



Review

Essential Oils as Potential Tools to Control *Listeria Monocytogenes* in Foods

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Abstract: Foodborne diseases are indisputable risks to human health, as they are directly affected by the consumption of food and beverages exposed to spoiling and pathogenic microorganisms. A commonly confronted issue by the food industry is the use of synthetic additives to control microbial growth on food products. Alternatively, plant essential oils (EOs) are natural compounds with antimicrobial effects against pathogens in edible and drinkable products. Based on bacteriostatic and bactericidal activities, this review describes the antimicrobial effects of plant EOs on *Listeria monocytogenes*, also indicating their potential applicability in different food products. Conclusively, it is determined that the Gram-positive bacteria have more susceptibilities to the EOs. In addition, the antibacterial efficacy of the EOs reported in the literature varied according to several parameters, including the seasonal harvest of plants, well-settled methodologies for extraction of EOs, experimental conditions for testing the antibacterial effect on *L. monocytogenes*, and matrix effects considering several types of foodstuffs. This later represents the main challenge to be addressed in future research aiming at specific applications of EOs in food products.

Keywords: essential oil, *Listeria monocytogenes*, bacteriostatic, bactericide, food industry

1. Introduction

Food and waterborne diseases are usually produced by ingesting pathogenically contaminated food or water [1]. It has been described that one per 10 individuals gets sick due to consuming contaminated food, that is, about 600 million people around the world [2]. Food and water contamination is a major threat, causing a high mortality rate for example 420,000 deaths per year [2]. Around 40% of infections together with the annual 125,000 deaths have been attributed to the preschool children [2]. In context, low- and middle-income countries from South or Southeast Asia, and Sub-Saharan Africa have shown higher contamination levels, accounting for 53% of foodborne diseases and 75% of deaths [3]. It is estimated that around 80% of food contamination has caused by the pathogenic microbes [2]. Similarly, 90% of the diseases were related to diarrhea [3]. In addition to these pathogens, the *Norovirus*, *Campylobacter*, agents of *Listeria* genus, tapeworms (mainly pork tapeworms that cause epilepsy), fish-associated fluke, and roundworms [3] can

also be responsible for prescribed complications. Amongst them, however, *Listeria* genus for example *L. monocytogenes* is a prominent cause of water and foodborne issues [4-6].

The bacterial genus *Listeria* comprised ten species [7]. These include *Listeria grayi*, *L. innocua*, *L. seeligeri*, *L. welshimer*, *L. ivanovii* and *L. monocytogenes*, while the last two species are highly pathogenic [8]. Ubiquitously, they can be found in soil, water, animals [7, 9], as well as diverse dairy products and the products conserved in different conditions [4]. Moreover, they capably grow in varied temperature-e.g., 0 °C to 45 °C [10-11] and saline environments [7, 9, 12], indicating their higher resistant nature and survival in these conditions. *L. monocytogenes* is a major cause of listeriosis, a disease leading encephalitis, sepsis, meningitis and abortion in immunocompromised individuals, elderly, children and pregnant women [10, 13], thus causing a high mortality rate [7, 9, 12]. These species are commonly occurring in water, foods that need refrigeration, vegetables, and ready-to-eat foods that can be considered at high risk of listeriosis [7, 9]. Then, their proper monitoring and control are of high relevance to be considered.

Nevertheless, *L. monocytogenes* is a gram-positive pathogenic bacterium of sanitary interest that occurs in different products, including those of animal origin such as meat and milk, among others [14]. Besides a wide environmental dispersal, such spp. has the ability to form biofilms [15-16]. In food industry, decontamination has mostly carried out by using sanitizers, which can compose oxidative agents (halogen-based compounds, peracetic acid, ozone, hydrogen peroxide, etc.), iodophors or surface-active compounds (acidic anionic, quaternary ammonia, etc.) [15, 17]. A great concern in food industry is the resistance of *L. monocytogenes* to the aforementioned agents, thus perpetuating recurrent cases of contamination and infection. Donaghy et al. [18] highlight the influence of adequate and indiscriminate uses of antimicrobial agents in animal products as a collaborative factor for the emergence of resistance to biocides by gene mutations. Cooper et al. [19], determined that 59% of the environmental isolates have gene expression cassette (bcrABC) that resists quaternary ammonium compounds, and this cassette can be transferred to bacteria. In addition, the presence of biofilms that comprised fats, carbohydrates and proteins, also contribute to bacterial resistance, limiting sanitizers action [20], therefore searching for products with antimicrobial action and developing new disinfection strategies, such as EOs can be a considerable approach.

