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ORIGINAL ARTICLE

Antioxidant activity and chemical composition of essential oils of three aromatic plants from La Rioja province

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Abstract

Context: The use of many traditional medicinal plants is often hampered by the absence of a proper biochemical characterization, which is essential to identify the bioactive compounds present in it. The essential oils (EOs) of three native species from the La Rioja province were analyzed: *Lippia turbinata* Griseb and *Lippia integrifolia* (Griseb.) Hieron (Verbenaceae), and *Clinopodium gilliesii* (Benth.) Kuntze (Lamiaceae).

Objective: The aim of this study was to evaluate their EOs antioxidant activity (AA) and their chemical composition.

Materials and methods: EOs were analyzed by gas chromatography-mass spectrometry (GC-MS). To enhance the aqueous solubilization of the EOs, EO-water emulsions were prepared (concentration range of $0.1-6 \text{ mg mL}^{-1}$). AA was determined using ABTS, DPPH, and peroxyl radical scavenging assays, as well as by the β -carotene bleaching test.

Results: Piperitenone oxide was a major constituent in *L. turbinata*, pulegone and piperitenone oxide in *C. gilliesii*, and β -caryophyllene in *L. integrifolia. Lippia turbinata* EO was the most active ABTS and DPPH radical scavenger (SC₅₀ values of 0.40 ± 0.14 and 0.74 ± 0.08 mg mL⁻¹, respectively). *Clinopodium gilliesii* EO exhibited the highest hydrogen peroxide scavenging activity (SC₂₅ value = 1.52 ± 0.27 mg mL⁻¹). In the β -carotene assay, *L. turbinata* EO was more effective at inhibiting lipid peroxidation than the other two oils (IC₂₅ value = 0.15 ± 0.04 mg mL⁻¹).

Conclusion: Our results suggest that the AA observed can be justified by the presence of oxygenated monoterpenes, mainly piperitenone oxide. Finally, *L. turbinata* EO might be used as a safe natural antioxidant and food preservative in the food and cosmetic industries.

Introduction

In animal tissues, oxidants can cause cellular damage through the peroxidation of unsaturated fatty acids, denaturation of proteins, and reactions with nucleic acids and carbohydrates. These damages have been involved in the pathogenesis of several human disorders such as cancer, neurodegenerative disorders, rheumatoid arthritis, and ageing (Pham-Huy et al., 2008). In this sense, natural products with antioxidant properties could have great importance as therapeutic agents in a several diseases, since they are effective as radical scavengers and inhibitors of lipid peroxidation (Sen et al., 2010).

In recent years, due to toxicological concerns associated with the use of synthetic substances in food, and increasing awareness about natural foods, there has been a growing interest in the use of natural substances as food preservatives

Keywords

Biological activity, *Clinopodium gilliesii*, *Lippia integrifolia*, *Lippia turbinata*, piperitenone oxide

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and antioxidants (Peschel et al., 2006). In this context, many aromatic plants, and particularly their essential oils (EOs), are being evaluated for antioxidant activity (AA) (Martins et al., 2014; Mohamed et al., 2013; Quiroga et al., 2011).

Lippia turbinata, Clinopodium gilliesii, and *Lippia integrifolia* are three popular aromatic species used widely in many Argentinean regions. In this sense, the province of La Rioja has a rich folk medicine tradition using an ample variety of medicinal and aromatic plants.

Lippia turbinata Griseb (Verbenaceae) or "poleo" is a native shrub from South America, commonly found in the west-central region of Argentina. It is well known for its aromatic-medicinal properties such as its digestive and antispasmodic effects to treat gastrointestinal disorders. It is also used in dyspepsia, oliguria, and dysmenorrhea (Alonso & Desmarchelier, 2006; Pascual et al., 2001).

Clinopodium gilliesii (Benth.) Kuntze (Lamiaceae) known popularly as "muña-muña" is a native herb which grows in the provinces of Salta, Jujuy, Córdoba, Catamarca, Tucumán, La Rioja, San Luis, and San Juan. It is used in traditional medicine to treat gastric troubles, stomach aches, and female

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sterility; furthermore, it is also known as an aphrodisiac (Bustos et al., 1996; Viturro et al., 2000).

