Biological properties of terpinolene evidenced by in silico, in vitro and in vivo tests: a systematic review.

Isis Oliveira Menezes , Jackelyne Roberta Scherf ,
Anita Oliveira Brito Pereira Bezerra Martins ,
Andreza Guedes Barbosa Ramos ,
Jullyana de Souza Siqueira Quintans ,
Henrique Douglas Melo Coutinho , Jaime Ribeiro-Filho ,
Irwin Rose Alencar de Menezes

PII: S0944-7113(21)00311-1
DOI: https://doi.org/10.1016/j.phymed.2021.153768
Reference: PHYMED 153768

To appear in: *Phytomedicine*

Received date: 8 March 2021
Revised date: 16 August 2021
Accepted date: 16 September 2021

Please cite this article as: Isis Oliveira Menezes , Jackelyne Roberta Scherf , Anita Oliveira Brito Pereira Bezerra Martins , Andreza Guedes Barbosa Ramos , Jullyana de Souza Siqueira Quintans , Henrique Douglas Melo Coutinho , Jaime Ribeiro-Filho , Irwin Rose Alencar de Menezes , Biological properties of terpinolene evidenced by in silico, in vitro and in vivo tests: a systematic review., *Phytomedicine* (2021), doi: https://doi.org/10.1016/j.phymed.2021.153768

Review

Biological properties of terpinolene evidenced by *in silico*, *in vitro* and *in vivo* tests: a systematic review.

Isis Oliveira Menezes[1], Jackelyne Roberta Scherf[2], Anita Oliveira Brito Pereira Bezerra Martins[1], Andreza Guedes Barbosa Ramos[1], Jullyana de Souza Siqueira Quintans[3], Henrique Douglas Melo Coutinho[4], Jaime Ribeiro-Filho[5] and Irwin Rose Alencar de Menezes[1,*] irwin.alencar@urca.br

Conceptualization, I.O.M, I.R.A.M.; writing—original draft preparation, I.O.M, J.R.S and J.R.-F; writing—review and editing, I.O.M, J.R.S, A.O.B.P.B.M., A.G.B.R., H.D.M.C., J.S.S.Q. J.R.-F and I.R.A.M.; visualization, I.O.M.; supervision, I.R.A.M., J.R.S, A.O.B.P.B.M., A.G.B.R., H.D.M.C, J.S.S.Q. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

[1]Laboratory of Pharmacology and Molecular Chemistry - LFQM, Regional University of Cariri - URCA, Crato 63105-000, CE, Brazil
[2]Antibiotics department - DB, Federal University of Pernambuco - UFPE, Recife 50670-901, PE, Brazil
[3]Department of Physiology - DFS, Federal University of Sergipe - UFS, São Cristóvão 49100-000, SE, Brazil
[4]Microbiology and Molecular Biology Laboratory - LMBM, Regional University of Cariri - URCA, Crato 63105-000, CE, Brazil
[5]Laboratory of Investigation in Genetics and Translational Hematology, Gonçalo Moniz Institute (IGM), Oswaldo Cruz Foundation (Fiocruz), Salvador 40296-710, BA, Brazil
*Correspondence:

Abstract

Background: Terpinolene, a monoterpene that is naturally found in a variety of herbs, is widely used as a flavoring agent in the industry. Although it's well established in the literature that terpinolene is an important component of plant extracts, the biological properties and the potential therapeutic use of this compound remain poorly explored.

Purpose: This work aimed to answer the following guiding question: "What are the biological activities of terpinolene demonstrated through *in silico*, *in vitro*, and *in vivo* assays?".

Study design and methodology: A systematic review was carried out in four electronic databases (Embase, Web of Science, Scopus, and PubMed) according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, using the following search terms: terpinolene "AND" activity "OR" properties "OR" therapeutic "OR" treatment. This search included manuscripts published between 1960 and June 2020. Study selection was conducted by two independent reviewers according to predefined criteria.

Results: The initial search found a total of 2449 articles. However, only 57 of them were selected as they met the inclusion criteria and answered the guiding question. The analysis of these studies indicated that terpinolene presents a series of biological effects, from which the antioxidant, larvicide, and insecticide activities stand out. Despite the evidence demonstrating that terpinolene has the potential to be used in a broad pharmacological context, the mechanisms underlying its cellular and molecular effects remain to be better elucidated. In addition, the *in vivo* efficacy and safety of the administration of this compound have been poorly evaluated through either preclinical and clinical trials. Therefore, this study highlights the importance of characterizing the biological aspects and mechanisms of action of this natural compound.

Conclusion: The data summarized in the present systematic review demonstrates the pharmacological potential of terpinolene. Nevertheless, most studies included in this review provide a superficial characterization of terpinolene biological effects and therefore, further research elucidating its mechanism of action and potential therapeutic benefits through preclinical and clinical trials are required. Nevertheless, due to its wide range of different biological activities, terpinolene will certainly attract the interest of scientific research, which could significantly contribute to the development of new products with both therapeutic and environmental applications.

Graphical Abstract Graphical Abstract

.

Keywords

Terpinolene. Biological properties. Systematic review. PRISMA guidelines, Abbreviations, AchE: Acetylcholinesterase, CFA: Complete Freud's Adjuvant, DPPH: 1,1-diphenyl-2-picrylhydrazyl (free radical elimination test), EC50: Concentration of a drug that gives half-maximal response, EtBr: Ethidium bromide absorption assay, FRAP: Ferric reducing / antioxidant power, HEWLs: hen egg white lysozyme, IC50: Concentration of drug required for 50% inhibition, iNOs: inducible nitric oxide synthase, IUPAC: International Union of Pure and Applied Chemistry, LC50: Lethal concentration of 50% animal exposed, LC90: Lethal concentration of 90% animal exposed, LDH: Lactate dehydrogenase , MDA: malondialdehyde, MIC: Minimum Inhibitory concentration, MTT: Cell Viability Assay, NBT: Nitro blue atrazolium reduction assay, NDMA: N-nitrosodimethylamine, NF-kB: Nuclear factor kappa light chain enhancer of activated B cells, NO: Nitric Oxide, PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses, RoB: Risk of Bias, ROS: Reactive oxygen species, RNS: Reactive nitrogen species, SCGE: Single cell gel electrophoresis, SEM: Scanning electron microscopy, TBA: thiobaribituric acid , TBARS: Thiobarbituric acid reactive species, ThT: Thioflavin T, TNF- α: Tumor necrosis fator alpha, TOC: Total oxidant capacity, TOS: Total oxidant status

1. Introduction

The therapeutic underestimation of chemical compounds derived from plant species directly impacts the development of new drugs. While nature provides a wide variety of herbs at low cost and whose consumption viability is recognized by several ethnopharmacological data, the bioactivity of isolated compounds needs to be confirmed through biological tests. In this context, computational (*in silico*), *in vitro*, and *in vivo* tests are fundamental tools in the scientific investigation of innovative therapies, contributing to guiding the therapeutic use of natural products (Geris, 2014; Tang et al., 2006).

Plants use metabolic processes to maintain their survival and specialized functions, which are divided into primary and secondary metabolism (Famiani et al., 2019). The primary metabolism is characterized by a set of vital processes responsible for the organism's essential maintenance through the generation of amino acids, simple sugars, lipids, and nucleic acids. On the other hand, the secondary metabolism comprises the generation of compounds that assist in growth and adaptation to stress conditions using substrates originated by the primary metabolism (Famiani et al., 2019; Felipe and Bicas, 2017; Keeling and Bohlmann, 2006; Kroymann, 2011).

Terpenes are structurally diverse secondary metabolites whose chemical and biological properties have been widely investigated in scientific research (Casanova and Costa, 2017). This class of metabolites has several subgroups, including monoterpenes, which have the simplest structure among them, comprising about 90% of the composition of essential oils (Bakkali et al., 2008; Guimarães et al., 2013). Terpinolene is a 10-carbon monoterpene composed of a cyclic main chain formed by two linked isoprene units to which methyl and propane are linked (Molecular formula: $C_{10}H_{16}$; IUPAC: 1-methyl-4-propan-2-ylidenecyclohexene (Figure 1)). With regard to the physicochemical aspects, terpinolene is a water-white to light amber-colored liquid with low water solubility (9.5 mg/L at 25 °C) and high liposolubility (estimated log Kow = 4.47). Due to its low molecular mass (136.23 g/mol) and high volatility, terpinolene is widely used in the production of fragrances (Ghasemi et al., 2009; Guimarães et al., 2013). This compound is widely found in the chemical composition of aromatic plants (Gasiński et al., 2020; Petrović et al., 2018), especially those of Asian origin such as blackcurrant (*Ribes rubrum*) and saffron (*Curcuma longa*) (Tisserand and Young, 2014).

Evidence has placed terpinolene as a bioactive compound with significant pharmacological activities, among which the antifungal (Davis et al., 2018; Pinto et al., 2020), antioxidant (de Christo Scherer et al., 2019; Lu et al., 2019), and insecticide (Liu et al., 2020; Pavela et al., 2018; Ribeiro et al., 2019; Ribeiro et al., 2020) are highlighted. Additionally, computational predictions by Bosc and collaborators (2019) indicate that terpinolene has a high confidence level (90%) and significant activity threshold (>6) for the following targets: Muscarinic acetylcholine receptor M1(Rattus norvegicus), Prostanoid EP4 receptor (Rattus norvegicus), Serotonin 3a (5-HT3a) receptor (Rattus norvegicus), and Proto-oncogene tyrosine-protein kinase ROS (Homo sapiens) ("Compound Report Card," n.d.). However, few precinical studies have investigated its biological activities through *in silico*, *in vitro*, and *in vivo* experiments. Additionally, there is currently no clinical trial supporting the potential of terpinolene as a therapeutic compound.

Therefore, this systematic review aims to report the biological activities of terpinolene demonstrated through preclinical studies, attempting to contribute to further research addressing the therapeutic potential of this monoterpene.

2. Results

2.1. Selection of studies

The initial literature search using the previously described keywords found a total of 2908 articles in the selected databases, including 261 articles in Embase, 381 articles in Medline, 4,618 articles in Scopus, 189 articles in PubMed, and 459 articles in the Web of Science. After removing 1992 duplicates of articles indexed in two or more databases and applying the inclusion and exclusion criteria, 76 articles were selected for the final analysis. Finally, following the individual verification of full texts, a total of 57 articles were obtained and included in the present review (figure 2).

2.2. Data extraction and analysis

Considering the type of study, most articles were performed exclusively *in vitro* (n = 38), followed by studies carried out exclusively *in vivo* (n = 15), both *in vitro* and *in vivo* (n = 2), both *in silico* and *in vitro* (n = 1) and exclusively *in silico* (n = 1). Concerning the procedures used to perform the analyses, only *in silico* studies (3%), were carried out using the same method: molecular docking. Differently, *in vitro* (67.1%) and *in vivo* (29.9%) studies were carried out following different protocols. To better describe the most relevant aspects of the articles, they were organized according to the type of study, as shown in tables 1, 2, and 3. Those articles using two or more types of preclinical trials were presented in different tables according to the corresponding type of assay.

The first article reporting terpinolene biological activity was published in 1967. Since then, although the number of publications has gradually fluctuated over the years, a growing number of publications from 2009 indicates increasing interest in this subject in the last years (figure 3a). Most of the investigations were carried out in the Asian continent, where China (6 publications), Taiwan (6 publications), Japan (5 publications), and Korea (5 publications) stand out as leading countries in this field of research. In the Americas, Brazil stands out with ten publications on this issue. In comparison with the Americas, Europe demonstrates a similar interest in the topic (figure 3b).

The selected studies were separated and grouped into three experimental categories: *in silico* (Table 1), *in vitro* (Table 2), and *in vivo* (Table 3). Also, those articles that use 2 types of preclinical assays for terpinolene activity analysis were presented in 2 distinct tables obeying the corresponding type of assay. The articles analyzed in this research revealed that terpinolene has several pharmacological activities reported in the literature, including anti-cholinesterase, sedative, cytotoxic, cytoprotective, anti-inflammatory, antispasmodic, antiproliferative, antinociceptive, lysozyme ligand and n-nitrosamine inhibitor, and P-glycoprotein. As reported for other terpenes, the antioxidant and toxic (insecticide/larvicide) properties stand out as the most investigated activities of terpinolene (figure 4).

With regard to the toxic activity of terpinolene, most articles reported its cytotoxic and insecticidal properties. The data repoting terpinolene cytotoxicity indicate that this compound present low toxicity when compared with other monoterpenes. Accordingly, in vitro assays using human lymphocytes showed that terpinolene present a concentration- dependent cytotoxicity, causing significant decreased on cell viability at concentrations greater than 100 mg/L, which was not associated with the occurrence of genotoxic effects (Turkez et al., 2015). A study investigating the toxicicty of orally administered terpinolene in rats found a LD_{50} of 4390 mg/Kg, which characterizes compounds with low systemic toxicity. The same study showed that a single topical application of this compound at a dose of 5000 mg/Kg resulted in transitory erythema and edema during the first few days of observation (Opdyke, 1976). Thus, based on the available literature data, terpinolene can be considered a safe drug for both topical and systemic use.

2.3. Methodological quality /risk of bias of the studies

This systematic review is a pioneering study listing the different properties of terpinolene in a biological context. Then only *in vivo* trials were used tocompose this study had their risk of bias analyzed in order to determine the reliability of their methods. It is worth mentioning that *in vivo* methods are of crucial importance in the evaluation of the pharmacokinetic (e.g., absorption, metabolism, bioavailability) and pharmacodynamic (e.g., potency, affinity, selectivity) parameters of a given compound, which allow the identification of the cellular or biochemical events modulated by the drug such as gene transcription, protein expression and metabolic changes associated to disease status. To analyze the methodological quality and risk of bias, we used the SYRCLE's RoB tool based on the Cochrane

RoB tool (Higgins et al., 2011; Hooijmans et al., 2014) adjusted for specific aspects of experimental studies in non-human animals. This methodology evaluates the risk of bias from the answer to 10 questions, including selection bias, performance bias, friction bias, detection bias, and other biases (Hooijmans et al., 2014). Some underlying evidence indicates that the animal environment and experimental conditions, such as lighting, humidity, temperature, etc., can influence the results of the study because they promote behavioral and biochemical changes (Claassen, 2013; Hooijmans et al., 2014; Johnston and Nevalainen, 2002). Group allocation (random selection) and blind treatments are also relevant for the methodological quality. Here, the articles were evaluated both qualitatively (figure 5) and quantitatively (figure 6), allowing the reproducibility in the choice of risk criteria of this study. The classification was initially performed independently by two researchers (IOM and JRS) in agreement with the kappa index = 0.748. Subsequently, the possible divergences in the classification of the studies were resolved by consensus and expressed in figures 5 and 6.