EOs have natural capability of preserving foods, but accordingly they can alter their sensory characteristics. Yet they also offer antimicrobial potentials, such as the EO from lemongrass, thyme, oregano and lime [21], lavender [22], sweet basil [23], mint [24] and cinnamon [25]. They can be used to preserve multiple products, containing meat, eggs, milk, plant-based products, and also in food packaging. In addition to food compositions, the process of inactivating microorganisms is dependent on the concentration and type of EOs [22]. EOs due to hydrophobicity, enable the partitioning of targeted lipids of the cell membrane and mitochondria, rendering them permeable and leading to the cell leakage [26]. Besides food preservation, some constraints have been predicted along the EOs application. These limitations incorporate intense aroma, high reactivity, hydrophobicity, reduced solubility, and possible negative interaction with food's carbohydrate and fat contents, leading to alterations in organoleptic properties [27]. The present review aimed to discuss the bacteriostatic and bactericidal activities of plant EOs on *Listeria monocytogenes*, also indicating their applicability in different food products.

2. Main characteristics of essential oils

Essential oils are complex mixtures of various bioactive metabolites that are obtained from leaf, flower, stem, seed and fruit of certain plants [22, 27]. These are low molecular weight metabolites, mainly lipophilic short-chain aliphatic hydrocarbons, phenylpropanoids, terpenoids and phenolic compounds [27]. EOs have mostly been attained through a variety of methods such as hydro- and steam-distillation, solvent extraction, and maceration, etc. Hence, these strategies are shown to represent poor efficiency, and dependent on longer extraction time, where chemical loss (e.g., volatile) and degradation of some composition can occur, yet solvent can generate toxicity [28]. The related issues have been overdue by alternative methods of supercritical fluid, microwave and ultrasound [28]. It is quite evident that using various methods for EOs extraction will result in product diversity, and subsequently, with varied antimicrobial actions. Although, as extensively used, this method may not be suitable in the case of food, because solvents can cause toxic effects, variations in the chemical profile of EO and can interfere with the flavor and aroma of extract. As for the palatability that EO confers to food, its usability depends not only on concentrations, but also on the sensory effects they provide.

There are many factors that can influence the chemical composition of plants. One of these factors is the seasonal shift along with the EO contents, which can markedly influence the analysis of the resultant efficiency. By comparing the major chemical compounds of EOs from *Campomanesia aurea*, collected in April and October, there can be seen changes in the concentration of metabolites, since some compounds revealed greater or lesser amounts in the samples [29]. For example, a significant increase in the proportion of some compounds was observed, mainly for α -cadinol and terpinolene when comparing October with April, while other compounds such as p-cymene had a decrease in their levels [29].

Botanical characteristics can be another factor that affects chemical composition of plants. According to Silva et al. [12], the main chemical components of tea tree are 43.1% to 22.8% terpinen-4-ol and γ -terpinene, respectively. A high level of d-limonene in oranges (around 95%), is the compound in significantly higher amounts than the others [30]. EOs from thyme and oregano have been used on cellulosic pads to promote antimicrobial activity against specific meat bacterial species (*Pseudomonas putida*, *Pseudomonas fragi*, *Pseudomonas fluorescens*, *Enterococcus faecalis* and *Lactococcus lactis*), and some common foodborne pathogens (*Salmonella enterica*, *Campylobacter jejuni* and *Staphylococcus aureus*). This antimicrobial activity is attributed to the difference in the reported concentrations of carvacrol (22.9%), thymol (69.2%), linalool (11.1%), and α - and β -pinene (5.02%) [31]. This difference in chemical profile can influence the antibacterial efficiency of the plant, and pathogen susceptibility to EOs. The main EO found in ginger is trans cinnamaldehyde [32], which is also the major component of cinnamon, while citral and carvacrol have been observed as principal chemical compounds from the EO of thyme [33].

