ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Peroxyl Radical Trapping Antioxidant Activity of Essential Oils and Their Phenolic Components

This is the final peer-reviewed author’s accepted manuscript (postprint) of the following publication:

Published Version:
Pan, W., Velasco Abadia, A., Guo, Y., Gabbanini, S., Baschieri, A., Amorati, R., et al. (2024). Peroxyl Radical Trapping Antioxidant Activity of Essential Oils and Their Phenolic Components. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 72(43), 23832-23843 [10.1021/acs.jafc.4c04580].

Availability:
This version is available at: https://hdl.handle.net/11585/1008694 since: 2025-03-19

Published:
DOI: http://doi.org/10.1021/acs.jafc.4c04580

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

22 April 2026

Accepted version of the paper:
W. Pan, A. Velasco Abadia, Y. Guo, S. Gabbanini, A. Baschieri, R. Amorati, L. Valgimigli.
Peroxyl Radical Trapping Antioxidant Activity of Essential oils and their Phenolic Components.
J. Agric. Food Chem. 2024, 72, 23832-23843.

Peroxyl Radical Trapping Antioxidant Activity of Essential Oils and Their Phenolic Components

Wenkai Pan, Albert Velasco Abadia, Yafang Guo, Simone Gabbanini, Andrea Baschieri,
Riccardo Amorati, and Luca Valgimigli*

ABSTRACT: Essential oils (EOs) are gaining importance as sustainable food antioxidants, but kinetic data on peroxyl radical trapping are missing. Thirteen EOs from 11 botanical species were studied in the inhibited autoxidation of cumene by oxygen-uptake kinetics. EOs of *Juniperus oxycedrus*, *Syzygium aromaticum*, *Thymus vulgaris*, *Thymbra capitata*, *Betula alba*, *Pimenta racemosa*, and *Satureja montana*, containing 23−86% phenolic components by gas chromatography/mass spectrometry (GC−MS) analysis, afforded inhibition rate constants k_{inh} in the order of 10^4 M^{-1} s^{-1} at 30 °C similar to reference butylhydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol) (BHT). They matched or outperformed BHT in the protection of olive oil. The EOs *Daucus carota* and *Cedrus atlantica* with <1% phenols and those of *Apium graveolens* and *Tagetes minuta* with no phenolics had no chain-breaking activity. Key components carvacrol, thymol, eugenol, dihydroeugenol, umbelliferone, conyferyl alcohol, *o*-cresol, *m*-cresol, *p*-cresol, 4-allylphenol, 2,3-xylenol, 2,4-xylenol, and phenol had k_{inh} in the range of 10^3−10^4 M^{-1} s^{-1} and, along with EOs containing them, could potentially replace BHT in the protection of food products.

KEYWORDS: *rate constants, synergy, olive oil, sustainable foods, Pimenta racemosa, Betula alba, Juniperus oxycedrus, Thymbra capitata, Satureja montana*

INTRODUCTION

Plant essential oils (EOs) continue attracting major research interest concerning their biological properties, several of which have been associated with a purported antioxidant activity.[1−4] Yet, the main interest related to the antioxidant activity of essential oils is perhaps their potential use as natural food preservatives[5,6] to counteract lipid peroxidation.[7] In this regard, their importance as food aromas,[8] combined with the antimicrobial activity that is being highlighted by a wealth of studies,[9−11] is the ideal complement of a possible antioxidant activity, making them highly interesting multifunctional natural food additives,[12] able to respond to the evolving demand for sustainable products and lifestyles.[13]

Despite the interest, only limited data are currently available on the actual ability of EOs to act as antioxidants and protect lipids from peroxidation.[12,14] Indeed, most studies have relied on rapid assays such as the discoloration of persistent radicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), or on other assays that, despite their popularity, do not actually test the ability of the potential antioxidant to protect a substrate from oxidation; hence, they are unable to quantify it.[15,16] Although exceptions might be observed in some food products under some circumstances,[17] the most effective mechanism for the antioxidant protection of lipids or other oxidizable materials is the, so-called, chain breaking.[18,19] This is based on the reactivity of the antioxidant with alkylperoxyl radicals, which are the carriers of the radical chain, thereby sparing the oxidizable material. The activity is best quantified by determining the inhibition constant k_{inh}, i.e., the rate constant for trapping peroxyl radicals by the antioxidant. This value should be orders of magnitude higher than the rate constant for reaction of the same radicals with the oxidizable substrate—the rate constant of chain propagation k_p—so to guarantee that, even when used at a very low concentration, the antioxidant would outcompete the substrate, e.g., the unsaturated fatty acid chain of vegetable oils, in reacting with peroxyl radicals, thereby preserving it.[19] Therefore, the knowledge of k_{inh} is key in assessing the performance of any antioxidant, including EOs, as well as to enable their use in rational antioxidant strategies, as opposed to time-consuming trial-and-error approaches.[15,16,18,19]

Unfortunately, while different experimental approaches have been validated to measure k_{inh} for pure molecular antioxidants, be them synthetic or of plant origin,[16,20] the task becomes much more difficult for complex mixtures like essential oils, often containing several potential antioxidant molecules, which might differently contribute to the overall radical reactivity and antioxidant (or even prooxidant) behavior.[21] This has precluded obtaining k_{inh} values for essential oils.

In the preliminary work, we have proposed a simplified experimental approach to handle such a complexity and measure the chain-breaking activity of raw EOs, upon

experimentally isolating it from other types of redox activities.[12] This was based on focusing on the characteristic phenolic components, treating them as a single molecule, and testing the EO at a very low concentration to protect a kinetically well-known oxidizable substrate in controlled autoxidation studies.[12] Despite its simplicity, the approach proved to be effective and to open to truly quantitative evaluation of EOs' antioxidant performance. However, it was only tested on a limited number of EOs, each with a relatively simple structure and containing one or two, out of overall only three phenolic components.[12]

In this work, we wish to report on our much-extended investigation on 13 new specimens from 11 botanical sources, boosting overall 27 phenolic components and whose total phenolic content ranged from ~0 to 86%. EOs were selected among those interesting as food aromas. Red thyme (*Thymus vulgaris*, L.) and clove bud (*S. aromaticum*, L.) are among the richest in phenolic terpenoids and phenylpropanoids, respectively. Although we had previously studied them,[12] we have included in this study two (different) additional specimens each, since their composition is subjected to major natural variability. Winter savory (*Satureja montana*, L.) and Spanish oregano (*Thymbra capitata*, L., Cav.) EOs were also included for the expected high phenolic content. Birch (*Betula alba*, L.), cade (*Juniperus oxycedrus*, L.), cedarwood (*Cedrus atlantica*, Endl.), and bay St. Thomas (*Pimenta racemosa*, Mill.) EOs were selected for their interest as smokey-woodsy aromas in food technology, along with aromatic carrot seeds (*Daucus carota*, L.), celery (*Apium graveolens*, L.), and marigold (*Tagetes minuta*, L.) EOs. The aim of this work was to validate our quantitative approach, based on controlled inhibited autoxidations of reference substrates, in affording EOs' k_{inh} values and allowing full rationalization of their antioxidant activity. Several individual phenolic components were also investigated since no k_{inh} values were available in the literature to allow comparison with raw EOs and evaluate on quantitative grounds any possible synergic interplay among components. Additionally, a selection of EOs was further tested in the protection of olive oil triglycerides in an accelerated oxidation test, in comparison with the benchmark synthetic antioxidant butylhydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol) (BHT), chosen for its widespread use in food products, to prove their real-life usefulness as alternative food preservatives.

Antioxidant protection of food products is sometimes further complicated by the partition or confinement of the antioxidant in heterogeneous systems;[13] these aspects are outside the scope of the current investigation and will not be discussed.

■ MATERIALS AND METHODS

Materials. The essential oils of Cade (*J. oxycedrus*, L.; origin = Italy), Clove bud 1 (*S. aromaticum*, L.; origin = Madagascar), Red Thyme 2 (*T. vulgaris*, L.; origin = Spain), and Spanish oregano (*T. capitata*, L. Cav.; origin = Italy) were from Maraschi & Quirici (Turin, Italy); the oils of Birch (*B. alba*, L.; origin = North America), Bay St. Thomas (*P. racemosa* (Mill.), J.W. Moore; origin = Caribbean), Winter Savory (*S. montana*, L.; origin = Italy), and Wild Marigold (*T. minuta*, L.; origin = South America) were from Esperis S.p.A. (Milan, Italy); the oil of Carrot seeds (*D. carota*, L.; origin = Europe) was from Silvestris & Szilas Ltd. (Kerepes, Hungary); the oil of Red Thyme 1 (*T. vulgaris*, L.; origin = Italy) was from G.E.I. Nectar (Settimo Torinese, Italy); the oils of Clove bud 2 (*Syzygium aromaticum*, L.; origin = Zanzibar) and Celery (*A. graveolens*, L.; origin = Spain) were from Muller & Koster (Milan, Italy); the oil of Cedarwood Atlas (*C. atlantica*, Endl.; origin = Morocco) was from Pell Wall (Stone, U.K.); they were all provided of a certificate of origin. 2,2-Azobis(isobutyronitrile) (AIBN, Merck, Milan Italy) was recrystallized from methanol. 2,2,5,7,8-Pentamethyl-6-chromanol (PMHC, Merck, Milan Italy) was recrystallized from hexane. Cumene (≥98%, Merck, Milan Italy) was percolated twice through activated basic alumina and once through silica to remove impurities and traces of hydroperoxides.[21,22] 2,4-Dimethylphenol (2,4-xylenol, 98%), 2,3-dimethylphenol (2,3-xylenol, 98%), 2,5-dimethylphenol (2,5-xylenol, ≥99%), 2-methylphenol (*o*-cresol, ≥99%), 3-methylphenol (*m*-cresol, ≥98%), 2-methoxy-4-propylphenol (dihydroeugenol, ≥99%), eugenol (≥98%), coniferyl alcohol (98%), umbelliferone (99%), thymol (Ph.Eur.99−101%), and carvacrol (99%), all from Merck (Milan, Italy), and 4-allylphenol (chavicol, ≥98%) from TCI (Tokyo, Japan), were used without further purification. Solvents were of the highest grade commercially available (Merck, VWR, Milan, Italy) and were used as received. (*d*)-*α*-Tocopherol and stripped olive oil (with native phenolic components removed) were available from previous studies.[23]

