

## Research Article

# Chemical Composition and In Vitro and In Silico Biological Activities of *Myrciaria tenella* (DC.) O.Berg (Myrtaceae) Essential Oil from Brazil

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*Myrciaria tenella* O.Berg, a native plant species of Brazil, exhibits pharmacological applications, including antitumor activity. In this study, we isolated the essential oil (EO) of *M. tenella* and identified its phytochemical profile. In addition, we determined the in vitro and in silico cytotoxic activities of EO in nontumor and tumor cell lines (gingival fibroblasts and oral squamous cell carcinoma, respectively) and its free radical scavenging activity (i.e., antioxidant activity) using ABTS and DPPH assays. The EO of *M. tenella* primarily constitutes hydrocarbons and oxygenated sesquiterpenes, with (*E*)-caryophyllene (33.95%),  $\delta$ -cadinene (7.4%), caryophyllene oxide (4.74%), and viridiflorene (4.49%) as its four major components. EO effectively suppressed the cell viability of CAL-27 tumor cells to below 70% at concentrations of 125 and 250  $\mu$ g/mL and exhibited a free radical inhibition potential of  $75.63 \pm 0.41\%$  and  $28.46 \pm 0.36\%$ , respectively, in the DPPH and ABTS assays. This chemical and biological potential may be attributed to the major compounds present in EO, as well as the molecular coupling simulations conducted, which revealed the anticancer mechanism of EO in the sesquiterpenes (*E*)-caryophyllene,  $\delta$ -cadinene, caryophyllene oxide, and viridiflorene.

## 1. Introduction

Aromatic plants have been used in ancient civilizations to treat various diseases [1]. To date, researchers have extracted various pharmacologically active molecules from plant species, which are cost-effective, less toxic, and more accessible compared with synthetic drugs [2, 3]. The volatile compounds present in essential oils (EOs) are biosynthesized as secondary metabolites of aromatic plants. These are complex, lipophilic, and volatile mixtures that can be extracted from buds, fruits, flowers, roots, leaves, and branches. Empirical data on EOs reveal their pharmaceutical potential, emphasizing the need to explore their bioactivities [4].

The genus *Myrciaria* of the plant family Myrtaceae comprises approximately 30 species that are distributed predominantly among countries on the American continent [5]. Twenty-two species of *Myrciaria*, including *Myrciaria tenella* O.Berg, are native to Brazil. The leaves of *M. tenella*, known as cambui-açu or jabuticaba-macia, exhibit anti-inflammatory, antibacterial, and astringent properties and are widely used by traditional communities [6, 7]. In vivo and in vitro studies have shown that *M. tenella* metabolites reduce edema, capture free radicals that trigger oxidative stress, and exhibit antimicrobial activities. *M. tenella*, originating from the Amazon, exhibits antioxidant potential by suppressing the overproduction of reactive oxygen species, which trigger diseases and degenerative processes [8]. Among these processes, the triggering of cancer, which is associated with the excess of free radicals in the body, is noteworthy [9]. Therefore, it is feasible to explore new alternatives, particularly in natural products, aligning with studies on *M. tenella* that have reported its antiproliferative activity against cancer cell lines [10–12].

In vitro tests enable researchers to analyze the cellular behavior of drugs in a controlled environment, which can help minimize their side effects [13]. According to the ISO 10993-5 guidelines published in 2009, in vitro cytotoxicity tests must expose cells directly or indirectly to the desired substance [14, 15]. Such exposure is crucial to evaluate drug biocompatibility and cell viability, including essential cellular functions, such as growth, reproduction, and metabolism [16].

The cytotoxic activity of the EO of *M. tenella* has not been investigated in cells obtained from the human oral cavity thus far. Therefore, we conducted in vitro studies on the EO of *M. tenella* to determine its antioxidant activity against ABTS and DPPH free radicals along with cytotoxicity in human gingival fibroblasts (a nontumor cell line) and oral squamous cell carcinoma cells (CAL-27).

## 2. Materials and Methods

**2.1. Collection and Identification of Plants.** In October 2018, leaves of *M. tenella* were gathered in the city of Magalhães Barata, PA, located at a latitude of 00°47'38" S and a longitude of 47°35'55" W. The collected sample, identified as MG 237466, was officially registered and is now housed in a collection of aromatic plants within the herbarium of the Museu Paraense Emílio Goeldi in Pará, Brazil.