3. *In-vitro* and *in-situ* anti-listerial activity of essential oils (EOs)

The effect of the EOs is largely studied on microbial species of food interest, e.g., *Escherichia coli* O157:H7, *Salmonella spp.*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus* and *L. monocytogenes* [27]. The last spp. has been a major interest in many experiments intended to determine the efficiency of the EOs. Several EOs have excellent antimicrobial activity [22]. The potential inhibitory mechanisms regarding microbial growth and reproduction have been shown altered due to the chemical effect of terpenoids, phenolic acids, flavonoids and phenylpropanoids [27]. An effect on leakage has been observed for the cytoplasmic materials, using limonene (20 mL/L) against *L. monocytogenes* [34]. The treatment with limonene at the same concentration effectively inhibited ATP content and ATPase activity [34]. A strong activity (minimum inhibitory concentration [MIC]: 0.70 mL/L) against *L. monocytogenes* by EOs with high amounts of naringenin has been determined [35].

It has been observed that the EOs are more effective on food contaminating gram-positive bacteria, when compared with the gram-negative bacteria [36]. However, this effect might occur due to the hydrophobic properties. Although, some components of the EOs capably break the related bacterial cytoplasmic barrier. The cytoplasmic membrane allows macromolecules to penetrate, causing their intracellular materials for example electrolytes, ATP, proteins and DNA can leak out, causing functional disruptions [27, 36, 37]. The bioactivity of the EOs is defined by the presence of individual chemical compositions [22]. In this aspect, higher antimicrobial activities are attributed mostly to phenol compositions followed by other chemical substances in the given sequence; phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons [38]. For example, the use of cinnamon EO against *E. coli* and *S. aureus* changes the synthesis of major components of the bacterial cell wall and disrupts the integrity and permeability of the plasma membrane [39]. The same effect was observed for thymol, menthol and carvacrol in EOs against different gram-positive bacteria [27]. Studies involving EOs with mechanistic actions against listeriosis demonstrate high potentiality considering their antibacterial effects, as observed in Table 1.

The EOs obtained from *Campomanesia aurea* O. Berg (Myrtaceae) were used against *L. monocytogenes* by showing better bacteriostatic effect at the concentration range of 10-20 mg/mL [29]. This study evaluated the effectiveness of the EOs in the months of October and April. The EOs that comprised a small amount (e.g., 28 metabolites, 4.44%) of key monoterpene (e.g., terpinolene of 3.43%) and sesquiterpene (α -cadinol of 12.79%) in Oct. presented better activities, when compared to those (p-cymene of 8.33% and α -cadinol of 10.72%) characterized in April (e.g., 31 metabolites, 6.15%) [29]. Further evaluation of the EOs from *C. aurea* can be of high relevance, if they are tested against *L. monocytogenes* in all seasons of the year by considering classy extraction procedures, seasonal shift, ecological and further environmental parameters, respectively.

Table 1. Antimicrobial activity of essential oils against *Listeria monocytogenes* in different medium and food matrices.