According to the present analysis, none of the in vivo studies included in the present review presented a low risk of bias. From a total of 17 studies, seven had a high risk of bias in any of the judging questions, while 30% of the manuscripts evaluated presented an unclear risk of bias. According to the score shown in figure 7, an analysis of the manuscripts using in vivo experiments found 5.29%, 42.35%, and 52.35% of articles presenting a high, uncertain, and low risk of bias, respectively. Although more than 50% of the studies have shown a low risk of bias, it is noteworthy mentioning that the number of questions answered with unclear risk was high, which might be due to the lack of information attested during the reading of manuscripts.

With regard to the judging questions, it was observed that questions 1, 2, 4, and 10 presented the lowest risk of bias, as only one article (6%) did not adequately describe the sequence of events. All articles were apparently carried out following the methodology proposed with an understandable and precise description. Additionally, almost all of them (94 %) were free from other bias problems such as contamination by drugs, the addition of new animals to replace unwanted animals in the groups, the influence of funders, or errors in analysis units.

All selected studies correctly described the objectives, methodology, outcomes, and main findings obtained. However, no information on blinding strategy was provided. Nevertheless, no detailed evidence was found in any of the studies that indicated that they were more prone to errors or manipulation of results than those using a blinding methodology (Bebarta et al., 2003; Hooijmans et al., 2014).

2.4. Computationally predicted molecular targets for terpinolene

In order to contribute to further research investigating the potential therapeutic properties of terpinolene, we used target prediction based on physicochemical parameters and structure similarity to evaluate other potential molecular targets for this monoterpene. Our analysis showed that the most likely targets for terpinolene interaction belong to the following categories: 1) Family A G protein-coupled receptors: Cannabinoid receptor 2, Adenosine A1 receptor, Prostanoid EP4 receptor, Acetylcholine receptor; 2) Nuclear receptors: Peroxisome proliferator-activated receptor alpha (PPAR-α), Estrogen and Androgen Receptors; 3) Araquidonate oxidoreductase enzymes: 5-lipoxygenase; Cyclooxygenase-1; 4) Orphan receptor tyrosine kinase (RTK): Proto-oncogene tyrosine-protein kinase ROS and 5) Other enzymes: Alkaline phosphatase, tissue-nonspecific isozyme, Adenosine deaminase, Aminopeptidase N (Figure 7). These findings corroborate the evidence demonstrated by the studies included in the present review and encourage the development of further research to better characterize the pharmacological properties and potential mechanisms of action by this compound in a preclinical context.

3. Discussion

Terpenes are the largest group of natural bioactive compounds. Studies have shown that the production of these compounds is both influenced by genetic factors and the environmental conditions to which the plant is exposed. In this context, evidence has suggested that the fact that terpenes are produced as part of the defense mechanism of plants in response to stressful stimuli contributes significantly to their wide variety of biological effects. Among the terpenes, monoterpenes are widely used flavoring agents with significant biological activity (Felipe and Bicas, 2017), which has stimulated the development of studies aiming to characterize their pharmacological properties, as well as determining their potential applications for human benefit.

Terpenes have been acknowledged as efficient therapeutic alternatives in the treatment of numerous conditions in the Mediterranean, Ayurvedic, and Chinese Medicine, which represent millenary treatment systems based on the use of natural substances and spices following an evidence-based approach guided by traditional knowledge (Khan, 2014). Asian countries such as India and China have a vast and rich record of medicinal plants that have been scientifically validated and widely used by the population for the treatment of many diseases, corroborating the interest of their scientists in natural product research (Jamshidi-Kia et al., 2018). Despite the outstanding Brazilian biodiversity and the existence of populations where the traditional medicine culture is transmitted from generation to generation, the use of herbal medicines and related therapies by the general population is still under development, which is in part motivated by the growing difficulty of access to commercial medicines (Valli and Bolzani, 2019). Importantly, natural product research using species of the Brazilian biodiversity has identified a significant number of molecules with the potential to be used in drug development (Paduch et al., 2007).

The present review included preclinical studies performed either *in silico*, *in vitro*, and *in vivo*, in order to cover the therapeutic potential and other biological activities of terpinolene. Special attention should be given to the small number of computational tests (in silico), despite the fact that this type of study present advantages such as lower demand for physical resources, low execution cost, fast results, selection of new and likely targets based on machine learning, and potential for fingerprint-based molecular interaction (Agamah et al., 2020). However, only preclinical studies using *in vivo* animal models followed by clinical trials will provide consistent information regarding the pharmacokinetic parameters (including absorption, distribution, metabolism, and excretion) required during the stages of drug development (Jaroch et al., 2018). Next, we discuss the properties of terpinolene in sections organized according to the main biological activities reported in this review.

3.1. Toxicity and Cytotoxicity

Insecticides are substances used to kill insects, which has a direct impact on human health due to the elimination of disease vectors. However, many of these agents are synthetic compounds that pollute the environment causing toxic effects to various organisms (Ansari et al., 2014; Beard et al., 2003). Thus, a growing number of researchers have searched for bioecological alternatives to combat vectors without causing environmental damage, among which plant-derived natural products stand out as promising insecticides (Ansari et al., 2014). In this context, studies have demonstrated the effectiveness of terpinolene in the elimination of disease vectors due both to its insecticide and larvicide properties (Monro, 1971).

Several of the pesticide categories (acaricides, fungicides, insecticides, herbicides, and larvicides) share properties reported for terpinolene in the present review (Coutinho et al., 2005). Accordingly, terpinolene was found to present toxic effects against a variety of organisms, especially against insects (Ali et al., 2015; Chang et al., 2012; do Nascimento et al., 2018; Liang et al., 2018; Liu et al., 2020; Park et al., 2003; Pavela et al., 2018; Ribeiro et al., 2020; N. C. Ribeiro et al., 2019; Wang et al., 2009; Zhang et al., 2017, 2016), larvae (Ali et al., 2015; Cheng et al., 2009a, 2009b; Conti et al., 2012; da Silva et al., 2016; Kweka et al., 2016; Pavela, 2015; Perumalsamy et al., 2009), and mites (Born et al., 2018; N. de C. Ribeiro et al., 2019; N. C. Ribeiro et al., 2019). Studies have reported the insecticide effect of terpinolene against *Culex quinquefasciatus* (with letal activity detremined by LC_{50} of 25.7 µL/L and LC_{90} of 50.1 µL/L) (Pavela et al., 2018), *Bemisia tabaci* (2 µL/L) (Ribeiro et al., 2020), *Bacopa caroliniana* (20 µL/L) (Liu et al., 2020), *Rhyzopertha dominica* (5 µL/L) (do Nascimento et al., 2018), *Musca domestica* (1.25 µL/L)(Zhang et al., 2017), *Callosobruchus chinensis* (0.18 mg/cm^2), and *Sitophilus oryzae* (0.05 mg/cm^2) (Park et al., 2003), *Tribolium castaneum* and *Lipocelis bostrychophila*(Liang et al., 2018), *Drosophila melanogaster* (Zhang et al., 2016), *Aedes aegypti* (da Silva et al., 2016), *Anopheles quadrimaculatus* (Ali et al., 2015), *A. albopictus* (Cheng et al., 2009b; Conti et al., 2012; Gu et al., 2009), *Culex quinquefasciatus* (Pavela, 2015), *Anopheles gambia* (Kweka et al., 2016), *Culex pipens pallens*, and *Ochlerotatus Togoi* (Perumalsamy et al., 2009) and *Blattella germanica* (Chang et al., 2012).

Corroborating the findings of the present review, literature data regarding the biological activities of other non-oxygenated monocyclic monoterpenes presenting the same molecular mass as terpinolene indicate that they share comparable insecticidal activities, as demonstrated for limonene (Liang et al., 2018; Ribeiro et al., 2020). Additionaly, p-cymene showed slightly higher toxicity than terpinolene against *Cx. quinquefasciatus* larvae (LC_{50} of 20.6 µl/l e LC_{90} µl/L = 25.8) (Pavela et al., 2018). Wang et al., (2009) demonstrated that like terpinolene, monoterpene terpinene presented a significant activity against *Sitophilus zeamaise* (fumigant assay) and a study carried out by Chang et al. (2012), demonstrated the high toxicity of terpinolene, p-cymene, o-cymene, and m-cymene against *Blattella Germanica.*

Unlike the large number of *in vitro* studies, the acaricide potential of terpinolene using *in vivo* models remains poorly investigated. Nevertheless, this monoterpene proved to be efficient against *Tetranychus urticae* at concentrations ranging from 0.0002 μL/L (Born et al., 2018) to 0.2 μL/L (N. de C. Ribeiro et al., 2019; N. C. Ribeiro et al., 2019) , which is comparable to results obtained with monoterpenes limonene and p-cymene against *Tetranychus urticae* (Born et al., 2018; N. C. Ribeiro et al., 2019).

Regarding the mechanisms of action underlying the insecticidal activity of terpinolene, da Silva et al. (2016) demonstrated that this monoterpene interferes with the activity of L4 gut proteases, including trypsin-like enzymes (serine proteases are involved in insect digestion processes) of *A. aegypti* in addition to suggesting the involvement of acetylcholine-related mechanisms in the toxicity to several insect species.

When analyzing the influence of drug exposure reported in the studies, it was observed that the insecticidal effect of terpinolene is influenced by the time of exposure (tending to decrease the effect over the course of hours), method of exposure (inhalation/vapor action seemed to be, comparatively, the most effective), and environmental conditions (closed environments drastically increase the insecticidal action). Therefore, it is believed that increased exposure in closed environments, for a shorter time and in a way that facilitates exposure to this airborne route tends to optimize the desired toxicity. Also, studies have demonstrated that terpinolene's insecticide potential is associated with its volatility and power of induction of cell death by mitochondrial apoptosis and ROS generation causing oxidative stress (Monro, 1971). Together, these findings suggest that terpinolene could be used in the development of anti-vector products.

The cytotoxic profile of terpinolene has been investigated and characterized on human cells (Aydin et al., 2013; de Christo Scherer et al., 2019; Morshedi et al., 2014; Turkez et al., 2015) in order to determine its *in vitro* safety, as well as assess its potential therapeutic uses, *e.g.* against cancer. Accordingly, our target prediction study identified the Proto-oncogene tyrosine-protein kinase ROS (which has been shown to play key roles in signal transduction and cellular communication, as well as is associated with a variety of cancers) as a potential target for terpinolene, suggesting that this compound could have beneficial roles in cancer. This is corroborated by previous research demonstrating that the monoterpene, at concentrations above 50 mg/L, has antiproliferative activity against neuroblastoma cells (N2a) (Aydin et al., 2013), which is possibly related to the inhibition of n-nitrosamine (*in silico*) (Sawamura et al., 1999). Blood cells treated with terpinolene at concentrations ranging from 150 mg/L to 200 mg/L released increased levels of LDH, indicating that the compound is toxic at this concentration range (Turkez et al., 2015). On the other hand, terpinolene presented a cytoprotective profile in PC12 (Rat pheochromocytoma) cells (Morshedi et al., 2014) and failed to induce significant cytotoxic effects against L929 fibroblasts and RAW macrophages cells (de Christo Scherer et al., 2019).

Interestingly, terpinolene caused a marked increase in intracellular production of ROS in cancer cells, resulting in increased expression of apoptotic markers such as the BCL2-associated X protein (BAX), Poly ADP (Adenosine Diphosphate)-Ribose Polymerase (cleaved-PARP), and pro-caspase-8 without promoting genotoxic effects (Kig et al., 2021). Another study, using the unicellular organism *Schizosaccharomyces pombe* showed that terpinolene toxicity was correlated with oxidative stress and reduction of the mitochondrial transmembrane potential (Agus et al., 2018).

3.2. Antioxidant activity

In this review, a total of 11 scientific studies reported the *in vitro* antioxidant activity of terpinolene (Aydin et al., 2013; Choi et al., 2000; de Christo Scherer et al., 2019; Dorman et al., 2000; Emami et al., 2011; Grassmann et al., 2003; Graßmann et al., 2005; Kim et al., 2004; Lu et al., 2019; Ruberto and Baratta, 2000; Turkez et al., 2015), most of which through the elimination of the DPPH free radical, inhibition of thiobarbituric acid reactive species (TBARS) (Dorman et al., 2000; Lu et al., 2019; Ruberto and Baratta, 2000), and inhibition of LDL oxidation (Grassmann et al., 2003; Graßmann et al., 2005). Studies have shown high terpinolene concentrations have a protective role against oxidative stimuli by increasing the total antioxidant capacity via induction of Akt1 expression. However, the study of Boulebd (2021) demonstrated that the hydroperoxyl radical scavenging activity exhibited by terpinolene is strongly influenced by the environment, which at least in part, explains the balance between ROS generation and the antioxidant capacity of terpinolene (Boulebd, 2021).

A study by Lu et al., (2019) demonstrated that terpinolene concentration-dependently promoted a reduction of total oxidant levels and an increase in the antioxidant substances, which was comparable to the effectiveness of butylated hydroxytoluene (positive control used) based on the results obtained using the DPPH and TBARS methods. The same study also demonstrated that monoterpene γ-terpinene inhibited lipid peroxidation to the same extent as terpinolene (over 80% inhibition, comparable to the standard antioxidant control). On the other hand, the monoterpene (+)-limonene exhibited no significant activity when evaluated through different methods, i.e., exhibited low DPPH radical-scavenging ability, low protective capacity against lipid from oxidation (Emami et al., 2011).