**2.2. Physical and Chemical Characterization of the Sample.** The plant material was cold-dried in a temperature-controlled room with air conditioning. The sample was finely ground using a knife mill (Model TE-631/3; Tecnal, Brazil). The moisture content of the plant material was determined using a moisture analyzer (ID50, Marte Científica, Brazil) based on infrared spectroscopy. The extraction yield of EO was calculated according to the procedure described by Santos et al. [17].

**2.3. Essential Oil Isolation.** To isolate EO from *M. tenella* leaves, hydrodistillation was carried out using a modified Clevenger-type apparatus, which utilized 151.42 g of the sample. Extraction was performed for 3 h at 100°C. Subsequently, anhydrous Na<sub>2</sub>SO<sub>4</sub> was added to the EO and the resulting mixture was centrifuged to eliminate moisture. The purified EO was carefully stored in an amber-type glass ampoule in a refrigerated environment at 5°C to maintain its chemical composition.

**2.4. Chemical Evaluation of Essential Oil.** To identify the chemical composition, the protocols already described in a previous study were used. The brand and model of the gas chromatograph (CG-MS and GC-FID), chromatographic column, and other procedures are available in the literature [18]. A comparison of the linear retention index and mass spectra of the reference standards was performed, which aligned with the information reported by Adams [19].

**2.5. Cell Culture.** In this study, CAL-27 strain (derived from human tongue squamous cell carcinoma) and human gingival fibroblasts obtained from the Cell Culture Laboratory at UFPA's Faculty of Dentistry were used. Thawed cells were cultured in 25 cm<sup>2</sup> flasks with DMEM-F12 medium supplemented with 10% fetal bovine serum. The cultures were maintained at 37°C in an incubator with 5% CO<sub>2</sub>. Daily monitoring and proliferation assessments were performed using an inverted microscope. All the cell manipulations adhered to biosafety standards and were performed in a laminar flow hood. Growth was observed daily, and the culture medium was refreshed every 2–3 days considering cell metabolism. The protocols used in this study were in accordance with those previously reported by Guimarães et al. [20].

**2.6. In Vitro Cytotoxicity Assay.** For cytotoxicity analysis, gingival fibroblast lines and CAL-27 cells were seeded in 96-well culture plates at a concentration of  $1 \times 10^3$  cells/well and incubated at 37°C in a humid environment with 5% CO<sub>2</sub> for 24 and 48 h for adhesion and cell proliferation, respectively. DMSO (LGC Biotechnology, São Paulo, Brazil) was used as a solvent to ensure solubilization of the oily compound in the culture medium. Subsequently, the compound was diluted to 125, 250, 500, and 1000 µg/mL in the culture medium (Sigma, St. Louis, MO, USA). The control group (no treatment) contained only the culture

medium, and 0.01% DMSO was used as the solvent control to eliminate the hypothesis that DMSO could interfere with the results. The cells were then exposed to oil for 24 and 48 h [21].

**2.7. Cell Viability Analysis.** Cell viability was assessed using the MTT assay following a previously reported method [20].

### 2.8. Antioxidant Assays

**2.8.1. DPPH Assay.** The scavenging capacity (SC) of the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical by *M. tenella* EO was evaluated using the method outlined by Blois [22]. Trolox served as the positive control, and deionized water was used as the blank control. The samples were assessed in quintuplicate using an ultraviolet-visible (UV-vis) light spectrophotometer (800XI, FEMTO; São Paulo, Brazil). The percentage of inhibition was determined from the observed absorbance and calculated using the formula described in the literature [23].

**2.8.2. ABTS Assay.** Chemical interactions between EO and ABTS<sup>•+</sup> radicals were determined according to the procedures outlined in the literature [24, 25]. A UV-vis spectrophotometer (800XI, FEMTO; São Paulo, Brazil) was used at a wavelength of 734 nm. Deionized water served as the blank control, whereas Trolox served as the positive control. The percentage of inhibition was determined using the observed absorbance values, which were calculated using the formula described in the literature [23].