Source of EO	Extract dilution	Solvent	Tested level	Medium or food type	Assay conditions	Reduction (Log CFU/mL or g)	MIC (mg/mL)	MBC (mg/mL)	Ref.
Campomanesia aurea	1/20	H ₂ O	0.4-4 mg/ml	OAB	<i>L. monocytogenes</i> conc. 1.5×10^8 CFU/ml, incubated at 37 °C for 24 h	NI	10	NI	[29]
Eugenia anomala Psidium salutare	1/10	CH ₃ Cl C ₄ H ₈ O ₂ C ₆ H ₁₄	50, 25, 12,5, 6,25 and 3,125 µg/mL	NI	<i>L. monocytogenes</i> conc. 1.5×10^8 CFU/mL, incubation at 37 °C for 24 h	NI	1,250 312.5	10,000 625	[40]
Cymbopogon flexuosus	1/10	CH ₂ Cl ₂	0.195%, 0.39% and 0.78%	TSB/ BHI	<i>L. monocytogenes</i> conc. 10^8 CFU/mL, incubated at 37 °C for 18 h.	NI	3.9	3.9	[41]
Citrus sinensis	1/10	H ₂ O	0.3-07 µL/g	BHI/ AMH	<i>L. monocytogenes</i> conc. 10^8 CFU/g, incubated at 37 °C for 24 h.	NI	2.6	5.21	[30]
Thymus vulgaris	1/10	C ₂ H ₅ OH	40, 70, 100, 200 mg/mL	BHI	<i>L. monocytogenes</i> conc. 4 log CFU/mL, incubated at 37 °C for 24 h.	NI	200	NI	[33]
Zingiber officinale	1/24	H ₂ O	12%	Minas cheese	1.5×10^8 CFU/g 37 °C for 24 h 12 days storage	4.39 to 3.62	2.3	4.7	[32]
Melaleuca alternifolia	0.8/100	H ₂ O	1.5% (v/w)	Ground meat	1.5×10^8 CFU/mL 4.6×10^4 CFU/mL 9.2×10^3 CFU/mL 1.2×10^2 CFU/mL & Storage at 4 °C for 14 days	No effect 2.41 No growth	0.1	0.15	[12]
Thymus capitata	0.02; 0.06; 0.1; 1; 1.5; 2 and 3%	10% DMSO	0.01% (v/w), 0.05% (v/w), 0.25% (v/w) & 1.25% (v/w)	Minced meat	<i>L. monocytogenes</i> inoculation at conc. 10^5 CFU/g Samples stored at 7 °C were examined for <i>L. monocytogenes</i> at days 0, 3, 6, 9, 12, and 15	NI Ni 1.45 1.13	5.0	NI	[42]
Zataria multiflora Boiss	NI	H ₂ O (Peptone)	0.3; 0.5; 1 and 2 mL/100 g beef	Beef Meat	10^3 CFU of <i>L. monocytogenes</i> /g Stored at 7 °C for 3, 5, 7 and 9 days	After 9 days storage: 4.13 (0.3%) 3.83 (0.5%) 3.13 (1%) 2.70 (2%)	625	1250	[43]
Rosmarinus officinalis L. Thymus vulgaris L.	0.1%	H ₂ O (Peptone)	1.25% (Rosemary, v/w) 0.08% (Thyme, v/w)	Beef Meat	1×10^8 CFU/mL <i>L. monocytogenes</i> . Stored at 2 or 8 °C for 1, 2, 3, 7, 14, 21 and 28 days	After 28 days of storage: Thyme: 5.05 Rosemary: 3.16	3.9 (Thyme) 62.5 (Rosemary)	NI	[44]
Juniperus communis Satureja montana	1/30	C ₂ H ₅ OH	0.125%; 0.25%; 0.5% (<i>J. communis</i>) 0.0625%; 0.125%; 0.25% (<i>S. montana</i>)	Beef Meat	6 log CFU/mL of <i>L. monocytogenes</i>	After 17 days storage: <i>J. communis</i> : 3.00 <i>S. montana</i> : 2.80 <i>J. communis</i> + <i>S. montana</i> : 2.50	0.1	<i>J. communis</i> : 0.4 <i>S. montana</i> : 0.1	[45]

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; N: no bactericidal effect observed; NI: Not informed; Conc.: concentration; OAB: oxford agar base; AMH: agar Muller-Hinton; TSB: tryptic soy broth; BHI: brain heart infusion.

The EOs from *Eugenia anomala* and *Psidium salutare* (Myrtaceae) were tested at the level of 100 µg/mL that represented adequate antilisteral consequences [40]. In line with these assessments for *L. monocytogenes* and other agents (e.g., *E. anomala*, *P. salutare* and *E. coli*), limited solvent-based extraction requires further elaboration. Because in-depth extraction methodologies of the stated EOs with a complete set of active metabolites can complement the food industry. Simply because the chemical profiles in these species have not been explored, yet they have definitely proven efficient antimicrobial (MIC: 312.5 mg/mL) capacities against the given microorganisms [40].

