Multidimensional complexity of natural complex substances: the case of essential oils

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EFEO Conference, Grasse, 30th May 2024

Natural complex substances from plants

Citrus $\xrightarrow{\text{Cold pressure}}$ EO

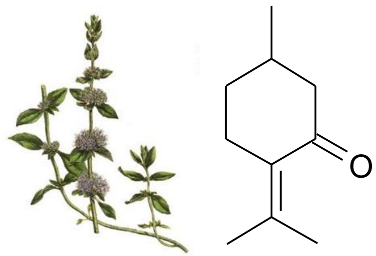
Plant $\xrightarrow{\text{Distillation}}$ EO

Hydrodistillation

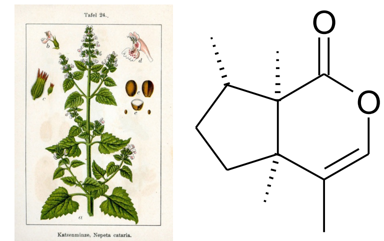
Steam distillation

Plant $\xrightarrow{\text{Extraction}}$ Extract

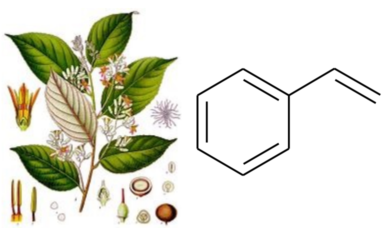
Chemical diversity found in EO



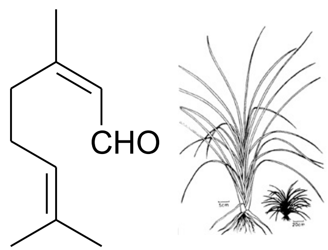
Pulegone



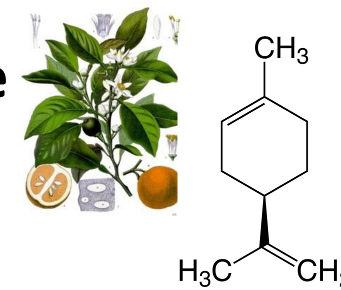
Nepeta lactone



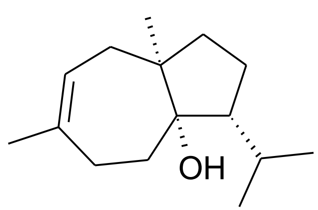
Styrene



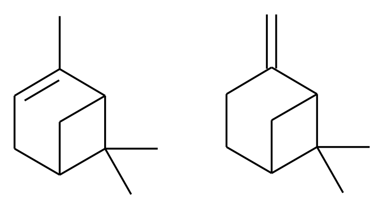