**2.9. Molecular Modeling.** (*E*)-Caryophyllene,  $\delta$ -cadinene, caryophyllene oxide, and viridiflorene were drawn using GaussView 5.5 software and optimized at the B3LYP/6-31G\* level [26, 27] using Gaussian 09 software [28]. The DNA structure used as the molecular target was retrieved from the Protein Data Bank (ID:1BNA) [29]. DNA-ligand docking studies were performed using AutoDock MGLTools 1.5.7 and AutoDock Vina 1.2.0 programs [30–32]. Docking was performed on the entire DNA structure using a grid box of 25 × 32 × 47 and a spacing of 1 Å. The lowest-energy conformation was considered to be the binding mode.

## 3. Results and Discussion

**3.1. Yield and Chemical Profile of the Essential Oil of *Myrciaria tenella*.** Table 1 shows the yield and main chemical compounds in the EO of *M. tenella*. The EO yield was 0.74% (w/w), which was lower than that found in two samples of *M. tenella* (specimen A: 2.1%; specimen B: 1.2%) by da Costa et al. [33]. This difference in yield may be attributed to environmental conditions and the collection period [34].

Fifty-eight chemical constituents, accounting for 99.76% of the volatile compounds, were quantified in the EO of *M. tenella*. The volatile components were categorized as hydrocarbon (78.49%) and oxygenated (21.27%) sesquiterpenes.

TABLE 1: Chemical compounds identified in the species in the essential oil of *Myrciaria tenella*.

		<i>Myrciaria tenella</i>	
IR <sub>L</sub>	IR <sub>C</sub>	Yield (%) Chemical constituents	0.74 Area (%)
1335	1340	$\delta$ -Elemene	0.03
1345	1353	$\alpha$ -Cubebene	0.24
1373	1375	$\alpha$ -Ylangene	0.52
1374	1380	$\alpha$ -Copaene	2.02
1387	1389	$\beta$ -Bourbonene	0.09
1390	1394	Sativene	0.07
1400	1400	$\beta$ -Longipinene	0.07
1409	1414	$\alpha$ -Gurjunene	0.16
1417	1431	( <i>E</i> )-Caryophyllene	33.95
1430	1436	$\beta$ -Copaene	2.57
1434	1438	$\gamma$ -Elemene	0.4
1480	1441	$\alpha$ -Maaliene	0.1
1439	1445	Aromadendrene	2.23
1458	1448	<i>allo</i> -aromadendrene	0.37
1448	1451	<i>cis</i> -muurolo-3,5-diene	0.15
1451	1456	<i>trans</i> -muurolo-3,5-diene	0.44
1452	1460	$\alpha$ -Humulene	3.23
1464	1466	9- <i>epi</i> -( <i>E</i> )-caryophyllene	0.5
1471	1474	4,5-di- <i>epi</i> -aristolochene	0.03
1471	1478	Dauca-5,8-diene	0.67
1484	1482	Germacrene D	3.86
1483	1485	$\alpha$ -Amorphene	0.72
1489	1492	$\beta$ -Selinene	1.92
1492	1496	<i>cis</i> - $\beta$ -guaiene	0.69
1496	1500	Viridiflorene	4.49
1500	1505	$\alpha$ -Muurolole	1.61
1511	1513	$\delta$ -Amorphene	2.49
1513	1521	$\gamma$ -Cadinene	3.31
1520	1524	7- <i>epi</i> - $\alpha$ -selinene	0.07
1522	1531	$\delta$ -Cadinene	7.4
1528	1532	Zonarene	0.38
1533	1538	<i>trans</i> -cadin-1,4-diene	0.62
1540	1542	Selina-4(15),7(11)-diene	0.4
1537	1543	$\alpha$ -Cadinene	0.69
1544	1549	$\alpha$ -Calacorene	1.67
1562	1558	<i>epi</i> -longipinanol	0.06
1559	1563	Germacrene B	0.06
1567	1565	Longipinanol	0.36
1564	1568	$\beta$ -Calacorene	0.27
1566	1573	Maaliol	0.12
1571	1576	Caryolan-8-ol	0.08
1577	1584	Spathulenol	0.66
1582	1591	Caryophyllene oxide	4.74
1592	1598	Viridiflorol	1.33
1600	1608	Rosifoliol	0.33
1602	1610	Ledol	0.16
1608	1615	Humulene epoxide II	0.2
1618	1621	1,10-di- <i>epi</i> -cubenol	1.12
1629	1629	Eremoligenol	0.64
1627	1635	1- <i>epi</i> -cubenol	2.35
1645	1639	Cubenol	1.18
1639	1643	Caryophylla-4(12),8(13)-dien-5- $\beta$ -ol	0.54
1640	1649	<i>epi</i> - $\alpha$ -muurolol	2.83
1644	1652	$\alpha$ -Muurolol	0.99
1652	1658	Himachalol	0.39
1652	1662	$\alpha$ -Cadinol	2.8
1685	1676	Germacra-4(15),5,10(14)-trien-1- $\alpha$ -ol	0.30

