular methylated flavonoids from Helichrysum nitens’, Phytochemistry 1988, 27, 753–755.

[46] L. Jing, L. He, P. Fan, Z. Jia, H. Ma, ‘Chemical Constituents of Saussurea involucrata with Anti-Hypoxia Activity’, Chem. Nat. Compd. 2016, 52, 487–489.

[47] S.-H. Nam, Y.-M. Kim, M. K. Walsh, Y.-J. Kee, K.-Y. Yang, J.-A Ko, S. Han, T. T. H. Nguyen, J. Y. Kim, D. Kim, ‘Synthesis and Functional Characterization of Caffeic Acid Glucoside Using Leuconostoc mesenteroides Dextranase’, J. Agric. Food Chem. 2017, 65, 2743–2750.

[48] R. Hahn, A. Nahrstedt, ‘Hydroxycinnamic Acid Derivatives, Caffeoylmalic and New Caffeoylaldonic Acid Esters, from Chelidonium majus’, Planta Med. 1993, 59, 71–75.

[49] U. Kuczkowiak, F. Peterait, A. Nahrstedt, ‘Hydroxycinnamic Acid Derivatives Obtained from a Commercial Crataegus Extract and from Authentic Crataegus spp.’, Sci. Pharm. 2014, 82, 835–846.

[50] S.-J. Lee, H.-J. Jang, Y. Kim, H.-M. Oh, S. Lee, K. Jung, Y.-H. Kim, W.-S. Lee, S.-W. Lee, M.-C. Rho, ‘Inhibitory effects of IL-6-induced STAT3 activation of bio-active compounds derived from Salvia plebeia R.B., Process Biochem. 2016, 51, 2222–2229.

[51] M. L. Uriburu Monasterio, R. L. Gil, V. E. Sosa, J. R. de la Fuente, ‘Caffeoyl esters of threonic acid and its lactone from Viguiera pazensis’, J. Argent. Chem. Soc. 2008, 96, 55–61.

[52] B. J. Belda, Y. Lee, J. P. V. Heuvel, ‘Conjugated linoleic acids and inflammation: isomer- and tissue-specific responses’, Clin. Lipidol. 2010, 5, 699–717.

[53] E. Sommella, G. Pepe, F. Pagano, G. C. Tenore, S. Marzocco, M. Manfra, G. Calabrese, R. P. Aquino, P. Campiglia, ‘UHPLC profiling and effects on LPS-stimulated J774 A.1 macrophages of flavonoids from bergamot (Citrus bergamia) juice, an underestimated waste product with high anti-inflammatory potential’, J. Funct. Foods 2014, 7, 641–649.

[54] G. Pepe, E. Sommella, D. Cianciarulo, C. Ostacolo, M. Manfra, V. Di Sarno, S. Musella, M. Russo, A. Messore, B. Parrino, A. Bertamino, G. Autore, S. Marzocco, P. Campiglia, ‘Polyphenolic Extract from Tarocco (Citrus sinensis L. Osbeck) Clone “Lempsso” Exerts Anti-Inflammatory and Antioxidant Effects via NF-kB and Nrf-2 Activation in Murine Macrophages’, Nutrients 2018, 10, 1961.

[55] L. Pompermaier, S. Marzocco, S. Adesso, M. Monizi, S. Schaiger, C. Neinhuis, H. Stuppner, T. Lautenschläger, ‘Medicinal plants of northern Angola and their anti-inflammatory properties’, J. Ethnopharmacol. 2018, 216, 26–36.

Received July 14, 2020
Accepted November 25, 2020