Free radicals such as reactive oxygen species (ROS) are naturally produced in various organisms, both at normal physiological conditions and stressful situations (Dallaqua and Damasceno, 2011; Halliwell and Gutteridge, 2015). However, it has been consistently demonstrated that pathological ROS generation is associated with the development of chronic diseases, as observed in Alzheimer's (Mecocci et al., 1994), which has stimulated the discovery of antioxidant substances capable of inhibiting the generation or neutralizing the effects of free radicals (Halliwell and Gutteridge, 2015). Given the significance of its antioxidant activity, it is assumed that terpinolene can be a potential drug candidate for the treatment of pathological processes caused by oxidative stress.

3.3. Antimicrobial activities

According to the consulted literature, terpinolene has antimicrobial activities such as a parasite, antifungal, antibacterial, virucide, and trypanocide. In this context, terpinolene showed activity against *Trypanosoma brucei* with an EC_{50}= 0.035 μg/mL (0.26 μM), similar to effect observed for the non-oxygenated monoterpene limonene (Ngahang Kamte et al., 2018). Its fungicide effects were demonstrated in studies with *Leptographium abietinum* (Davis et al., 2018), *Candida tropicalis* (32 mg/mL), *C. utilis* (8 mg/mL), *C. albicans* (at concentrations above 32 mg/mL), *Botrytis cinereac*, and *Sclerotium cepivorum*. Importantly, evidence has indicated that the mechanism of action underlying terpinolene effects involves direct damage to the fungal membrane and organelles (Pontin et al., 2015; Shin, 2004; Yu et al., 2015). Additionally, Pinto and collaborators' (2020) showed the interference of terpinolene in the plasma membrane of the fungus Microsporum canis LM216 (dermatophytes fungi strains) promoting cytotoxicity mechanisms associated to increased K^+ influx.

Regarding the antibacterial effects of terpinolene, studies have demonstrated that this monoterpene inhibited the growth of *Microcystis aeruginosa* (at concentrations above 1,079 mM), a harmful freshwater cyanobacteria of economic and ecological importance (Zhao et al., 2020). Lee et al. (2013). Furthermore, the compound demonstrated its effectiveness against *Propionibacterium acnes* and *S. aureus*, which are important causative agents of skin infections. The compared the effectiveness of other terpenes, were observed that alfa-terpinene and limonene showed a moderate antibacterial action, while p-cymene exhibited low antibacterial activity.

Interestingly, while Kim et al. (2006) stated that terpinolene showed little or no significant effect against *B. bifidum*, *B. longum*, *L. acidophilus*, *E. coli*, and *C. perfringens*, Ngan et al. (2012) found intense antibacterial activity against the same strains, except for *E. coli*. However, since no MIC value for terpinolene was reported in the work of Kim et al. (2006), a complete interpretation of their results is not possible. Finally, in addition to being active against nine enteric pathogenic bacteria (*B. fragilis*, *B. thetaiotaomicron*, *C. perfringens*, *C. paraputrificum*, *K. pneumoniae*, *E. coli*, *S. Typhimurium*, *C. difficile*, *C. butyricum*, and *S. aureus*),terpinolene inhibited the growth acidophilic bacteria playing important roles on the intestinal flora balance such as *B. adolescentes*, *B. bifidum*, *B. breve*, *B. infantis*, *B. longum*, *L. acidophilus*, *L. casei* (Ngan et al., 2012).

Terpinolene was found to cause inhibition of photosynthesis and nitrogen metabolism through the enzymatic inhibition of nitrate reductase and glutamine synthetase, in addition to inducing the oxidative stress of the algae Microcystis aeruginosa (Zhao et al., 2020).

Finally, consistent evidence has demonstrated the antiviral properties of this monoterpene against influenza A virus, PR8 subtype H1N1, Herpes simplex virus type 1 (HSV-1) and 2 (HSV-2), Echovirus 9 (Hill strain), Poliovirus 1 (Sabin strain), Coxsackievirus B1 and Adenovirus 2. For the influenza A/PR/8 virus subtype H1N1, this compound showed antiproliferative effects, without however inhibiting neuraminidase expression or virus fixation in the cells (Garozzo et al., 2011, 2009).

3.4. Other pharmacological effects

Among the less reported activities, terpinolene was found to induce a fibrinolytic effect by apparent disruption of fibrillation formation in hen egg white lysozyme (HEWL), corroborating with the finding that p-cymene presented similar effect , decreasing the ThT fluorescence intensity. On the other hand, limonene was found to induce fibrillation and increased ThT fluorescence intensity by more than 50% (Morshedi et al., 2014). Terpinolene can inhibit P-glycoprotein (P-gp)-mediated transport and interact with P-gp substrates during intestinal absorption processes, which is also observed for alpha-terpinene (Yoshida et al., 2006).

Terpinolene was also found to inhibit acetylcholinesterase (AChE) *in silico* (Politi et al., 2017), *in vitro* (Bonesi et al., 2010) e *in vivo* (IC_{50} values < 10 μL/mL) (Liu et al., 2020), in adition to inhibiting butyrylcholinesterase (BChE) *in vitro* (Bonesi et al., 2010). Comparable results were demonstrated by limonene with regard to the inhibition of AchE (IC_{50}=225.9) and BchE (IC_{50}=456.2) (Menichini et al., 2009). Other monoterpenes, including p-cymene, γ-terpinene, (+)-limonene, and (-)-limonene inhibited AChE activity by 30% to 40% (Miyazawa et al., 1997). Importantly, it has been suggested that compounds with anti- AChE activity have the potential to be used in the development of a drug against Alzheimer's Disease (Seifi Nahavandi et al., 2020).

Previous research demonstrated that terpinolene has anti-inflammatory and antinociceptive effects that are related to interference with serotonergic pathways in the central nervous system (CNS). It was proposed that the mechanisms underlying these effects involve inhibition of serotonin receptors (5HT-2A) (Macedo et al., 2016), as demonstrated by an increase in the mechanical threshold (as measured by Randall Selitto paw pressure test) (Macedo et al., 2016). Other proposed mechanisms are the interaction with 5HT-3 receptor channels (expressed in an adrenergic cell line N1E-115) and the inhibition of calcium influx inhibition via GABA-mediated signaling (Riyazi et al., 2007).

A study by Ito and Ito (2011) demonstrated that the monoterpene showed an effect similar to that chlorpromazine, prolonging the pentobarbital-induced sleep time through an antagonistic action in dopaminergic, noradrenergic, and serotonin neurons. A study by Koyama and Heinbockel (2020) suggested that the mechanisms of action of essential oils and terpenes are intrinsically related to their multiple roles in the olfactory/respiratory system (Kobayakawa et al., 2007; Mori and Sakano, 2011; Soria-Gómez et al., 2014). Accordingly, a clinical study of terpinolene found significantly reduced tension, enhanced relaxation, and stable states of brain function following the treatment, especially in prefrontal regions, which is possibly due to the modulation of olfactory receptors, one of the largest families of G-protein-coupled receptors (Sowndhararajan et al., 2015).

This systematic review is a pioneering study listing the different properties of terpinolene in a biological context. The *in vivo* trials that compose this study had their risk of bias analyzed to determine the reliability of their methods. It is worth mentioning that *in vivo* methods are crucial for the evaluation of pharmacokinetic (e.g., absorption, metabolism, bioavailability) and pharmacodynamic (e.g., potency, affinity, selectivity) parameters, as well as to elucidate the cellular and biochemical events underlying the mechanism of action of a given compound such as gene transcription, protein expression, mediator product and other metabolic changes associated to disease status. Finally, with regard to the biological properties of terpinolene, this review highlights its insecticidal and antioxidant effects as the most promising activities demonstrated through preclinical studies. Other terpinolene properties, such as cytotoxicity, genotoxicity, and oxidative potential are discussed in conformity with the corresponding literature.

In general, terpenes are capable of inducing ROS generation, contributing to lipid peroxidation, oxidative damage, and increased cytotoxic markers. Here, we suggest that the prooxidant and cytotoxic effects of terpenes could be explored in drug development in the context of anticancer and antiparasitic research. On the other hand, the antioxidant activity of terpinolene are directly linked to its cytoprotective effects, which could be useful in preventing cell damage caused by oxidative stress (mediated by both ROS and RNS), as well as having potential beneficial effects on neurodegenerative diseases such as Alzheimer's. Accordingly, evidence raised by the present study indicates that terpinolene may have a wide range of pharmacological effects.

Finally, before the small number of preclinical trials reporting the pharmacokinetic profile of terpinolene, we encourage the development of research addressing this issue. Although some studies have mentioned the possibility of using terpinolene in the production of food, insecticides, and medicines, given the lack of scientific data proving its effectiveness, this theme deserves further investigation through *in vivo* and clinical studies.

4. Materials and Methods

A systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and and the Cochrane Handbook for Systematic Reviews guidelines. The systematic review protocol used in this aim was registered at the International Prospective Register of Systematic Reviews (PROSPERO).

4.1. Strategy of research

Bibliographic research was conducted in four electronic databases: Embase, Medline/PubMed, Scopus, and Web of Science. In each database, different combinations of the following descriptors were used: terpinolene "AND" activity "OR" properties "OR" therapeutic "OR" treatment. All manuscripts published in English from 1960 to 12 September 2020 were considered and analyzed according to the other steps of the review.

4.2. Inclusion and exclusion criteria

The following inclusion criteria were adopted: (1) articles using *in silico, in vitro*, or *in vivo* methods, (2) the intervention was performed using only terpinolene as a treatment, (3) the control group was placebo or non-exposed control group, (4) the articles should discuss the biological activity of terpinolene, and (5) only primary (original) papers were considered.

The following criteria were used to exclude articles: (1) papers showing only clinical studies, (2) articles presenting mixtures of compounds whose activity was not attributed to terpinolene alone, (3) manuscripts published in other language except English, portuguese and espanhol, (4) articles with unavailable full texts, , (5) articles without the selected descriptors, (6) duplicated studies, and (7) review articles.

4.3. Selection of studies

The first step of our work was to conduct a search in the electronic databases using the selected descriptors. The list of articles containing full information (title, abstract, and keywords) was downloaded (specific programs were not used for the screening of articles), duplicates were removed, and inclusion and exclusion criteria were applied by independent researchers (IOM and JRS) using the PRISMA guidelines to assess the eligibility criteria for each article. Any divergence in the selection of eligible studies was resolved by consensus. The articles selected in the initial screening, as well as those whose preliminary analysis left doubts, had the full texts analyzed and documented in a PRISMA flowchart.

4.4 Data extraction and analysis

Data extraction was carried out by two researchers (IOM and JRS) independently using a predetermined extraction table, and disagreements were resolved by consensus. The reported activities are subdivided into *in silico*, *in vitro*, and *in vivo*. For each subgroup, a table was elaborated with information on (1) authors, (2) year, (3) country, (4) method, (5) administration route, (6) dose and/or concentration tested, (7) main results, and (8) biological activity. For each table, the compatible extraction data for each type of study was addressed.

4.5 Prediction of biological activity profiles

The SwissTargetPrediction online tool (http://swisstargetprediction.ch/) was used to predict small molecules working as potential targets for terpinolene according to their 2D or 3D similarity with the ligand.

4.6. Evaluation of the methodological quality of the study/risk of bias

The risk of bias and methodological quality of the selected *in vivo* studies (non-human animals) were manually analyzed by two researchers (IOM and JRS) independently using the SYRCLE's Risk of Bias (RoB) methodology (Hooijmans et al., 2014) and the final validation of the risk assessment was performed by two independent researchers using the kappa index. This tool was not used to analyze *in vitro* or *in silico* studies since there is no validated tool for this type of research. After the analysis, the studies were classified into the following categories: "low risk of bias", "high risk of bias" and "clear risk of bias".

4.7. Data synthesis

The results were presented through a narrative synthesis since it was not possible to conduct a meta-analysis due to the great heterogeneity of the studies addressed in this review.

5. Conclusions

Our analysis of the literature revealed that most studies addressing the biological activities of terpinolene were conducted using *in vitro* tests. However, it is observed that the *in vivo* assays describe in more detail its biological properties at the molecular level, which is useful for elucidating the mechanisms of action of this monoterpene. According to the studies presented in this review, terpinolene is an isolated compound with the potential to be used in the development of commercial formulations with repellent effects as well in the composition of insecticides, both as the active principle or as an adjuvant. It is worth mentioning, however, that terpinolene has a series of pharmacological effects that need to be better investigated to establish its potential therapeutic applications, as well as the mechanisms underlying its biological actions. Thus, future research should consider exploring the possibility of new studies *in vitro*, *in vivo*, and clinical studies for therapeutic applications of this compound with a better perspective in understanding its potential benefits to human health.

Funding

This study was financed in part by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Cearense Foundation to Support Scientific and Technological Development (FUNCAP) - finance code BPI, National Council for Scientific and Technological Development (CNPq) – finance code 304291/2017-0, and Financier of Studies and Projects - Brasil (FINEP). This article is a contribution of the National Institute of Science and Technology - Ethnobiology, Bioprospecting and Nature Conservation/CNPq/FACEPE.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgments

The authors acknowledge the financial support of the Brazilian agencies CNPq, CAPES and Funcap, and the support of the Regional University of Cariri (URCA).

References

Agamah, F.E., Mazandu, G.K., Hassan, R., Bope, C.D., Thomford, N.E., Ghansah, A., Chimusa, E.R., 2020. Computational/in silico methods in drug target and lead prediction. Brief. Bioinform. 21, 1663–1675. https://doi.org/10.1093/bib/bbz103

Agus, H.H., Sarp, C., Cemiloglu, M., 2018. Oxidative stress and mitochondrial impairment mediated apoptotic cell death induced by terpinolene in Schizosaccharomyces pombe. Toxicol. Res. (Camb). 7, 848–858. https://doi.org/10.1039/C8TX00100F

Ali, A., Tabanca, N., Ozek, G., Ozek, T., Aytac, Z., Bernier, U.R., Agramonte, N.M., Baser, K.H.C., Khan, I.A., 2015. Essential Oils of Echinophora lamondiana (Apiales: Umbelliferae): A Relationship Between Chemical Profile and Biting Deterrence and Larvicidal Activity Against Mosquitoes (Diptera: Culicidae). J. Med. Entomol. 52, 93–100. https://doi.org/10.1093/jme/tju014

Ansari, M.S., Moraiet, M.A., Ahmad, S., 2014. Insecticides: Impact on the Environment and Human Health, in: Environmental Deterioration and Human Health. Springer Netherlands, Dordrecht, pp. 99–123. https://doi.org/10.1007/978-94-007-7890-0_6

Aydin, E., Türkez, H., Taşdemir, Ş., 2013. Anticancer and Antioxidant Properties of Terpinolene in Rat Brain Cells. Arch. Ind. Hyg. Toxicol. 64, 415–424. https://doi.org/10.2478/10004-1254-64-2013-2365

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils–a review. Food Chem. Toxicol. 46, 446–475.

