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Inhibitory activity of nine essential oils on nitric oxide production by human leukocytes

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Introduction

Nitric oxide (NO) plays a key role in the production of reactive nitrogen species (RNS), which have cytotoxic properties against pathogenic microbes and, at the same time, can damage host tissues [1]. Some essential oils have antioxidant properties and their consumption can influence immune cell functions [2,3]. In order to widen our knowledge of the antioxidant properties of essential oils we studied their effect on NO production induced by lipopolysaccharide (LPS) in human blood leukocytes.

Essential oils and substances tested

Nine essential oils were investigated in addition to eugenol, thymol, carvacrol and nutmeg terpenes. The essential oils investigated, all obtained from commercial sources, were from:

- **Clove leaves** (*Syzygium aromaticum* (L.) Merr. & L.M. Perry)
- **Juniper berries** (*Juniperus communis* L.)
- **Lemon** (*Citrus limon* (L.) Burm. f.)
- **Lemon grass** (*Cymbopogon martinii* Roxb. Wats.)
- **Nutmeg** (*Myristica fragrans* Houtt.)
- **Rosemary** (*Rosmarinus officinalis* L.)
- **Spanish oregano** (*Thymbra capitata* Griseb.)
- **Tarragon** (*Artemisia dracunculus* L.)
- **Thyme** (*Thymus zygis* L.)

Methods

Analysis of the essential oils

Essential oils composition was analysed by GC-FID and GC-MS using a Supelcowax 10™ capillary column (60 m, 0.25 mm i.d., 0.25 µm film) and different chromatographic conditions depending on the essential oil. Retention indices and MS were used for identification. Quantification was performed from GC-FID peak areas by the normalisation procedure.

Leukocyte isolation and NO analysis

Buffy coats isolated from human whole blood provided by healthy volunteers were used as the source for leukocytes. After purification, leukocytes were placed in 96-well microtiter plates with different concentrations of the essential oils or substances for 10 min, and were stimulated with LPS for 1h. Nitrite accumulation, an indicator of NO production, was measured in the cell free supernatant by the Griess reaction (15 min incubation at room temperature) [3]. Absorbance was then measured at 540 nm in a microplate reader (BioRad Benchmark Plus). The amount of nitrite in the samples was calculated from a sodium nitrite standard. Cell free supernatant from leukocytes not exposed to LPS was used as negative control. L-NMMA was used for positive control treatment. Results are expressed as percentage of inhibition calculated versus NO produced by cells treated only with LPS.

Results and discussion

A summary of results is shown in **Table 1.** Results showed that clove oil and its major constituent, eugenol, were the most active inhibitors of the nitric oxide production by human leukocytes stimulated by LPS (IC_{50}=39.8±6.3 µg/mL and IC_{50}=19.0±1.8 µg/mL, respectively) (**Figure 1**). The activity of eugenol was roughly twice that of the L-NMMA (positive control, IC_{50}= 38.2±1.4 µg/mL) (**Figure 2**).

Carvacrol, the main constituent of Spanish oregano, displayed a similar activity to the clove oil, with an IC_{50} of 39.3±6.8 µg/mL. However, Spanish oregano oil was considered inactive (IC_{50}>45 µg/mL). It is remarkable that thymol (an isomer of carvacrol) (**Figure 2**) and thyme oil (**Figure 3**) showed no activity.

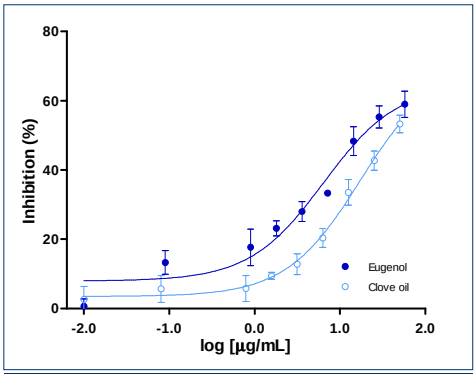

Figure 1. Inhibitory activity (%, mean±SD, n=4) of clove oil and eugenol on NO production in human leukocytes stimulated with LPS.

Other essential oils, such as rosemary, lemon and lemon grass oils, did not cause any NO decrease. Nutmeg, tarragon and juniper berry oils, as well as nutmeg terpenes, were also considered inactive (IC_{50}>45 µg/mL).

A good antioxidant activity of clove oil and eugenol has also been previously found by our group, both on the DPPH scavenging capacity and on the intracellular generation of reactive oxygen species by human leukocytes stimulated with PMA [4].

In conclusion, the present work confirms the good antioxidant profile of clove oil.

Table 1. Inhibitory activity (IC_{50}) of the essential oils and some constituents on NO production in human leukocytes stimulated with LPS. L-NMMA was used as positive control.

Essential oil or substance	IC_{50} (µg/mL ± SD)
Clove leaves	39.8±6.3
Juniper berries	No activity
Lemon	No activity
Lemon grass	No activity
Nutmeg	No activity
Nutmeg terpenes	No activity
Rosemary	No activity
Spanish oregano	No activity
Tarragon	No activity
Thyme	No activity
Carvacrol	39.3±6.8
Eugenol	19.0±1.8
Thymol	No activity
L-NMMA	38.2±1.4

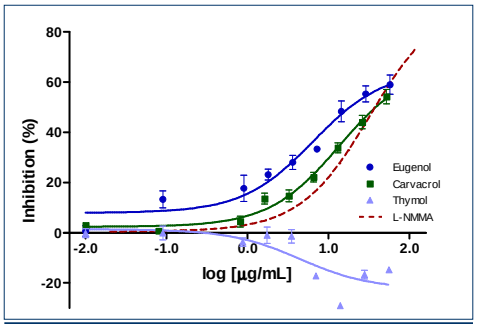

Figure 2. Inhibitory activity (%, mean±SD, n=4) of eugenol, carvacrol, thymol and L-NMMA (positive control) on NO production human leukocytes stimulated with LPS.

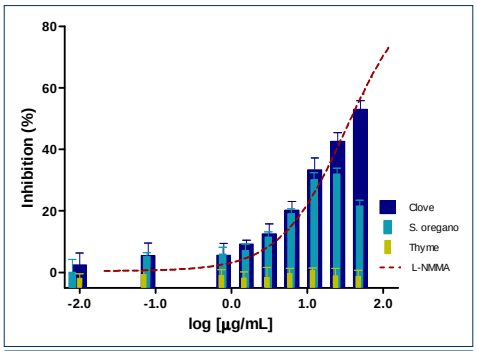

Figure 3. Inhibitory activity (%, mean±SD, n=4) of clove, Spanish oregano and thyme oils, and L-NMMA (positive control) on NO production in human leukocytes stimulated with LPS.

Acknowledgements

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