Antimicrobial Mechanisms of Citral

Subjects: Biotechnology & Applied Microbiology

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Citral is a monoterpene constituted by two isomers known as neral and geranial. It is present in different plant sources and recognized as safe (GRAS) by the Food and Drug Administration (FDA). Investigations have demonstrated that this compound exhibited several biological activities, such as antibacterial, antifungal, antibiofilm, antiparasitic, antiproliferative, anti-inflammatory, and antioxidant properties, by in vitro and in vivo assays. Additionally, when incorporated into different food matrices, citral can reduce the microbial load of pathogenic microorganisms and extend the shelf life.

Keywords: citral ; biological activities ; food additive ; pharmaceuticals

1. Introduction

Citral is a linear monoterpene aldehyde, present in more than 85% of lemongrass essential oils. This compound is also found in a wide diversity of plant leaves and fruits, such as limes, oranges, lemons, tomatoes, myrtle trees, and African basil [1]. Citral is a mixture of two isomers named neral (cis-3,7-dimethyl-2,6-octadien-1-al) and geranial (*trans*-3,7-dimethyl-2,6-octadien-1-al). Typically, commercial compositions between both compounds are complementary, and this mixture ranges from 48 to 52% of each one [2]. Additionally, this compound is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and is commonly used as a citrus base flavoring in different products [3].

Citral has exhibited a broad spectrum of biological activities, where it has been described as an effective antimicrobial agent against different Gram-positive and Gram-negative bacteria, fungi, and parasites of clinical relevance [4][5]. Recent studies have shown that citral has an inhibitory effect against planktonic cells and can also affect biofilms produced by a different microorganism of clinical and food relevance [6][7]. Furthermore, other biological activities have also been demonstrated, such as its antiproliferative effect against human and murine cancer cell lines and anti-inflammatory and antihypertensive properties [8][9][10]. In addition, the capability to stabilize synthetic and biological free radicals and reduce activity has also been reported [11][12].

On the other hand, different studies have analyzed the effect of citral as a food additive incorporated into meat products, fish, fruits, vegetables, juices, and bread [13][14][15][16][17]. Overall, it has been observed that citral effectively reduced the growth and proliferation of pathogenic and deteriorative microorganisms and delayed oxidative processes [13][14][15][16]. In turn, citral extended the shelf life of the added foods. These results promote the possibility of the food industry's effective and safe use of citral based on studies demonstrating its low cytotoxicity [18].

2. Biological Activities

2.1. Antibacterial Activity

One of citral's most relevant biological activities is its antibacterial effect, evidenced against Gram-positive and Gram-negative bacteria (**Table 1**). In this way, citral obtained from the *Litsea cubeba* (L.) plant was used in the formulation of food packaging films, showing that citral coatings achieved bactericidal effects against *Escherichia coli* (2.1 log) and *Staphylococcus aureus* (4.3 log) at concentrations of 20%. Headspace equilibrium concentrations of 1.8 µg/mL air were found for 20% citral coatings, resulting in a 3.8 log reduction against *E. coli* [19]. Citral, also obtained from *C. flexulosus*, was used against four strains of *Acinetobacter baumannii* with resistance to antimicrobials (MDR = multi-drug resistant), finding that all the tested strains were susceptible to citral with zones of inhibition that varied between 17 and 80 mm. Additionally, citral's minimum inhibitory concentration (MIC = 0.14% *v*/*v*) and the minimum bactericidal concentration (MBC = 0.3% *v*/*v*) were determined. The ability of citral to inhibit and kill MDR *A. baumannii* highlights its potential for use in treating drug-resistant infections.

Table 1. Antibacterial and antifungal activities of citral.