Citral



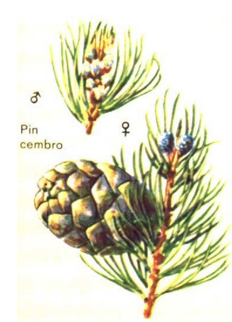
Limonene



Carotol



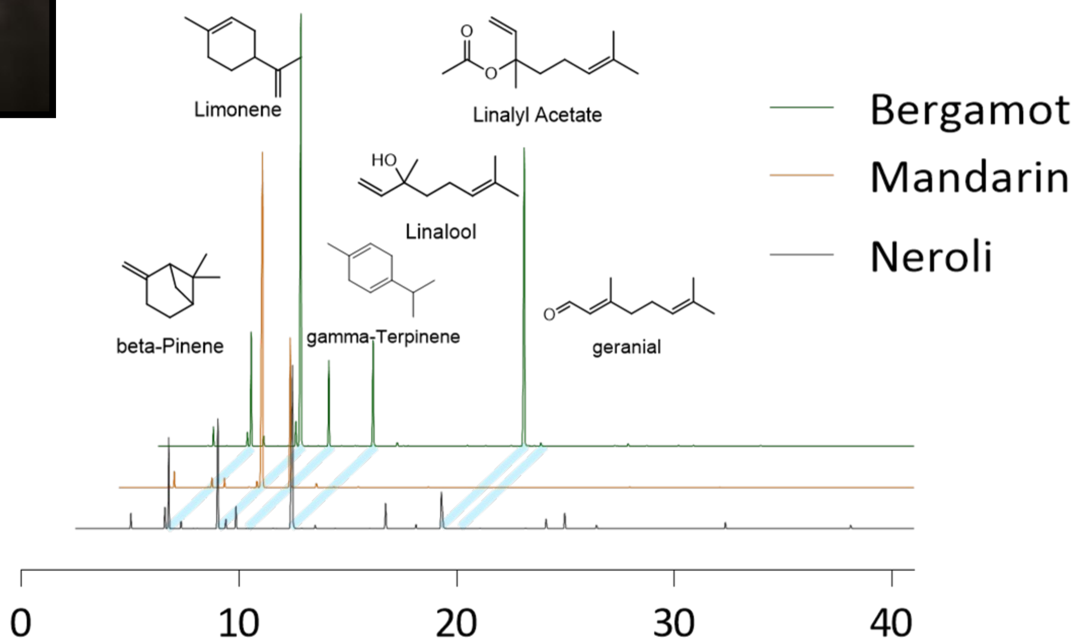
Pinenes



Complexity of composition

Citrus —Cold pressure→ EO

GCxMS TIC

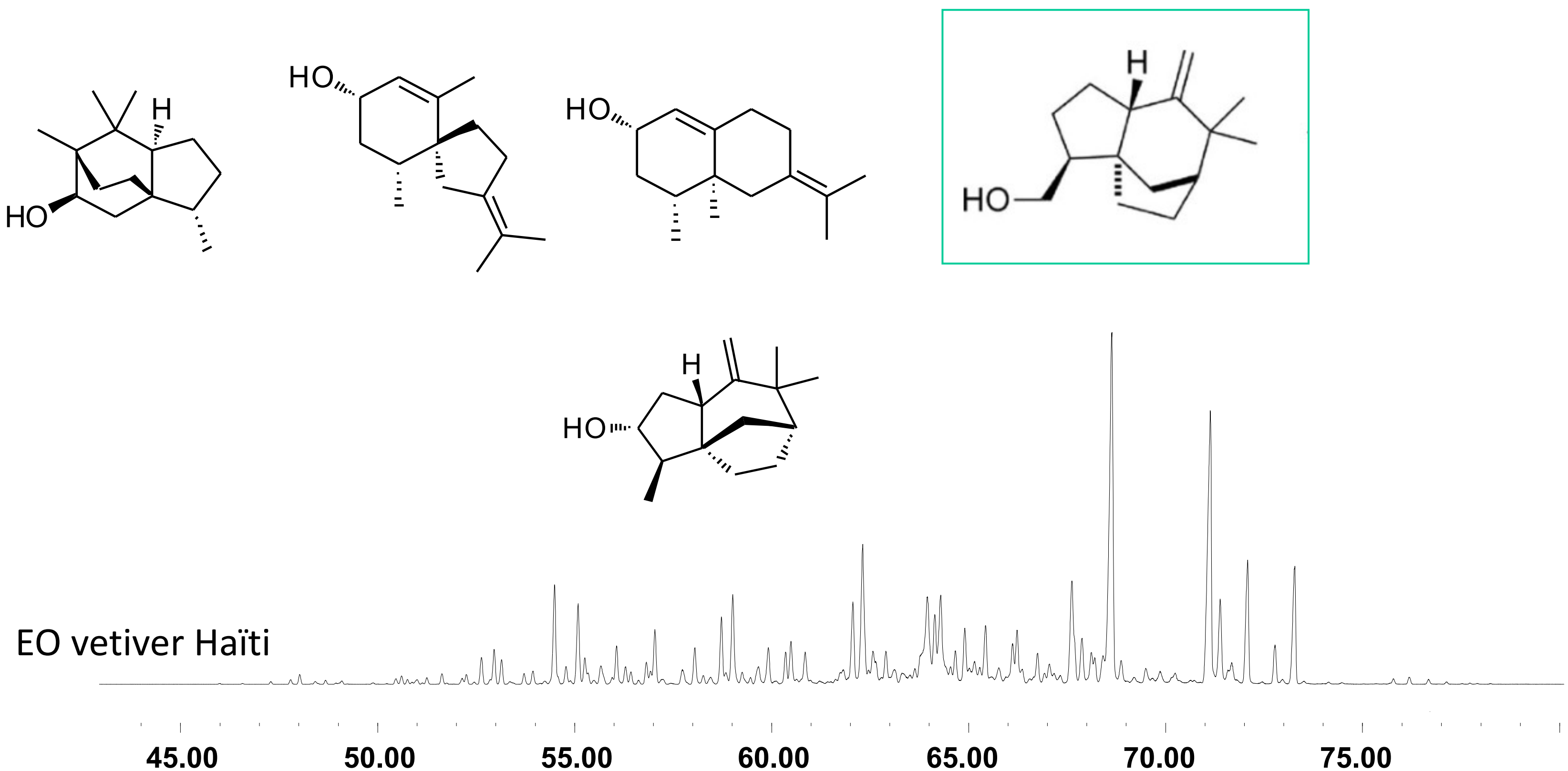


T_r min

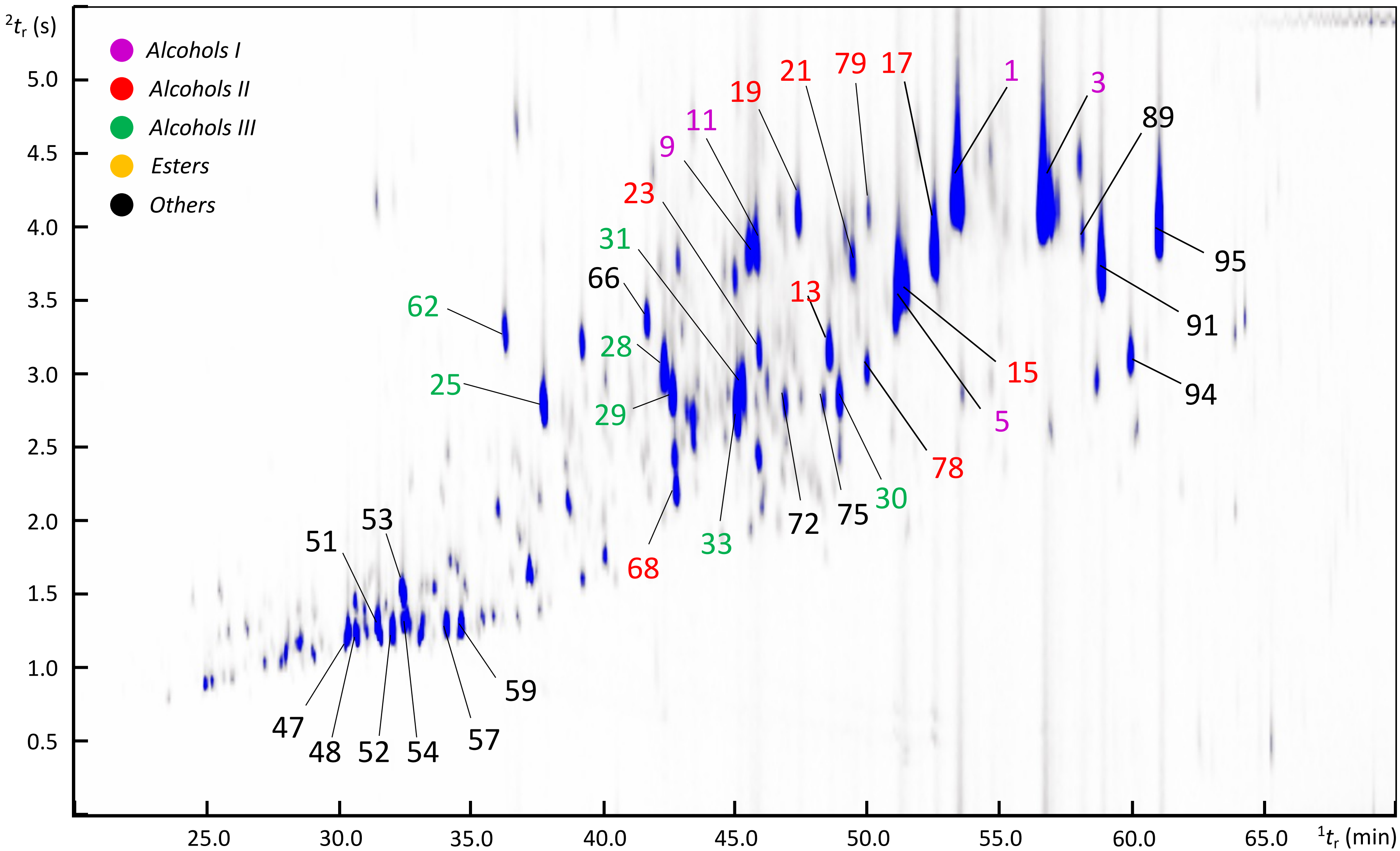
M. Pierson. X. Fernandez. S. Antoniotti. *Sci. Rep.* **2021**. 11. Article number: 11096.