Lippia integrifolia (Griseb.) Hieron (Verbenaceae) is a shrub, known popularly as "incayuyo", which grows in the Northwestern region of Argentina. It is traditionally used to treat dyspepsia, indigestions, and stomachaches, as a diuretic, emmenagogue, and tonic agent (Alonso & Desmarchelier, 2006).

The biological activity of these plants has been investigated by several researchers. Although most research has been done with extracts, a few studies have assessed the activity of their EOs. The antibacterial (González & Marioli, 2010), antifungal (Bluma & Etcheverry, 2008; Passone & Etcheverry, 2014), insecticidal (Gleiser & Zygadlo, 2007), virucidal (García et al., 2003), and antioxidant (Quiroga et al., 2013) activities of *L. turbinata* EO have been reported, as well as the antifungal, antibacterial (Lima et al., 2011), and antiradical (Cabana et al., 2013) properties of *C. gilliesii*, and the antibacterial, antifungal (Lima et al., 2011), and insectrepellent (Gleiser et al., 2011; Lima et al., 2011) activities of *L. integrifolia* EO.

In the present study, we compared the AA between the EOs extracted from *L. turbinata*, *C. gilliesii*, and *L. integrifolia*, and also analyzed their chemical composition. To our knowledge, this is the first report on both the chemical composition and the antioxidant potential of EOs from *L. turbinata*, *C. gilliesii*, and *L. integrifolia* collected in the La Rioja province (Valle Antinaco-Los Colorados).

Materials and methods

Plant materials and isolation of EOs

The plant specimens, L. turbinata, C. gilliesii, and L. integrifolia, were collected in February 2013 from Valle Antinaco-Los Colorados, province of La Rioja, Argentina (1100–1620 m.a.s.l). Sample copies are kept in the personal collection of the team in the Universidad Nacional de Chilecito. The plants were botanically identified by Lic. Cs. Biol. Gloria S Jamie (Universidad Nacional de Tucumán, Tucumán, Argentina) according to the reference material present in Miguel Lillo Institute Herbarium, Tucumán, Argentina (LIL) and specific literature. The following are the voucher data of Cfr (examined reference material) with studied material: L. turbinata Griseb. Cfr L. turbinata Griseb. Prov. La Rioja LIL 27589, L. integrifolia (Griseb.) Hieron. Cfr L. integrifolia (Griseb.) Hieron. Prov. La Rioja LILL 609723, and C. gilliesii (Benth.) Kuntze Cfr Syn. Satureja parvifolia (Phil.) Mold. Prov. La Rioja LILL 420243.

The air-dried aerial parts of plant samples were submitted to hydrodistillation for 3 h using a Clevenger apparatus. The EOs obtained were stored at 4 °C for subsequent experiments. Yields of the samples were 1.0% for *L. turbinata*, 1.2% for *C. gilliesii* and 1.4% for *L. integrifolia*.

Analysis of EO compounds

The chemical composition of the EOs was analyzed using a gas chromatography-mass spectrometry (GC-MS) carried out on a Shimadzu GC-2010 GCMS-QP2010 (Shimadzu Corporation, Kyoto, Japan) fitted with a DB 5-fused silica column $(30 \text{ m} \times 0.25 \text{ mm} \text{ i.d.}, \text{ a film thickness of } 0.25 \text{ µm})$. The initial oven temperature was set at $60 \,^{\circ}\text{C}$, then it was increased to $250 \,^{\circ}\text{C}$ at $4 \,^{\circ}\text{C} \,^{\min^{-1}}$, and the final temperature was maintained for 5 min. Carrier gas, helium, was adjusted to a linear velocity of 34 cm s^{-1} (1 mL min⁻¹). The injector temperature was $250 \,^{\circ}\text{C}$, the injection volume of 1 µL, and the split ratio of 1:10. The identity of the oil components was established by comparison of their MS spectra with those reported in Wiley8 and Nist08 libraries and the relative percentage of the individual compounds in each EO was calculated using the total ion current from the MS detector signal.