Microorganism	Dose (MIC)	Effect	Ref.
Bacteria			
V. parahaemolyticus	**0.125 mg/mL**	**Inhibited bacterial growth, causing damage to bacterial membrane and cell wall.**	[20]
S. aureus DMST 4745 ***S. aureus*** ***S. agalactiae*** ***B. cereus*** ***E. coli***	**0.62–1.25 µL/mL** **0.62–1.25 µL/mL** **0.31–0.62 µL/mL** **0.15 µL/mL** **1.25–2.5 µL/mL**	**Citral possessed bacteriostatic and bactericidal actions at different concentrations.**	[21]
***E. coli* MG1655**	**300 µL/L**	**It inactivated at least 2.5 $\log_{10}$ cycles of exponentially growing cells in 3 h under aerobic conditions.**	[22]
L. monocytogenes ***S. aureus*** ***E. coli***	**200 µL/mL** **500 µL/mL** **500 µL/mL**	**Growth inhibition.**	[23]
L. monocytogenes ***L. innocua***	**0.125 mL/mL** **0.125 mL/mL**	**Microbial growth of both *Listeria* species was reduced by almost 2 $\log_{10}$ CFU/mL.**	[24]
L. innocua ***L. monocytogenes***	**100 µL/mL**	**Citral in the culture medium of both bacteria provided a reduction of bacitracin from 32 µg/mL to 4 µg/mL, and the colistin changed from 96 and 128 µg/mL for *L. monocytogenes* and *L. innocua*, respectively, to 16 µg/mL, for both species.**	[25]
***Salmonella* Typhimurium**	**3.1 mM**	**Citral at subinhibitory concentrations (1, 2, and 3 mM) could induce bacterial adaptation and acquire tolerance to inactivation processes.**	[26]
Fungi			
B. dothidea ***P. macrospore*** ***B. cinerea***	**0.2 µL/mL** **0.2 µL/mL** **0.4 µL/mL**	**At 0.4 µL/mL, citral entirely inhibited the growth of all the tested fungi. When concentration reached 0.2 µL/mL, citral inhibited the growth of *B. dothidea* best, followed by *P. macrospore* and *B. cinerea.***	[27]
C. sakazakii	**0.8 mg/mL**	**Growth inhibition and cell damage.**	[28]
	3600 µM	**Concentrations below 225 µM (1/16 MIC) exhibited no inhibition against *C. sakazakii* ATCC 29544.**	
Penicillium roqueforti	**0.17 mg/mL**	**Citral combination with eugenol damaged the cell membrane, caused a collapse of mitochondria, and inhibited energy production.**	[16]
Penicillium digitatum	**2.0 or 4.0 µL/mL**	**Citral altered the mitochondrial morphology, led to the leakage of ATP, and showed an inhibition of the TCA pathway of *P. digitatum* cells.**	[29]
S. cerevisiae	**2.0 mM**	**MIC: Results showed that yeast cells treated with 2 mM citral reached a 95% reduction in CFU/mL.**	[30]
Zygosaccharomyces rouxii.	**0.188 µL/mL**	**The minimum fungicidal concentration was 0.375 µL/mL.**	[31]
Candida albicans	**64 µg/mL**	**The minimum fungicidal concentration was 256 µg/mL. The MIC and the MFC of citral required only 4 h of exposure to effectively inhibit 99.9% of the inoculum.**	[32]
Aspegillus niger	**0.23 mg/mL**	**The combination of citral and eugenol had a synergistic inhibitory effect on *A. niger*.**	[16]

ATP: adenosine triphosphate; TCA: tricarboxylic acid cycle; MIC: minimal inhibitory concentration; MFC: minimal fungicidal concentration.

2.2. Antifungal Activity

Citral stands out for its broad-spectrum antifungal effect, and its mechanisms of action have been extensively studied (**Table 2**, **Figure 1**). Recently, *Alternaria alternata*, an important foodborne pathogen, was completely inhibited with a citral dose of 250 µL/L corresponding to the MIC. With a citral dose of ½ MIC, the biosynthesis of the polyketide mycotoxin (alternariol) and its derivative (alternariol monomethyl ether) was also suppressed up to 97% [33]. The transcriptomic profile revealed that citral provoked cell integrity disturbance, specifically by disrupting fungal spores and inhibiting the biosynthesis of ergosterol, a major structural constituent of fungal cell membranes. Interestingly, 41 over-expressed and 84 repressed proteins of *Penicillium digitatum* were identified by isobaric tags for the relative and absolute quantitation technique (iTRAQ) when 1.0 µL/L of citral was applied to the fungus for 30 min [34]. The authors suggested that the citral mechanism involved membrane damage of *P. digitatum* cells and the disruption of oxidative phosphorylation, which is the first way to produce energy in eukaryotic cells. In another study reported by Tang, Chen [35], citral also showed inhibitory action by downregulating the genes related to the sporulation and growth of *Aspergillus ochraceus* and *A. flavus*. While on *A. ochraceus*, the synthesis of proteins associated with the mycelial growth was altered, achieving a fungal complete inhibition at 200 µL/L and the reduction of production of mycotoxin ochratoxin A, a 2B-categorized mycotoxin related to cancer in humans, with a dose of 75 µL/L of citral [27]. Given the broad antifungal activity of citral, its potential as an innovative barrier for controlling fungi in agri-food systems is confirmed. However, further research is needed to explore its incorporation into different products. Factors such as vehicles, specific doses, and bioactivity in each system must be considered during the study.