Complexity of composition

Monodimensional GC/MS SQP



I. Notar Francesco. J.-J. Filippi. S. Antoniotti. *ChemPlusChem*. **2017**. *82*. 3. 407-415.



EO Vetiver Haiti – GCxGC-MS TQP: **better resolution + better sensibility**

Complexity of natural variability

Botanical

- Species
- Subspecies
- Hybrids
- Cultivars

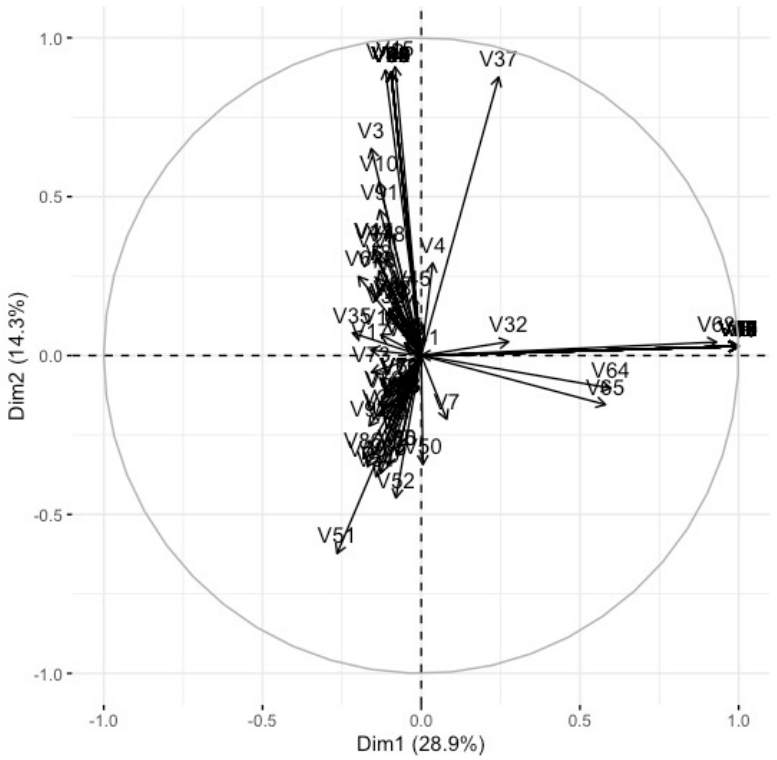
Geographical origin

- **Chemotypes**

Climate

Agricultural practices

Technical variations



Statistical analysis (PCA) of the chemical composition (96 variables) for 14 essential oils of *Ocimum gratissimum*.

