

# Effect of essential oils on the activity of human neutrophil myeloperoxidase *in vitro*

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## Introduction

Myeloperoxidase (MPO) (EC.1.11.1.7), together with its substrate hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and a halide, has proved to be a powerful antimicrobial system, and as such, MPO is a key component of innate immune defense and the oxygen-dependent microbicidal activity of phagocytes. However, more recent evidence has extended this view by demonstrating that MPO is also intimately involved in cellular homeostasis and is an important factor in the initiation and progression of various inflammatory diseases. Hence, the present work was designed to examine the effect of fifteen essential oils (EO) and four pure volatile substances on MPO activity and MPO cellular release as an important part of their anti-oxidative and anti-inflammatory properties.

## Material and methods

### Samples

The essential oils and compounds investigated were obtained from commercial sources and are listed in **Table 1**.

### Analysis of the essential oils

Essential oils composition was analysed by GC-FID and GC-MS using a Supelcowax 10<sup>TM</sup> 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 MPO tests

Buffy coats isolated from human whole blood provided by healthy volunteers were used as the source for leukocytes. Two different tests were used to measure MPO relative inhibition.

**Test A.** Enzyme inhibition in absence of cells: bacterial lipopolysaccharides (LPS) stimulated neutrophils were centrifuged to obtain a cell free supernatant rich in MPO where the inhibitory effect of various EO was studied.

**Test B.** MPO extracellular release from neutrophils and enzyme inhibition were tested at the same time using a human neutrophils preparation. Freshly isolated neutrophils were treated with the EO or maintained untreated in the case of stimulated controls. Later, cells were stimulated with LPS. MPO activity was assessed by oxidation of *o*-dianisidine dihydrochloride by H<sub>2</sub>O<sub>2</sub>, using quercetin as positive control. Absorbance was determined at 460 nm. Relative inhibition (%) was calculated from absorbance values of treated samples in relation to untreated controls [1-3]. An analysis of variance followed by a Dunnet's test was used to compare means of untreated controls to the treatments. Results were considered significant at *p*<0.05. IC<sub>50</sub> was calculated by interpolation in a concentration / effect curve. For data analysis, software Graph Pad Prism Version 5.0 for Windows was used.

## References

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## Acknowledgements

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## Results and discussion

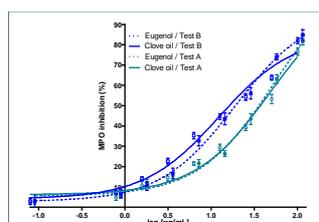
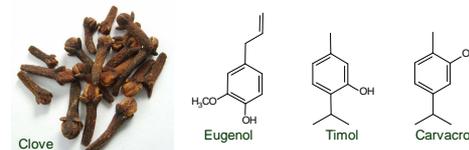
Results are shown in **Table 1**. Inhibitory activity was mainly detected in the phenol rich EO (clove, red thyme and Spanish oregano) and the corresponding main constituents (eugenol, thymol and carvacrol).

Clove EO, with IC<sub>50</sub> of 37.2±1.0 µg/mL (test A) and 16.3±1.3 µg/mL (test B), and eugenol, with IC<sub>50</sub> of 35.9±2.4 µg/mL (test A) and 19.2±2.0 µg/mL (test B), showed the highest activity. Lower IC<sub>50</sub> values in test A than in test B (**Figure 1**) suggest that the inhibition mechanism for clove and eugenol involves direct enzyme inhibition and that it could also be linked to a modification in the extracellular release of MPO.

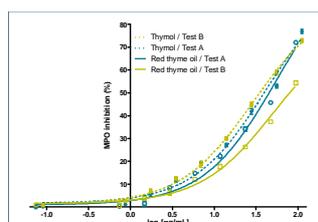
Thymol, red thyme EO (**Figure 2**) and Spanish oregano EO had similar results in both tests, which might indicate that enzyme inhibition was the main mechanism involved.

Finally, in some cases such as rosemary EO (**Figure 3**) and carvacrol, lower inhibitory activity was observed in presence of neutrophils.

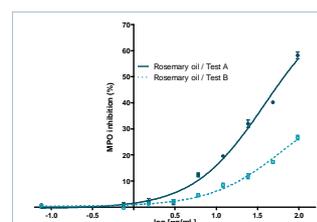
In conclusion, results suggest that some essential oils not only act as MPO inhibitors but also as modulators of MPO cellular release. Our findings provide new insights into the antioxidant and immunomodulating properties of essential oils previously described by our group [4-7].