Beard, J., Sladden, T., Morgan, G., Berry, G., Brooks, L., McMichael, A., 2003. Health impacts of pesticide exposure in a cohort of outdoor workers. Environ. Health Perspect. 111, 724–730. https://doi.org/10.1289/EHP.5885

Bebarta, V., Luyten, D., Heard, K., 2003. Emergency Medicine Animal Research: Does Use of Randomization and Blinding Affect the Results? Acad. Emerg. Med. 10, 684–687. https://doi.org/10.1111/j.1553-2712.2003.tb00056.x

Bonesi, M., Menichini, F.F., Tundis, R., Loizzo, M.R., Conforti, F., Passalacqua, N.G., Statti, G.A., Menichini, F.F., 2010. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of Pinus species essential oils and their constituents. J. Enzyme Inhib. Med. Chem. 25, 622–8. https://doi.org/10.3109/14756360903389856

Born, F. de S., da Camara, C.A.G., de Melo, J.P.R., de Moraes, M.M., 2018. Acaricidal property of the essential oil from Lippia gracilis against Tetranychus urticae and a natural enemy, Neoseiulus californicus, under greenhouse conditions. Exp. Appl. Acarol. 75, 491–502. https://doi.org/10.1007/s10493-018-0286-3

Bosc, N., Atkinson, F., Felix, E., Gaulton, A., Hersey, A., Leach, A.R., 2019. Large scale comparison of QSAR and conformal prediction methods and their applications in drug discovery. J. Cheminform. 11, 1–16. https://doi.org/10.1186/S13321-018-0325-4

Boulebd, H., 2021. Are thymol, rosefuran, terpinolene and umbelliferone good scavengers of peroxyl radicals? Phytochemistry 184, 112670. https://doi.org/10.1016/J.PHYTOCHEM.2021.112670

Casanova, L.M., Costa, S.S., 2017. Interações sinérgicas em produtos naturais: potencial terapêutico e desafios. Rev. Virtual Quim. 9, 575–595.

Chang, K.-S., Shin, E.-H., Park, C., Ahn, Y.-J., 2012. Contact and Fumigant Toxicity of Cyperus rotundus Steam Distillate Constituents and Related Compounds to Insecticide-Susceptible and -Resistant Blattella germanica. J. Med. Entomol. 49, 631–639. https://doi.org/10.1603/ME11060

Cheng, S.-S., Chang, H.-T., Lin, C.-Y., Chen, P.-S., Huang, C.-G., Chen, W.-J., Chang, S.-T., 2009a. Insecticidal activities of leaf and twig essential oils from Clausena excavata against Aedes aegypti and Aedes albopictus larvae. Pest Manag. Sci. 65, 339–343. https://doi.org/10.1002/ps.1693

Cheng, S.-S., Chua, M.-T., Chang, E.-H., Huang, C.-G., Chen, W.-J., Chang, S.-T., 2009b. Variations in insecticidal activity and chemical compositions of leaf essential oils from Cryptomeria japonica at different ages. Bioresour. Technol. 100, 465–470. https://doi.org/10.1016/j.biortech.2007.11.060

Choi, H.S., Sun Song, H., Ukeda, H., Sawamura, M., 2000. Radical-scavenging activities of citrus essential oils and their components: Detection using 1,1-diphenyl-2-picrylhydrazyl. J. Agric. Food Chem. 48, 4156–4161. https://doi.org/10.1021/jf000227d

Claassen, V., 2013. Neglected factors in pharmacology and neuroscience research: biopharmaceutics, animal characteristics, maintenance, testing conditions. Elsevier.

Compound Report Card [WWW Document], n.d. URL https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL454697/ (accessed 7.13.21).

Conti, B., Benelli, G., Flamini, G., Cioni, P.L., Profeti, R., Ceccarini, L., Macchia, M., Canale, A., 2012. Larvicidal and repellent activity of Hyptis suaveolens (Lamiaceae) essential oil against the mosquito Aedes albopictus Skuse (Diptera: Culicidae). Parasitol. Res. 110, 2013–2021. https://doi.org/10.1007/s00436-011-2730-8

Coutinho, C.F.B., Tanimoto, S.T., Galli, A., Garbellini, G.S., Takayama, M., do Amaral, R.B., Mazo, L.H., Avaca, L.A., Machado, S.A.S., 2005. PESTICIDAS: MECANISMO DE AÇÃO, DEGRADAÇÃO E TOXIDEZ. Pestic. Rev. ecotoxicologia e meio Ambient. 15, 65–72.

da Silva, M.F.R., Bezerra-Silva, P.C., de Lira, C.S., de Lima Albuquerque, B.N., Agra Neto, A.C., Pontual, E.V., Maciel, J.R., Paiva, P.M.G., Navarro, D.M. do A.F., 2016. Composition and biological activities of the essential oil of Piper corcovadensis (Miq.) C. DC (Piperaceae). Exp. Parasitol. 165, 64–70. https://doi.org/10.1016/j.exppara.2016.03.017

Dallaqua, B., Damasceno, D.C., 2011. Comprovação do efeito antioxidante de plantas medicinais utilizadas no tratamento do diabetes mellitus em animais: Artigo de atualização. Rev. Bras. Plantas Med. 13, 367–373. https://doi.org/10.1590/s1516-05722011000300017

Davis, T.S., Horne, F.B., Yetter, J.C., Stewart, J.E., 2018. Engelmann Spruce Chemotypes in Colorado and their Effects on Symbiotic Fungi Associated with the North American Spruce Beetle. J. Chem. Ecol. 44, 601–610. https://doi.org/10.1007/s10886-018-0961-1

de Christo Scherer, M.M., Marques, F.M., Figueira, M.M., Peisino, M.C.O., Schmitt, E.F.P., Kondratyuk, T.P., Endringer, D.C., Scherer, R., Fronza, M., 2019. Wound healing activity of terpinolene and α-phellandrene by attenuating inflammation and oxidative stress in vitro. J. Tissue Viability 28, 94–99. https://doi.org/10.1016/j.jtv.2019.02.003

do Nascimento, A.F., da Camara, C.A.G., de Moraes, M.M., 2018. Fumigant activity of schinus terebinthifolius essential oil and its selected constituents against rhyzopertha dominica. Rev. Fac. Nac. Agron. Medellin 71, 8359–8366. https://doi.org/10.15446/rfna.v71n1.62743

Dorman, H.J.D., Figueiredo, A.C., Barroso, J.G., Deans, S.G., 2000. In vitro evaluation of antioxidant activity of essential oils and their components. Flavour Fragr. J. 15, 12–16.

Emami, S.A., Asili, J., Malekian, M., Hassanzadeh, M.K., 2011. Antioxidant effects of the essential oils of different parts of platycladus orientalis l. (franco) and their components. J. Essent. Oil-Bearing Plants 14, 334–344. https://doi.org/10.1080/0972060X.2011.10643943

Famiani, F., Rischer, H., Osorio, S., Es, S., Vallarino, J.G., Pott, D.M., 2019. From Central to Specialized Metabolism: An Overview of Some Secondary Compounds Derived From the Primary Metabolism for Their Role in Conferring Nutritional and Organoleptic Characteristics to Fruit 10, 835. https://doi.org/10.3389/fpls.2019.00835

Felipe, L.O., Bicas, J.L., 2017. Terpenos, aromas e a química dos compostos naturais. Química Nov na Esc 39, 120–130.

Garozzo, A., Timpanaro, R., Bisignano, B., Furneri, P.M., Bisignano, G., Castro, A., 2009. In vitro antiviral activity of Melaleuca alternifolia essential oil. Lett. Appl. Microbiol. 49, 806–808. https://doi.org/10.1111/j.1472-765X.2009.02740.x

Garozzo, A., Timpanaro, R., Stivala, A., Bisignano, G., Castro, A., 2011. Activity of Melaleuca alternifolia (tea tree) oil on Influenza virus A/PR/8: study on the mechanism of action. Antiviral Res. 89, 83–8. https://doi.org/10.1016/j.antiviral.2010.11.010

Gasiński, A., Kawa-Rygielska, J., Szumny, A., Czubaszek, A., Gąsior, J., Pietrzak, W., 2020. Volatile compounds content, physicochemical parameters, and antioxidant activity of beers with addition of mango fruit (Mangifera Indica). Molecules 25, 3033.

Geris, L., 2014. Regenerative orthopaedics: in vitro, in vivo … in silico. Int. Orthop. 38, 1771–1778. https://doi.org/10.1007/s00264-014-2419-6

Ghasemi, Y., Mohagheghzadeh, A., Moshavash, M., Ostovan, Z., Rasoul-Amini, S., Morowvat, M.H., Ghoshoon, M.B., Raee, M.J., Mosavi-Azam, S.B., 2009. Biotransformation of monoterpenes by Oocystis pusilla. World J. Microbiol.

Biotechnol. 25, 1301.

Graßmann, J., Hippeli, S., Spitzenberger, R., Elstner, E.F., 2005. The monoterpene terpinolene from the oil of Pinus mugo L. in concert with α-tocopherol and β-carotene effectively prevents oxidation of LDL. Phytomedicine 12, 416–423. https://doi.org/10.1016/j.phymed.2003.10.005

Grassmann, J., Hippeli, S., Vollmann, R., Elstner, E.F., 2003. Antioxidative Properties of the Essential Oil from Pinus mugo. J. Agric. Food Chem. 51, 7576–7582. https://doi.org/10.1021/jf030496e

Gu, H.-J., Cheng, S.-S., Lin, C.-Y., Huang, C.-G., Chen, W.-J., Chang, S.-T., 2009. Repellency of Essential Oils of Cryptomeria japonica (Pinaceae) against Adults of the Mosquitoes Aedes aegypti and Aedes albopictus (Diptera:Culicidae). J. Agric. Food Chem. 57, 11127–11133. https://doi.org/10.1021/jf9024486

Guimarães, A.G., Quintans, J.S.S., Quintans-Júnior, L.J., 2013. Monoterpenes with analgesic activity—a systematic review. Phyther. Res. 27, 1–15.

Halliwell, B., Gutteridge, J.M.C., 2015. Free Radicals in Biology and Medicine, Free Radicals in Biology and Medicine. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001

Higgins, J.P.T., Altman, D.G., Gøtzsche, P.C., Jüni, P., Moher, D., Oxman, A.D., Savović, J., Schulz, K.F., Weeks, L., Sterne, J.A.C., 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Bmj 343, d5928.

Hooijmans, C.R., Rovers, M.M., De Vries, R.B.M., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 43. https://doi.org/10.1186/1471-2288-14-43

Ito, K., Ito, M., 2011. Sedative effects of vapor inhalation of the essential oil of Microtoena patchoulii and its related compounds. J. Nat. Med. 65, 336–343. https://doi.org/10.1007/s11418-010-0502-x

Jamshidi-Kia, F., Lorigooini, Z., Amini-Khoei, H., 2018. Medicinal plants: Past history and future perspective. J. Herbmed Pharmacol. J Herbmed Pharmacol 7, 1–7. https://doi.org/10.15171/jhp.2018.01

Jaroch, K., Jaroch, A., Bojko, B., 2018. Cell cultures in drug discovery and development: The need of reliable in vitro-in vivo extrapolation for pharmacodynamics and pharmacokinetics assessment. J. Pharm. Biomed. Anal. 147, 297–312. https://doi.org/10.1016/j.jpba.2017.07.023

Johnston, N.A., Nevalainen, T., 2002. Impact of the biotic and abiotic environment on animal experiments. Handb. Lab. Anim. Sci. Essent. Princ. Pract. 1, 311.

Keeling, C.I., Bohlmann, J., 2006. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytol. 170, 657–675. https://doi.org/10.1111/j.1469-8137.2006.01716.x

Khan, H., 2014. Medicinal Plants in Light of History: Recognized Therapeutic Modality. J. Evidence-Based Complement. Altern. Med. 19, 216–219. https://doi.org/10.1177/2156587214533346

Kig, C., Mertoglu, E., Caliskan, A., Hincal Agus, H., Onay Ucar, E., Guler, V., 2021. Selective and oxidative stress-mediated cell death of MCF-7 cell line induced by terpinolene. Biol. 2021 1–10. https://doi.org/10.1007/S11756-021-00803-Z

Kim, H.-J., Chen, F., Wu, C., Wang, X., Chung, H.Y., Jin, Z., 2004. Evaluation of Antioxidant Activity of Australian Tea Tree (Melaleuca alternifolia) Oil and Its Components. J. Agric. Food Chem. 52, 2849–2854. https://doi.org/10.1021/jf035377d

Kim, H.G., Cho, J.H., Jeong, E.Y., Lim, J.H., Lee, S.H., Lee, H.S., 2006. Growth-Inhibiting Activity of Active Component Isolated from Terminalia chebula Fruits against Intestinal Bacteria. J. Food Prot. 69, 2205–2209. https://doi.org/10.4315/0362-028X-69.9.2205

Kobayakawa, K., Kobayakawa, R., Matsumoto, H., Oka, Y., Imai, T., Ikawa, M., Okabe, M., Ikeda, T., Itohara, S., Kikusui, T., Mori, K., Sakano, H., 2007. Innate versus learned odour processing in the mouse olfactory bulb. Nature 450, 503–8. https://doi.org/10.1038/nature06281

Koyama, S., Heinbockel, T., 2020. The Effects of Essential Oils and Terpenes in Relation to Their Routes of Intake and Application. Int. J. Mol. Sci. 21, 1558. https://doi.org/10.3390/ijms21051558

Kroymann, J., 2011. Natural diversity and adaptation in plant secondary metabolism. Curr. Opin. Plant Biol. 14, 246–251. https://doi.org/10.1016/j.pbi.2011.03.021