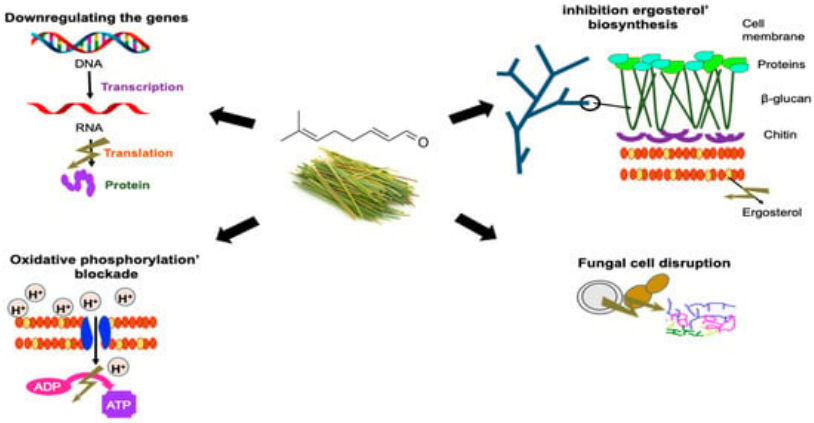

Figure 1. Mechanisms of action of citral as an antifungal agent [27][29][30].

Table 2. Antifungal mechanisms of action proposed for citral.

Microorganism	Mechanism	Ref.
***Saccharomyces cerevisiae* BY4741**	**Loss of membrane and cell wall integrity results in a typical apoptotic/necrotic cell death. However, yeast cells that escape this first cell membrane disruption, particularly evident in sub-lethal concentration, die by metacaspase-mediated apoptosis induced by the accumulation of intracellular ROS.**	**[30]**
B. dothidea	**Changes in the morphological characteristics of fungal hyphae, resulting in loss of cell content and distortion of the mycelium. Increase in membrane permeability, with increases in extracellular electrical conductivity and a decrease in soluble protein content. A decrease in the range of ergosterol levels showed that citral altered the physiology of the cell membrane. Reduction in the level of enzymes associated with respiration, resulting in the disruption of energy metabolism.**	**[27]**
Aspegillus ochraceus	**Citral downregulated ochratoxin biosynthetic genes, including *pks* and *nrps*, but slightly upregulated global regulatory factors *veA*, *velB*, and *laeA*.**	**[27]**
Aspegillus niger	**Direct damage to the cell membranes of *A. niger* may explain the antimicrobial activity of citral combined with eugenol. Among the two components, eugenol is mainly responsible for the permeability of damaged cell membranes, whereas citral mainly causes membrane lipid peroxidation, which leads to a burst in ROS.**	**[16]**
Penicillium roqueforti.	**The combination of citral and eugenol destroyed the integrity of the cell membrane and internal structures and degraded the cell content. The combination induced membrane lipid peroxidation and promoted the ability to destroy the cell membrane. The combined agents eventually caused leakage of cell contents and, ultimately, cell death.**	**[16]**
Penicillium digitatum	**Citral can affect the mitochondrial morphology and function of *P. digitatum*, inhibiting the respiratory metabolism, decreasing the activities of TCA-related enzymes, and changing the TCA metabolic abilities.**	**[29]**

Microorganism	Mechanism	Ref.
Zigosachamomyces rouxii	The antifungal effect can be attributed to the alteration of the integrity and permeability of the cell membrane, which can cause irreversible damage to the cell wall and membrane. They can also destroy yeast proteins and inhibit their synthesis.	[31]

2.3. Antiproliferative Effect against Cancer Cells

The effect of citral against the proliferation of cancer cells has been extensively reported (**Table 3**). Studies on colorectal cancer cells showed that citral inhibits the growth of HCT116 and HT29 cell lines in a dose- and time-dependent manner without inducing effect in the non-cancerous colon cells CCD841-CoN [9]. Citral reduces the proliferation of the colon cancer cells Caco-2 notoriously [9]. Mohd Izham, Hussin [36] reported that the nano-emulsifying drug delivery system loading with citral (CIT-SNEDDS) was more effective than citral in inhibiting the proliferation of SW620 colorectal adenocarcinoma cells after 72 h of treatment; however, at short times (24 and 48 h), citral was more effective than CIT-SNEDDS to reduce the growth of this cell line. In HT29 cells, citral was more effective than CIT-SNEDDS in reducing cell proliferation. On the other hand, citral was more effective than the antineoplastic drug Cisplatin in reducing the proliferation of the human stomach cancer cells AGS; shrunken cells and generation of shapeless cells were observed after the treatment of AGS cells with citral, which could be correlated with the activation of cell death by apoptosis, where contraction, cell rounding, and the formation of membrane blebs are characteristic. Furthermore, citral showed less cytotoxicity than Cisplatin against the lung fibroblast non-cancerous MRC-5 [9].

Table 3. Antiproliferative activity of citral.