Analysis of the Essential Oils. GC−MS analysis was carried out on a Star 3400 CX gas chromatograph (Varian, Palo Alto, CA) equipped with two detectors: a Saturn 2000 ion-trap mass spectrometer detector (Varian) and a flame ionization detector (FID;H2/Air), each connected to a Zebron ZB-5 column (5% phenyl−95% dimethyl-polysiloxane, 30 m, 0.25 mm, 0.25 m) from Phenomenex (Bologna, Italy), each fed by one of two identical split/splitless 1078 Universal Capillary Injectors (Varian). Injection (0.4 μL) was operated by a CombiPAL 2-axis autosampler (CTC Analytics, Zwingen, Switzerland). The carrier was helium at 1.25 mL/min, and the split ratio was 1:16. The injection and transfer line temperature was 250 °C, while the column had the following programming: 50−220 °C at 2 °C/min. Mass spectra were collected in electron impact (EI+) mode at 70 eV, with a range of 40−650 m/z and used to build the TIC chromatogram. Compounds' identification was based on matching the MS spectrum with the NIST14 library and with a self-built EOs/terpenes library and then confirming the identity with Kovat's retention index.[24,25] Semiquantitative analysis of each oil was based on the relative % area of the chromatographic peak over the total area in the chromatogram, by means of 3 repeated injections in gas chromatography flame ionization detector (GC-FID), under identical settings as those used in gas chromatography/mass spectrometry (GC−MS). The concentration (in g/L) of the phenolic components was estimated from the above % values assuming equal relative response factor (RRF) for all components.[12]

Inhibited Autoxidation Kinetics. The antioxidant activity of EOs and their phenolic components were analyzed by determining their kinetics of reaction with alkylperoxyl radicals during inhibited autoxidation studies.[26] Cumene was used as the reference oxidizable substrate ($k_p = 0.34$ M^{-1} s^{-1}, $2k_t = 4.5 \times 10^4$ M^{-1} s^{-1} in PhCl at 30 °C).[12,26]

Oxygen consumption during the autoxidation of the substrate was measured in a differential oxygen-uptake apparatus built in our laboratory, based on a Validyne DP15 pressure transducer (Northridge, CA), which has been previously described.[27−29] The reaction was thermally initiated at constant rate (R_i in the range $(1-5) \times 10^{-9}$ M^{-1} s^{-1}) by the decomposition of AIBN (0.01−0.1 M), and tocopherol's mimic 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) was used as a reference antioxidant.[16,18] In a typical experiment, an air-saturated solution of cumene (50% v/v, 3.6 M) in chlorobenzene (PhCl) containing 0.05 M AIBN (sample) was equilibrated at 30 °C with an identical solution (reference) containing an excess of PMHC (25 mM) to block any radical chain and the consumption of oxygen. After reaching a constant O_2 consumption in the sample, a stock solution of the antioxidant to be tested (e.g., the EO or some phenolic component) was injected in the sample flask at a final concentration (typically 1−10 μM for pure molecules or 1−5 μL/L for EOs) adjusted to allow some oxygen consumption even during inhibition, i.e., a chain length of at least 8−10.[27−29] Oxygen consumption in the sample was measured from the difference in pressure between the two channels upon calibration of the apparatus. The slope of the oxygen

Table 1. Phenolic Components Found in the Investigated Essential Oil Specimens, Reported as % Peak Area in Gas-Chromatogram (Average ± SD, n = 3)

essential oil	d (g/mL)	phenolic component	% (GC)	essential oil	d (g/mL)	phenolic component	% (GC)
birch (*B. alba*, L.)	1.042	phenol	1.88 ± 0.06	cade (*J. oxycedrus*, L.)	1.012	phenol	1.10 ± 0.04
		o-cresol	2.03 ± 0.08			*o*-cresol	0.99 ± 0.03
		m-cresol	1.45 ± 0.02			*m*-cresol	0.72 ± 0.06
		p-cresol	2.96 ± 0.07			*p*-cresol	2.34 ± 0.10
		guaiacol	8.93 ± 0.16			guaiacol	2.70 ± 0.08
		2,6-xylenol	0.34 ± 0.02			2,6-xylenol	0.26 ± 0.02
		2,5-xylenol	1.36 ± 0.05			2,5-xylenol	0.78 ± 0.03
		2,4-xylenol	0.86 ± 0.02			2,4-xylenol	0.55 ± 0.03
		4-ethylphenol	0.24 ± 0.02			4-ethylphenol	0.26 ± 0.02
		3-ethylphenol	0.23 ± 0.01			3-ethylphenol	0.22 ± 0.01
		3,5-xylenol	0.59 ± 0.03			3,5-xylenol	0.10 ± 0.03
		3-methylguaiacol	0.44 ± 0.02			3-methylguaiacol	0.25 ± 0.04
		2,3-xylenol	0.25 ± 0.01			2,3-xylenol	0.20 ± 0.03
		4-methoxy-3-methylphenol	0.44 ± 0.05			4-methoxy-3-methylphenol	0.19 ± 0.01
		p-creosol (4-methylguaiacol)	14.73 ± 0.43			*p*-creosol (4-methylguaiacol)	5.04 ± 0.12
		3,4-xylenol	0.15 ± 0.02			3,4-xylenol	0.27 ± 0.04
		1,2-benzenediol	1.17 ± 0.16			1,2-benzenediol	0.56 ± 0.02
		6-ethyl-*o*-cresol	0.29 ± 0.01			6-ethyl-*o*-cresol	0.27 ± 0.01
		4-ethylguaiacol	7.92 ± 0.09			4-ethylguaiacol	3.68 ± 0.09
		syringol	2.10 ± 0.06			eugenol	0.59 ± 0.05
		eugenol	1.22 ± 0.03			dihydroeugenol	1.32 ± 0.07
		dihydroeugenol	2.07 ± 0.07			**total**	**22.67**
		total	**51.65**	winter savory (*S. montana*, L.)	0.921	thymol	18.70 ± 0.41
bay St. Thomas (*P. racemosa*, Mill.)	0.972	4-allylphenol	8.68 ± 0.17			carvacrol	23.51 ± 0.55
		eugenol	51.25 ± 1.04			eugenol	1.23 ± 0.05
		total	**59.93**			**total**	**43.44**
carrot seeds (*D. carota*, L.)	0.886	umbelliferone	0.86 ± 0.04	Spanish oregano (*T. capitatua*, L., Cav.)	0.920	carvacrol	69.43 ± 1.19
		total	**0.86**			**total**	**69.43**
cedarwood (*C. atlantica*, Gaussen)	0.922	coniferyl alcohol	0.88 ± 0.07	red thyme 1 (*T. vulgaris*, L.)	0.914	thymol	3.44 ± 0.08
						carvacrol	36.39 ± 0.42
		total	**0.88**			**total**	**39.83**
celery (*A. graveolens*, L.)	0.882	ND	ND	red thyme 2 (*T. vulgaris*, L.)	0.910	thymol	53.77 ± 0.88
						carvacrol	3.50 ± 0.06
		total	**~ 0**			**total**	**57.27**
clove bud 1 (*S. aromaticum*, L.)	1.050	eugenol	86.02 ± 1.72				
		total	**86.02**	wild marigold (*T. minuta*, L.)	0.892	ND	ND
clove bud 2 (*S. aromaticum*, L.)	1.044	eugenol	73.90 ± 1.54			**total**	**~ 0**
		total	**73.90**				

consumption during the inhibited period afforded k_{inh} values using eq 1, where n is the stoichiometric coefficient of the antioxidant (assumed as $n = 2$ if not known) and k_p was the rate constant for chain propagation of the substrate.[16] For pure phenolic components, n was determined from the duration of the inhibited period τ using eq 2, where [AH] is the concentration of the antioxidant.[7,27]

The rate of initiation R_i was determined, for each experimental setting, by preliminary measurements using PMHC as the reference antioxidant also in the sample (at a concentration of 1−5 μM) using eq 2. In those cases in which a clear inhibition period was not observed, the value of k_{inh} for the antioxidant was determined from the reduced slope of oxygen uptake in the presence of the inhibitor, R_{inh}, compared to the uninhibited rate of oxygen uptake in its absence, R_0, via eq 3, where $2k_t$ is the chain-termination constant of the oxidizable substrate. The duration of the inhibition period (τ) was determined graphically as the crosspoint between the initial tangent of the inhibited period and the final tangent of the not-inhibited period.[12,29] Details on the meaning and applicability, as well as on the derivation of eqs 1 and 3, are provided in the Supporting Information.

$$-\frac{d[O_2]_{inh}}{dt} = \frac{k_p[RH]R_i}{nk_{inh}[AH]} \quad (1)$$

$$R_i = \frac{n[AH]}{\tau} \quad (2)$$

$$\frac{R_0}{R_{inh}} - \frac{R_{inh}}{R_0} = \frac{nk_{inh}[AH]}{\sqrt{2k_tR_i}} \quad (3)$$

Autoxidation Studies with Stripped Olive Oil. The autoxidation of olive oil was investigated using the same equipment and protocols on triglycerides from which native antioxidants had been removed to avoid interference with the kinetics.[23] Triglycerides had a measured density of 0.9074 g/mL (at 30 °C) and an average M.W. of 880.10[23] and were tested in a PhCl solution at a concentration of 25−50% v/v (0.26−0.52 M). Autoxidation was initiated by AIBN (0.05−0.1 M) at 30 °C and inhibited by 5−25 μL/L of the tested EOs or by 1−10 μM BHT or α-tocopherol as reference antioxidants.