Kweka, E.J., Lima, T.C., Marciale, C.M., de Sousa, D.P., 2016. Larvicidal efficacy of monoterpenes against the larvae of Anopheles gambiae. Asian Pac. J. Trop. Biomed. 6, 290–294. https://doi.org/10.1016/j.apjtb.2016.03.001

Lee, C.J., Chen, L.W., Chen, L.G., Chang, T.L., Huang, C.W., Huang, M.C., Wang, C.C., 2013. Correlations of the components of tea tree oil with its antibacterial effects and skin irritation. J. Food Drug Anal. 21, 169–176.

https://doi.org/10.1016/j.jfda.2013.05.007
Liang, J.Y., Guo, S.S., Zhang, W.J., Geng, Z.F., Deng, Z.W., Du, S.S., Zhang, J., 2018. Fumigant and repellent activities of essential oil extracted from Artemisia dubia and its main compounds against two stored product pests. Nat. Prod. Res. 32, 1234–1238. https://doi.org/10.1080/14786419.2017.1331227
Liu, T.T., Chao, L.K.P., Hong, K.S., Huang, Y.J., Yang, T.S., 2020. Composition and insecticidal activity of essential oil of Bacopa caroliniana and interactive effects of individual compounds on the activity. Insects 11. https://doi.org/10.3390/insects11010023
Lu, Q., Huang, N., Peng, Y., Zhu, C., Pan, S., 2019. Peel oils from three Citrus species: volatile constituents, antioxidant activities and related contributions of individual components. J. Food Sci. Technol. 56, 4492–4502. https://doi.org/10.1007/s13197-019-03937-w
Macedo, E.M.A., Santos, W.C., Sousa Neto, B.P., Lopes, E.M., Piauilino, C.A., Cunha, F.V.M., Sousa, D.P., Oliveira, F.A., Almeida, F.R.C., 2016. Association of terpinolene and diclofenac presents antinociceptive and anti-inflammatory synergistic effects in a model of chronic inflammation. Brazilian J. Med. Biol. Res. 49, 1–10. https://doi.org/10.1590/1414-431X20165103
Mecocci, P., MacGarvey, U., Beal, M.F., 1994. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann. Neurol. 36, 747–751. https://doi.org/10.1002/ana.410360510
Menichini, Federica, Tundis, R., Loizzo, M.R., Bonesi, M., Marrelli, M., Statti, G.A., Menichini, Francesco, Conforti, F., 2009. Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from Pimpinella anisoides V Brig . (Apiaceae). Fitoterapia 80, 297–300. https://doi.org/10.1016/j.fitote.2009.03.008
Miyazawa, M., Watanabe, H., Kameoka, H., 1997. Inhibition of Acetylcholinesterase Activity by Monoterpenoids with a p -Menthane Skeleton. J. Agric. Food Chem. 45, 677–679. https://doi.org/10.1021/jf960398b
Monro, H.A.U., 1971. Manual of Fumigation for Insect Control. Anzeiger für Schädlingskd. und Pflanzenschutz 44, 176–176. https://doi.org/10.1007/BF02026755
Mori, K., Sakano, H., 2011. How is the olfactory map formed and interpreted in the mammalian brain? Annu. Rev. Neurosci. 34, 467–99. https://doi.org/10.1146/annurev-neuro-112210-112917
Morshedi, D., Kesejini, T.S., Aliakbari, F., Karami-Osboo, R., Shakibaei, M., Marvian, A.T., Khalifeh, M., Soroosh, M., 2014. Identification and characterization of a compound from Cuminum cyminum essential oil with antifibrilation and cytotoxic effect. Res. Pharm. Sci. 9, 431–43.
Ngahang Kamte, S.L., Ranjbarian, F., Cianfaglione, K., Sut, S., Dall'Acqua, S., Bruno, M., Afshar, F.H., Iannarelli, R., Benelli, G., Cappellacci, L., Hofer, A., Maggi, F., Petrelli, R., 2018. Identification of highly effective antitrypanosomal compounds in essential oils from the Apiaceae family. Ecotoxicol. Environ. Saf. 156, 154–165. https://doi.org/10.1016/j.ecoenv.2018.03.032
Ngan, L.T.M., Moon, J.K., Kim, J.H., Shibamoto, T., Ahn, Y.J., 2012. Growth-inhibiting effects of Paeonia lactiflora root steam distillate constituents and structurally related compounds on human intestinal bacteria. World J. Microbiol. Biotechnol. 28, 1575–1583. https://doi.org/10.1007/s11274-011-0961-6
Opdyke, D.L.J., 1976. Terpinolene. Food Cosmet. Toxicol. 14, 877–878. https://doi.org/10.1016/S0015-6264(76)80163-0
Paduch, R., Kandefer-Szerszeń, M., Trytek, M., Fiedurek, J., 2007. Terpenes: Substances useful in human healthcare. Arch. Immunol. Ther. Exp. (Warsz). https://doi.org/10.1007/s00005-007-0039-1
Park, I.-K., Lee, S.-G., Choi, D.-H., Park, J.-D., Ahn, Y.-J., 2003. Insecticidal activities of constituents identified in the essential oil from leaves of Chamaecyparis obtusa against Callosobruchus chinensis (L.) and Sitophilus oryzae (L.). J. Stored Prod. Res. 39, 375–384. https://doi.org/10.1016/S0022-474X(02)00030-9
Pavela, R., 2015. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against Culex quinquefasciatus Say larvae. Parasitol. Res. 114, 3835–3853.
Pavela, R., Maggi, F., Cianfaglione, K., Bruno, M., Benelli, G., 2018. Larvicidal Activity of Essential Oils of Five Apiaceae Taxa and Some of Their Main Constituents Against Culex quinquefasciatus. Chem. Biodivers. 15, e1700382. https://doi.org/10.1002/cbdv.201700382
Perumalsamy, H., Kim, N.J., Ahn, Y.J., 2009. Larvicidal activity of compounds isolated from asarum heterotropoides against culex pipiens pallens, aedes aegypti, and ochlerotatus togoi (diptera: Culicidae). J. Med. Entomol. 46, 1420–1423. https://doi.org/10.1603/033.046.0624
Petrović, G.M., Stamenković, J.G., Mitić, V.D., Stojanović, G.S., Zlatković, B.K., Jovanović, O.Č., 2018. Chemical Composition and Antioxidant Activity of the Athamanta turbith ssp. haynaldii Volatiles. Nat. Prod. Commun. 13, 1934578X1801300634.
Pinto, Â.V., Oliveira, J.C., Costa de Medeiros, C.A., Silva, S.L., Pereira, F.O., 2020. Potentiation of antifungal activity of

terbinafine by dihydrojasmone and terpinolene against dermatophytes. Lett. Appl. Microbiol. lam.13371. https://doi.org/10.1111/lam.13371

Politi, F.A.S., Nascimento, J.D., da Silva, A.A., Moro, I.J., Garcia, M.L., Guido, R.V.C., Pietro, R.C.L.R., Godinho, A.F., Furlan, M., 2017. Insecticidal activity of an essential oil of Tagetes patula L. (Asteraceae) on common bed bug Cimex lectularius L. and molecular docking of major compounds at the catalytic site of ClAChE1. Parasitol. Res. 116, 415–424. https://doi.org/10.1007/s00436-016-5305-x

Pontin, M., Bottini, R., Burba, J.L., Piccoli, P., 2015. Allium sativum produces terpenes with fungistatic properties in response to infection with Sclerotium cepivorum. Phytochemistry 115, 152–160. https://doi.org/10.1016/j.phytochem.2015.02.003

Ribeiro, N. de C., da Camara, C.A.G., de Melo, J.P.R., de Moraes, M.M., 2020. INSECTICIDAL POTENTIAL OF CITRUS AND MANGO ESSENTIAL OILS AND SELECTED CONSTITUENTS ON SILVERLEAF WHITEFLY1. Rev. Caatinga 33, 90–99. https://doi.org/10.1590/1983-21252020v33n110rc

Ribeiro, N. de C., da Camara, C.A.G., de Melo, J.P.R., de Moraes, M.M., 2019. Effect of the essential oil from the latex of the fruit mangifera indica l. On tetranychus urticae koch (acari,tetranychidae). Acarologia 59, 335–347. https://doi.org/10.24349/acarologia/20194333

Ribeiro, N.C., da Camara, C.A.G., Melo, J.P.R., de Moraes, M.M., 2019. Acaricidal properties of essential oils from agro-industrial waste products from citric fruit against Tetranychus urticae. J. Appl. Entomol. 143, 731–743. https://doi.org/10.1111/jen.12642

Riyazi, A., Hensel, A., Bauer, K., Geißler, N., Schaaf, S., Verspohl, E., 2007. The Effect of the Volatile Oil from Ginger Rhizomes (Z ingiber officinale), its Fractions and Isolated Compounds on the 5-HT 3 Receptor Complex and the Serotoninergic System of the Rat Ileum. Planta Med. 73, 355–362. https://doi.org/10.1055/s-2007-967171

Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 69, 167–174. https://doi.org/10.1016/S0308-8146(99)00247-2

Sawamura, M., Sun, S.H., Ozaki, K., Ishikawa, J., Ukeda, H., 1999. Inhibitory Effects of Citrus Essential Oils and Their Components on the Formation of N-Nitrosodimethylamine. J. Agric. Food Chem. 47, 4868–4872. https://doi.org/10.1021/jf9903206

Seifi Nahavandi, B., Yaghmaei, P., Ahmadian, S., Ebrahim-Habibi, A., Ghobeh, M., 2020. Effects of Terpinolene and Physical Activity on Memory and Learning in a Model of Alzheimer's Disease among Rats. Qom Univ. Med. Sci. J. 14, 25–33. https://doi.org/10.52547/QUMS.14.10.25

Shin, S., 2004. In vitro inhibitory activities of essential oils from Oenanthe javanica DC against Candida and Streptococcus species. Nat. Prod. Sci. 10, 325–329.

Soria-Gómez, E., Bellocchio, L., Reguero, L., Lepousez, G., Martin, C., Bendahmane, M., Ruehle, S., Remmers, F., Desprez, T., Matias, I., Wiesner, T., Cannich, A., Nissant, A., Wadleigh, A., Pape, H.-C., Chiarlone, A.P., Quarta, C., Verrier, D., Vincent, P., Massa, F., Lutz, B., Guzmán, M., Gurden, H., Ferreira, G., Lledo, P.-M., Grandes, P., Marsicano, G., 2014. The endocannabinoid system controls food intake via olfactory processes. Nat. Neurosci. 17, 407–415. https://doi.org/10.1038/nn.3647

Sowndhararajan, K., Cho, H., Yu, B., Kim, S., 2015. Effect of olfactory stimulation of isomeric aroma compounds, (+)-limonene and terpinolene on human electroencephalographic activity. Eur. J. Integr. Med. 7, 561–566. https://doi.org/10.1016/j.eujim.2015.08.006

Tang, Y., Zhu, W., Chen, K., Jiang, H., 2006. New technologies in computer-aided drug design: Toward target identification and new chemical entity discovery. Drug Discov. Today Technol. https://doi.org/10.1016/j.ddtec.2006.09.004

Terpinolene | C10H16 - PubChem [WWW Document], 2021. URL https://pubchem.ncbi.nlm.nih.gov/compound/Terpinolene#section=3D-Conformer&fullscreen=true (accessed 2.1.21).

Tisserand, R., Young, R., 2014. Essential Oil Safety, Essential Oil Safety: A Guide for Health Care Professionals: Second Edition. Elsevier. https://doi.org/10.1016/C2009-0-52351-3

Turkez, H., Aydın, E., Geyikoglu, F., Cetin, D., 2015. Genotoxic and oxidative damage potentials in human lymphocytes after exposure to terpinolene in vitro. Cytotechnology 67, 409–418. https://doi.org/10.1007/s10616-014-9698-z

Valli, M., Bolzani, V.S., 2019. Natural products: Perspectives and challenges for use of brazilian plant species in the bioeconomy. An. Acad. Bras. Cienc. 91. https://doi.org/10.1590/0001-3765201920190208

Wang, J.L., Li, Y., Lei, C.L., 2009. Evaluation of monoterpenes for the control of Tribolium castaneum (Herbst) and

Sitophilus zeamaise Motschulsky. Nat. Prod. Res. 23, 1080–1088. https://doi.org/10.1080/14786410802267759
Yoshida, N., Koizumi, M., Adachi, I., Kawakami, J., 2006. Inhibition of P-glycoprotein-mediated transport by terpenoids contained in herbal medicines and natural products. Food Chem. Toxicol. 44, 2033–2039. https://doi.org/10.1016/j.fct.2006.07.003
Yu, D., Wang, J., Shao, X., Xu, F., Wang, H., 2015. Antifungal modes of action of tea tree oil and its two characteristic components against m cinerea. J. Appl. Microbiol. 119, 1253–1262. https://doi.org/10.1111/jam.12939
Zhang, Z., Xie, Y., Wang, Y., Lin, Z., Wang, L., Li, G., 2017. Toxicities of monoterpenes against housefly, Musca domestica L. (Diptera: Muscidae). Environ. Sci. Pollut. Res. 24, 24708–24713. https://doi.org/10.1007/s11356-017-0219-4
Zhang, Z., Yang, T., Zhang, Y., Wang, L., Xie, Y., 2016. Fumigant toxicity of monoterpenes against fruitfly, Drosophila melanogaster. Ind. Crops Prod. 81, 147–151. https://doi.org/10.1016/j.indcrop.2015.11.076
Zhao, P., Wang, Y., Huang, W., He, L., Lin, Z., Zhou, J., He, Q., 2020. Toxic effects of terpinolene on Microcystis aeruginosa: Physiological, metabolism, gene transcription, and growth effects. Sci. Total Environ. 719, 137376. https://doi.org/10.1016/j.scitotenv.2020.137376

Table 1. *In silico* terpinolene studies

Author	Year	Country	Method	Results	Biological Activity	Reference
POLITI et al	2017	Brazil	Molecular docking	Polar and hydrophobic interactions with the catalytic site of ClAChE1 (competitive inhibitor).	Acetylcholinesterase inhibitor	(Politi et al. 2017)
MORSHEDI et al	2014	Iran	Molecular docking	Amino acids, E35, W62, W63 (near RII), and V109, A 110 (RIV) were the residues with the highest proximity to terpinolene.	Binding to HEWLs (chicken egg white lysozyme)	(Morshedi et al. 2014)