Compound/ Extract	Doses	Effect	Ref.
Citral	145.32 µg/mL 85.47 µg/mL 52.63 µg/mL	Inhibition of HCT116 cell proliferation (IC_{50}: 24, 48, and 72 h)	[37]
Citral	181.21 µg/mL 143.61 µg/mL 91.5 µg/mL	Inhibition of HT29 cell proliferation (IC_{50}: 24, 48, and 72 h)	[37]
Citral	3.125–200 µM	Inhibition of CCD841-CoN cell (IC_{50} not detected at 200 µM)	[37]
Citral	3.7 µg/mL	Inhibition of Caco-2 cell proliferation (IC_{50}: 72 h)	[38]
CIT-SNEDDS	38.50 µg/mL 23.75 µg/mL 16.50 µg/mL	Inhibition of SW620 cell proliferation (IC_{50}: 24, 48, and 72 h)	[36]
CIT-SNEDDS	44.10 µg/mL 36.60 µg/mL 34.10 µg/mL	Inhibition of HT29 cell proliferation (IC_{50}: 24, 48, and 72 h)	[36]
Citral	31.25 µg/mL 23.30 µg/mL 22.50 µg/mL	Inhibition of SW620 cell proliferation (IC_{50}: 24, 48, and 72 h)	[36]
Citral	28.33 µg/mL 22.00 µg/mL 21.77 µg/mL	Inhibition of HT29 cell proliferation (IC_{50}: 24, 48, and 72 h)	[36]
Citral	<25 µg/mL	Inhibition of AGS cell proliferation (IC_{50}: 48 h)	[39]
Citral	>75 µg/mL	Inhibition of MRC-5 cell proliferation (IC_{50}: 48 h)	
Citral	1.04 µM	Inhibition of B16F10 cell proliferation (IC_{50}: 24 h)	[40]
Citral	11.7 µM	Inhibition of SK-MEL-147 cell proliferation (IC_{50}: 24 h)	
Citral	13.4 µM	Inhibition of UACC-257 cell proliferation (IC_{50}: 24 h)	
Citral	50.3 µM	Inhibition of HaCaT cell proliferation (IC_{50}: 24 h)	
Citral	2.5 µM	Inhibition of NIH-3T3 cell proliferation (IC_{50}: 72 h)	
Citral	7 µg/mL	Inhibition of HepG2 cell proliferation (IC_{50}: 72 h)	[38]
Citral	1.3 µg/mL	Inhibition of MCF-7 cell proliferation (IC_{50}: 72 h)	

Compound/ Extract	Doses	Effect	Ref.
Citral	71.90 μM 57.11 μM 50.20 μM	Inhibition of KKU-M213 cell proliferation (IC_{50}: 24, 48, and 72 h)	[41]
Citral	94.43 μM 75.06 μM 58.92 μM	Inhibition of HuCCA-1 cell proliferation (IC_{50}: 24, 48, and 72 h)	[41]
Citral	87.53 72.17 69.22	Inhibition of MMNK-1 cell proliferation (IC_{50}: 24, 48, and 72 h)	[41]
Citral	10 μg/mL	Inhibition of PC-3 cell proliferation (IC_{50}: 72 h)	[42]
Citral	12.5 μg/mL	Inhibition of PC-3M cell proliferation (IC_{50}: 72 h)	[42]
Citral	>75 μg/mL	Inhibition of MRC-5 cell proliferation (IC_{50}: 72 h)	[42]
Citral	238 μM	Inhibition of PaCa-2 cell proliferation (IC_{50}: 72 h)	[43]
Citral	300 μM	Inhibition of DeFew cell proliferation (IC_{50}: 72 h)	[43]
Citral	5, 10, 20, 40 μg/mL	Inhibit colony formation and migration of AGS (96 h)	[39]
Citral	5, 10, 20, 30, 40 μg/mL	Inhibit colony formation and migration PC-3 (96 h)	[42]
Citral	17.5 and 35 μM	Increase the surviving fraction of KKU-M213 in 106.75 and 115.64% (168 h)	[41]
Citral	23.5 and 47 μM	Decrease the surviving fraction of HU-CCA-1 in 76.35 and 57.71% (168 h)	[41]
Citral	24 and 48 μM	Decrease the surviving fraction of MMNK-1 in 98.46 and 85.26% (168 h)	[41]
Citral	0.25, 0.375, 0.50 mM 0.25, 0.375, 0.50 mM	Decrease the clonogenicity of HaCaT in 0.3, 4, and 7% (3 h) Decrease the clonogenicity of HaCaT in 22, 28, and 30% (8 h)	[44]
Citral		Decrease the clonogenicity of M624 in 20, 38, and 50% (3 h)	[44]
Citral	50 μM 100 μM 200 μM	Early apoptosis (17.1%), late apoptosis (3.1%) in HCT116 (24 h) Early apoptosis (14.2%), late apoptosis (15.1%) in HCT116 (24 h) Early apoptosis (26.2%), late apoptosis (25.8%) in HCT116 (24 h)	[37]
Citral	50 μM 100 μM 200 μM	Early apoptosis (22.3%), late apoptosis (16.1%) in HCT116 (48 h) Early apoptosis (26.2%), late apoptosis (24.6%) in HCT116 (48 h) Early apoptosis (32.1%), late apoptosis (37.5%) in HCT116 (48 h)	[37]
Citral	50 μM 100 μM 200 μM	Early apoptosis (6.5%), late apoptosis (3.9%) in HT29 (24 h) Early apoptosis (8.5%), late apoptosis (14.2%) in HT29 (24 h) Early apoptosis (8.4%), late apoptosis (24.9%) in HT29 (24 h)	[37]
Citral	50 μM 100 μM 200 μM	Early apoptosis (14.5%), late apoptosis (7.1%) in HT29 (48 h) Early apoptosis (22.7%), late apoptosis (17.8%) in HT29 (48 h) Early apoptosis (30.5%), late apoptosis (23.5%) in HT29 (48 h)	[37]
Citral	10 and 20 μg/mL	Induce early and late apoptosis in AGS	[39]
Citral	1 μM	Apoptosis induction by annexin V-FITC/PI staining in B16F10 (24 h)	[40]
Citral	0.5, 1, and 2 μM	Apoptosis induction by TUNEL assay in B16F10 (24 h)	[40]
Citral	10 μg/mL 20 μg/mL	Early apoptosis (44.1%), late apoptosis (52.6%) in PC-3 (48 h) Early apoptosis (62.2%), late apoptosis (38.4%) in PC-3 (48 h)	[42]
Citral	50, 100, and 200 μM	Disruption of MMP (19.5, 38.8 and 60.9%) in HCT116 (24 h)	[37]
Citral	50, 100, and 200 μM	Disruption of MMP (34.9, 56.4 and 77.3%) in HCT116 (48 h)	[37]