**Figure 1.** Inhibitory activity (% mean ± SD, n=4) of clove oil and eugenol on MPO by two different tests: without cells (A) and with cells (B).



**Figure 2.** Inhibitory activity (% mean ± SD, n=4) of red thyme oil and thymol on MPO by two different tests: without cells (A) and with cells (B).



**Figure 3.** Inhibitory activity (% mean ± SD, n=4) of rosemary oil on MPO by two different tests: without cells (A) and with cells (B).

**Table 1.** Inhibition (%) at the highest concentration tested (1.0 × 10<sup>-2</sup> % v/v<sup>a</sup>) and IC<sub>50</sub> (µg/mL) on myeloperoxidase activity of the fifteen essential oils and four compounds studied, in two different tests.

| Essential oil / compound                          | Test A (cell absence) <sup>b</sup> |                          | Test B (human neutrophils) <sup>b</sup> |                          |
|---|------------------------------------|--------------------------|---|--------------------------|
|   | Inhibition (%)                     | IC <sub>50</sub> (µg/mL) | Inhibition (%)                          | IC <sub>50</sub> (µg/mL) |
| Clove ( <i>Syzygium aromaticum</i> )              | 76.7±1.8 * <sup>c</sup>            | 37.2±1.0                 | 81.9±1.4 *                              | 16.3±1.2                 |
| Eugenol   | 81.4±1.5 *                         | 35.9±2.4                 | 84.7±2.7 *                              | 19.2±2.0                 |
| Red thyme ( <i>Thymus zygis</i> )                 | 72.1±1.0 *                         | 45.3±2.1                 | 54.4±1.9 *                              | 80.2±6.6                 |
| Thymol  | 76.9±1.8 *                         | 39.7±2.6                 | 72.9±1.8 *                              | 35.0±1.0                 |
| Spanish oregano ( <i>Coridothymus capitatus</i> ) | 60.7±1.4 *                         | 58.9±5.3                 | 57.6±2.2 *                              | 74.0±7.6                 |
| Carvacrol   | 80.9±1.0 *                         | 35.9±1.4                 | 67.0±1.2 *                              | 50.8±3.4                 |
| Rosemary ( <i>Rosmarinus officinalis</i> )        | 58.2±2.6 *                         | 67.1±5.3                 | 26.6±1.7 *                              | na                       |
| Nutmeg ( <i>Myristica fragans</i> )               | 41.8±1.5 *                         | na <sup>d</sup>          | 26.0±1.1 *                              | na                       |
| Niaouli ( <i>Melaleuca quinquenervia</i> )        | 50.9±2.0 *                         | na                       | 28.0±1.6 *                              | na                       |
| Tea tree ( <i>Melaleuca alternifolia</i> )        | 41.0±3.0 *                         | na                       | 10.2±0.8 *                              | na                       |
| Cajeput ( <i>Melaleuca cajuputi</i> )             | 15.9±1.2 *                         | na                       | 17.1±1.8 *                              | na                       |
| Tarragon ( <i>Artemisia dracunculus</i> )         | 41.3±1.5 *                         | na                       | 16.3±1.0 *                              | na                       |
| Coriander ( <i>Coriandrum sativum</i> )           | 30.8±1.2 *                         | na                       | 22.7±1.1 *                              | na                       |
| Juniper ( <i>Juniperus communis</i> )             | 49.0±1.5 *                         | na                       | 32.4±1.0 *                              | na                       |
| Bay laurel ( <i>Laurus nobilis</i> )              | 44.0±1.3 *                         | na                       | 39.9±1.8 *                              | na                       |
| Palmarosa ( <i>Cymbopogon martinii</i> )          | 29.3±1.4 *                         | na                       | 23.6±1.3 *                              | na                       |
| Bornyl acetate                                    | 10.4±1.0 *                         | na                       | 20.5±1.0 *                              | na                       |
| Lemon ( <i>Citrus limon</i> )                     | 25.1±0.9 *                         | na                       | 22.7±1.7 *                              | na                       |
| Ginger ( <i>Zingiber officinale</i> )             | 15.2±0.9 *                         | na                       | 8.5±1.4 *                               | na                       |
| Quercetin   | na                                 | 1.0±0.3                  | na                                      | 2.8±0.8                  |

a) Equivalent to 90-115 µg/mL depending on the EO or compound tested

b) Mean ± SD, n=4

c) \* : significant difference vs untreated control (*p*<0.05)

d) na: not available.