TABLE 1: Continued.

		<i>Myrciaria tenella</i>	
IR <sub>L</sub>	IR <sub>C</sub>	Yield (%)	0.74
		Chemical constituents	Area (%)
1700	1702	Eudesm-7(11)-en-4-ol	0.09
		Hydrocarbon sesquiterpenes	78.49
		Oxygenated sesquiterpenes	21.27
		Total	99.76

IRL: Adams literature retention index [19]; CRI: retention index calculated from a homologous series of *n*-alkanes (C8–C40) in a DB5-MS column; \*relative area (%) calculated based on peak areas.

The major volatile compounds were (*E*)-caryophyllene (33.95%),  $\delta$ -cadinene (7.4%), caryophyllene oxide (4.74%), and viridiflorene (4.49%). Our results differed from those reported by Gonçalves et al. [34], who investigated a specimen of *M. tenella* collected in Restinga da Marambaia, Rio de Janeiro, Brazil, and reported that the main volatile compounds were  $\beta$ -pinene (21.46%),  $\alpha$ -pinene (19.43%), and (*E*)-caryophyllene (10.89%).

According to a research study by Apel et al. [35], certain EOs derived from *Myrciaria* species are abundant in terpenoids, as exemplified by the EO of *M. plinioides* [35]. This particular EO is comprised of significant quantities of nonoxygenated and oxygenated monoterpenes and sesquiterpenes. In a separate study by Andrade et al. [36], the EO of *M. tenella* from Acará, Pará, Brazil, revealed (*E*)-caryophyllene (32.0%),  $\delta$ -cadinene (5.1%), and caryophyllene oxide (5.0%) as the predominant volatile compounds. Another specimen of *M. tenella*, collected from Mogi Guaçu, São Paulo, Brazil, contained (*E*)-caryophyllene (25.1%) and spathulenol (9.7%), according to Apel et al. [35]. The EO from *M. tenella* leaves from Rodeio Bonito, RS, Brazil, contains  $\alpha$ -pinene (31.5%),  $\beta$ -pinene (19.2%), and 1,8-cineole (6.6%) as the major constituents [11]. The main compounds identified in the EO of *M. tenella* in this study exhibited noteworthy bioactivity. For instance, (*E*)-caryophyllene exhibited antimicrobial properties against both Gram-positive and Gram-negative bacteria [37], suggesting its potential use as a disinfectant and preservative. In addition,  $\delta$ -cadinene inhibited the growth of *Streptococcus pneumoniae*, a bacterium responsible for respiratory infections, with an MIC of 31.25  $\mu$ g/mL [38]. Furthermore, caryophyllene oxide exhibited anti-inflammatory and analgesic activities, as reported by Chavan et al. [39]. These findings highlight the diverse and potentially beneficial properties of the main constituents found in the EO obtained from *M. tenella*.

**3.2. Cell Viability.** According to the ISO 10993-5 standard, a compound is considered toxic when cell viability in its presence is less than 70% compared with the control group [40]. At 125 and 250  $\mu$ g/mL, the EO of *M. tenella* was not toxic to gingival fibroblasts during a 24-h period, as shown in Figure 1. After 48 h, despite the decrease in the percentage of viable gingival fibroblasts, it remained nontoxic at both concentrations. This compound maintains the viability of

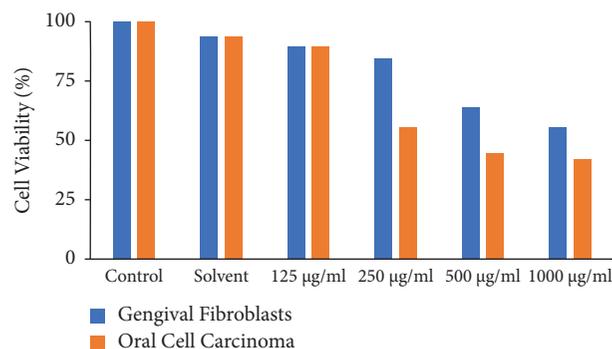


FIGURE 1: Cell viability of gingival fibroblasts and oral squamous cell carcinoma cells at different concentrations of *M. tenella* essential oil (EO) during a 24-h period.

this cell group, which is crucial for oral tissue regeneration and repair processes.