Compound/ Extract	Doses	Effect	Ref.
Citral	50, 100, and 200 µM	Disruption of MMP (20.4, 28.2 and 41.9%) in HT29 (24 h)	[37]
Citral	50, 100, and 200 µM	Disruption of MMP (24.5, 43.9 and 59.9%) in HT29 (24 h)	[37]
Citral	50, 100, and 200 µM	Increase intracellular ROS level (1.26, 2.07, and 3.19 folds) in HCT116 (4 h)	[37]
Citral	50, 100, and 200 µM	Increase intracellular ROS level (1.21, 1.39, and 2.25 folds) in HC29 (4 h)	[37]
Citral	50, 100, and 200 µM	Decrease intracellular GSH level in HCT116 (4 h)	[37]
Citral	50, 100, and 200 µM	Decrease intracellular GSH level in HT29 (4 h)	[37]
Citral	1 µM	Autophagic vacuole induction formation in B16F10 (24 h)	[37]
Citral	0.5, 1, and 2 µM	DNA damage in B16F10 (24 h)	[40]
Citral	2.5 µM	Reduction of malondialdehyde level in B16F10 (24 h)	[40]
Citral	10 and 20 µg/mL	Inhibition of lipid droplet accumulation in PC-3 (48 h)	[42]
Citral	50, 100, and 200 µM	Down-expression of Bcl-2 and Bcl-xL proteins in HCT116 (24 h) High expression of Bax, p53, and caspase-3 proteins in HCT116 (24 h)	[37]
Citral	50, 100, and 200 µM	Down-expression of Bcl-2 and Bcl-xL proteins in HT29 (24 h) High expression of Bax, p53, and caspase-3 proteins in HT29 (24 h)	[37]
Citral	0.5 and 1 µM	Down-expression of ERK1/2, PI3K, AkT in HCT116 (24 h) High expression of p53 in HCT116 (24 h)	[40]
Citral	1 µM	Increase cytoplasmatic NF-κB in B16F10 (24 h)	[40]
Citral	1 µM	Decrease nuclear translocation of NF-κB in B16F10 (24 h)	[40]
Citral	0.25, 0.375, 0.5 mM	Caspase-3 activation in M624 (3 h)	[44]
Citral	0.25, 0.375, 0.5 mM	Caspase-3 activation in HaCaT (3 h)	[44]
Citral	20 µg/mL	Down-expression of HMGR, SREPB1, and ACC proteins in PC-3 (48 h) Up-expression of AMPαK in PC-3 (48 h)	[44]
Citral	5, 10, and 20 µg/mL	Down-expression of BCl-2 in PC-3 (48 h) and high expression of BAX proteins in PC-3 (48 h)	[44]
Citral	Not reported	mRNA upregulate in AGS (48 h): MAPK, Nf-κB, PI3K-Akt, p53, and other signaling pathways. Spliceosoma, apoptosis, and prostate cancer, among others.	[39]
	Not reported	mRNA downregulate in AGS: NF-κB, PI3K-Akt, p53, PPAR, among other signaling pathways. Cell cycle, fatty acid metabolism, and proteoglycans in cancer, among others.	[39]
Citral	5, 10, and 20 µg/mL	Down-expression of *HMCR*, *ACC*, *FASN*, and *SREPB1* mRNAs in PC-3 (48 h)	[42]

CIT-SNEDDS: Nano-emulsifying drug delivery system loading with citral. IC_{50}: half-maximal inhibitory concentration.