Statistical Analysis. Each measurement was performed in triplicate, and each antioxidant or essential oil was tested at least at two different concentrations; results are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

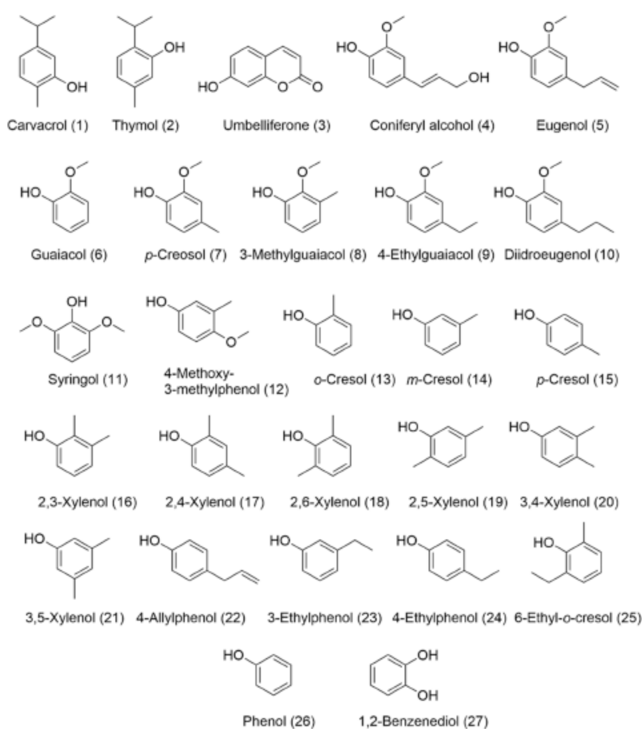
Composition of the Essential Oils. In order to rationalize their antioxidant behavior, it was necessary to preliminarily determine the actual composition of the investigated essential oils. It is well-known that EOs' composition is subjected to significant variability due to epigenetic and environmental factors, even for oils from the same botanical species, beside the influence of collection time and extraction technique.[30−32]

We subjected the EOs to GC−MS analysis and identified the components by a combined evaluation of the MS spectrum and Kovats' retention index.[24] Mass spectra were matched to the NIST14 library and to an internal library of terpenoids and EO's components. Since our focus was on the chain-braking antioxidant activity of EOs, components showing less than 0.1% area in TIC chromatogram were not investigated, as they were judged unlikely to afford any significant contribution to the EO's overall antioxidant behavior. In the absence of individual standards for all of the many identified components, semiquantitative analysis was performed by three repeated injections using flame ionization detection (FID), under identical columns and instrumental settings as used for the GC−MS analysis, assuming a similar relative response factor (RRF) for all components when expressed as a g/L concentration in the EO, which is justified by the proportionality of the FID response to the number of carbons in the analyte,[33] and was sufficient for the purpose of this investigation.[12] Detailed results are reported in the Supporting Information (Tables S1−S13), while the phenolic components found in oils are collected in Table 1 and their structure is displayed in Chart 1.

The two specimens of red thyme (*T. vulgaris*) EO showed a significantly different composition. Sample 1 (origin = Italy) had *p*-cymene as the main component (43.4%), followed by phenolic carvacrol (36.4%) and camphene (6.2%), while the characteristic phenolic thymol was only 3.4% (Table S11): it can tentatively be classified in the *p*-cymene/carvacrol/thymol chemotype, according to Satyal et al.[32] Instead, sample 2 (origin = Spain) belonged to chemotype thymol[32] showing such a component as largely prevailing (55.7%), while carvacrol was only 3.5%, being the only other phenolic component among 21 identified, accounting for ∼98% of the oil (Table S12). Carvacrol (23.5%) and thymol (18.7%) were also the main components of winter savory (*S. montana*) EO, which also listed smaller amounts of eugenol (1.2%) among the phenolic components that summed up 43.4% of the oil (Table 1). Other characteristic (nonphenolic) components were linalool, *p*-cymene, eucalyptol, limonene, and *γ*-terpinene, among 18 identified components accounting for 99% of the oil (Table S9). The EO composition was in range with the very major variability encountered in this species.[34,35] The highest levels of carvacrol (69.4%) were found in Spanish oregano (*T. capitata*), where it represented the only phenolic among 19 overall identified components (Table S10), in line with previous reports.[36] Clove bud EOs had the highest phenolic content among the investigated oils, and eugenol was the only contributor representing, respectively, 86 and 73.9% of the EO for specimens 1 and 2 (Table 1). In both cases, the composition was very simple, as expected,[12] albeit different (Tables S7 and S8). Bay St. Thomas (*P. racemosa*) EO also boosted large amounts of eugenol as the main component (51.25%), along with the structurally related *p*-allylphenol (chavicol, 8.68%) summing up at ∼60% phenolics among 15 overall identified components (Table S3). The composition of our specimen was in line with some previous reports;[37,38] however, it was at odds with another study that found only trace amounts of phenolics, although it reported a prominent antioxidant activity.[39] Birch (*B. alba*) buds EO had a more complex composition, with 40 identified components (Table S1), among which it boosted a wealth of 22 phenolic components representing an overall ∼52% of the oil (Table 1). *p*-Cresol (4-methylguaiacol) and guaiacol were the most abundant (14.7 and 8.9%, respectively) followed by 4-ethylguaiacol (7.9%), the cresols (*ortho*, *meta*, and *para* in the range of ∼1.5−3%), syringol (2.1%), eugenol and dihydroeugenol (1.2 and 2.1%, respectively), phenol itself (1.9%), 1,2-benzenediol (catechol, 1.2%), and a variety of xylenols and related disubstituted phenols, each at a level of ∼1% or lower (see Table 1 and Chart 1 for structures). Most of the available literature on *Betula* species EOs focused on sesquiterpenoids or specific components held responsible for the anti-inflammatory and antimicrobial properties of the oil;[40] however, our phenolic and overall composition is in accord with a very recent study on *B. alba* buds EO in pest control.[41] Cade EO obtained from the distillation of twigs and bark of *J. oxycedrus* had an even more complex composition with 48 identified components among which sesquiterpenes *trans*-calamenene and *β*-cadinene were prevailing (Table S2). Its phenolic composition somewhat copied that of birch, with the absence of syringol, although its overall concentration was approximately halved (22.7% of the oil) and with a different relative ratio (Table 1). It varied with the composition of other *J. oxycedrus* oils obtained from leaves or fruits, rich in terpenes and sesquiterpenes but poor in phenolics.[42]

At variance with the above oils, cedarwood atlas (*C. atlantica*, Gaussen) EO showed negligible content in phenolics: coniferyl alcohol was the only identified, weighting less than

Chart 1. Structure of the Phenolic Components Identified in the Studied EOs



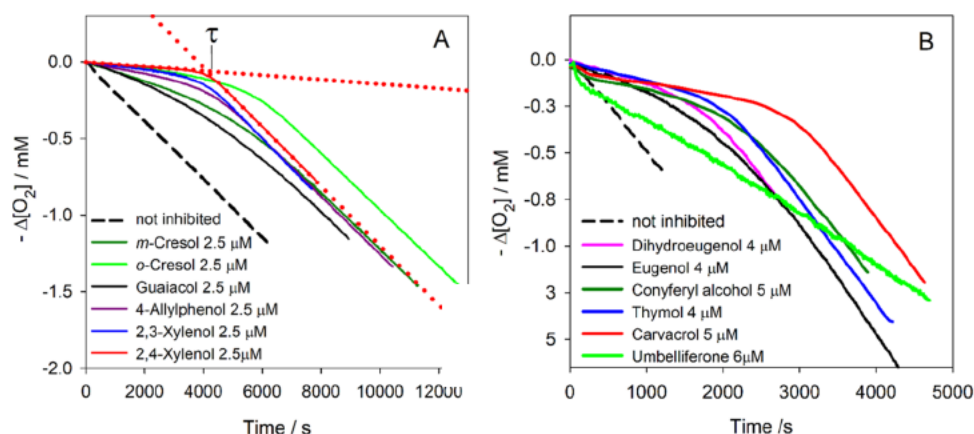


Figure 1. Examples of oxygen consumption plots during the autoxidation of 3.6 M cumene in PhCl at 30 °C initiated by (A) 0.025 M AIBN in the absence of antioxidants (dashed line) or in the presence of EO phenolic components as indicated in the legend; (B) 0.1 M AIBN in the absence of antioxidants (dashed line) or in the presence of EO phenolic components as indicated in the legend. Regression of inhibited ad uninhibited periods (dotted lines) and the length of the inhibited period τ are shown for representative compounds.