Preparation of EO emulsions

To enhance the aqueous solubilization of the EOs, stock EO–water emulsions were prepared in two concentrations: 4 mg mL^{-1} and 10 mg mL^{-1} by weighing 20 and 50 mg of each oil and adding them to 5 mL of distilled water. The tubes were then shaken using a 40 kHz ultrasonic TESTLAB-BU-01 (Shimadzu Corporation, Kyoto, Japan) for 30 min. The emulsions were diluted and used in a concentration range of $0.1-6 \text{ mg mL}^{-1}$.

DPPH and ABTS radical scavenging assay

DPPH radical scavenging activity was measured as described by Farasat et al. (2013) with minor modifications. Briefly, 95 μ L of DPPH solution was added to 105 μ L of each EO aliquot previously dissolved in ethanol over a concentration range of 0.1–8.5 mg mL⁻¹. The absorbance of the reaction mixture at 520 nm was measured using a microplate reader (Biotek EL 808, BioTek Instruments, Inc., Shoreline, WA). The SC₅₀ values (concentration of sample required to scavenge 50% DPPH radicals) were calculated using the regression equation prepared from the different EOs concentrations.

The radical scavenging capacity of the samples for the ABTS radical cation was determined as described by Re et al. (1999) with some modifications. About $50\,\mu\text{L}$ of ABTS solution was added to $150\,\mu\text{L}$ of EO emulsions (a concentration range of $0.1-5\,\text{mg}\,\text{mL}^{-1}$). The absorbance was measured at 734 nm (microplate reader Biotek EL 808, BioTek Instruments, Inc., Shoreline, WA) 6 min after initial mixing. Results were expressed as SC₅₀ values (sample concentration required to scavenge 50% ABTS radicals).

Hydrogen peroxide scavenging assay

The ability of the EOs to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). About 100 μ L of each EO emulsion (0.5–6 mg mL⁻¹) were added to the reaction mixture containing 900 μ L of 2 mM hydrogen peroxide. After 10 min, the absorbance was read against a blank at 230 nm (Spectrophotometer Shimadzu UV-240PC, Shimadzu Corporation, Kyoto, Japan). The SC₅₀ values (concentration that is required to scavenge 50% H₂O₂ radicals) were calculated.

β-Carotene–linoleic acid assay

AA based on the β -carotene bleaching test was evaluated by measuring the inhibition of the bleaching of β -carotene by the

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peroxides generated during linoleic acid oxidation (Ordoñez et al., 2006). A stock solution of β -carotene–linoleic acid mixture was prepared adding 1 mg β -carotene, 1 mL chloroform, 20 µL linoleic acid, and 200 µL Tween 40. Chloroform was completely evaporated and 40 mL of oxygenated distilled water was added to form a yellowish emulsion. About 100 µL of the EO in methanol solution (0.05–1.8 mg mL⁻¹) was added to 1 mL of β -carotene–linoleic acid stock solution. The absorbance was measured at 470 nm (Spectrophotometer Shimadzu UV-240PC, Shimadzu Corporation, Kyoto, Japan) at 0 min, and after 30, 60, 90, and 120 min incubation in a water bath at 50 °C. IC₅₀ values (concentration required to inhibit 50% β -carotene bleaching) were determined from graphs with AA plotted against EO concentration.

Statistical analysis

The data are presented as mean \pm standard deviation of three determinations. Statistical significance was analyzed by the one-way analysis of variance followed by the Tukey test (MINITAB software version 15 for Windows, SPSS Inc., Chicago, IL). *p* Values < 0.05 were regarded as significant.

Results and discussion

Chemical composition of the EOs

Since the biological activity of EOs is dictated by the sum of its components, we first analyzed their chemical composition. The compositions of *L. turbinata*, *C. gilliesii*, and *L. integrifollia* oils were determined by comparing mass spectra of oil components with those from Wiley and NIST08 libraries, plus two other criteria: retention index (Kovat's Index) and comparison with literature data (Adams, 2001). Volatile oils are very complex compound mixtures in which a variety of mono- and sesquiterpenes can be found. Ten compounds were identified in the EO from *L. turbinata*, 12 from *C. gilliesii* and 13 from *L. integrifolia*, representing 86.6, 78.0, and 68.4% of each total oil, respectively (Table 1).