2.4. Anti-Inflammatory

The anti-inflammatory potential showed by citral has been previously reported (**Table 4**). Martins, Selis [10] evaluated the anti-inflammatory activity of citral in male mice infected with *S. aureus*. *This bacterium* is one of the most pathogenic species of the staphylococci group and causes several diseases, such as skin diseases, bacteremia, septic arthritis, and respiratory infections. *S. aureus* triggers inflammation and recruitment of neutrophils, critical responses for pathogen clearance but associated with substantial tissue damage. Results indicated that citral inhibited the expression of NO synthase and some features of acute inflammation, such as monocyte numbers and the gene transcription of the pro-

inflammatory cytokine TNF-α. The anti-inflammatory activity of citral in RAW 264.7 cells (mouse macrophages, Abelson murine leukemia virus-induced tumor) in the presence and absence of lipopolysaccharide (LPS) was evaluated by Zielińska, Martins-Gomes [45]. Citral inhibited NO production in 84 and 99% at the lowest (5 µg/mL) and highest tested concentrations (20 µg/mL).

Table 4. Anti-inflammatory activity of citral, its isomers, and citral-rich EOs.

Citral/EO Citral Rich/Constituent	Concentration	Animal/Cell Line Tested	Results	Ref.
Citral	5–100 µg/well	Peritoneal macrophage of male BALB/c mice	50 and 100 µg of citral significantly inhibited IL-1β and IL-10 release and LPS activation. IL-6 production by macrophages significantly decreased at citral concentrations of 5, 10, 25, 50, and 100 µg/well).	[46]
Citral	0.36, 0.15, and 0.06 g/kg	MRSA-infected mice	Citral significantly reduced the levels of TNF-α, IL-6, IL-1β, malondialdehyde, and hydroxyl radicals. Increased superoxide dismutase and glutathione enzyme levels. Reduced the lung inflammatory infiltrates infected by MRSA.	[47]
Citral	300 mg/kg	Diabetes-induced rats	Gene expression of IL-6 and TNF-α in the liver were significantly downregulated.	[48]
Citral	50–300 mg/kg	Paw edema-induced mice	Reversed paw edema formation in mice induced by LPS and zymosan, inducers of TLR4 and TLR2 signaling.	[49]
Citral	300 mg/kg	Eutrophic and obese mice	Citral reduced TNF-α and serum leptin concentration after the LPS challenge. IL6 levels in the hypothalamus of obese mice were reduced.	[50]
Citral	125, 250, and 500 mg/kg	Male Swiss mice	Citral reduced NO production and inhibited neutrophil migration in liver.	[51]
Citral	10, 20, and 40 mg/kg 3, 6, and 12 µg/mL	Mice with LPS-induced acute lung injury Alveolar macrophages	On in vivo LPS-induced acute lung injury, citral reduced TNF-α, IL-6, and IL-1β production. In vitro, citral inhibited the production of TNF-α, IL-6, and IL-1β in alveolar macrophages. The mechanism was associated with PPAR-γ activation.	[52]
Citral, neral, and geranial	66 µM	Murine J774A.1 macrophages	Citral inhibited TNF-α and IL-6. Pure neral inhibited TNF-α secretion by 60–80%, whereas geranial 57–75%. Both neral and geranial reduced IL-6 secretion of LPS-stimulated macrophages and the expression of inflammatory mediators IL-1β, iNOS, COX-2, and NLRP-3.	[53]
Citral-rich fractions of Citrus lemon EO	0.005, 0.01, and 0.02%	Murine macrophage RAW264.7 cell line	Reduced the expression of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in LPS-induced macrophages.	[54]
Cymbopogon citratus EO	0.1%	Pre-inflamed human dermal fibroblasts	Significantly inhibited the production of the inflammatory biomarkers: vascular cell adhesion molecule 1 (VCAM-1), interferon gamma-induced protein 10 (IP-10), interferon-inducible T-cell alpha chemoattractant (I-TAC), and monokine induced by gamma interferon (MIG).	[55]
Myrcia ovata EO	200 and 300 mg/kg	Male Swiss mice with induced acute inflammation	Reduced leukocyte extravasation and inhibited TNF-α production by 50% and 69% at both concentrations, as well as IL-1β production by 47%.	[56]