Table 2. Rate Constant (k_{inh}) and Stoichiometric Factor (n) for Trapping Peroxyl Radicals (at 30 °C) by Phenolic EO Components Measured in This Study (Average ± SD), from the Literature, or Estimated (Values in Parentheses) from Data for Related Compounds

		PhCl		MeCN	
no.	compound	k_{inh} (10^3 M^{-1} s^{-1})	n	k_{inh} (10^3 M^{-1} s^{-1})	n
1	carvacrol	14 ± 1	2.2 ± 0.2	2.9 ± 0.3	2.0 ± 0.2
2	thymol	13 ± 2	2.0 ± 0.1	2.6 ± 0.2	1.8 ± 0.2
3	umbelliferone	1.9 ± 0.3	2.1 ± 0.2	0.2 ± 0.04	
4	coniferyl alcohol	10 ± 0.5	2.1 ± 0.1	9.0 ± 0.3	2.2 ± 0.3
5	eugenol	5.4 ± 0.5	2.0 ± 0.1	4.6 ± 0.5	2.0 ± 0.1
6	guaiacol	3.8 ± 0.5	2.1 ± 0.1	2.3 ± 0.4	1.9 ± 0.2
7	*p*-creosol[a]	12	2		
8	3-methylguaiacol[c]	(8)			
9	4-ethylguaiacol[c]	(12)			
10	dihydroeugenol	11 ± 2	2.0 ± 0.1		
11	syringol[a]	18	2		
12	4-methoxy-3-methylphenol[c]	(40)			
13	*o*-cresol	8.9 ± 1.2	2.1 ± 0.1	0.8 ± 0.1	
14	*m*-cresol	3.9 ± 0.5	2.2 ± 0.1	0.5 ± 0.1	
15	*p*-cresol[b]	15	2		
16	2,3-xylenol	14 ± 2	1.8 ± 0.2	0.9 ± 0.1	2.0 ± 0.3
17	2,4-xylenol	35 ± 5	1.8 ± 0.2	1.5 ± 0.5	2.1 ± 0.2
18	2,6-xylenol[b]	25	2		
19	2,5-xylenol[c]	(14)			
20	3,4-xylenol[c]	(20)			
21	3,5-xylenol[c]	(8)			
22	4-allylphenol	9.1 ± 1.1	1.9 ± 0.2	1.5 ± 0.5	1.8 ± 0.3
23	3-ethylphenol[c]	(3.9)			
24	4-ethylphenol[c]	(10)			
25	6-ethyl-*o*-cresol[c]	(25)			
26	phenol	2.8 ± 0.5	2.2 ± 0.2	0.4 ± 0.1	
27	1,2-benzenediol[b]	550	2		

[a]Data from ref 47. [b]Data from ref 48. [c]Estimated from the current experimental data and literature, as described in refs 47 and48.

1% (Table 1), while sesquiterpenes *β*-himachalene (49.2%) and thujopsene (22.9%) were the main components (Table S5), and (*E*)-atlantone was another characteristic component, in line with previous studies.[43] Carrot seeds (*D. carota*) EO had a very minor content of umbelliferone (0.86%) as the only phenolic, while, in line with the previous literature, carotol (26.7%) was the main component,[44] and terpenes *α*-pinene (7.2%), *β*-pinene (13.41%), sabinene (13.4%), *α*-terpinene (11.1%), and *γ*-terpinene (4.4%) were characteristic components (Table S4).

No phenolic component could be identified in celery (*A. graveolens*) EO, where limonene (64.6%), *β*-selinene, and sedanolide were the characteristic components (Table S6), as expected.[45] Wild marigold (*T. minuta*) EO also showed no phenolic component: it had limonene, *β*-(*Z*)-ocimene, and dehydrotagetone as the characteristic terpenoids (Table S13),

in line with previous studies that, however, reported a significant antioxidant activity of the oil.[46]

Antioxidant Activity of the Individual Phenolic Components. Only limited data are available in the literature on the antioxidant activity of the phenolic components identified in the essential oils; hence, we investigated a representative selection of them (13 compounds) on the inhibited autoxidation of cumene as a reference substrate (Figure 1).

Results of our measurements are collected in Table 2 along with the available data from the scientific literature for the other phenolic components identified in our EOs.[47,48] Analysis of the kinetic constants k_{inh} and the stoichiometric factors n allows some general considerations. All of the phenolic components showed a stoichiometric factor n of 2 within experimental error, in line with the well-established knowledge that they quench two peroxyl radicals: one by rate-determining formal H atom transfer from the phenolic PhO-H to $ROO^•$ to afford the hydroperoxide ROOH and the phenoxyl radical $PhO^•$, which traps a second $ROO^•$, generally by fast addition into the aromatic ring.[48] The value of k_{inh} for most compounds bearing electron-donating (ED) substituents (e.g., alkyls) on the aromatic ring was higher than the value for unsubstituted phenol (26; $k_{inh} = 2.8 \times 10^3$ M^{-1} s^{-1}), and the actual kinetic advantage was maximum for phenols substituted in conjugated *ortho* and *para* positions, and lower in *meta*, owing to (different) reduction of the O−H-bond dissociation enthalpy (BDE_{OH}) of the phenolic O−H.[19,47,48]

Indeed, frequently encountered isomeric carvacrol (1) and thymol (2) had a k_{inh} of $\sim 1.4 \times 10^4$ M^{-1} s^{-1}, which was similar to that of most xylenols bearing one methyl in 2 or 4 positions and one in 3 or 5. 2,4-Xylenol (17) having both substituents in the conjugated positions had a higher inhibition constant, while the related 2,6-xylenol (18) was slightly less effective than the former due to a higher steric hindrance around the phenolic −OH.[48] The same reasoning explains the slightly lower performance of the singly substituted cresols (13−15) and their relative ranking. Compounds bearing a methoxy substituent in the *ortho* position deserve further discussion. While the strongly ED MeO- group would lower the BDE_{OH} and increase the reactivity—e.g., when it is in the *para* position—in the *ortho* position, it also "blocks" the phenolic −OH in an intermolecular H-bond (see Scheme 1), which affects the reactivity in the opposite direction, and the two effects are approximately counterbalancing.[47] Thus, eugenol, guaiacol, syringol, and the related phenols have lower k_{inh} than the ED character of the substituents might suggest. Occurrence of the intramolecular H-bonding with an *ortho* substituent, however, has the opposite effect in catechol (1,2-benzenediol, 27) as the BDE_{OH} of the second −OH group is massively decreased, thereby boosting the reactivity with peroxyl radicals.[19,48]

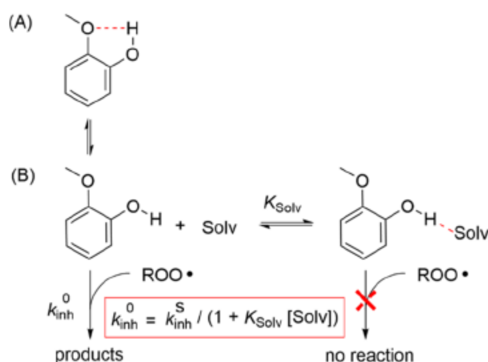
Extension of the conjugated system of the aromatic ring into the substituents, as in coniferyl alcohol, (4) also improves the antioxidant performance (compared to eugenol); however, it has the opposite effect if such an extended π system is conjugated with an electron-withdrawing (EW) group as in umbelliferone (5) since EW substituents increase the BDE_{OH} and decrease k_{inh}.[19,48]

Since the contribution of each substituent as a function of its position in the ring in determining the BDE_{OH} is known for many substituents and approximately additive,[48] and since there exist empirical linear free-energy correlations between BDE and k_{inh},[19,47] by combining the current and available literature kinetic data, it was possible to estimate the missing data for all compounds listed in Table 2, as illustrated by Lucarini and Pedulli,[48] and in our previous work.[19,47] Estimated values are reported in parentheses in Table 2.

It is interesting to note that most EO components had a k_{inh} $\sim 1 \times 10^4$ M^{-1} s^{-1} or higher, which favorably compares with the known value $k_{inh} = 1.1 \times 10^4$ M^{-1} s^{-1} for ubiquitous food antioxidant BHT (often labeled E321 as the food additive).[48] This indicates that many EO key components would offer similar or better antioxidant protection in food products.

One feature largely affecting the antioxidant protection by phenols is the occurrence of intermolecular H-bonding to the medium (e.g., the solvent or the substrate to protect), which diminishes the reactivity of the antioxidant as illustrated in Scheme 1.[26] This is relevant in the protection of natural lipids like triglycerides as they are good H-bond acceptors (HBAs).[18,23] Sensitivity to such a medium effect depends on the H-bond-donating (HBD) ability of the antioxidant, and it varies among phenols; hence, for a representative selection of them, we remeasured the reactivity in HBA solvent acetonitrile (Table 2). It is interesting to note that while for most compounds the solvent effect was very relevant with $k_{inh}^{PhCl}/k_{inh}^{MeCN}$ in the range 3-to-10, for *ortho*-methoxy-substituted phenols (e.g., eugenol, conyferyl alcohol, guaiacol), it was negligible, as the phenolic OH is already engaged in a more stable intramolecular H-bond. Thus, the relative antioxidant potency of EO components could vary depending on the medium, with possible consequences in lipids' protection (*vide infra*).