L. Gilles, S. Antoniotti. *Chem. Biodiv*. **2023**, e202300252.

Complexity of properties

CASE OF ROSE ESSENTIAL OIL

GC/FID, selection of compounds from more than 200

Components	*R. Centifolia* 2021	*R. Damascena* 2020
Linalool	1.03	0.85
cis-Rose oxide	0.07	0.44
Phenylethanol	0.09	1.04
trans-Rose oxide	0.04	0.10
Citronellol	9.22	25.45
Nerol	4.36	9.95
Geraniol	17.6	21.44
Eugenol	0.74	0.91
Methyleugenol	0.56	1.36
Heptadecane C17	1.07	2.22
Nonadecane C19	8.10	14.64
Heneicosane C21	6.31	5.00
Tricosane C23	5.90	0.84
Pentacosane C25	2.86	0.20
Rose furan	<0.2	<0.2
β-damascenone	<0.2	<0.2
β-ionone	<0.1	<0.1

D. V. Antonova *et al*. *Journal of Essential Oil Research* **2020,** 33;2, 171-181. M. Mileva. *Biomolecules* **2021,** 11, 127.
http://www.leffingwell.com/rose.htm

Complexity of properties

CASE OF ROSE ESSENTIAL OIL

GC/FID, selection of compounds from more than 200

Components	*R. Centifolia* 2021	*R. Damascena* 2020	*R. Damascena* >1% (=81%)
Linalool	1.03	0.85	-
cis-Rose oxide	0.07	0.44	-
Phenylethanol	0.09	1.04	1.2
trans-Rose oxide	0.04	0.10	-
Citronellol	9.22	25.45	31.4
Nerol	4.36	9.95	12.3
Geraniol	17.6	21.44	26.4
Eugenol	0.74	0.91	-
Methyleugenol	0.56	1.36	1.7
Heptadecane C17	1.07	2.22	2.7
Nonadecane C19	8.10	14.64	18.0
Heneicosane C21	6.31	5.00	6.2
Tricosane C23	5.90	0.84	-
Pentacosane C25	2.86	0.20	-
Rose furan	<0.2	<0.2	-
β-damascenone	<0.2	<0.2	-
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D. V. Antonova *et al*. *Journal of Essential Oil Research* **2020,** 33;2, 171-181. M. Mileva. *Biomolecules* **2021,** 11, 127.
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Complexity of properties

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Components	*R. Centifolia* 2021	*R. Damascena* 2020	*R. Damascena* >1% (=81%)	Odor unit (%)
Linalool	1.03	0.85	-	1.0
cis-Rose oxide	0.07	0.44	-	3.3
Phenylethanol	0.09	1.04	1.2	0.016
trans-Rose oxide	0.04	0.10	-	0.8
Citronellol	9.22	25.45	31.4	4.3
Nerol	4.36	9.95	12.3	0.1
Geraniol	17.6	21.44	26.4	0.8
Eugenol	0.74	0.91	-	0.18
Methyleugenol	0.56	1.36	1.7	0.013
Heptadecane C17	1.07	2.22	2.7	-
Nonadecane C19	8.10	14.64	18.0	-
Heneicosane C21	6.31	5.00	6.2	-
Tricosane C23	5.90	0.84	-	-
Pentacosane C25	2.86	0.20	-	-
Rose furan	<0.2	<0.2	-	0.003
β-damascenone	<0.2	<0.2	-	70.0
β-ionone	<0.1	<0.1	-	19.2