2.5. Antiparasitic

Several studies have demonstrated the effect of citral against different stages of trypanosomatids (**Figure 2**). Santoro, Cardoso [8] observed that citral affected the growth of the epimastigotes and trypomastigotes of *Trypanosoma cruzi* with an IC_{50} of 42 µg/mL and 142 µg/mL after 24 h of incubation, respectively. Furthermore, 50 µg/mL resulted in 100% lysis of trypomastigotes, while 30 µg/mL citral induced a rounded body, mitochondrial swelling, kDNA alterations, and cytoplasmic vacuoles in epimastigotes [8][57][58]. Moreno, Leal [59] also demonstrated that citral exhibited an inhibitory effect against *T. cruzi*, affecting growth in different stages such as epimastigotes (14 µg/mL), trypomastigotes (22 µg/mL), and amastigotes

(74 µg/mL). Rojas Armas, Palacios Agüero [60] determined citral's in vivo antiparasitic effect against *T. cruzi* inoculated in mice. They observed that a dose of 300 mg/kg of citral decreased the proliferation of the parasite at 16, 18, 20, and 22 days post-infection. Furthermore, the same dose of the compound evaluated after 28 days post-infection decreased the amastigote nests in the heart (67.7%) and the infected animal's inflammatory response (51.7%).

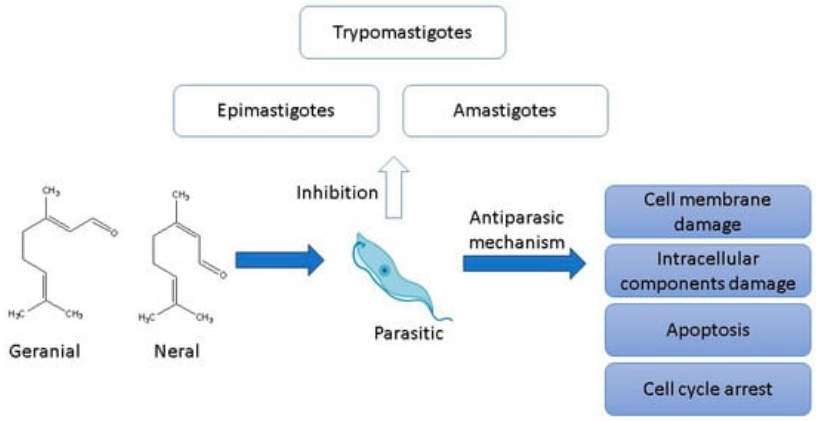

Figure 2. Antiparasitic effect of citral [60][61][62].

2.6. Antioxidant

The antioxidant activity of citral has been previously explored (**Figure 3**). In this way, different studies have demonstrated that citral can scavenge the DPPH radical, presenting IC_{50} values between 6.9 and 3700 µg/mL [11][63][64]. A study performed by Xu, Zhu [65] found that citral exhibited the ability to stabilize the DPPH radical (IC_{50}: 67.31 mg/mL) and reduce power (concentration of 2 to 10 mg/mL of citral showed an absorbance value between 0.15 and 0.55; high concentration means high absorbance). Bouzenna, Hfaiedh [66] also reported the capacity of citral to stabilize the DPPH radical (EC_{50}: 263.33 µg/mL) and reduce metals (FRAP, EC_{50}: 125 µg/mL), as well as the capacity to inhibit the oxidation of linoleic acid (β-carotene/linoleate system, inhibition of 71.27%). In the same regard, Guimarães, dasGraças Cardoso [67] tested the capacity of citral to inhibit the DPPH radical and linoleic acid oxidation at a concentration between 5 and 100 µg/L. DPPH results showed that citral had a low antioxidant effect (inhibition lowest to 1.10%). However, the evaluated compound could inhibit linoleic acid oxidation (inhibition between 5.6 and 38%). Wang, Jiang [12] studied the antioxidant potential of citral by several methods. The reduction potential showed a dose-dependent effect, presenting absorbance values between 0.8 and 1.9 at a dose between 0.05 and 0.40 mg/mL. In addition, citral effectively stabilizes the superoxide (IC_{50}: 0.67 mg/mL) and hydroxyl (IC_{50}: 0.5 mg/mL) radicals. Additionally, citral presented the potential to inhibit lipid peroxidation based on the ferric thiocyanate method, where the analyzed compound at 1 mg/mL reduced linoleic acid oxidation by 47% after 8 days of incubation compared to the control (without citral). The thiobarbituric acid (TBA) method demonstrated that citral reduced malonaldehyde formation by 81%, compared with control (without citral).