The major compounds identified by GC-MS in the *L. turbinata* EO were piperitenone oxide (63.0%) and limonene (7.2%). While the major components in *C. gilliesii* EO were pulegone (19.2%), piperitenone oxide (14.7%), isopulegone (12.0%), and menthone (9.7%). GC-MS analysis of *L. integrifolia* EO showed that β -caryophyllene (15.3%) and terpinen-4-ol (8.7%) were its major constituents (Table 1).

Monoterpenes were dominant in the *L. turbinata* and *C. gilliesii* EOs in contrast to the *L. integrifolia* oil, in which sesquiterpenes were the most abundant constituents. As shown in Table 1, monoterpenes (and their derivatives) represent 78.6% of *L. turbinata* EO, 71.4% being oxygenated monoterpenoids. In *C. gilliesii*, monoterpenes and monoterpenoids represent 74.5% of the overall EO, 74.0% being oxygenated monoterpenoids. About 43.1% of *L. integrifolia* EO was identified as sesquiterpenes (or their derivatives), 23.5% being hydrocarbon sesquiterpenes, and 19.6% oxygenated sesquiterpenoids.

Antioxidant activity of the EOs

EOs, as natural sources of antioxidants, have been evaluated for their activity as free radical (FR) scavengers. ABTS and DPPH are stable FR, which have been widely accepted as a tool for estimating FR scavenging activities of antioxidants (Miguel, 2010). The percentages of remaining ABTS and DPPH in the presence of all oils at different concentrations are shown in Figure 1. ABTS and DPPH scavenging activities of all the tested samples were observed to be dose dependent. The SC₅₀ values indicated that *L. turbinata* EO had the highest AA in ABTS and DPPH tests. In addition, *C. gilliesii* EO showed a stronger AA than *L. integrifolia* EO in these tests (Table 2).

The SC_{50} values obtained with the ABTS test were lower than those with the DPPH test (Table 2). The differences between the two results can be explained by the mechanism of the reaction involved. The ABTS radical reaction involves

Table 1. Main components of the essential oils from L. turbinata, C. gilliesii, and L. integrifolia.

		L. turbinate	2		C. gilliesii			L. integrifolia
Compound	KI ^a	(%)	Compound	KI ^a	(%)	Compound	KI ^a	(%)
Monoterpenes		7.2	Monoterpenes		0.5	Monoterpenes		7.0
Limonene	1024	7.2	Limonene	1024	0.5	Sabinene	969	1.7
Oxygenated monoterpenoids		71.4	Oxygenated monoterpenoids		74.0	<i>p</i> -Cymene	1020	3.5
cis-Verbenol	1137	1.0	β-Ocimene	1041	1.2	Limonene	1024	1.8
Carvone	1239	1.1	β-Linalool	1086	1.8	Oxygenated monoterpenoids		18.3
cis-Piperitone oxide	1250	2.4	Menthone	1136	9.7	cis-Sabinene hydrate	1065	6.9
Bornylacetate	1287	1.2	Isomenthone	1146	6.2	trans-Verbenol + camphor	1140/1141	2.7
Piperitenone	1340	1.6	Isopulegone	1148	12.0	Terpinen-4-ol	1174	8.7
Piperitenone oxide	1366	63.0	Pulegone	1215	19.2	Sesquiterpenes		23.5
Methyleugenol	1403	1.1	cis-Carvone oxide	1268	9.2	β-Caryophyllene	1417	15.3
Sesquiterpenes		2.4	Piperitenone oxide	1366	14.7	Bicyclogermacrene	1500	3.3
β-Caryophyllene	1417	2.4	Sesquiterpenes		2.8	β-Bisabolene	1505	4.9
Oxygenated sesquiterpenoids		5.6	β-Caryophyllene	1417	1.2	Oxygenated sesquiterpenoids		19.6
Caryophyllene oxide	1582	5.6	Bicyclogermacrene	1494	1.6	Muurol-5-en-4a-ol	1559	1.7
5 1 5			Oxygenated sesquiterpenoids		0.7	trans-Davanone	1564	4.9
			1,6-Germacradien-5-ol	1585	0.7	Spathulenol	1577	6.5
			-			Caryophyllene oxide	1582	6.5
Total		86.6			78.0	× 1 ×		68.4

^aKI, Kovat's index; relative retention index.

electron transfer and takes place at a much faster rate compared with DPPH radicals whose degree of discoloration is attributed to the hydrogen-donating ability of tested compounds (Afoulous et al., 2013; Bayala et al., 2014).