Scheme 1. Intramolecular (A) and Intermolecular (B) H-Bonding of Phenols Affecting Their Antioxidant Activity



Antioxidant Activity of Whole Essential Oils. On turning to study the antioxidant activity of raw essential oils, we focused exclusively on the chain-breaking mechanism, aiming at determining their k_{inh} for trapping peroxyl radicals. To isolate such a mechanism from others, such as the preventive and the termination-enhancing, the latter requiring rather large concentrations of the antioxidant to be appreciated,[15,16,21] we set to study the inhibited autoxidation of cumene initiated by the thermal decomposition of AIBN, using very low concentrations of the EO (1−5 ppm or μL/L), so to reproduce the same experimental approach used for the isolated components and allow direct comparison.[16] As shown in Figure 2, several EOs were able to produce neat inhibition of cumene autoxidation. This was the case for EOs of bay St. Thomas, birch, cade, red thyme, Spanish oregano, winter savory, and clove, which were rich in phenolic components,

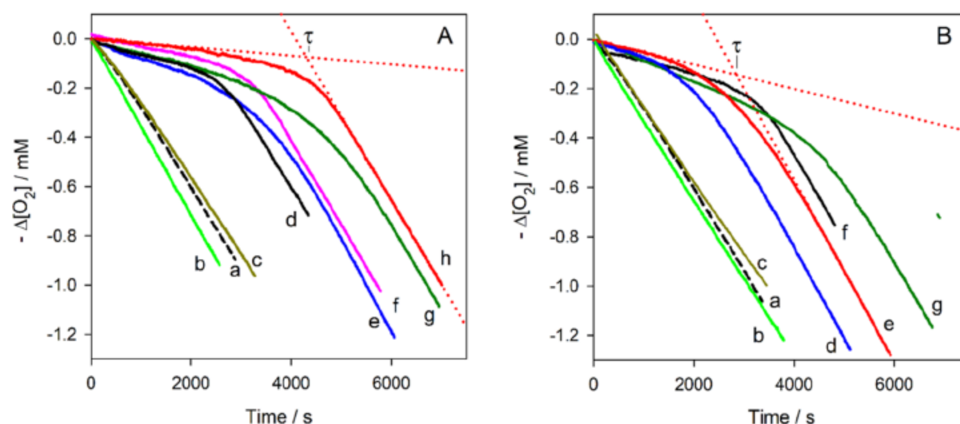


Figure 2. Examples of oxygen consumption plots during the autoxidation of 3.6 M cumene in PhCl at 30 °C initiated by 0.1 M AIBN in the absence of inhibitors (a, dashed) or in the presence of the EOs of (panel A): *A. graveolens* 5 μL/L (b), *D. carota* 5 μL/L (c), *T. vulgaris* (sample 1) 2.5 μL/L (d), *P. racemosa* 2.5 μL/L (e), *S. montana* 2.5 μL/L (f), *S. aramaticum* (sample 1) 2.5 μL/L (g), and *T. capitata* 2.5 μL/L (h); (panel B): *T. minuta* 5 μL/L (b), *C. atlantica* 5 μL/L (c), *J. oxycedrus* 2.5 μL/L (d), *B. alba* 2.5 μL/L (e), *T. vulgaris* (sample 2) 2.5 μL/L (f), and *S. aramaticum* (sample 2) 2.5 μL/L (g). Regressions of inhibited and uninhibited periods are shown for representative examples (dotted lines).

Table 3. Antioxidant Activity of EO Samples from Inhibited Cumene Autoxidation Studies at 30 °C in Chlorobenzene: EO Concentrations, Concentration of All Phenolic Components from GC Analysis, Apparent Total Phenolic Concentration, and Apparent k_{inh} Values (Average ± SD)

essential oil	concn (μL/L)[a]	$\sum[PhOH]_{GC}$ (M)[b]	$[AH]_{app}$ (M)[c]	k_{inh} (M^{-1} s^{-1})[d]
birch (*B. alba*, L.)	2.5	9.7×10^{-6}	$(9.8 \pm 0.1) \times 10^{-6}$	$(1.0 \pm 0.1) \times 10^{4}$
cade (*J. oxycedrus*, L.)	2.5	4.2×10^{-6}	$(5.5 \pm 0.3) \times 10^{-6}$	$(1.2 \pm 0.2) \times 10^{4}$
bay St. Thomas (*P. racemosa*, Mill.)	2.5	7.8×10^{-6}	$(7.5 \pm 0.3) \times 10^{-6}$	$(8.9 \pm 0.5) \times 10^{3}$
winter savory (*S. montana*, L.)	2.5	7.2×10^{-6}	$(7.9 \pm 0.2) \times 10^{-6}$	$(1.4 \pm 0.2) \times 10^{4}$
Spanish oregano (*T. capitatua*, L., Cav.)	2.5	1.2×10^{-5}	$(1.1 \pm 0.1) \times 10^{-5}$	$(1.5 \pm 0.2) \times 10^{4}$
red thyme 1 (*T. vulgaris*, L.)	2.5	6.6×10^{-6}	$(6.6 \pm 0.3) \times 10^{-6}$	$(1.4 \pm 0.3) \times 10^{4}$
red thyme 2 (*T. vulgaris*, L.)	1.0	4.0×10^{-6}	$(4.0 \pm 0.1) \times 10^{-6}$	$(1.5 \pm 0.3) \times 10^{4}$
clove bud 1 (*S. aromaticum*, L.)	1.0	5.2×10^{-6}	$(5.8 \pm 0.4) \times 10^{-6}$	$(7.0 \pm 0.5) \times 10^{3}$
clove bud 2 (*S. aromaticum*, L.)	5.0	2.3×10^{-5}	$(2.2 \pm 0.2) \times 10^{-5}$	$(5.5 \pm 0.3) \times 10^{3}$
carrot seeds (*D. carota*, L.)	2.5	1.3×10^{-7}	~0	ND[e]
cedarwood atlas (*C. atlantica*, Gaussen)	5.0	2.6×10^{-7}	~0	ND[e]
celery (*A. graveolens*, L.)	5.0	0	~0	ND[e]
wild marigold (*T. minuta*, L.)	5.0	0	~0	ND[e]

[a]Reference concentration used in the calculations; similar results were observed at the other tested concentrations. [b]Calculated as the sum of all phenolic components from GC analysis (data from Table 1). [c]Determined from the length of inhibited period in autoxidation experiments assuming that $n = 2$ for all antioxidants. [d]Apparent inhibition rate constant from autoxidation experiments. [e]No inhibition could be measured up to a concentration of 12.5 μL/L.

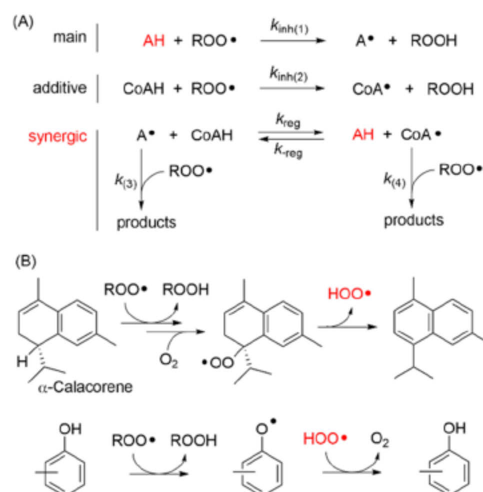
while no significant inhibition was observed with the EOs of celery and wild marigold, both containing no phenolic components, or with the EOs of cedarwood and carrot seeds containing, respectively, coniferyl alcohol and umbelliferone, albeit at a very low concentration.

Concerning the phenol-rich EOs, to quantify their antioxidant performance, we tested the approach proposed in our preliminary report.[12] Since all of the studied phenolic components showed the expected stoichiometric factor $n \sim 2$ (see Table 2), to a first approximation, we treated each EO as containing a single phenolic component, which simplified the kinetic treatment allowing the use of eqs 1−3. The duration of the inhibited period τ was analyzed by eq 2 to afford $[AH]_{app}$, the apparent total antioxidant concentration in the autoxidizing mixture, and, for each experimental setting, we matched such a value with $\sum[PhOH]_{GC}$, the sum of all phenolic components contributed by the EO, determined by GC and GC−MS analyses of the EO (Table 1), under the simplified hypothesis that only the phenolic components were contributing to inhibition, behaving as a single component. Results are collected in Table 3 for a representative set of experiments, showing a single concentration of each EO. There is excellent matching of $[AH]_{app}$, and $\sum[PhOH]_{GC}$, for the majority of oils (minor exceptions are noted in the following), confirming that our simplified hypothesis is generally correct: only the phenolic components of EOs contribute to the chain-breaking antioxidant activity, and all such components apparently contribute; hence, their sum is key in determining the overall antioxidant behavior. This general statement is supported by the lack of inhibition shown by wild marigold and celery, while the similar lack of inhibition observed for carrot seeds and cedarwood is easily explained by the very minor content in phenolics, <1% in each oil, which contributes submicromolar concentrations of chain-breaking compounds in the autoxidizing mixture under our experimental settings, making any activity negligible. It should be noted that the lack of chain-breaking activity shown here does not imply that other antioxidant mechanisms such as the termination-enhancing,[15,21] would not occur under different settings.

Analysis of the rate of consumption of O_2 during the inhibition period offered further insights. Following the simplified approach to treat the sum of all phenolic components as a single molecule, by eq 1 we could determine the apparent inhibition constant k_{inh} for whole EOs, which are collected in Table 3. Interestingly, they were of the same order of magnitude (10^4 M^{-1} s^{-1}) previously determined for most phenolic components (Table 2), which prompts a comparison of the apparent k_{inh} of each oil with that of its specific components. Both red thyme specimens had k_{inh} similar within the experimental error to that of thymol and carvacrol; furthermore, the $[AH]_{app}$ well matched the expected value on the basis of GC analysis, proving that their antioxidant behavior is entirely due to their phenolic content. Spanish oregano that contained only carvacrol as the main component had k_{inh} and $[AH]_{app}$ entirely dictated by such a component. Winter savory containing both thymol and carvacrol with the minor presence of eugenol had antioxidant behavior that copied that of the main and most active components; however, its duration was slightly higher (10%) than predicted on the basis of the sum of phenolic components. The two clove bud EO specimens, with different contents of eugenol as the main component, also behaved mostly as a source of such a component as the only antioxidant. However, sample 1 (but not sample 2) had both k_{inh} and $[AH]_{app}$ slightly exceeding the expected value based on the eugenol content. Bay St. Thomas containing prevalently eugenol, with a lower content in more effective 4-allylphenol, had k_{inh} coincident with that of 4-allylphenol, and $[AH]_{app}$ corresponded to the sum of the two components. The scenario was more complex for birch and cade EOs. Both birch and cade were very good antioxidants, with similar apparent k_{inh}, which was roughly average among the values of the same very complex set of phenolic components. However, while birch had $[AH]_{app}$ matching well to the sum of components, cade EO exceeded the expected value by about 30%. The higher proportion of effective xylenols and *ortho*- and *para*-substituted phenols (*o*- and *p*-cresol, 4-ethylphenol, etc.), the similar proportion of highly effective 1,2-benzenediol, and the lower proportion of less effective guaiacol and derivatives apparently justify the slightly higher k_{inh} of cade EO; however, understanding the longer than expected duration of protection requires the involvement of some synergic interplay among components.