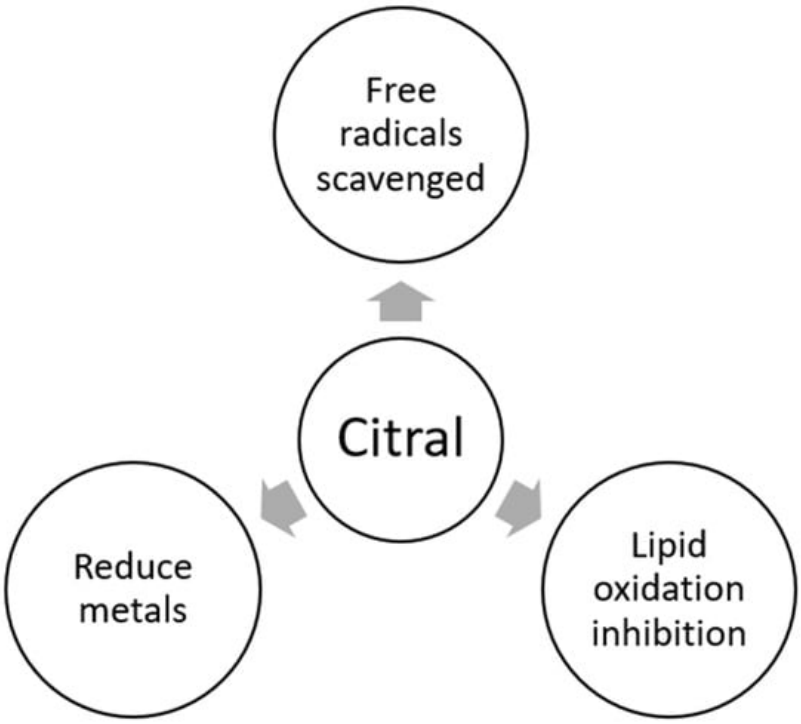

Figure 3. Antioxidant activity of citral [12][67].

3. Use as Possible Food Additive and Pharmaceutical

Citral's inclusion in the US Environmental Protection Agency (EPA)'s GRAS list as a biopesticide has made it a versatile natural preservative for various food products. This not only extends shelf life but also aligns with consumer demand for healthier, eco-friendly, and clean-label options. hen et al. [68] reported that liposome–citral nanoencapsulates (105.7–238.0 nm) significantly improved the quality of fresh Shatangju mandarins compared to free citral-treated samples by reducing their weight loss and microbial spoilage after storage at 25 °C and 60–70% relative humidity (RH) for 26 d. Nanoemulsions containing citral have also shown outstanding effects when incorporated into coatings as vehicles, such as the case of the study reported by Machado [69], where alginate-based coatings that include citral nanoemulsions, in an optimal concentration between 0.1–0.5%, were a good barrier against microbial attack, while the quality parameters of the fruit were positively affected (e.g., color and respiration rate) during storage for 12 d at 4 °C and 90% RH.

Furthermore, citral's antifungal activity has been potentiated when combined with other EOs. For example, a nanoemulsion blending clove (CO) and lemongrass (LGO) oils as eugenol and citral sources effectively disrupted the membrane of the highly invasive *Fusarium oxysporum* f.sp. *lycopersici* fungus. When tested individually, this combination exhibited the lowest MIC value at 3.9 mg/L, compared to 31.3 for CO and 62.5 mg/L for LGO [70]. Similarly, combining citral and eugenol at concentrations of 60 and 170 mg/L resulted in more substantial damage to *P. roqueforti* than when these compounds were used separately, leading to the cell content destruction and, consequently, the death of the fungus [16]. The combination of EOs proves to be a cost-effective strategy, achieving superior results with lower component concentrations. Furthermore, developing nanosystems, such as nanoemulsions and nanoencapsulates, facilitates their incorporation into food products, ensuring prolonged bioactivity.

In vitro and in vivo studies have shown that citral is a potent agent with many biological activities. However, citral's ADME-Tox properties (absorption, distribution, metabolism, excretion, and toxicity) have been poorly understood. An in silico study showed that citral isomers (*cis*-citral and *trans*-citral) have acceptable drug-likeness properties and do not present any violations of Lipinski's rules (molecular weight <500 daltons, Log*Po/w* value <5, <5 hydrogen bond donors, <10 hydrogen bond acceptors) which guarantees their high absorption when administered orally, however, their low solubility in water limits its distribution. Another important factor to consider is that the plasmatic concentration of citral isomers can be reduced due to the high capacity of both compounds to bind to plasmatic proteins [42]. On the other hand, citral isomers could present a high plasma half-life (T1/2) because both compounds do not show an inhibitory effect on CYP2D6. Citral isomers have an acceptable partition coefficient (*cis*-citral Log *Po/w* = 2.74, *trans*-citral Log *Po/w* = 2.71), suggesting both compounds can enter the cell and recognize their therapeutic targets. In addition, the predictive carcinogenicity effect in rodents is variable for the citral isomers (*cis*-citral toxicity (R) = negative, *trans*-citral toxicity (R) = positive); therefore, it is important to consider the concentration of individual isomers during the preclinical evaluations [9].

These results suggest that citral isomers have an acceptable capacity to be absorbed through the gastrointestinal tract and enter the target cells. However, several research studies do not consider the low distribution and bioavailability of this compound, so it is necessary to focus on the design of formulations that guarantee the compound´s good distribution and bioavailability.

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