

Antioxidant and complement modulating activities of five essential oils

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Introduction

Antioxidant consumption has shown to lessen the decline in immune functions caused by aging, and it appears to be associated with enhancement of both macrophage and lymphocyte functions. The human complement system is also an important component of innate immunity. Complement derived products mediate functions contributing to pathogen elimination. However, inappropriate activation of the system contributes to the pathogenesis of immunological and inflammatory diseases. The purpose of this study was to investigate five essential oils for their activity on the complement system and their antioxidant properties by *in vitro* assays. All five essential oils were obtained from commercial sources.

Material and 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 temperature gradients 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. Quercetin was used as active reference substance.

Activity on human complement system

The activity on the classical pathway of the complement system was determined in human pooled serum, used as source of complement. The hemolytic assay established by Klerx *et al.* [2] was followed and the amount of hemoglobin released was measured at 405 nm. Quercetin was used as active reference substance.

Results and discussion

The main chemical data of the essential oils assayed are shown in **Table 1**. The free radical scavenging activity and the activity on the complement system of the essential oils and some of their main constituents are shown in **Table 2**.

In conclusion, among the five oils tested, the clove essential oil showed the best combination of free radical scavenging and complement inhibitory activities

Table 1. Chemical characteristics of the essential oils assayed.

Essential oil	Lemon	Spanish oregano	Clove	Thyme	Rosemary
Plant source	<i>Citrus limon</i> L. Burman fil. Fresh fruit peel	<i>Thymbra capitata</i> Griseb. Flowering aerial parts	<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry = <i>Eugenia caryophyllata</i> Thunb. Leaves	<i>Thymus zygis</i> L. Flowering aerial parts	<i>Rosmarinus officinalis</i> L. Flowering aerial parts
Identified % (n° compounds)	99.9 % (17)	99.8 % (16)	99.7 % (6)	98.4 % (16)	99.9 % (17)
Main components	Limonene (71.6%) β-Pinene (12.2%) γ-Terpinene (6.9%)	Carvacrol (73.5%) p-Cymene (8.1%) γ-Terpinene (5.5%)	Eugenol (86.2%) β-Caryophyllene (13.4%)	Thymol (56.6%) p-Cymene (28.4 %) γ-Terpinene (5.8%)	α-Pinene (9.9%) β-Pinene (9.7%) 1,8-Cineole (49.7%) Camphor (9.6 %)



Only clove oil showed antioxidant activity in the DPPH assay ($IC_{50}=13.2 \pm 2.9 \mu\text{g/mL}$), indicating that this oil has free radical scavenging. This activity can be attributed to eugenol, which is the major component (86.2 %) and showed an ($IC_{50}=11.7 \pm 0.5 \mu\text{g/mL}$). Results on inhibition of the classical pathway of the complement system, showed that thyme and clove oils have a similar weak activity ($IC_{50} = 61.4 \pm 10.6 \mu\text{g/mL}$ and $72.7 \pm 4.1 \mu\text{g/mL}$, respectively). Spanish oregano, rosemary and lemon oils showed no inhibitory effect on the complement system activated by the classical pathway.

References

1. Malenčić D *et al.* (2000) Screening for antioxidant properties of *Salvia reflexa* Hornem. *Phytother Res* 14: 546-548. 2. Klerx JP *et al.* (1983) Microassay for colorimetric estimation of complement activity in guinea pig, human and mouse serum. *J Immunol Methods* 63: 215-220.

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Table 2. Activity of the essential oils and some constituents on free radical scavenging (DPPH) and complement system (classical pathway).

Essential oil or substance	Free radical scavenging activity IC_{50} ($\mu\text{g/mL} \pm \text{SD}$)	Activity on complement system IC_{50} ($\mu\text{g/mL} \pm \text{SD}$)
Clove	13.2 ± 2.9	72.7 ± 4.1
Rosemary	n.a.	n.a.
Lemon	n.a.	n.a.
Thyme	n.a.	61.4 ± 10.6
Spanish oregano	n.a.	151.6 ± 48.7
Eugenol	11.7 ± 0.6	70.1 ± 3.6
Carvacrol	500.7 ± 35.4	73.3 ± 13.1
Thymol	448.0 ± 33.6	149.7 ± 10.2
Quercetin	10.5 ± 4.6	33.7 ± 4.3
	n = 4 n.a.: no activity between 0 and 600 µg/mL.	n = 6 n.a.: no activity between 0 and 400 µg/mL.