Is There Synergism Among EO Components? Synergism among antioxidants is an important contributor to the activity of biomolecules and has often been claimed in relation to essential oils.[7,15] It is normally based on the regeneration of a main antioxidant from its oxidized products by other molecules acting as coantioxidants or sacrificial reducing agents.[19,27,49] Synergism among phenolic compounds normally implies that the fastest reacts first with one peroxyl radical to afford the corresponding phenoxyl radical that, before it can trap the second peroxyl radical, is reduced back by another (coantioxidant) phenol, which would be of more modest efficacy on its own, thereby regenerating and extending the full antioxidant power (see Scheme 2A).[19] If this mechanism occurred with full efficiency, it would imply that the mixture has similar k_{inh} as the best antioxidant component(s), with a duration that corresponds to the sum of contributing coantioxidants.[19] Since this mechanism is based on the kinetic competition among reactions, its efficiency is dictated by the relative rate of reaction of phenoxyl radicals with the other phenolic components or with peroxyl radicals and hence also

Scheme 2. Synergy (A) Between a Phenolic Main Antioxidant (AH) and Coantioxidant (CoAH) and (B) Between Phenolic and Nonphenolic Antioxidants Using *α*-Calacorene as an Example



on the relative concentration of the competing species. Clearly, in EOs such as thyme, synergism between phenolic components cannot be distinguished from simple additive behavior, as carvacrol and thymol have identical reactivity. Similar is the case for winter savory, where weaker eugenol only accounts for about 1%. However, synergy is clearly occurring in Bay St. Thomas EO whose k_{inh} matches the least abundant but more reactive 4-allylphenol, which is then regenerated by eugenol acting as a sacrificial coantioxidant (Scheme 2). Indeed, a single inhibited period is observed in Figure 2, with a slope representing 4-allyphenol and a duration representing the sum of the two phenols. Instead, an additive contribution would have produced a bimodal inhibition: a faster very short phase due to 4-allylphenol, followed by a more modest protection (steeper O_2 uptake plot) of longer duration due to eugenol.[19,27] Although the complex phenolic pattern does now allow us to unequivocally demonstrate its occurrence, our kinetic data suggest that synergism might actually be operating with cade EO and to some lower extent with birch EO. Both showed a single inhibited period with k_{inh} close to the phenolic fraction with the higher reactivity (e.g., the xylenols) despite the fact that the less reactive cresols, guaiacols, and phenol are more abundant. These last are likely working as coantioxidants regenerating the formers. Clearly, however, the efficiency of synergism is not complete, k_{inh}, of the oils and is not matching that of the single most effective component 1,2-benzenediol, and it varies with the relative ratio of components, being more pronounced in cade EO. Synergy among phenols, however, does not justify a duration of protection exceeding that expected from their sum, as observed in a few cases.

In cade EO, allyl-type cadinenes or proaromatics like *α*-calacorene could also possibly contribute to different mechanisms, such as by undergoing co-oxidation with the release of hydroperoxyl radicals ($HOO^•$), similarly to the established behavior of *γ*-terpinene.[50] In turn, $HOO^•$ might act as a sacrificial reducing agent and regenerate the phenolic components, extending their antioxidant protection—a mechanism demonstrated to occur between *γ*-terpinene and phenolic or polyphenolic antioxidants (Scheme 2B).[7,28] A similar mechanism likely takes place in winter savory whose

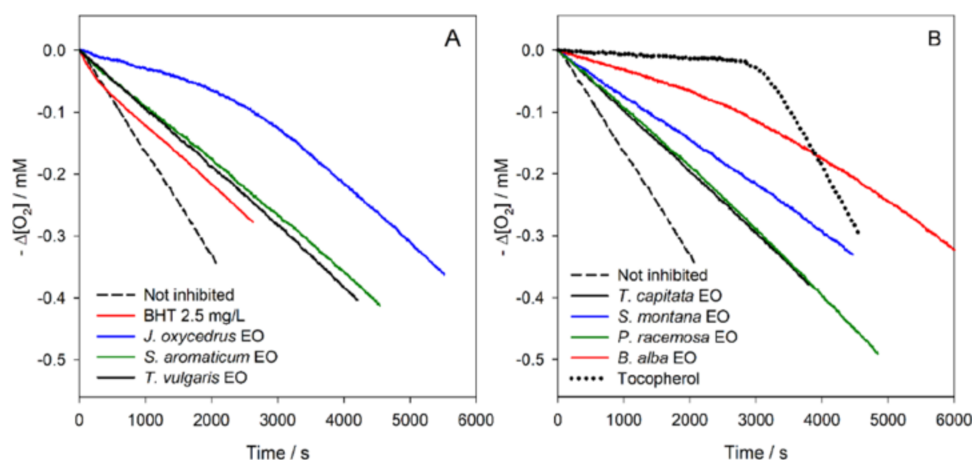


Figure 3. (A and B) Examples of oxygen consumption plots during the autoxidation of olive oil (50% v/v in PhCl) at 30 °C initiated by 0.1 M AIBN in the absence of inhibitors (dashed) or in the presence of 5 μL/L of selected EOs as indicated in the legend. Inhibition by 2.5 mg/L BHT (panel A, red full) and 5 μM (2.2 mg/L) α-tocopherol (panel B, dotted) is shown for comparison.

duration of inhibition slightly exceeds the expected value: beside γ -terpinene, it contains carvone and linalool. This last along with abundant *p*-cymene might also produce faster radical-chain termination, which might contribute to some very minor extent to the overall antioxidant activity.[21]

It should be noted that the contribution of synergy between phenolic and nonphenolic components, as well as that of other antioxidant mechanisms, is of quite limited magnitude in our experiments (only 10-to-30% of $[AH]_{app}$ extension), by their own design, as we aimed at favoring the chain-breaking mechanism, as we have previously discussed. Additional studies under different settings might be needed to clarify the relevance of such mechanisms.

Antioxidant Protection of Olive Oil by Whole Essential Oils. To assess the ability of essential oils to protect real food products and lipids, beside model oxidizable substrates, we studied the autoxidation of olive oil as representative triglyceride, upon removal of the native antioxidants so to unmask the effect of essential oils.[18] Only a selection of seven EOs that had shown good antioxidant behavior in protecting cumene were tested in the protection of olive oil: red thyme 1, clove bud 2, winter savory, Spanish oregano, birch, cade, and Bay St. Thomas.

All of them showed a clear antioxidant activity toward olive oil, already when used at the modest concentration of 5 μL/L; however, they were able to afford less marked protection of the triglyceride compared to model cumene, owing to much larger oxidizability and ~100-folds faster chain propagation rate of olive oil compared to cumene,[23] which makes antioxidant protection more challenging.[23] Results, summarized visually in Figure 3, were, however, surprising in that also the relative efficacy of the EOs changed compared with experiments with cumene. Only birch and cade EOs could afford a clear inhibition period, Bay St. Thomas, Spanish oregano, and red thyme could only slow down the rate of oxygen consumption without a neat inhibition period, while winter savory and clove bud EOs had some intermediate performance. For all oils affording only a slow-down of the autoxidation rate, a neat inhibition period could be observed upon increasing the concentration of use (e.g., by 5-folds), as exemplified in Figure S20 (Supporting Information) for Bay St. Thomas EO.

In the protection of olive oil, the ranking of antioxidant efficacy was Cade EO > Birch EO > Winter Savory > Clove ≥ Red Thyme ≈ Spanish Oregano ≈ Bay St. Thomas.

This change in the relative ranking can be rationalized by considering the medium effects on the antioxidant kinetics of the phenolic components. Since triglycerides are good H-bond acceptors,[18,23] they hamper the antioxidant performance of carvacrol, thymol, 4-allylphenol, and other strong H-bond donor (HBD) phenols more markedly than that of eugenol and guaiacol derivatives, which are marginally sensitive to medium effects owing to the occurrence of intermolecular H-bonding (see Scheme 1 and the discussion therein). Indeed, we have noted that the kinetic constants k_{inh} measured in HBA solvent acetonitrile are very close to that measured in PhCl for guaiacol, eugenol, and dihydroeugenol, while it is strongly reduced for most other phenols lacking an *ortho*-methoxy or significant steric hindrance in the *ortho* positions. Such a medium effect would also extend to the synergic interplay among coantioxidants in the same EO.[19] Additionally, we have previously suggested that during the autoxidation of complex lipids like triglycerides, minor amounts of $HOO^{\bullet}$ radical could be released as a side reaction,[23] which would trigger nonconventional antioxidant mechanism similar to the one previously mentioned to explain synergism (*vide supra*). Clearly, medium effects and the need for testing in actual lipids cannot be overlooked in planning antioxidant strategies.

It is interesting to note that the absolute performance of most EOs was similar to or higher than that produced by reference antioxidant BHT at a concentration similar to that of their EO phenolic components, although it was clearly lower than the protection offered by α-tocopherol (Figure 3).

