

Antioxidant activity of eleven essential oils by two different *in vitro* assays

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Introduction

Essential oils may have antioxidant properties and their consumption can influence immune cell functions. Also, their use in food industry may serve to replace synthetic antioxidant food additives. In order to contribute to a better knowledge of their antioxidant mechanisms, eleven essential oils, eugenol and carvacrol were investigated using two different *in vitro* models: the intracellular generation of reactive oxygen species (ROS) in human leukocytes and the scavenging of free radicals.

Essential oils

The essential oils investigated, all obtained from commercial sources, were from:

- Clove leaves (*Syzygium aromaticum* (L.) Merr. et L.M. Perry)
- Leaves and branches of niauli (*Melaleuca* sp.)
- Tarragon (*Artemisia dracunculus* L.)
- Coriander (*Coriandrum sativum* L.)
- Tea tree leaves and branches (*Melaleuca alternifolia* L.)
- Juniper berries (*Juniperus communis* L.)
- Ginger roots (*Zingiber officinale* Roscoe)
- Rosemary (*Rosmarinus officinalis* L.)
- Cayeputi aerial parts (*Melaleuca cajuputi* Powell)
- Lemon (*Citrus limon* (L.) Burman fil.)
- Spanish oregano (*Thymbra capitata* Griseb.)



Methods

Analysis of the essential oils

Essential oils composition was analysed by GC-FID and GC-MS using a Supelcowax 10TM 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.

Free radical scavenging activity assay

Free radical scavenging activity was evaluated according to the method described by Malenčić *et al.* [1], in which neutralization by antioxidants of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is measured through absorbance at 515 nm (96-well microplate).

Results and discussion

Main results are shown in Table 1 and in Figures 1 and 2.

Clove oil and ginger oil were the most efficient inhibitors of intracellular generation of ROS after activation by PMA, ($IC_{50} = 7.5 \pm 1.6$ µg/mL and 8.9 ± 5.8 µg/mL, respectively). Moreover, the main constituent of clove oil, eugenol (86.2%), has also strong inhibition of ROS generation ($IC_{50} = 1.6 \pm 0.3$ µg/mL). The other essential oils assayed and carvacrol (main component from Spanish oregano, 72.7%) were considered inactive ($IC_{50} > 45$ µg/mL).

No radical scavenging activity was observed for the essential oils and compounds investigated between 0.1 µg/mL to 200 µg/mL, except for clove oil and eugenol. Their IC_{50} were 13.2 ± 2.9 µg/mL and 11.7 ± 0.6 µg/mL, respectively, as we already reported [3].

Then, niauli, tarragon, coriander, juniper, tea tree, rosemary, cayeputi, lemon and Spanish oregano oils showed no antioxidant effect in both methods.

Activity on the intracellular production of ROS

Activity on the intracellular production of ROS in human polymorphonuclear leukocytes (PMNs) stimulated by PMA (phorbol myristate acetate, 10 µM) was determined by flow cytometry according to Pérez-García *et al.* [2] with modifications (96-well microplate), using 2',7'-dichlorofluorescein diacetate (DCFH-DA) as fluorescence probe. Viability was measured by propidium iodide exclusion. To stabilize the cells, they were resuspended with HBBS containing 1% paraformaldehyde. Samples of essential oils were prepared with HBBS containing 1% of E-484 (glyceryl polyethyleneglycol ricinoleate). Fluorescence was measured by Cytomics FC 500 MPL (Beckman coulter). Data analysis has been carried out with the software Summit v. 4.2 (Dako Cytomation).

Clove essential oil and eugenol displayed intense activity in both tests, suggesting that their **antioxidant activity** is related both, to **enzymatic mechanisms** and to **free radical scavenging activity**.

Ginger essential oil has also strong inhibition of ROS production, suggesting that their antioxidant activity is only related to **enzymatic mechanisms**.

Table 1. IC_{50} (µg/mL ± SD) of the active essential oils.