Table 2. *In vitro* terpinolene studies

Author	Year	Country	Method	Concentration	Results	Biological Activity	Reference
ZHAO et al	2020	China	Analysis of: Antibacterial activity against *C. vulgaris* Oxidative damage Cellular morphology Photosynthetic activity Antioxidant capacity Nitrogen metabolism	0.551, 0.881, 1.079, 1.233, and 1.470 mM	Inhibited algae growth and the photosynthetic activity of *C. vulgaris.* Induced concentration-dependent changes in the microstructure	Herbicide	(Zhao et al. 2020)

			Abundance of transcribed genes				
LIU et al	2020	Taiwan	Analysis of AchE inhibition through calorimetry	The work cites the effective concentrations but does not explain which concentrations were tested.	IC $_{50}$ = 1.10 ± 0.17 (μL/mL) - Terpinolene exhibited the best inhibitory activity among the tested compounds	AchE inhibition *(in vitro)*	(Liu et al. 2020)
PINTO et al	2020	Brazil	Antifungal activity against *Microsporum canis* LM 216, *Trichophyton interdigitale* H6 (ATCC MYA-3108) and *T. interdigitale* Δ mdr2 Effect on membrane functionality Analysis of K^+ efflux by turbidimetry	1024 μg/mL to 1 μg/mL	MIC: 128 μg/mL against *T. interdigitale* Δ mdr2 Terpinolene MIC > 1,024 μg/mL). Increased K+: efflux ($p < 0.05$), affecting membrane functions	Antifungal	(Pinto et al. 2020)
LU et al	2019	India	DPPH free radical elimination test Thiobarbituric acid reactive species (TBARS) generation test	100 μL/mL (concentration used to obtain EC_{50} values)	Strong antioxidant activity: EC_{50} (DPPH): 65.77 ± 4.98. EC_{50} (TBARS) < 5 Caused 70% inhibition of lipid peroxidation, increasing antioxidant activity	Antioxidant	(Lu et al. 2019)
De CHRISTO et al	2019	Brazil	Ferric reducing / antioxidant power (FRAP) NO quantification	Wound healing 10, 100 e 200 μM NF-kB	Weak FRAP activity IC_{50} for NO production: 409,4 ± 1,6	Wound healing, anti-inflammatory, cytoprotective, and	(de Christo Scherer et al. 2019)

			ABTS cationic radical elimination assay Cytotoxicity evaluation: MTT test Wound healing activity: Fibroblast proliferation and migration iNOs expression Determination of intracellular superoxide anion: Nitro blue atrazolium reduction assay (NBT) Cytokine quantification (ELISA) NF-kB activity	activity 1-100 μM Other tests: 1.0- 200 μM	IC_{50} for ABTS: 497.4 ± 14.5 μM Non-cytotoxic to fibroblasts L929 and RAW 267.7 macrophages (200 μM). Proliferative effects were observed in L929 cells: 121.5 ± 3.2% at 200 μM. Wound healing activity: Increased proliferation and migration of fibroblasts. 36.3 ± 4.8% (maximum stimulating effect) at 200 μM Suppressed NO production in RAW 264.7 macrophages: 41.3 ± 1.4% at 200 μM; No effects on LPS-stimulated cells Inhibition of intracellular superoxide production: 82.1 ± 3.5% (100 μM) and 82.6 ± 3.5% (200 μM) Reduced production of	antioxidant	

					IL-6 and TNF-α Inhibition (14.3 ± 2.5%) of TNF- α induced NF-κB activity at 100 μM		
PAVELA et al.	2018	Czech Republic	Evaluation of insecticide activity against *Culex quinquefasciatus*	5.0 to 200.0 μL/L	LC_{50}: 25.7 μl /L LC_{90}: 50.1 μl / L X^2: 0.043	Inseticide	(Pavela et al. 2018)
NGAHANG KAMTE et al.	2018	Itália	Antiparasitic activity against *Trypanosoma brucei*	Tested concentrations not mentioned	Active against *Trypanosoma brucei* EC_{50}= 0.035 μg/mL (0.26 μM)	Tripanocide	(Ngahang Kamte et al. 2018)
DAVIS et al.	2018	USA	Antifungal activity against *L. abietinum*	1, 5, and 10%	Complete inibition of fungal growth at 5% and 10%.	Antifungal	(Davis et al. 2018)
ANDRÉS et al.	2017	Spain	Nematicide activity against *Meloidogyne javanica* Phytotoxic activity	Nematicide activity: 1.0 and 0.5 mg/mL Phytotoxic activity: 0.4 and 0.2 mg / mL	Inactive against *Meloidogyne javanica* Weak phytotoxicity against *S. lycopersicum* (25%)	Nematicide	(Andrés et al. 2017)
KWEKA et al.	2016	Brazil	Larvicidal activity against *Anopheles gambiae* s.s.	200, 100, 50, 25 and 12.5 mg/L	LC_{50} at 12h: 493.38 mg/L 24h: 404.71 mg/L 48h: 343.79 mg/L 72h: 259.40 mg/L	Larvicide	(Kweka et al. 2016)
DA SILVA et al.	2016	Brazil	Larvicidal activity against *A. aegypti* Effects on oviposition Effects on	0.01 mg/mL (10 mg + 0,1 g Tween 80 + distilled water)	Larvicidal activity: LC_{50} = 31,16 ppm No effect on oviposition	Larvicide	(da Silva et al. 2016)

			intestinal enzymes (L_4) Zymography of intestinal proteases Effect on gut trypsin activity		Inhibited proteolytic polypeptides of intestinal enzymes L_4 Inhibited the activity of trypsin-like enzymes		
YU et al.	2015	China	Effect of terpinoleone on the mycelial growth of *Botrytis cinerea*	0.25, 0.5, 1.0, and 1.5 µl/mL	Strong and concentration-dependent antifungal activity	Antifungal	(Yu et al. 2015)
TURKEZ et al.	2015	Peru	Cytotoxicity to human blood cells Lactate dehydrogenase (LDH) release assay Cell Viability Assay (MTT) Cytogenetic assays Oxidation of nucleic acid Analysis of total antioxidant capacity and total oxidizing status Cell Viability Assay (MTT) Cytogenetic assays Oxidation of nucleic acid Analysis of total antioxidant capacity and total oxidizing status	10, 25, 50, 75, 100, 150 and 200 mg/L	Increased LDH release Reduction of cell viability at 150 and 200 mg/L. Increased lymphocyte counts in peripheral blood No changes in 8-OH-dG levels (nucleic acid oxidation) Total oxidant capacity (TOC) was decreased at 200 mg/L, stable at 100 and 150 mg/L) and increased at 10, 25, 50, and 75 mg/L). Total oxidant status (TOS) was increased at 150 and 200	Cytotoxic Antioxidant	(Turkez et al. 2015)
PONTIN et al.	2015	Argentina	Antifungal activity against	Antifungal activity and	Antifungal activity: 2.0	Antifungal	(Pontin et al. 2015)

			Sclerotium cepivorum (disk diffusion) Number of sclerotia produced by *S. cepivorum* Ethidium bromide absorption assay (EtBr) Scanning electron microscopy (SEM)	sclerotia count: 2, 3, 4 and 5 µg/disc	and 5.0 µg/disc. Strong inhibition of mycelial growth (about 93%) Reduction of sclerotia production by about 18% Possible disturbance of fungal membrane integrity by interference in Etbr absorption SEM: Hyphae with shorter branching, morphological changes, and partial distortion		
PAVELA	2015	Czech Republic	Acute toxicity against *Culex quinquefasciatus* larvae	5 to 250 µg/L	Mortality at 250 mg/L= 93.2±2.8 LC_{25}= 11 mg/L (9–15) LC_{50}= 21 mg/L (18–27) LC_{90}= 245 mg/L (206–278) Larval mortality (%) = 6.5	Larvicide	(Pavela 2015)
ALI et al.	2015	USA	Mosquito bite bioassays Larvicidal activity against *A. aegypti* and *A. quadrimaculatus*	Mosquito bite: 25 nmol/cm² Larvicidal assay: 1,5 a 0,0375 mg/cm²	*A. aegypti* CE_{50} ppm= 14.0 LV_{90} ppm= 21.4 *A.Quadrimaculatus* CE_{50} ppm= 20.9	Larvicide	(Ali et al. 2015)

					LV_{90} ppm= 36.8		
MORSHEDI et al.	2014	Irã	Fluorescence assay LDH release Flow cytometry	2%	Protection of PC12 cells from HEWL-induced cytotoxicity (chicken egg white lysozyme) Reduced fluorescence intensity, (prevented fibrillation over time) Prevention of cell death induced by HEWLs: flow cytometry analysis showed a decrease in cell death by terpinolene treatment	Cytoprotective	(Morshedi et al. 2014)
MADEMTZOGLOU et al.	2013	Greece	Activity against *Drosophila melanogaster* Somatic mutation and recombination test	2.5, 5.0, 7.5 and 10 µl/ml	Genotoxic potential could not be assessed, as terpinolene caused mortality of Drosophila (data not shown)		(Mademtzoglou et al. 2013)
LEE et al.	2013	Taiwan	Antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus*	0-80%	Inactive against *P. acnes*, Antibacterial activity against *S. aureus*. Reduction of 1.03±0.03 in disk diameter and e 6.25% in the MIC	Antibacterial	(Lee et al. 2013)

AYDIN; TÜRKEZ; TAŞDEMIR	2013	Turkey	Antiproliferative and/or cytotoxic properties: (MTT) Comet Assay Genotoxic damage potential (single cell gel electrophoresis (SCGE)) TAC and TOS analysis	10, 25, 50, 100, 200 and 400 mg/L	Potent antiproliferative agent for brain tumor cells (anti-cancer potential) Cytotoxic doses for neuronal cells: 100, 200, and 400 mg/L; Cytotoxic doses for neuroblastoma cells N2a 50, 100, 200, and 400 mg/L Comet assay: Non-genotoxic Primary neuron: Increase of TAC by 10, 25, and 50 mg/L and reduction by 400 mg/L; Increased TOS at 100, 200, and 400 mg/L N2a cells: Decreased TAC and increase TOS at 50, 100, 200, and 400 mg/L	Antiproliferative and antioxidant	(Aydin et al. 2013)
NGAN et al.	2012	Republic of Korea	Bacterial growth inhibition test MIC values (mg / mL): <0.1 (extremely high), 0.1–0.62 (high), 0.62–1.25 (moderate), 1.25–2.5 (low) and> 2.5 (no growth inhibition)	2,5 a 0,1 mg/mL	Antibacterial activity against all tested bacteria. MIC of 0.16 mg/mL against *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Clostridium perfringens*, *Clostridium paraputrificum*,	Antibacterial	(Ngan et al. 2012)

					Klebsiella pneumoniae); MIC of 0.31 mg/mL against *Escherichia coli, Salmonella typhimurium*); *Bifidobacterium adolescentes, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus*) MIC of 0.62 mg/mL against *Clostridium difficile, Staphylococcus aureus, Clostridium butyricum*) MIC of 1.25 mg/mL against *Lactobacillus casei*		
CONTI et al.	2012	Italy	Larvicidal activity against *Aedes albopictus*: WHO (1991)	48 ppm	Mortality (%): 43.33±0.76	Larvicide	(Conti et al. 2012)
GAROZZO et al.	2011	Italy	Activity against Influenza A/PR/8 virus subtype H1N1 in MDCK cells. Virucidal activity Virus fixation inhibition assay Haemagglutination inhibition assay Neuraminidase	0.005%	Inhibition of virus replication EC_{50}= 0.00125% (v/v) Inhibition of viral replication at a specific stage (initial stage of the viral cycle No interfere in	Antiviral	(Garozzo et al. 2011)

			inhibition		the cellular fixation of the virus or viral adsorption No significant inhibitory effect on neuraminidase inhibition		
EMAMI et al.	2011	Iran	Evaluation of *in vitro* antioxidant activity: Rapid TLC screening for antioxidants DPPH free radical scavenging activity Deoxyribose degradation test Non-enzymatic lipid peroxidation test	0.05; 0.1; 0.2; 0.5; 1; 2, and 4 µL/mL	Antioxidant activity o DPPH scavenging activity at 0.1 (3.83%), 0.5 (6.89%), 1 (9.64%), 2 (16.18%), and 4 µL / mL (30.16%) Inhibition of deoxyribose degradation at 0.1 (20.90), 0,2 (24.85), 0.5 (21.65), 1 µL/mL (21.65) Non-enzymatic lipid peroxidation test: 0.05 (52.77), 0.5 (38.64), 1 (32.16), 2 µL/mL (10.52)	Antioxidant	(Emami et al. 2011)
BONESI et al.	2010	Italy	Cholinesterase inhibition assay	10, 25, 50, 100 and 200 µg/mL	AchE CI_{50}= 156.4 µg/mL BchE CI_{50}= 147.1 µg/mL Concentration-dependent anti-cholinesterase activity	Cholinesterase inhibition (AchE and BchE)	(Bonesi et al. 2010)
PERUMALSAMY; KIM; AHN	2009	Republic of Korea	Larvicidal activity (Toxicity)	1 to 200 ppm	Toxic against *Cx. p. fallens* (LC_{50}= 11.85	Larvicide	(Perumalsamy et al. 2009)

					ppm) Presented the highest toxicity against *Ochlerotatus Togoi* (LC_{50}= 11.85 ppm)		
GAROZZO et al.	2009	Italy	Antiviral activity against polio type 1, ECHO 9, Coxsackie B1, adeno type 2, herpes simplex (HSV) type 1 and 2 viruses	0.1 % a 0,0001 %.	Inhibition of the influenza A-PR8 virus replication. The IC_{50} value (0. 0012) was lower than the CD_{50} (0. 012) of terpinolene Not effective against polio viruses 1, adeno 2, ECHO 9, Coxsackie B1, HSV-1, and HSV-2	Antiviral	(Garozzo et al. 2009)
CHENG; CHUA; et al.	2009	Taiwan	Larvicidal activity against *Aedes aegypti* and *Aedes albopictus*.	100, 50, 25, 12.5, and 6.25 µg/ml	Strong larvicidal effect against *A. albopictus* *A. aegypti:* LC_{50} = 32.1 µg/ml LC_{90} = 83.6 µg/ml *A. albopictus* LC_{50} = 22.0 µg/ml LC_{90} = 55.5 µg/ml	Larvicide	(Cheng, Chua, et al. 2009)
CHENG; CHANG; et al.	2009	Taiwan	Larvicidal activity against *Aedes aegypti* and *Aedes albopictus*.	50, 25, 12.5, and 6.25 µg/mL	Larvicidal activity *Aedes aegypti* LC_{50} = 32.1µg/ml LC_{90} > 50.0 µg/ml (not effective) *A. Albopictus* LC_{50} = 21.3 µg/ml	Larvicide	(Cheng, Chang, et al. 2009)