D. V. Antonova *et al*. *Journal of Essential Oil Research* **2020,** 33;2, 171-181. M. Mileva. *Biomolecules* **2021,** 11, 127.
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Heptadecane C17	1.07	2.22	2.7	-
Nonadecane C19	8.10	14.64	18.0	-
Heneicosane C21	6.31	5.00	6.2	-
Tricosane C23	5.90	0.84	-	-
Pentacosane C25	2.86	0.20	-	-
Rose furan	<0.2	<0.2	-	0.003
β-damascenone	<0.2	<0.2	-	70.0
β-ionone	<0.1	<0.1	-	19.2

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Complexity of properties

The mystery of mute flowers




Complexity of biological properties

How to measure antimicrobial activity ? By assaying the **MIC** : Minimum Inhibitory Concentration, in μg/mL, the lowest concentration of a substance that inhibits the growth of a strain of bacteria *in vitro*.

Complexity of biological properties

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Case of *Perilla frutescens* essential oil

Assayed for antimicrobial activity against *Staphyloccus aureus*

Nabeta, K.; Oda, T.; Fujimura, T.; Sugisawa, H. *Agric. Biol. Chem*. **1985**, 49, 276.

	EO
MIC S. aureus (µg/ml)	125

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

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	EO	perillaldehyde	limonene	β-caryophyllene	linalool	benzaldehyde	β-pinene	peillyl alcohol	isoeugenol	α-caryophyllene	Total
MIC S. aureus (μg/ml)	125	1000	125	>1000	>1000	>1000	125	1000	500	>1000	N.A.

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

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	EO	perillaldehyde	limonene	β-caryophyllene	linalool	benzaldehyde	β-pinene	peillyl alcohol	isoeugenol	α-caryophyllene	Total
MIC S. aureus (μg/ml)	125	1000	125	>1000	>1000	>1000	125	1000	500	>1000	N.A.
Ratio	N.A.	74	12.8	3.8	2.6	1.6	0.6	0.26	0.25	0.13	96.04

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

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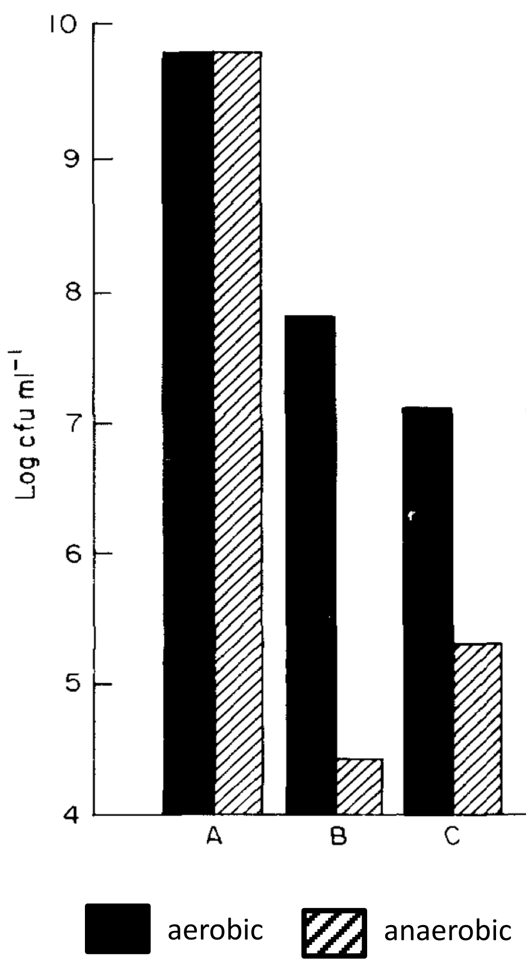
MIC S. aureus (μg/ml)	125	1000	125	>1000	>1000	>1000	125	1000	500	>1000	N.A.
Ratio	N.A.	74	12.8	3.8	2.6	1.6	0.6	0.26	0.25	0.13	96.04
Composite MIC	760.6	740	16	-	-	-	0.75	2.6	1.25	-	

It does not make sense to recalculate a composite MIC by linear combination of individual MIC weighted by their ratios.