Antimicrobial and antibiofilm activities have been observed regarding the main components of EOs extracted from leaves of *Cymbopogon flexuosus*. These activities are determined against *S. aureus*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium* and *Listeria monocytogenes*. EOs against *L. monocytogenes*, *S. aureus* and *S. Typhimurium* have represented MIC and MBC values of 3.9 µL/mL, showing satisfactory antimicrobial activity, even at the lowest concentration (1:10 w/v). For the antibiofilm activity, a significant reduction ($P < 0.05$) was observed for *S. typhimurium* and *S. aureus*. Biofilm biomass significantly reduced only for *S. aureus* and *P. aeruginosa* [41]. These bioactivities are attributed to the major metabolites-e.g., geranial and neral (isomeric mixture of citral) from the terpenoids family in *C. flexuosus*. Sufficient quantities for both geranial (41.8%) followed by neral (33.2%) were characterized. In addition, the proportions of the EOs have been dependent on leaf growth, inflorescence and biomass production. Comparatively, a high level of citral (75%) with good quality EOs was screened in the nascent leaf, compared to that in mature ones. However, the resistance of *L. monocytogenes* has been described at all concentrations, and this can be based on the variability in the antibacterial activities of metabolites that varies according to bacterial types and more settings [41], which should be considered when these EOs are introduced to the food industry.

In addition, the EOs from thyme (at 1:10 concentration w/v) have been tested and proven that these can capably produce lethal effects on *L. monocytogenes*, by suggesting combinations with other microbiological barriers in order to reduce the required amount of EO [33]. The EOs of *Zingiber officinale* (ginger) was efficient regarding antibacterial effects against *L. monocytogenes* in the food packaging of Minas cheese [32]. The use of corn starch film containing orange EO also showed high antibacterial activity against *S. aureus* and *L. monocytogenes* [30]. Regarding the concentrations used and the observed effects, some EOs proved to be effective, even at lower concentrations. *Melaleuca alternifolia* EO showed antimicrobial activity and preserved beef at low concentrations (0.8:100 (w/v)) [12]. The same result was found in a study analyzing cucumber juice, in which the growth of *L. monocytogenes* showed an inhibition halo of 13.6 to 34.3 mm with a concentration of 0.1% (v/v) [46]. Antimicrobial potential of EOs from *Melaleuca alternifolia* has been shown in inhibiting *L. monocytogenes* in meat product [12]. It has implied that the EOs of *M. alternifolia*, even at low concentrations, is a possible antimicrobial agent for beef preservation, and can be tested also in further food products [12].

Antimicrobial activity with a low concentration (MIC: 3.90 µL/mL) of the EOs was observed without efficient reduction of biofilm [41]. A moderate anti-listerial effect at MIC of 5.0 µL/mL was found for the EOs from *Juniperus communis* and *Satureja montana* [45]. The exposure to 100 mg/L of carvacrol to *L. monocytogenes* cells, reduced the viable cell load by one logarithmic cycle [33]. Analyzing *J. communis* and *S. montana* as antibacterial in beef meat, the results showed that after 17 days of storage the lowest CFU/g ($< 1 \log \text{CFU/mL}$) was in the association with *J. communis* 0.25% + *S. montana* 0.125%, diluted in ethanol (1 µL) and injected in a split-mode (1:30) [45]. But on the other hand, higher concentrations of EO from *Croton blanchetianus* on fresh meat were required to inhibit bacterial growth.

4. Applicability of essential oils against *Listeria monocytogenes* in the food industry

A key issue in the food industry is to deliver safer food to consumers. In this regard, the food industry has generally used synthetic preservers to increase food protection against damaging microorganisms. However, in terms of consumer choice, the food conserved with synthetic additives can be less satisfactory. An optimal safety grade for meeting the consumer's demand have motivated the food industry to employ natural food protectors such as the EOs.

There is a trend in finding natural solutions for food protection regarding healthier, organic and cleanly labeled foods [47]. In this aspect, the challenges are maintaining consumer's acceptance, low cost and good functionality [48],

which may be achieved through the use of plants' products such as the EOs. EOs have been greatly used in preserving food products because they provide antimicrobial features based on its chemical contents, such as phenolic acids, terpenes, aldehydes and flavonoids [49]. In line with that, the EOs have been evaluated against the occurrence of *L. monocytogenes* in the meat and poultry products [49]. In order to preserve food and enhance its shelf life, the EOs are applicable and can be used in multiple ways [50]. For example, they can be used either in pure or as formulation in different storage containers, knowingly the cardboard, tin, glass, polyethylene and natural materials [51]. However, besides many benefits, the EOs can negatively impact sensory characteristics of certain foods, even at low concentration [52]. For proper antimicrobial effects, the EOs should be implemented usually in higher concentration, and this can be a limiting factor [50]. However, to diminish such negative effect(s), alternatively EOs can be used in packaging, or as encapsulated polymers, entrapped sachets or in nano-emulsions [53-54].