					LC_{90} = 48.0 µg/ml		
RIYAZI et al.	2007	Germany	Evaluation of antispasmodic activity using isolated rat ileum in organ bath	1 mM	Antispasmodic effect on rat ileum by inhibiting maximum and biphasic contraction after 2.5 min at 1 mM through Interference with 5-HT_3 receptors	Antispasmodic	(Riyazi et al. 2007)
YOSHIDA et al.	2006	Japan	Effects of terpenoids in the accumulation of [3H] digoxin in LLC-GA5-COL150 cells	1 mM	Terpinolene inhibited by 50% the efflux of [3H] digoxin mediated by glycoprotein P LC_{50} = 481µM	P-glycoprotein inhibition	(Yoshida et al. 2006)
KIM et al.	2006	Korea	Classification of antimicrobial activity	2 mg/disk	No antibacterial activity observed	Bacteriostatic	(KIM et al. 2006)
GRASSMANN et al.	2005	Germany	Copper-induced LDL oxidation	0.01 a 0.25%	Terpinolene inhibits LDL oxidation at concentrations above 0. 01%	Antioxidant	(Graßmann et al. 2005)
SHIN	2004	Korea	Antifungal analysis through the disk diffusion test	4 to 64 mg/mL	*C. albicans* MIC: 64 mg/mL (ineffective) *C. tropicalis* MIC: 32 mg/mL *C. utilis* MIC: 8 mg/mL	Antifungal	(Shin 2004)
KIM et al.	2004	USA	Antioxidant capacity. 1 - DPPH free radical elimination test: 2 – Hexanal/hexanoic acid assay	0.19 mM and 180 mM	Weak inhibition of DPPH scavenging at 0.19 mM 65% inhibition of hexanal oxidation to hexanoic acid	Antioxidant	(H.-J. Kim et al. 2004)

					at 180 mM		
GRASSMANN et al.	2003	Germany	Copper-induced LDL oxidation	0.01 a 0.25%	Concentration-dependent inhibition of LDL oxidation. Increase in latency time up to 774 min.	Antioxidant	(Grassmann et al. 2003)
RUBERTO; BARATTA	2000	Italy	Thiobarbituric acid reactive species (TBARS) Determination of a diene conjugated formation from linoleic acid by spectrophotometry.	1000, 500, and 100 ppm 10^{-2}, 10^{-3}, and 10^{-4} M	Concentration-dependent antioxidant activity TBARS: 1000 ppm = 64.6 500 ppm = 56.3 100 ppm = 40.3 Diene formation rate 10^{-2} M = 78.3 10^{-3} M = 22.0 10^{-4} M = 12.2	Antioxidant	(Ruberto and Baratta 2000)
CHOI et al.	2000	Japan	Free radical (DPPH) scavenging activity	235.2 mg/mL	DPPH scavenging activity (87.4%, 235.2 mg Trolox E/mL) 3.5-fold stronger than Standard Trolox.	Antioxidant	(Choi et al. 2000)
DORMAN et al.	2000	Scotland	Thiobarbituric acid reactive species (TBARS)	0.05 – 25,000 ppm	Almost 100% antioxidant activity at the concentration of 10000 ppm.	Antioxidant	(Dorman et al. 2000)
SAWAMURA et al.	1999	Japan	N-nitrosodimethylamine (NDMA) generation	10 µL	50% inhibition of NDMA generation	NDMA inhibition	(Sawamura et al. 1999)
OH et al.	1967	USA	Antimicrobial activity	0.025 mL	Inhibition of sheep rumen microbe growth (-58%)	Antimicrobial	(Oh et al. 1967)

Table 3. *In vivo* terpinolene studies

Author	Year	Country	Method	Route of Administration	Concentration	Results	Biological Activity	Reference

RIBEIRO et al.	2020	Brazil	Fumigation against *Bemisia tabaci* Fecundity test	Fumigation	Fumigation: 2.0 to 6.0 µL/L air Fecundity test 2.0 µL/L air	Terpinolene was the 3rd most toxic compound and promoted a greater reduction in the number of eggs laid by *B. tabaci*	Inseticide	(RIBEIRO et al. 2020)
LIU et al.	2020	Taiwan	Toxicity against *Bacopa caroliniana*	Fumigation	20 µL	LC_{50} = 172 ± 6 (µL/mL). Insecticide impact (IT): IT = 39.76 Synergistic insecticide effects with all tested compounds	Insecticide	(Liu et al. 2020)
RIBEIROet al.a	2019	Brazil	Toxicity against *T. urticae* Fumigation test Residual contact test Fertility Bioassay	Fumigation and residual contact	Fumigation: 0.2 to 4.0 µl/L air Residual contact: 43 to 688 mg/mL Fertility: 0,4 µl / L de ar	Ensaio de Fumigation: Terpinolene was the most toxic compound. Residual contact test: Terpinolene had the lowest effect Fecundity bioassay: reduced the number of eggs laid by *T. urticae* by 24.53%	Acaricide	(N. C. Ribeiro et al. 2019)
RIBEIRO, et al.b	2019	Brazil	Toxicity against *T. urticae* Fumigation test Residual contact test Fertility Bioassay	Fumigation and residual contact	Fumigation: 0.2 to 4.0 µL/L air Residual contact: 44 to 689 µL/mL Fecundity: 0.2 µL/L	Terpinolene was the most toxic compound. LC_{50} (95%CI) = 2.07 (1.61-2.62); χ^2= 5.29 Low effect on residual contact	Insecticide and Repellent	(N. de C. Ribeiro et al. 2019)

						LC_{50} (95%CI) = 263.06(221.31-); χ^2= 5.21 Low reduction of fecundity		
DO NASCIMENTO; DA CAMARA; DE MORAES	2018	Brazil	Fumigation assay	Fumigation	5 to 90 µL/L.	High toxicity: 100% mortality by the last hour of testing	Insecyicide	(do Nascimento et al. 2018)
LIANG et al.	2018	China	Insecticide activity against *Tribolium castaneum* and *Lipocelis bostrychophila*	Fumigation	78, 63, 15, 73, 3, 15, 0.63, and 0.13 nL/cm^2	Toxic by fumigation against *T. castaneum* and *L. bostrychophila* Weak repellent activity against both insects	Insecticide	(Liang et al. 2018)
BORN et al.	2018	Brazil	Acaricide activity against *Tetranychus urticae* Fumigation and residual contact assays	Fumigation Residual contact	Fumigation: 0.0002 a 16.0 µL/L Residual contact: 0.1 to 800.0 µL/mL	Fumigation assay LC_{50} (95% CI) = 1.08 (0.62–1.63) Residual contact LC_{50} (95% CI)= 341.91 (206.91–520.84)	Acaricide	(Born et al. 2018)
ZHANG et al.	2017	China	Toxicity against *Musca domestica*	Fumigation	1.25, 2.5, 3.75, and e 5 µL/L	LC_{50} = 1,84 µl/L	Insecticide	(Z. Zhang et al. 2017)
ZHANG et al.	2016	China	Toxicity against *Drosophila melanogaster*	Fumigation	Not stated	LC_{50} = 0.09 µl/L (04-0.14) LC_{90} = 0.62 µl/L (0.44-1.15)	Insecticide	(Z. Zhang et al. 2016)
MACEDO et al.	2016	Brazil	CFA-induced inflammati	Oral	3.125, 6.25, 12.5, and 25 mg/Kg	Analgesic effect in the acute phase	Anti-inflammatory and	(Macedo et al. 2016)

			on in the rat Hyperalgesia Analysis of gastric lesions			Inhibition of CFA-induced paw edema Inhibition of leukocyte infiltration in the paw Involvement of serotoninergic pathways in the analgesic effect Absence of gastric lesions after 11 days of treatment	analgesic	
ALI et al.	2015	USA	Repellent activity against	Fumigation	25 nmol/cm²	Strong repellent activity against *A. aegypti* and *A. quadrimaculatus*	Larvicide and insecticide	(Ali et al. 2015)
ITO; ITO	2013	Japan	Sedative effect in ddY mice (olfactory deficiency) Mice with olfactory deficiency caused by zinc sulfate	Inhalation Intraperitoneal	Inhalation: Cotton soaked with 0.1mg terpinoleone/cage Intraperitoneal: 0.01 or 0.1 mg/kg	The motor activity of the mice was reduced to 67.8% after inhalation of terpinolene 0.1 mg/cage) Motor activity was reduced after i.p. administration: 31.3% (0.01 mg/kg) and 47.1% (0.1 mg/kg)	Sedative	(Ito and Ito 2013)
CHANG et al.	2012	Republic of Korea	Insecticide activity against KSS	Fumigation	Not stated	LD_{50} against *B. germanica:* Females:	Insecticide	(Chang et al. 2012)

			strain and two field-collected SEL and DJN colonies of *Blattella germanica* (L.) Residual contact Fumigation LD_{50} Determination			KSS = 0.44 mg/cm² ; SEL = 0.75 mg/cm²; and DJN = 0.84 mg/cm² KSS Males = 0.28 mg/cm²		
ITO; ITO	2011	Japan	Sedative activities in ddY mice Open field test using caffeine and phenobarbital	Inalation	0.004, 0.04, 0.4 and 4 mg	SNC suppression Spontaneous locomotor activity reduced at the doses of 0.04 and 0.4 mg Terpinolene (0.4 mg) antagonized caffeine-induced excitation, prolonging the sleep time of guinea pigs with outcomes comparable to those of chlorpromazine	Sedative	(Ito and Ito 2011)
WANG; LI; LEI	2009	China	Repellent activity against *T. castaneum* Contact toxicity test:	Topical Fumigation	2, 4, 6, 8, and 10 μL	Weak repellent activity against *Tribolium castaneum* and *Sitophilus*	Insecticide	(Wang et al. 2009)

			Adult fumigant toxicity test of *Sitophilus zeamaise*			*zeamaise* Moderate contact toxicity (LC_{50} between 51.41 and 66.38 µg / mg) Strong fumigant toxicity against *S. zeamaise:* LC_{50} = 1.30 (24h), 0.86 (48h), 0.67 (72h), 0.37 (96h)		
GU et al.	2009	Taiwan	Repellent activity against *Aedes aegypti* and *Aedes albopictus*	Inalation	1.92 µg/cm²	The article reports that terpinolene has repellent activity but does not report corresponding values.	Repellent	(Gu et al. 2009)
PARK et al.	2003	South Korea	Insecticide activity	Fumigation	0.1mg/cm²	*Callosobruchus chinensis* The dose of 0.05 mg/cm² caused 55% mortality, while the dose of 0.1 mg/cm² caused 87% mortality *Sitophilus oryzae* 0.18 mg/cm²: 52 to 72% mortality 0.26 mg/cm²: 93% to 95% mortality. Fumigation in closed	Insecticide	(Park et al. 2003)

						containers resulted in 100% mortality compared to the open container (2%).		

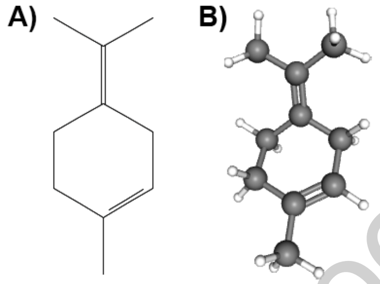

Figure 1: The chemical structure of terpinolene | $C_{10}H_{16}$ emphasizing the bidimensional (A) and tridimensional (B) atomics positions ("Terpinolene | C10H16 - PubChem" 2021).

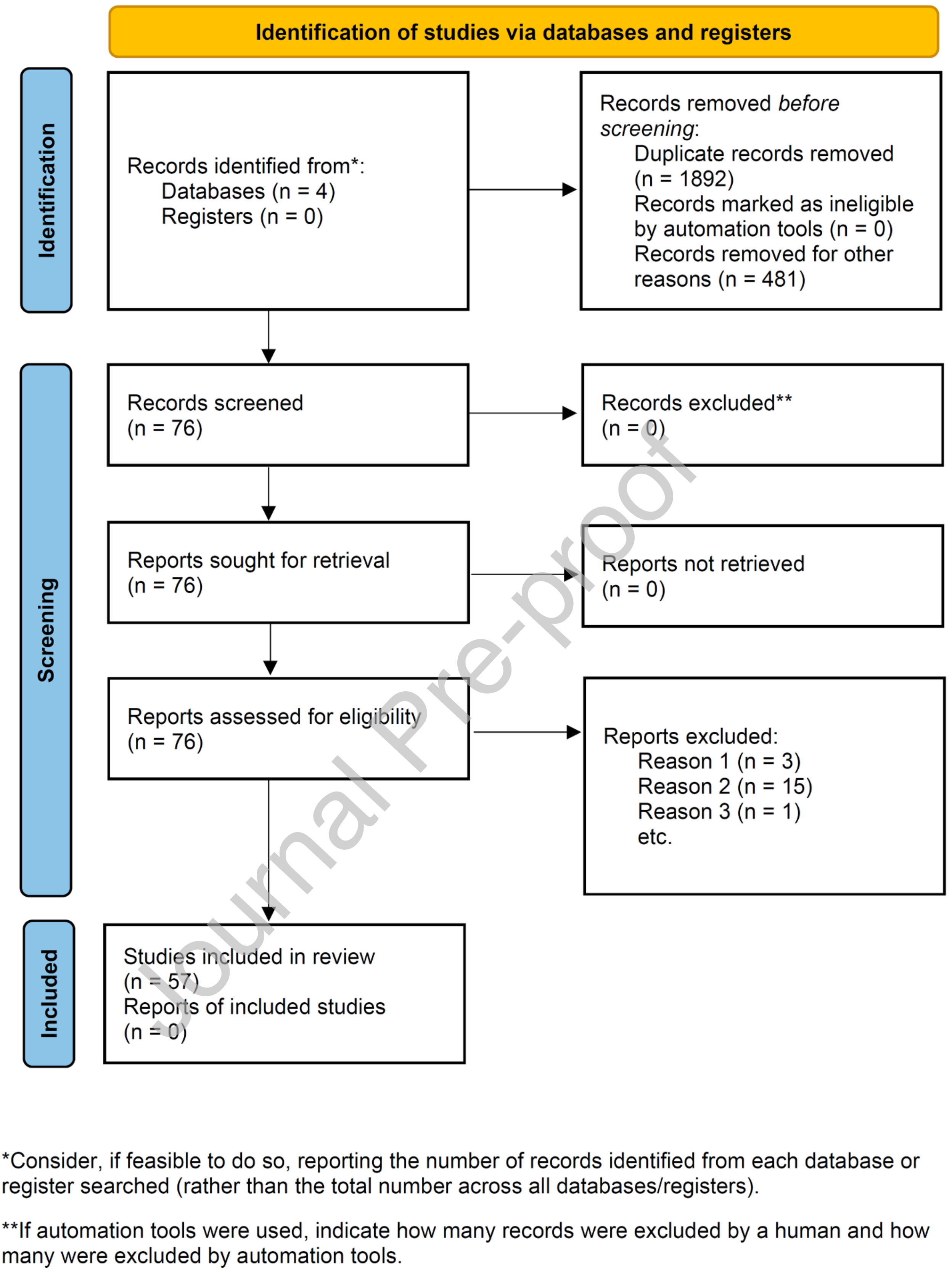

Figure 2. Flowchart detailing literature search according to the PRISMA statement. Reason 1:Articles with unavailable full texts, reason 2: Articles presenting mixtures of compounds whose activity is not attributed to terpinolene alone, reason 3: Does not deal with the action of terpinolene.