The whole substance is more active = **synergistic effect**

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

Complexity of biological properties



Case of *Thymus* sp. essential oil

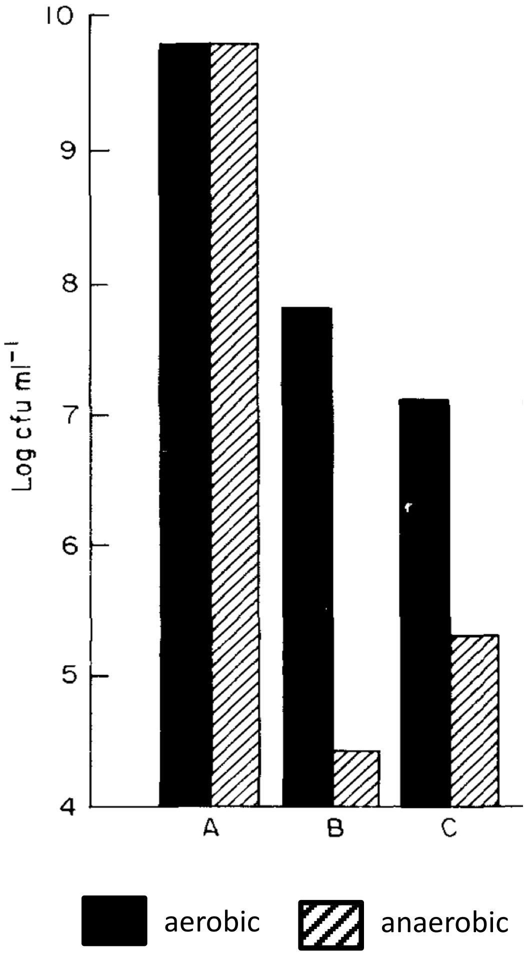
Assayed for antibacterial activity against *Salmonella enterica* T.

A: negative control (without substance)

Adapted from B. J. Juven, J. Kanner, F. Schved, H. Weisslowicz, *J. Appl. Bacteriol.* **1994**, *76,* 626.

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

Complexity of biological properties



Case of *Thymus* sp. essential oil

Assayed for antibacterial activity against *Salmonella enterica* T.

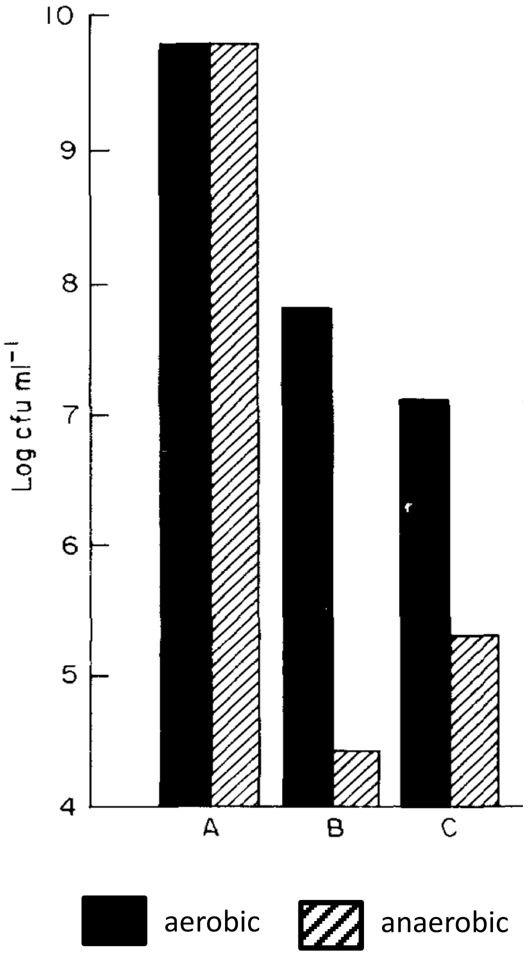
A: negative control (without substance)

B: thyme EO (350 μg/ml)

Adapted from B. J. Juven, J. Kanner, F. Schved, H. Weisslowicz, *J. Appl. Bacteriol.* **1994**, *76,* 626.