Advantages and Limitation of Our Investigation Method. Unlike other assays on essential oils as antioxidants,[15] our approach, while being relatively simple, offers reliable kinetic constants for peroxyl radical trapping, thereby allowing full quantitative assessment of antioxidant efficacy, which would be transferable lab-to-lab and favor the rational design of antioxidant strategies. It is also a potent tool to investigate the mechanistic aspects and synergism. Although it works reliably with essential oils protecting a homogeneous lipid, it may need further work to apply in the protection of heterogeneous food products, since partition and confinement of EO components would need to be considered. The

simplified approach of treating antioxidants in a mixture as a single molecule may not be applicable to other vegetable extracts, particularly in case they contain antioxidants with largely different structure and reactivity, or with different polarity and able to differently partition in heterogeneous systems. Specific adaptation and validation would appear necessary, or a more rigorous approach addressing the individual components should be preferred.[16]

In conclusion, 9 out of 13 tested EOs showed prominent antioxidant activity, which could be quantified with our kinetic approach affording k_{inh} values close to 1×10^4 M^{-1} s^{-1}, i.e., of the same magnitude of ubiquitous synthetic antioxidant BHT. Values of k_{inh} of the same magnitude were also measured for 13 representative phenolic EO components, which largely dictate the chain-breaking antioxidant behavior of the tested EOs. Indeed, EOs not containing significant amounts of phenolic components (e.g., *A. graveolens*, *D. carota*, *T. minuta*, and *C. atlantica*) did not show chain-breaking activity. This does not imply that other antioxidant mechanisms like the termination-enhancing[21] cannot be elicited; however, they would require higher testing doses to be relevant and were deliberately excluded in our experimental design. Synergic interaction among phenolic components was observed in some EOs such as *P. racemosa*, *S. montana*, *B. alba*, and particularly *J. oxycedrus*, allowing the EOs to perform as the fastest phenolic components, likely regenerated by the less active. An additional synergic interaction between phenolic and nonphenolic components was also apparent in some cases, particularly for *J. oxycedrus*, allowing a longer duration of the antioxidant activity than predicted on the basis of the sum of the phenolic components. Overall, our kinetic studies demonstrate the efficacy of phenol-rich EOs in breaking the autoxidation chain reaction both in model systems and in the protection of complex natural lipids like olive oil, being able to match or surpass the protection offered by BHT. This supports their use in place of this reference additive in food products or in high-value health products such as cosmetics and pharmaceuticals, to afford more sustainable alternatives, in line with the current demand.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c04580.

Chromatograms and composition tables of the investigated essential oils, example of autoxidation kinetics inhibited by phenolic components or EOs, and notes on kinetic equations and their derivation (PDF)

AUTHOR INFORMATION

Corresponding Author

Luca Valgimigli − *Department of Chemistry "G. Ciamician", University of Bologna, 40129 Bologna, Italy; Tecnopolo di Rimini, 47922 Rimini, Italy;* orcid.org/0000-0003-2229-1075; Email: luca.valgimigli@unibo.it

Authors

Wenkai Pan − *Department of Chemistry "G. Ciamician", University of Bologna, 40129 Bologna, Italy*

Albert Velasco Abadia − *Department of Chemistry "G. Ciamician", University of Bologna, 40129 Bologna, Italy*

Yafang Guo − *Department of Chemistry "G. Ciamician", University of Bologna, 40129 Bologna, Italy*

Simone Gabbanini − *R&D Division, BeC s.r.l., 47122 Forlì, Italy*

Andrea Baschieri − *Institute for Organic Synthesis and Photoreactivity (ISOF), National Research Council of Italy (CNR), I-40129 Bologna, Italy;* orcid.org/0000-0002-2108-8190

Riccardo Amorati − *Department of Chemistry "G. Ciamician", University of Bologna, 40129 Bologna, Italy;*

ACKNOWLEDGMENTS

The authors thank Simone Di Marzo and Romeo Pizzol for assistance with kinetic measurements.

ABBREVIATIONS

AIBN:2,2′-azobis(isobutyronitrile); BDE:bond dissociation enthalpy; BHT:butylhydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol); EO:essential oil; HBA:hydrogen-bond acceptor; HBD:hydrogen-bond donor; TIC:total ion count

REFERENCES

(1) Wang, K.; Chen, D.; Yu, B.; He, J.; Mao, X.; Huang, Z.; Yan, H.; Wu, A.; Luo, Y.; Zheng, P.; Yu, J.; Luo, J. Eugenol Alleviates TGEV-Induced Intestinal Injury via Suppressing ROS/NLRP3/GSDMD-Dependent Pyroptosis. *J. Agric. Food Chem.* **2023**, *71*, 1477−1487.

(2) Xiong, T.; Zeng, J.; Chen, L.; Wang, L.; Gao, J.; Huang, L.; Xu, J.; Wang, Y.; He, X. Anti-Inflammatory Terpenoids from the Rhizomes of Shell Ginger. *J. Agric. Food Chem.* **2024**, *72*, 424−436.

(3) Zhang, W.; Lin, H.; Cheng, W.; Huang, Z.; Zhang, W. Protective Effect and Mechanism of Plant-Based Monoterpenoids in Non-alcoholic Fatty Liver Diseases. *J. Agric. Food Chem.* **2022**, *70*, 4839−4859.

(4) Matera, R.; Lucchi, E.; Valgimigli, L. Plant Essential Oils as Healthy Functional Ingredients of Nutraceuticals and Diet Supplements: A Review. *Molecules* **2023**, *28*, 901.

(5) Tian, B.; Liu, J.; Yang, W.; Wan, J.-B. Biopolymer Food Packaging Films Incorporated with Essential Oils. *J. Agric. Food Chem.* **2023**, *71*, 1325−1347.

(6) Yang, T.; Li, Z.; Shu, J.; Fang, L.; He, X.; Li, Q.; Jiang, G.; Chen, S.; Yang, B. Rapid Detection of Potential Natural Food Preservatives and Identification of Artemisia Species via High-Sensitivity Photoionization Mass Spectrometry. *J. Agric. Food Chem.* **2023**, *71*, 6197−6209.