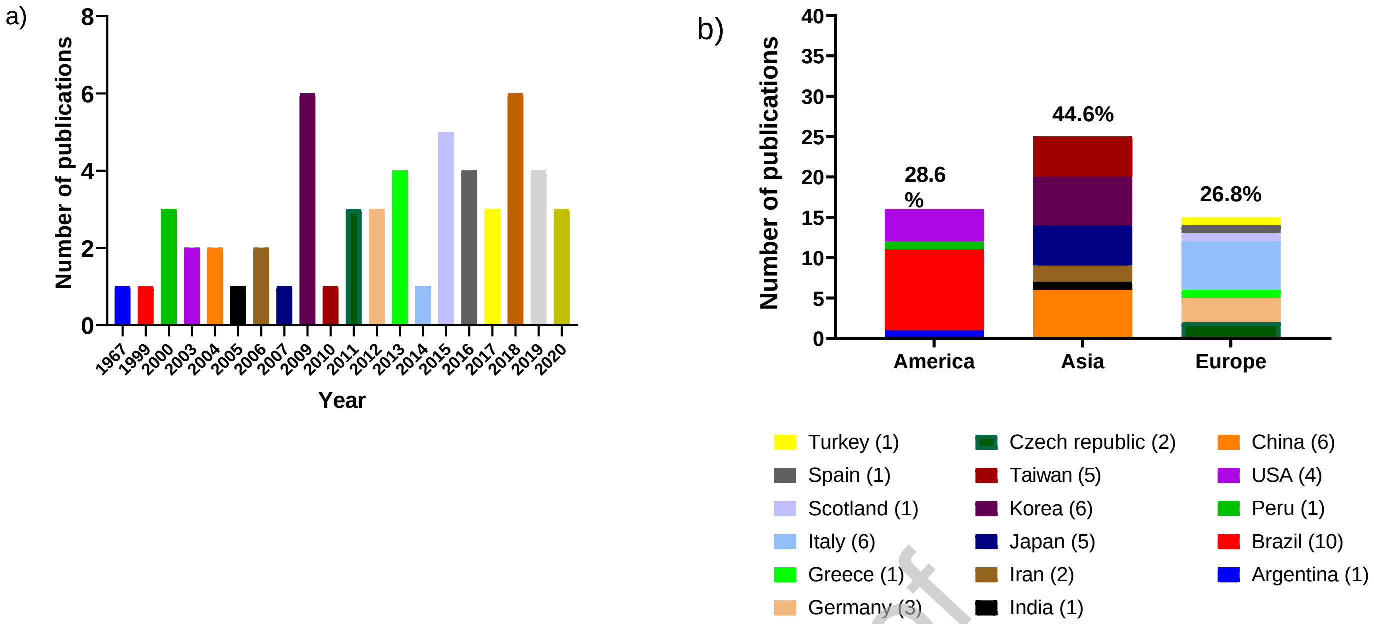

Figure 3. (a) Number of publications per year; (b) Geographical distribution of publications are represented as the number and percentage of total publications.

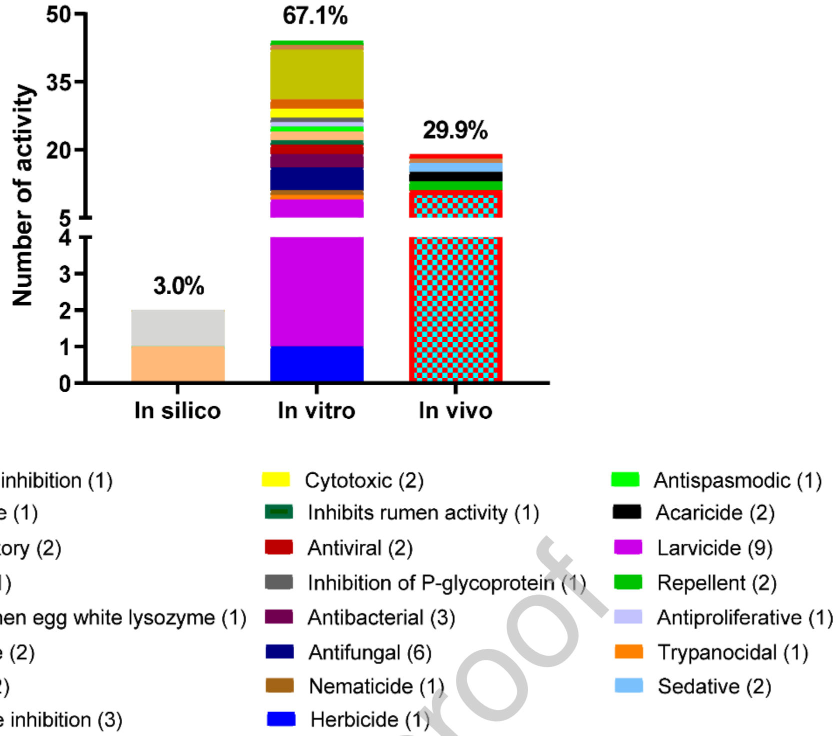

Figure 4. Type of study *versus* biological activity. Resulst are expressed as the number and percentage of publications reporting the corresponding biological activity.

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
RIBEIRO et al. (2020)	+	+	?	+	?	?	+	?	-	+
LUI el al. (2020)	-	+	?	+	?	?	?	?	+	+
RIBEIRO *et al.* (2019)a	+	+	?	+	?	+	?	?	+	+
RIBEIRO *et al.* (2019)b	+	+	?	+	?	+	+	?	+	+
DO NASCIMENTO (2018)	+	+	?	+	?	+	+	?	+	+
LIANG et al., (2018)	+	?	-	+	+	?	+	?	+	?
BORN et al., (2018)	+	+	-	+	?	+	?	?	?	+
ZHANG et al. (2017)	+	+	+	+	?	?	?	?	+	+
ZHANG et al. (2016)	+	+	?	+	?	?	+	?	+	+
MACEDO et al. (2016)	+	?	?	+	?	?	+	?	+	+
ALI et al. (2015)	+	+	?	+	?	?	?	?	+	+
ITO & ITO (2013)	+	+	-	?	?	?	-	?	+	+
CHANG et al. (2012)	+	+	?	+	?	?	?	?	+	+
ITO; ITO (2011)	+	+	-	?	?	?	-	?	?	+
WANG; LI & LEI (2009)	+	+	?	+	?	+	?	?	?	+
GU et al. (2009)	+	+	?	+	?	?	?	?	+	+
PARK et al., (2003)	+	+	?	+	?	+	-	?	+	+

Figure 5. Risk of bias summary. Each included study was analyzed by the authors following judging questions (Q1-Q10) and classified acording to their risk of bias. Yellow (?): unclear/uncertain risk of bias; red (-): high risk of bias; blue (+): low risk of bias. Q1: Was the allocation sequence adequately generated and applied?; Q2: Were the groups similar at baseline or were they adjusted for confounders in the analysis?; Q3: Was the allocation to the different groups adequately concealed?; Q4: Were the animals randomly housed during the experiment?; Q5: Were the caregivers and/or investigators blinded from the knowledge of which intervention each animal received during the experiment?; Q6: Were the animals randomly selected for outcome assessment?; Q7: Was the outcome assessor-blinded?; Q8: Were incomplete outcome data adequately addressed?; Q9: Are the study reports free of selective outcome reporting?; Q10: Was the study apparently free of other problems that could result in a high risk of bias?)

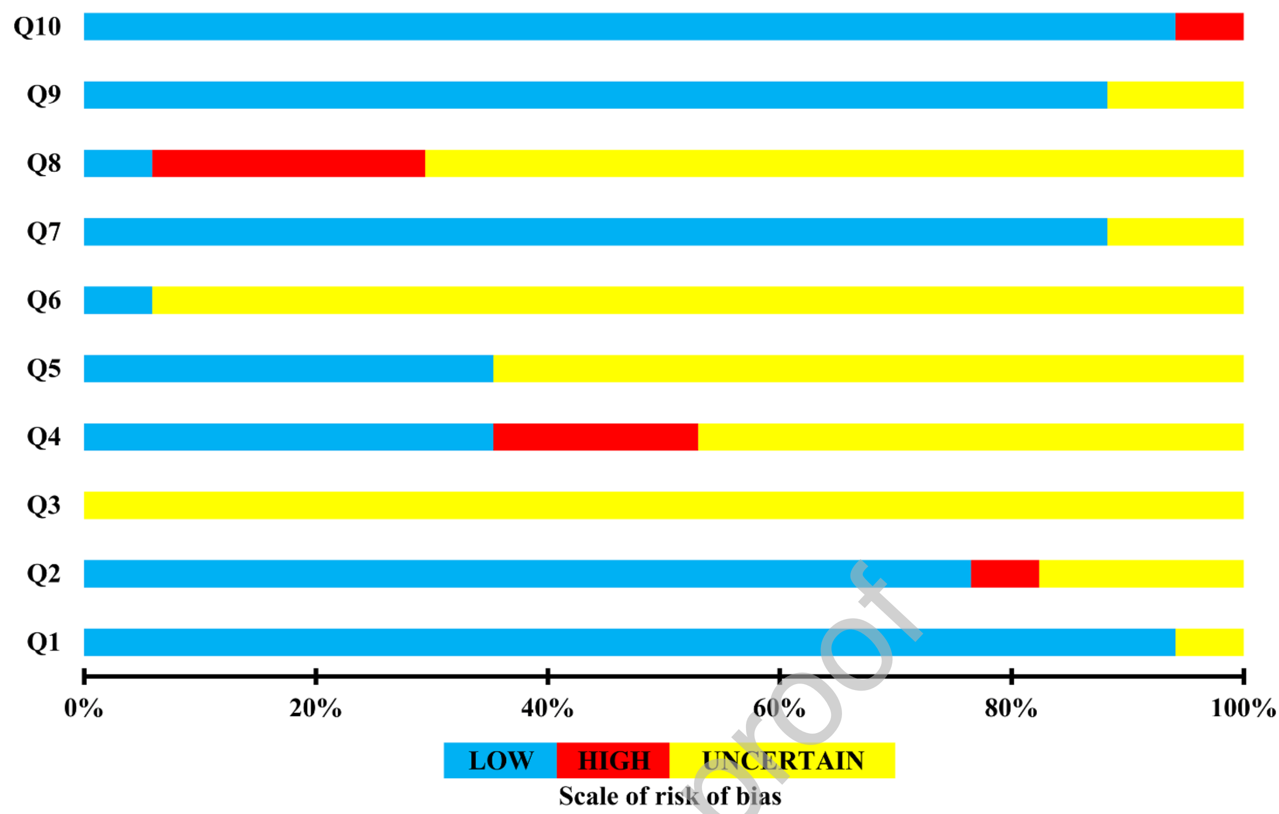

Figure 6. The risk of bias scale indicates the proportion of articles that met each criterion.

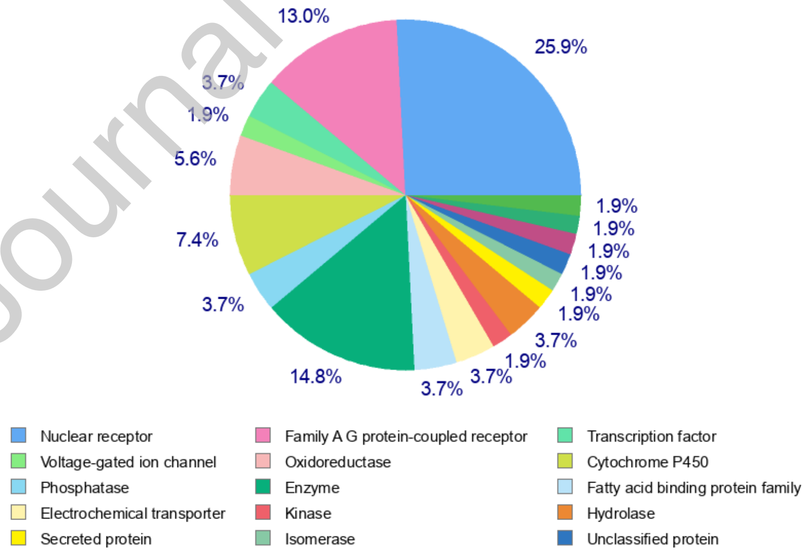

Figure 7. Predicted molecular targets for terpinolene. This data was obtained using the Swiss TargetPrediction computational tool.