Oral cancer, the second most prevalent malignant tumor within the head and neck region, is largely dominated by oral squamous cell carcinoma, accounting for over 90% of all cases, as highlighted by the National Cancer Institute [41]. Notably, EO of *M. tenella* exhibited pronounced antiproliferative effects on CAL-27 cells. In particular, at 24 h, a concentration of 250  $\mu$ g/mL reduced cell viability to below 70%, as shown in Figure 2. By the second day, all concentrations demonstrated toxicity, underscoring the potent antiproliferative nature of *M. tenella* in combating this malignant neoplasm. The cytotoxic attributes can be ascribed to the key compounds present in EO. Oliveira et al. [42] emphasized the cytotoxic potential of (*E*)-caryophyllene, which is a major component in EO of *M. tenella*.

Furthermore, Jun et al. [43] reported the cytotoxicity of caryophyllene oxide, another major compound in the EO of *M. tenella*. Caryophyllene, a natural sesquiterpene present in plants, exhibits potent anticancer effects and enhances drug efficacy. BCP, a phytocannabinoid, binds strongly to CB2 and lacks CB1 affinity. BCPO, an oxidative derivative, is independent of the endocannabinoid system. BCPO alters cancer-related pathways and selectively activates CB2, making BCP a potential natural analgesic. The correlation between anticancer and antioxidant properties highlights a crucial interplay in disease prevention and treatment. Antioxidants, by neutralizing harmful free radicals and reducing oxidative stress, contribute to lowering the risk of cancer development. Moreover, many natural compounds exhibiting potent antioxidant activity also demonstrate anticancer effects, suggesting a shared mechanism. These dual functionalities underscore the potential of antioxidant-rich diets and therapies in combating cancer and promoting overall health [42, 43]. Its dual anticancer and analgesic roles influence chemotherapy efficacy and are valuable in oncology [44]. Giacomo et al. [45] highlighted the anticarcinogenic activity of both caryophyllene oxide and (*E*)-caryophyllene against human colorectal adenocarcinoma (Caco-2) and T-cell leukemia (CCRF/CEM), which resulted in a significant reduction in cell viability. These findings collectively underscore the potential therapeutic significance

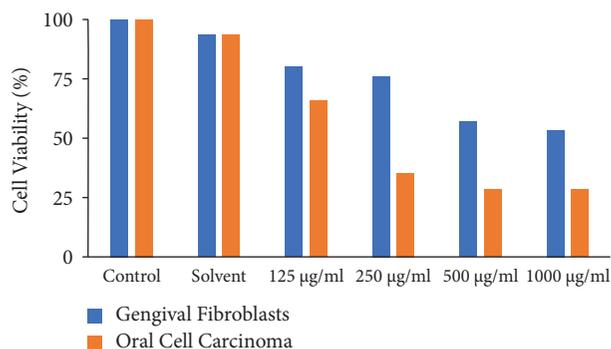


FIGURE 2: Cell viability of gingival fibroblasts and oral squamous cell carcinoma cells at different concentrations of *M. tenella* EO during a 48-h period.

of EO of *M. tenella* and its major constituents, particularly (*E*)-caryophyllene and caryophyllene oxide, in combating oral squamous cell carcinoma [46, 47].