Essential oil or substance	Intracellular ROS production	Free radical scavenging activity
Clove	7.5 ± 1.6	13.2 ± 2.9
Ginger	8.9 ± 5.8	No activity
Eugenol	1.6 ± 0.3	11.7 ± 0.6
Quercetin (Reference)	7.6 ± 2.9	10.5 ± 4.6

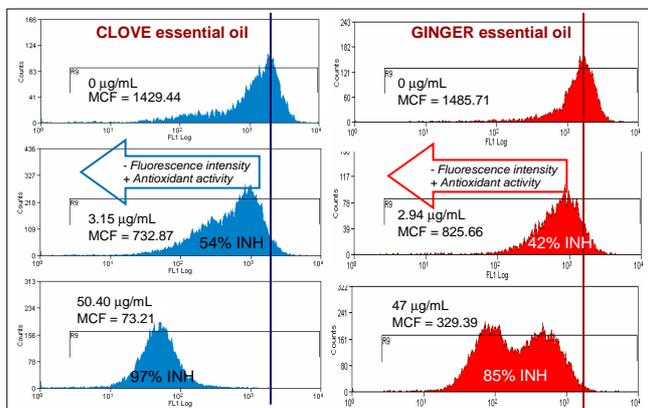


Figure 1. Histograms of fluorescence (FL1) distribution showing the effect of clove (left panel) and ginger (right panel) oils on ROS production in PMA stimulated PMNs. Intracellular fluorescence was measured by flow cytometry and plotted on a \log_{10} scale from channel numbers 0.1 to 1023 on the abscissa. A representative experiment is shown. MCF: mean channel of fluorescence. INH: Inhibition of ROS production.

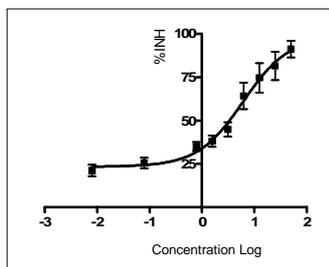


Figure 2. Inhibition (%) of intracellular ROS production in human PMNs due to clove essential oil at concentrations ranging from 0.008 µg/mL to 50.4 µg/mL.

Data of inhibition (%±SD) obtained at different concentrations follow: $21.22\% \pm 7.57$ (0.008 µg/mL); $25.60\% \pm 6.62$ (0.079 µg/mL); $35.07\% \pm 5.62$ (0.788 µg/mL); $38.08\% \pm 7.40$ (1.575 µg/mL); $44.84\% \pm 9.21$ (3.15 µg/mL); $64.23\% \pm 17.02$ (6.3 µg/mL); $74.66\% \pm 18.84$ (12.6 µg/mL); $81.48\% \pm 18.36$ (25.2 µg/mL); $91.12\% \pm 10.68$ (50.4 µg/mL).

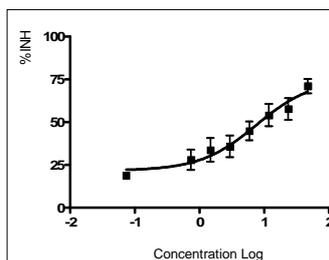


Figure 3. Inhibition (%) of intracellular ROS production in human PMNs due to ginger essential oil at concentrations ranging from 0.073 µg/mL to 47.0 µg/mL.

Data of inhibition (%±SD) obtained at different concentrations follow: $18.86\% \pm 3.74$ (0.07 µg/mL); $27.93\% \pm 13.27$ (0.73 µg/mL); $33.72\% \pm 15.57$ (1.47 µg/mL); $35.83\% \pm 14.22$ (2.94 µg/mL); $44.87\% \pm 1.94$ (5.87 µg/mL); $54.08\% \pm 14.45$ (11.75 µg/mL); $57.71\% \pm 14.18$ (23.5 µg/mL); $71.00\% \pm 9.18$ (47.0 µg/mL).

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