The most significant FR in biological systems results from oxygen. In this sense, we evaluated the effect of EOs on ROS

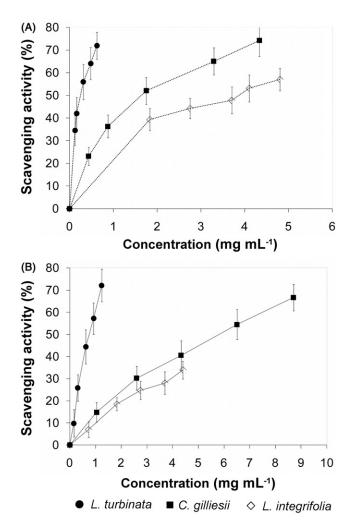


Figure 1. Free radical scavenging activity of essential oils from *L. turbinata*, *C. gilliesii*, and *L. integrifolia*. The activity was measured by the scavenging of ABTS (A) and DPPH (B) radicals. Values are means \pm SD, n = 3.

using the hydrogen peroxide scavenging test. Hydrogen peroxide is not reactive on its own, but, under appropriate conditions, it can form hydroxyl radicals. These radicals are the most reactive oxygen radical types as they can rapidly react with polyunsaturated fatty acids, resulting in the production of peroxyl radicals (Miguel, 2010; Sahin & Candan, 2013). When the EO hydrogen peroxide inhibition activity was measured, it was observed that *C. gilliesii* EO was more active than *L. integrifolia* EO, and the AA of *L. turbinata* EO was intermediate between them (Table 2).

The β -carotene–linoleic acid test is usually used to estimate the potential ability of the antioxidants to delay lipid peroxidation by reacting with the peroxyl radical propagating chains faster than the reaction of these radicals with proteins or fatty acid side chains (Ebrahimabadi et al., 2010). The inhibition of β -carotene oxidation in the presence of different EOs is shown in Figure 2. Considering the IC₂₅ or IC₅₀ values, the activity of *L. turbinata* EO was higher than that of both *C. gilliesii* and *L. integrifolia* oils (Table 2). The IC₂₅ values shown also demonstrate that *C. gilliesii* EO was more effective at inhibiting lipid peroxidation than *L. integrifolia* EO (Table 2).

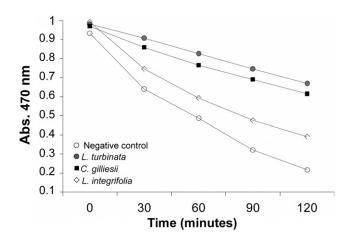


Figure 2. β -Carotene bleaching assay of *L. turbinata, C. gilliesii*, and *L. integrifolia* essential oils. The β -carotene oxidation induced by heat was monitored for 120 min. The maximum concentrations used were 1.13 mg mL⁻¹ for *L. turbinata*, 1.79 mg mL⁻¹ for *C. gilliesii*, and 1.38 mg mL⁻¹ for *L. Integrifolia*.

Table 2.	Antioxidant	activity of L	turbinata.	С.	gilliesii.	and L	integrifolia	essential oils.

	Free radical	scavenging	ROS sca	venging	Inhibition of lipid peroxidation β-Carotene–linoleic acid		
EO ^a	ABTS	DPPH	H ₂ O	\mathcal{D}_2			
	SC ₅₀ ^b		SC ₂₅ ^b	SC_{50}^{b}	IC ₂₅ ^c IC ₅₀ ^c		
L. turbinata C. gilliesii L. integrifolia Quercetin ^d		$\begin{array}{c} 0.74 \pm 0.08^{a} \\ 5.80 \underset{e}{\pm} 0.14^{b} \\ 0.0105 \pm 0.0015 \end{array}$		$ \frac{\stackrel{e}{e}}{4.56 \pm 0.46} $		0.99 ± 0.18^{a} 1.56 ± 0.22^{b} 0.0069 ± 0.0009	

Values are means \pm SD. Means in a column without a common letter differ (p < 0.05), a <b < c. ^aEO, essential oil.