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

Complexity of biological properties



Case of *Thymus* sp. essential oil

Assayed for antibacterial activity against *Salmonella enterica* T.

A: negative control (without substance)

B: thyme EO (350 μg/ml)

C: thymol (140 μg/ml)

41%

The whole substance is less active = **antagonist effect**

Adapted from B. J. Juven, J. Kanner, F. Schved, H. Weisslowicz, *J. Appl. Bacteriol.* **1994**, *76,* 626.

T. Nakatsu, A. T. Lupo, J. W. Chinn, R. K.L. Kang, *Studies in Natural Products Chemistry*, **2000**, 21, Part B, 571-631.

Complexity of biological properties

Other examples of combination of individual compounds of EO and their activities.

Pair combinations	Organism	Methods	Interaction	References
Thymol/carvacrol	*Staphylococcus Aureus*, *Pseudomonas. Aeruginosa*	Half dilution	Additive	Lambert *et al.* [23]
	Escherichia Coli	Checkerboard	Synergism	Pei *et al.* [54]
	S. aureus, *Bacillus. cereus*, *E coli*	Checkerboard	Antagonism	Gallucci *et al.* [55]
	S. aureus, *P. aeruginosa*	Mixture	Additive	Lambert *et al.* [23]
	E. coli	Checkerboard	Additive	Rivas *et al.* [56]
	Salmonella typhinurium	Mixture	Synergism	Zhou *et al.* [57]
Thymol/eugenol	*E. coli*	Checkerboard	Synergism	Pei *et al.* [54]
Carvacrol/eugenol	*E. coli*	Checkerboard	Synergism	Pei *et al.* [54]
	S. aureus, *B. cereus*, *E coli*	Checkerboard	Antagonism	Gallucci *et al.* [55]
Carvacrol/myrcene	*S. aureus*, *B. cereus*, *E coli*	Checkerboard	Antagonism	Gallucci *et al.* [55]
Carvacrol/Cymene	*B. cereus*	Mixture	Synergism	Ultee *et al.* [58]
Carvacrol/linalool Eugenol/linalool Eugenol/menthol	*Listeria monocytogenes*, *Enterobacter aerogenes*, *E. coli*, *P. aeruginosa*	Checkerboard	Synergism	Bassole *et al.* [30]
Menthol/Geraniol Menthol/Thymol	*S. aureus*, *B. cereus*		Synergism	Gallucci *et al.* [55]
Cinnamaldehyde/ Carvacrol	*E. coli*	Checkerboard	Additive	Pei *et al.* [54]
	S. typhinurium	Mixture	Synergism	Zhou *et al.* [57]
Cinnamaldehyde/ Thymol	*E. coli*	Checkerboard	Synergism	Pei *et al.* [54]
	S. typhinurium	Mixture	Synergism	Zhou *et al.* [57]
Cinnamaldehyde/ Eugenol	*Staphylococcus sp.*, *Micrococcus sp.*, *Bacillus sp.*, *and Enterobacter sp.*	Mixture	Additive	Moleyar et Narasimham [59]

I. H. N. Bassolé, H. R. Juliani. *Molecules* **2012**, 17, 3989-4006.

Summary

- Essential oils are **complex mixtures** of secondary metabolites with a high **chemical diversity**
- The chemical composition of a given essential oil is subjected to **natural variation**
- Essential oils have their own biological properties, dependant on the biological activity of their individual components but **not linearly correlated** to their activity and proportion
- Biological properties of essential oils should be assayed on essential oils of **known composition considered as a whole**