**3.3. Antioxidant Potential.** Figure 3 shows the antioxidant potential of EO of *M. tenella* against the ABTS<sup>•+</sup> and DPPH<sup>•</sup> radicals. The SC of the EO was defined as the decrease in the concentration of radicals. Trolox was used as a positive control. The benefits of EO in the medical and food fields can be attributed to their antioxidant properties [48]. Therefore, the antioxidant activity of EO of *M. tenella* was assessed via ABTS<sup>•+</sup> and DPPH<sup>•</sup> radical scavenging assays [49]. The EO of *M. tenella* exhibited a strong SC (75%) in the DPPH assay and good antioxidant activity (28%) in the ABTS assay compared with the Trolox standard. Moraes et al. [50] reported that the EO of *M. floribunda* exhibited excellent scavenging activity for both ABTS<sup>•+</sup> and DPPH<sup>•</sup> radicals. This result was attributed to the high levels of oxygenated monoterpene 1,8-cineole in the EO. The EO of *M. tenella* primarily comprises hydrocarbon sesquiterpenes with 33.95% (*E*)-caryophyllene content. In addition, the differences in the antioxidant activities of the EO of *M. tenella* in the DPPH and ABTS assays may correspond to the synergistic interactions between their components. This difference was partially attributed to the reaction medium (ethanol for the DPPH assay and phosphate-buffered saline for the ABTS assay) and reaction time (5 min for the ABTS assay and 30 min for the DPPH assay). All these factors facilitate radical scavenging kinetics [51, 52].

**3.4. Molecular Modeling.** MD studies have demonstrated that these compounds interact favorably with DNA. (*E*)-Caryophyllene,  $\delta$ -cadinene, caryophyllene oxide, and viridiflorene exhibited binding energies of  $-4.9$ ,  $-5.1$ ,  $-4.5$ , and  $-4.8$  kcal/mol, respectively.

Morávek et al. [53] found that small compounds, such as volatile compounds in EO of *M. tenella*, exhibited a greater binding affinity for the minor groove of DNA, aligning with our docking study results. This phenomenon could be partially attributed to the molecular size of the ligands, which renders the formation of a stable complex with the

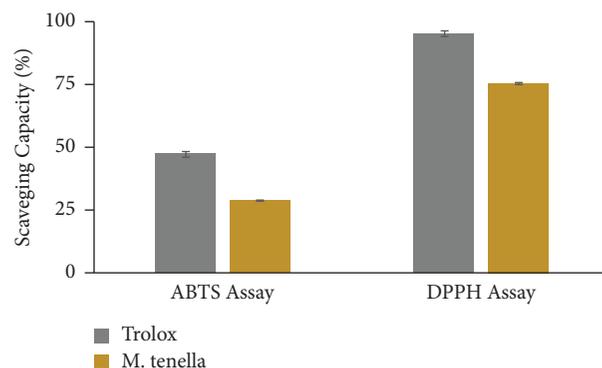


FIGURE 3: Scavenging capacity of EO of *M. tenella* for ABTS<sup>•+</sup> and DPPH<sup>•</sup> radicals.

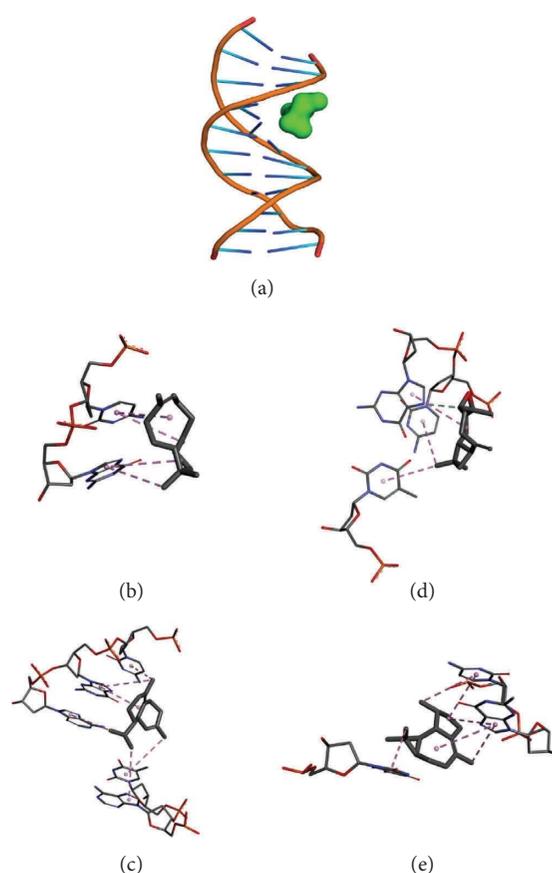


FIGURE 4: Schematic depicting the molecular interactions between DNA and ligands. (a) General representation of the binding site of the compounds. This is further elucidated through detailed illustrations of the molecular interactions facilitated by (b) (*E*)-caryophyllene, (c)  $\delta$ -cadinene, (d) caryophyllene oxide, and (e) viridiflorene. Each compound's specific mode of interaction with DNA is highlighted, providing insights into their respective roles in molecular binding processes.