^bSC, concentration required to scavenge 25 or 50% ABTS, DPPH radicals, or H₂O₂.

^cIC, concentration required to inhibit 25 or 50% β-carotene bleaching.

^dPositive control (mg mL⁻¹).

^eIn the range of concentrations tested, we were unable to determine SC_{50} and IC_{50} values. Results are expressed in mg mL⁻¹.

Antioxidant activity versus phytochemical composition

The results obtained revealed that *L. turbinata* EO is the best antioxidant agent as compared with the other two oils, due to its high ability to scavenge FR and ROS as well as its capacity of inhibiting lipid peroxidation. Additionally, the AA of *C. gilliesii* oil was higher than that of *L. integrifolia*.

The major compounds of *L. turbinata* and *C. gilliesii* oils are oxygenated monoterpenes while the major compounds of *L. integrifolia* EO are sesquiterpenes, which could explain the differences in the AA between them. In addition, the oxygenated monoterpenes found in these EOs may act as antioxidant agents.

The antioxidant properties of monoterpene in EOs have been referred to by several authors. Martins et al. (2014) suggested that the observed β -carotene antioxidant capacity in the Schinus molle L. (Anacardiaceae) EO can be justified by the presence of the monoterpenes. Edziri et al. (2010) reported that Retama raetam (Forssk.) Webb (Fabaceae) EO has a good AA (measured through the DPPH method), and they consider that this activity could be attributed to the relatively high percentage of monoterpenes present in the oil. Moreover, there are some reports that show that oxygenated monoterpenes, such as α -terpineol, linalool, carvacrol, piperitenone, and piperitenone oxide, were mainly responsible for the antioxidant potential of plant oils (Bicas et al., 2011; Maestri et al., 2006; Miguel, 2010). In this sense, Iqbal et al. (2013) reported that the most abundant constituents in Mentha longifolia L. (Lamiaceae) EO were piperitenone oxide (28.3%) and piperitenone (24.9%), and they showed that the AA of its EO was similar to the piperitenone activity.

Civitelli et al. (2014) reported that piperitenone oxide exerted a strong inhibitory effect against herpes simplex virus type 1 (HSV-1). HSV-1 infection is known to induce a prooxidative state, and this redox change in the cell is important for viral replication. These authors proposed that piperitenone oxide could interfere with some redox-sensitive cellular pathways exploited for viral replication. Considering the results reported in the literature, as well as our results, we can suggest that the observed AA in the oils studied can be mainly justified by the presence of oxygenated monoterpenes and piperitenone oxide.

According to our best knowledge, there is only one report on the antioxidant potential of *L. turbinata* EO (Quiroga et al., 2013). The authors have reported a good AA for this oil, finding α -limonene (76.80%) and 1,8-cineole (4.95%) as major components, while in our study, piperitenone oxide (63.0%) and limonene (7.2%) are identified as the principal components. This difference in oil composition is probably due to the existence of different chemotypes. Overall, the EO composition proved to exhibit considerable chemodiversity depending on the method of extraction and geographical origin (Lima et al., 2011), thus, the importance of analyzing plants from different locations.

In addition, comparing our results with those reported by Cabana et al. (2013), the antiradical activity (DPPH test) of *C. gilliesii* EO observed herein was weaker than that found by these authors. They described piperitenone oxide as the major compound of *C. gilliesii* EO (68.8%), while in our study, this

compound only represented 14.7% of the oil. Taking this into account, the difference in the piperitenone oxide amounts between these two *C. gilliesii* oils could explain their different AA.

Conclusion

The AA of EOs is a biological property of great interest because it may be used to preserve foods from the toxic effects of oxidants. Moreover, the oils that have the ability to scavenge FR may play an important role in the prevention of some diseases such as cancer, brain dysfunction, and inflammatory diseases. We found that *L. turbinata* oil was significantly more effective as antioxidant than the other oils studied. This oil is predominantly constituted by piperitenone oxide. These findings support the view that certain aromatic plants are potential sources of antioxidants. In this sense, the *L. turbinata* EO collected in the La Rioja province may be a good and safe source of natural antioxidants to be used as preservative, cosmeceutical, or nutraceutical.

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Declaration of interest

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