The bioactivity of EOs can be reduced by some components in food containing fats, carbohydrates and proteins [38]. High concentrations of fats or proteins in the food matrix can avoid bacterial effects, because these contents provide a protective layer and absorb EOs [55]. Similarly, the starch and sunflower oil exerted a negative effect on the bioactivity of oregano and thyme against *L. monocytogenes*, while proteins affected it in a positive way [38]. High levels of fat in the milk reduced the bioactivity of cinnamon and clove EOs against *L. monocytogenes* [56].

Additionally, packaging is an effective and simple way to promote food quality and safety, as they can prolong foods' shelf lives [57]. It is suggested that the use of EOs on packages can promote antimicrobial activity against *L. monocytogenes* [58]. The association between sustainable food packing using renewable sources and nanoparticles with antimicrobial properties play a key role in sustainability and performance of food packing [47, 59]. The use of ZnO nanoparticles associated with EO of oregano on cellulose nanofibrils films have promoted antimicrobial activity (89.6%) against *L. monocytogenes*, also enhancing barriers properties against oxygen and water [58]. Other studies with silver nanoparticles using chitosan-based films with blending essential oils (Asian formulation, Mediterranean formulation, citrus extract and cinnamon) showed strong antimicrobial activity against *L. monocytogenes* (3.9 log reduction) [60]. The chitosan nanoparticles with clove essential oil have antioxidant capacity and antimicrobial activity against *L. monocytogenes*, having a potential to be used in active packaging [61]. Other active packages made by lemon waste powder, obtained from lemon juice byproducts, incorporated with cellulose nanofibers can offer good antibacterial protection against *L. monocytogenes* [62]. Biofilms formed by micellar-encapsulated eugenol and carvacrol showed a potential to control the growth of *L. monocytogenes* on food contact surfaces [63].

Nanoencapsulation of EOs is a viable and efficient approach to enlarge physical stability of the active substances, avoiding the interactions with the food ingredients and increasing their bioactivity [54]. The nano-encapsulated *Eucalyptus staigeriana* essential oil demonstrated antimicrobial activity against *L. monocytogenes*, with a minimum bactericidal concentration of 2 or 3 g/L of oil [64]. Using *Origanum Dictamnus* L. (thymol and carvacrol) encapsulated with phosphatidylcholine enhanced the antimicrobial activities against *L. monocytogenes*, compared with free forms of EOs [65]. The nano-encapsulated lime EO also showed enhanced antibacterial activity against *L. monocytogenes* [66].

Nano-emulsion reduces the surface properties of oil droplets and surface charge because of the improvement in physical and dispersion stability of the emulsions [54, 67]. In this context, the nano-emulsion of eugenol with whey protein isolate and maltodextrin showed antimicrobial activity against *L. monocytogenes* with better distribution and solubility in the food system [67]. Also, the nano-emulsion of peppermint oil had antimicrobial activity against *L. monocytogenes* for up to 30 days of storage [68].

5. Conclusion

The use of EOs is a promising field with regard to the culture of food safety, since many of them are obtained from easily accessible herbs, and the process of acquiring them does not express a limitation. Studies even suggested that oils extracted by hydrodistillation can be used on industrial scale, but there are still limitations, such as the lack of studies on the toxicity of their compounds. For this reason, it is proposed that such compounds and solvents must be tested, to determine a maximum limit of their toxic effects in food products. The bacteriostatic and bactericidal activities of EOs against *L. monocytogenes* reported in the literature indicate great potential applicability of these compounds in different food products, as well as active components in functional food packages. However, the antibacterial efficacy of the EOs depends on several parameters, such as seasonal variations in the harvest of plants, extraction methodologies including

types of solvents, and experimental conditions for testing the antibacterial effect on *L. monocytogenes*, and interactions with the food matrix. Future studies on the interactions of EOs with different food matrices and their potential effects on sensory attributes are highly recommended, aiming at specific applications of EOs in food products.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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