

Supporting Information

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Composition of essential oils and ethanol extracts of the leaves of *Lippia* species: identification, quantitation and antioxidant capacity

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DPPH Assay

The free radical scavenging capacity of the substances was determined by using the DPPH (1,2-diphenyl-2-picrylhydrazyl) radical discoloration method.

The substances were diluted in methanol at concentrations between 0.01-1.0 mM. Twenty μL of the different concentrations were placed in 96 well plates in duplicate. The reaction was initiated by adding 180 μL of DPPH solution (20 $\mu\text{g}/\text{mL}$ in methanol). The absorbance was read at 515 nm over 45 min. with an universal Micro plate reader (Elx 800 Bio-Tek Instruments, Winooski, Vermont, USA) versus a control (20 μL methanol). The concentration of DPPH radical was calculated from a standard curve of DPPH between 1 and 100 $\mu\text{g}/\text{mL}$ measured simultaneously.

Steady-state levels were reached within the first 15 minutes. For comparison the IC_{50} values for each substance (concentration of each substance where 50% of the DPPH radical is scavenged) were calculated with the DPPH values at 15 min. for different concentrations using a Table curve program (Jandel Scientific, Chicago, IL).

FRAP Assay

FRAP the “ferric reducing ability of plasma”, means the ability to reduce an excess of Fe^{3+} /2,4,6-tripyridyl-s-triazine complex (TPTZ) to the blue colored Fe^{2+} form.

The dilution of the different substances was identical to that described for the DPPH assay. Ten μL of substance were incubated with 30 μL water and 300 μL FRAP reagent, consisting of 25 mL acetate buffer (300 mM sodium acetate buffer, $\text{pH} = 3.6$), 2.5 mL TPTZ (10 mM TPTZ in 40 mM HCl) and 2.5 mL FeCl_3 solution (20 mM $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ in water) at 37°C . All reagents were freshly prepared and warmed to 37°C

before measurement. A calibration curve of ferrous sulphate (0.01-1.0 mM) was used and the results expressed in mM Fe²⁺/L.

The reaction was measured every min. for 10 minutes. The reaction of all isolated substances reached a steady-state level after 5 minutes. A linear regression curve was generated at the 5 min. reaction time-point for different concentrations of the isolated substances with the Microcal Origin 5.0 program. Using these regression curves the EC₁ values were calculated as the concentrations of antioxidant (μM) giving an absorbance equivalent to a 1 mM Fe(II) solution.

ORAC Assay

3,3'-azo-bis-(2-amidinopropane)-dihydrochloride (AAPH) was used as a peroxy-radical generator and fluorescein (0.21 μM in ORAC buffer) as a redox-sensitive fluorescent indicator.

Aliquots (20 μL) of a 10 mM stock solution of Trolox in DMSO were stored at -20°C, melted and diluted with ORAC buffer to a final concentration of 20 μM immediately before use. The ORAC buffer contained 75 mM sodium hydrogen phosphate/potassium dihydrogenphosphate buffer at pH = 7.4. Freshly prepared sample solutions (1.0 mM) in methanol or DMSO were diluted in ORAC buffer to a final concentration of 20 μM. Ten μL of the sample, buffer or Trolox solution together with 170 μL of fluorescein solution were placed in quadruplicate in a 96 well-plate and incubated at 37°C for 10 minutes. The exterior wells of the plates were not used for experimental determinations. The reaction was initiated by addition of 20 μL of APPH solution (103.5 mg/2 mL buffer), freshly mixed on ice immediately before use.

The decline of fluorescence was measured at 37°C every 2 min. until completion at 122 min. using a Cytoflour 4000 fluorescent microplate reader (excitation wavelength Ex 530/25nm, emission wavelength Em 585/30 nm) (Perspective Biosystems, Minnesota, USA).

The net area under the curve (AUC) of the standards and samples was calculated. The final ORAC values were calculated using the regression equation between Trolox concentration and the net AUC and are expressed as relative ORAC units, where 1 ORAC unit equals the inhibition of the declining fluorescence produced by 1 μ M Trolox.