(7) Guo, Y.; Baschieri, A.; Amorati, R.; Valgimigli, L. Synergic antioxidant activity of γ-terpinene with phenols and polyphenols enabled by hydroperoxyl radicals. *Food Chem.* **2021**, *345*, No. 128468.
(8) Bora, P. K.; Borah, G.; Kalita, D.; Saikia, S. P.; Haldar, S. Mushroom-Mediated Reductive Bioconversion of Aldehyde-Rich Essential Oils for Aroma Alteration: A Rose-like Floral Bioflavor from Citronella Oil. *J. Agric. Food Chem.* **2023**, *71*, 1690−1700.
(9) Che, J.; Pan, F.; Chen, X.; Zhang, Y.; Tao, N.; Fu, Y. Screening of Oxygenated Aromatic Compounds for Potential Antifungal Activity against Geotrichum citri-aurantii through Structure−Activity Relationship Analysis. *J. Agric. Food Chem.* **2022**, *70*, 13787−13795.
(10) Ge, X.; Hu, Y.; Shen, H.; Liang, W.; Sun, Z.; Zhang, X.; Li, W. Pheophorbide-a as a Light Triggered Liposomal Switch: For the Controlled Release of Alpinia galanga (A. galanga) Essential Oil and Its Stability, Antioxidant, and Antibacterial Activity Assessment. *J. Agric. Food Chem.* **2023**, *71*, 1667−1678.
(11) Li, J.; Ye, J.; Zhou, R.; Gui, K.; Li, J.; Feng, J.; Ma, Z.; Lei, P.; Gao, Y. Systematic Study on Turpentine-Derived Amides from Natural Plant Monoterpenes as Potential Antifungal Candidates. Systematic Study on Turpentine-Derived Amides from Natural Plant Monoterpenes as Potential Antifungal Candidates. *J. Agric. Food Chem.* **2023**, *71*, 5507−5515.
(12) Guo, Y.; Pizzol, R.; Gabbanini, S.; Baschieri, A.; Amorati, R.; Valgimigli, L. Absolute Antioxidant Activity of Five Phenol-Rich Essential Oils. *Molecules* **2021**, *26*, 5237.
(13) Novais, C.; Molina, A. K.; Abreu, R. M. V.; Santo-Buelga, C.; Ferreira, I. C. F. R.; Carla Pereira, C.; Barros, L. Natural Food Colorants and Preservatives: A Review, a Demand, and a Challenge. *J. Agric. Food Chem.* **2022**, *70*, 2789−2805.
(14) López, P. L.; Juncos, N. S.; Grosso, N. R.; Olmedo, R. H. Use of Humulus lupulus and Origanum vulgare as Protection Agents Against Oxidative Deterioration in "Deep-Fried Process": Frying Model Assay with Sunflower Oil and High-Oleic Peanuts. *Food Bioprocess Technol.* **2024**, *17*, 1970−1984.
(15) Amorati, R.; Foti, M. C.; Valgimigli, L. Antioxidant activity of essential oils. *J. Agric. Food Chem.* **2013**, *61*, 10835−10847.
(16) Amorati, R.; Valgimigli, L. Methods to measure the antioxidant activity of phytochemicals and plant extracts. *J. Agric. Food Chem.* **2018**, *66*, 3324−3329.
(17) Olmedo, R.; Valeria, N.; Ruben, G. N. Antioxidant activity of fractions from oregano essential oils obtained by molecular distillation. *Food Chem.* **2014**, *156*, 212−219.
(18) Baschieri, A.; Pizzol, R.; Guo, Y.; Amorati, R.; Valgimigli, L. Calibration of squalene, p-cymene, and sunflower oil as standard oxidizable substrates for quantitative antioxidant testing. *J. Agric. Food Chem.* **2019**, *67*, 6902−6910.
(19) Valgimigli, L. Lipid Peroxidation and Antioxidant Protection. *Biomolecules* **2023**, *13*, 1291.
(20) Li, B.; Pratt, D. A. Methods for determining the efficacy of radical-trapping antioxidants. *Free Radical Biol. Med.* **2015**, *82*, 187−202.
(21) Baschieri, A.; Daci Ajvazi, M.; Folifack Tonfack, J. L.; Valgimigli, L.; Amorati, R. Explaining the antioxidant activity of some common non-phenolic components of essential oils. *Food Chem.* **2017**, *232*, 656−663.
(22) Baschieri, A.; Pulvirenti, L.; Muccilli, V.; Amorati, R.; Tringali, C. Chain-breaking antioxidant activity of hydroxylated and methoxylated magnolol derivatives: the role of H-bonds. *Org. Biomol. Chem.* **2017**, *15*, 6177.
(23) Guo, Y.; Pina, A.; Gabbanini, S.; Valgimigli, L. Absolute Kinetics of Peroxidation and Antioxidant Protection of Intact Triglyceride Vegetable Oils. *Food Chem.* **2024**, *452*, No. 139289.
(24) Costa, R.; De Fina, M. R.; Valentino, M. R.; Dugo, P.; Mondello, L. Reliable Identification of Terpenoids and Related Compounds by using Linear Retention Indices Interactively with Mass Spectrometry Search. *Nat. Prod. Commun.* **2007**, *2*, 413−418.
(25) Babushok, V. I.; Linstrom, P. J.; Zenkevich, I. G. Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, No. 043101.
(26) Lucarini, M.; Pedulli, G. F.; Valgimigli, L. Do Peroxyl Radicals Obey the Principle That Kinetic Solvent Effects on H-Atom Abstraction Are Independent of the Nature of the Abstracting Radical? *J. Org. Chem.* **1998**, *63* (13), 4497−4499.
(27) Johansson, H.; Shanks, D.; Engman, L.; Amorati, R.; Pedulli, G. F.; Valgimigli, L. Long-Lasting Antioxidant Protection: A Regenerable BHA Analogue. *J. Org. Chem.* **2010**, *75* (22), 7535−7541.
(28) Guo, Y.; Baschieri, A.; Mollica, F.; Valgimigli, L.; Cedrowski, J.; Litwinienko, G.; Amorati, R. Hydrogen Atom Transfer from HOO. to ortho-Quinones Explains the Antioxidant Activity of Polydopamine. *Angew. Chem., Int. Ed.* **2021**, *60*, 15220−15224.
(29) Zielinski, Z.; Presseau, N.; Amorati, R.; Valgimigli, L.; Pratt, D. A. Redox Chemistry of Selenenic Acids and the Insight It Brings on Transition State Geometry in the Reactions of Peroxyl Radicals. *J. Am. Chem. Soc.* **2014**, *136* (4), 1570−1578.
(30) Sampaio, B. L.; Edrada-Ebel, R.; Da Costa, F. Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: A model for environmental metabolomics of plants. *Sci. Rep.* **2016**, *6*, No. 29265.
(31) Koşar, M.; Demirci, B.; Demirci, F.; Can Baser, K. H. Effect of maturation on the composition and biological activity of the essential oil of a commercially important satureja species from turkey: Satureja cuneifolia Ten. (Lamiaceae). *J. Agric. Food Chem.* **2008**, *56*, 2260−2265.
(32) Satyal, P.; Murray, B. L.; McFeeters, R. L.; Setzer, W. N. Essential Oil Characterization of *Thymus vulgaris* from Various Geographical Locations. *Foods* **2016**, *5*, 70.
(33) de Saint Laumer, J.-Y.; Cicchetti, E.; Merle, P.; Egger, J.; Chaintreau, A. Quantification in Gas Chromatography: Prediction of Flame Ionization Detector Response Factors from Combustion Enthalpies and Molecular Structures. *Anal. Chem.* **2010**, *82*, 6457−6462.
(34) Maccelli, A.; Vitanza, L.; Imbriano, A.; Fraschetti, C.; Filippi, A.; Goldoni, P.; Maurizi, L.; Ammendolia, M. G.; Crestoni, M. E.; Fornarini, S.; et al. *Satureja montana* L. Essential Oils: Chemical Profiles/Phytochemical Screening, Antimicrobial Activity and O/W NanoEmulsion Formulations. *Pharmaceutics* **2020**, *12*, 7.
(35) Dodoš, T.; Janković, S.; Marin, P. D.; Rajčević, N. Essential Oil Composition and Micromorphological Traits of *Satureja montana* L., *S. subspicata* Bartel ex Vis., and *S. kitaibelii* Wierzb. Ex Heuff. Plant Organs. *Plants* **2021**, *10*, 511.
(36) Faleiro, L.; Miguel, G.; Gomes, S.; Costa, L.; Venâncio, F.; Teixeira, A.; Figueiredo, A. C.; Barroso, J. G.; Pedro, L. G. Antibacterial and Antioxidant Activities of Essential Oils Isolated from *Thymbra capitata* L. (Cav.) and Origanum vulgare L. *J. Agric. Food Chem.* **2005**, *53* (21), 8162−8168.
(37) Ayoub, I. M.; Abdel-Aziz, M. M.; Elhady, S. S.; Bagalagel, A. A.; Malatani, R. T.; Elkady, W. M. Valorization of Pimenta racemose Essential Oils and Extracts: GC-MS and LC-MS Phytochemical Profiling and Evaluation of Helicobacter pylori Inhibitory Activity. *Molecules* **2022**, *27*, 7965.
(38) Pragadheesh, V. S.; Yadav, A.; Singh, S. C.; Gupta, N.; Chanotiya, C. S. Leaf Essential Oil of Cultivated *Pimenta racemosa* (Mill.) J.W. Moore from North India: Distribution of Phenylpropanoids and Chiral Terpenoids. *Med. Aromat. Plants* **2013**, *2*, No. 1000118.
(39) Al-Gendy, A. A.; Moharram, F. A.; Zarka, M. A. Chemical composition, antioxidant, cytotoxic and antimicrobial activities of *Pimenta racemosa* (Mill.) J.W. Moore flower essential oil. *J. Pharmacogn. Phytochem.* **2017**, *6*, 312−319.
(40) Vinod, M.; Singh, M.; Pradhan, M.; Iyer, S. K.; Tripathi, D. K. Phytochemical Constituents and Pharmacological Activities of *Betula alba* Linn.—A Review. *Int. J. PharmTech Res.* **2012**, *4*, 643−647.
(41) Gugliuzzo, A.; Francardi, V.; Simoni, S.; Roversi, P. F.; Ferrati, M.; Spinozzi, E.; Perinelli, D. R.; Bonacucina, G.; Maggi, F.; Tortorici, S.; Garzia, G. T.; Biondi, A.; Rizzo, R. Role of plant essential oil nanoemulsions on host colonization by the invasive ambrosia beetle Xylosandrus compactus. *Ind. Crops Prod.* **2023**, *195*, No. 116437.

(42) Salido, S.; Altarejos, J.; Nogueras, M.; Sanchez, A.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. Chemical studies of essential oils of *Juniperus oxycedrus* ssp. badia. *J. Ethnopharmacol.* **2002**, *81*, 129−134.
(43) Uehara, A.; Tommis, B.; Belhassen, E.; Satrani, B.; Ghanmi, B.; Baldovini, M. N. Odor-active constituents of *Cedrus atlantica* wood essential oil. *Phytochemistry* **2017**, *144*, 208−215.
(44) Verma, R. S.; Padalia, R. C.; Amit Chauhan, A. Chemical composition variability of essential oil during ontogenesis of *Daucus carota* L. subsp. sativus (Hoffm.) Arcang. *Ind. Crops Prod.* **2014**, *52*, 809−814.
(45) Salimi, F.; Fattahi, M.; Hamzei, J. Phenolic contents, composition and antioxidant activity of essential oils obtained from Iranian populations of *Apium graveolens*, and their canonical correlation with environmental factors. *Biochem. Syst. Ecol.* **2022**, *101*, No. 104394.
(46) de Oliveira, D. H.; Abib, P. B.; Giacomini, R. X.; Lenardão, E. J.; Schiedeck, G.; Wilhelm, E. A.; Luchese, C.; Savegnago, L.; Jacob, R. G. Antioxidant and antifungal activities of the flowers' essential oil of *Tagetes minuta*, (Z)-tagetone and thiotagetone. *J. Essent. Oil Res.* **2019**, *31*, 160−169.
(47) Amorati, R.; Menichetti, S.; Mileo, E.; Pedulli, G. F.; Viglianisi, C. Hydrogen-Atom Transfer Reactions from ortho-Alkoxy-Substituted Phenols: An Experimental Approach. *Chem. - Eur. J.* **2009**, *15*, 4402−4410.
(48) Lucarini, M.; Pedulli, G. F. Free radical intermediates in the inhibition of the autoxidation reaction. *Chem. Soc. Rev.* **2010**, *39*, 2106−2119.
(49) Valgimigli, L.; Lucarini, M.; Pedulli, G. F.; Ingold, K. U. Does β-carotene really protect vitamin E from oxidation? *J. Am. Chem. Soc.* **1997**, *119*, 8095−8096.
(50) Foti, M. C.; Ingold, K. U. Mechanism of Inhibition of lipid peroxidation by γ-terpinene, an unusual and potentially useful hydrocarbon antioxidant. *J. Agric. Food Chem.* **2003**, *51*, 2758−2765.