major groove of DNA difficult. However, the compounds exhibited only a few chemical interactions with the minor groove of the DNA. In this study, these interactions were sufficient to form a stable and energetically favorable complex, according to the binding affinity energy results

TABLE 2: Binding energy values ( $\Delta G_{\text{bind}}$ ) of the drug-receptor systems.

Compounds	$\Delta E_{\text{ele}}$	$\Delta E_{\text{vdW}}$	$\Delta G_{\text{GB}}$	$\Delta G_{\text{NP}}$	$\Delta G_{\text{bind}}$
( <i>E</i> )-Caryophyllene	-5.41	-9.65	5.45	-3.76	-13.37
$\delta$ -Cadinene	-7.86	-6.27	3.43	-4.11	-14.81
Caryophyllene oxide	-5.98	-8.32	6.26	-6.72	-14.76
Viridiflorene	-6.57	-5.15	3.13	-4.23	-12.82

$\Delta E_{\text{vdW}}$ , van der Waals contributions;  $\Delta E_{\text{electrostatic}}$ , electrostatic energy;  $\Delta G_{\text{GB}}$ , polar solvation free energy;  $\Delta G_{\text{nonpol}}$ , nonpolar solvation free energy. All values are in kcal/mol.

reported by Cascaes et al. [54]. The observed chemical interactions were hydrophobic, and electrostatic interactions were not observed because of the absence of polarizable and ionizable groups in the ligands (Figure 4).

The lowest-energy complexes from the docking results were selected as the starting point for the MD simulations, in which the complexes exhibited favorable binding energy values (Table 2). Van der Waals interactions significantly contributed to the formation of these complexes in addition to the favorable contributions of electrostatic and nonpolar interactions.

#### 4. Conclusions

In this study, the essential oil (EO) of *M. tenella* has revealed significant advancements on various fronts. The identification of the EO's chemical profile highlighted its main components, especially hydrocarbon and oxygenated sesquiterpenes, such as (*E*)-caryophyllene,  $\delta$ -cadinene, caryophyllene oxide, and viridiflorene. Additionally, its antioxidant activity was demonstrated against DPPH and ABTS radicals, indicating its relevance as an antioxidant agent. The evaluation of its cytotoxicity in tumor and nontumor cells revealed a concentration and test period dependency, with promising results in gingival fibroblast cells, suggesting its potential utility in treating oral cavity-related diseases. The in silico analysis underscored the ability of the EOs' major constituents to interact with molecular targets, corroborating its cytotoxic activity. These findings indicate that the EO of *M. tenella* holds therapeutic potential, although further investigations are necessary to validate its efficacy and safety in clinical applications. In summary, this research represents a promising step toward the development of economically viable and accessible therapeutic alternatives.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare they have no conflicts of interest.

#### Authors' Contributions

Oberdan Oliveira Ferreira, Vanessa Guimarães Costa, and Jorddy Nevez Cruz conceptualized the study and performed investigation. Maria Sueli da Silva Kataoka, João de Jesus Viana Pinheiro, Lidiane Diniz do Nascimento, Everton Luiz

Pompeu Varela, Márcia Moraes Cascaes, Sandro Percário, Suraj N. Mali, and Oberdan Oliveira Ferreira proposed the methodology. Tatiany Oliveira de Alencar Menezes, Mozaniel Santana de Oliveira, and Eloisa Helena de Aguiar Andrade validated the data, visualized the data, and performed formal analysis. Lidiane Diniz do Nascimento, Everton Luiz Pompeu Varela, Márcia Moraes Cascaes, Sandro Percário, and Suraj N. Mali provided resources. Oberdan Oliveira Ferreira curated the data. Eloisa Helena de Aguiar Andrade supervised the data and administered the project. All authors participated in result discussions and approved the final manuscript.

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