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Original Article

Essential oil and its major compounds from oil camphor inhibit human lung and breast cancer cell growth by cell-cycle arresting

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Abstract: Recent studies suggested that essential oils from some medical plants exhibit substantial antitumor activities, especially for lung cancer and breast cancer cells. *Cinnamomum longepaniculatum* (oil camphor) is a plant widely distributed in China and used as folk medicine throughout the world. However, the antitumor activities of essential oil and its major compounds from *C. longepaniculatum* and the inhibitory mechanisms have not been reported before. In this report, we found that *C. longepaniculatum* essential oil and its major compounds terpinene-4-ol, α-terpineol and safrole induced substantial apoptosis or necrosis of both human A549 lung cancer and MCF-7 breast cancer cells. However, 1,8-cineole did not show obvious inhibitory effects on both A549 and MCF-7 cells growth. After the treatments, the cell size and quantity were reduced, the cells were gradually rounded-up, and the nuclei became pyknotic in a dose-dependent manner. *C. longepaniculatum* essential oil showed significant *in vitro* antiproliferative activity towards A549 and MCF-7 cancer cells by increasing the proportion of their sub-G_1 phase. Western blotting indicated that essential oil compounds (especially the safrole) induced up-regulation of cell-cycle-related proteins P53 and P21. Among the major compounds, the costs of safrole were the lowest for both A549 and MCF-7 cell lines. Therefore, the essential oil from *C. longepaniculatum* and its key ingredients exhibit various degrees of inhibitory activity on the growth of A549 lung cancer cells and MCF-7 breast cancer cells by cell-cycle arresting. Among them, terpinene-4-ol, α-terpineol and safrole manifested the most potent suppressive effect.

Keywords: *Cinnamomum longepaniculatum*, essential oil, cancer cell, cell cycle, p53/p21

Introduction

Malignant tumors are a life-threatening form of cancer that causes serious human health concerns worldwide. Chemotherapy is one of the major approaches to treating cancers, but its clinical use has been limited due to severe side effects. Therefore, the studies aimed at the identification of natural medicines with high efficiencies but low toxicities have become the hot field in cancer research. Recently, it has been shown that many plant essential oils play an important role in the induction of apoptosis in cancer cells [1, 2]. *Cinnamomum longepaniculatum* is a plant, that identically to *Cinnamomum camphora*, belongs to the genus *Cinnamomum* of the family Lauraceae. Moreover, *C. longepaniculatum* is a Chinese endemic species, which is an important natural spice plant in China [3]. Its leaves are rich in essential oil (3.8-4.5%), including more than 40 components, such as 1,8-cineole, γ-terpinene, terpinene-4-ol, α-terpineol, and safrole [4]. The essential oil from the leaves of *C. longepaniculatum* has been shown to exert a variety of biological activities, such as antimicrobial effects [5-7], antitumor actions [8-10], and analgesic influence [11]. Previous researches demon-

strated that the essential oil of *C. longepaniculatum* and its major components exhibit various degrees of inhibitory effects on human BEL-7402 liver cancer cells. However, their impact on human lung and breast cancer cells is still unknown. In the current study, we used the ATP-based tumor chemosensitivity assay (ATP-TCA) to investigate the effect of the essential oil from *C. longepaniculatum* and its major components on A549 and MCF-7 cell growth, aiming to provide the experimental basis for the development of the relevant natural products and the enhancement of their clinical use as antitumor and healthy food.

Materials and methods

Cancer cells

Cell lines of human A549 lung cancer and human MCF-7 breast cancer were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. The cell survival rate was more than 95%. Human A549 lung cancer cells and human MCF-7 breast cancer cells were cultured in RPMI1-640 medium containing 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 U/mL) at 37°C in an incubator with an atmosphere containing 5% CO_2 [10].

Plant materials and reagents

Leaves of *C. longepaniculatum* were collected from a plant base in Sichuan Province of China in august 2013. 1,8-cineole, γ-terpinene, terpinene-4-ol, α-terpineol, and safrole were provided by Yibin Shiping spices Co., Ltd. (Yibin, China). ATP-TCA kits were purchased from Beijing Jinzijing Biological Pharmaceutical Co., Ltd. (Beijing, China).

Extraction of essential oil and sample preparation

1 kg of air-dried *C. longepaniculatum* leaves were subjected to distill for 4 h to obtain essential oil according to steam distillation method described previously [12]. The essential oil was separated from the condensate water and dried over anhydrous sodium sulfate.

Samples were prepared to a stock solution of a concentration 0.5 g/mL by using 95% ethanol for essential oil of *C. longepaniculatum* and 1,8-cineole or dimethylsulfoxid (DMSO) for γ-terpinene, terpinene-4-ol, α-terpineol, and safrole, and then were diluted with RPMI1-640 containing 5% FBS into different concentrations, including 200.00, 100.00, 50.00, 25.00, 12.50, and 6.25 μg/mL. The drugs were sterilized with 0.22 μm membrane filter and stored at 4°C.

Gas Chromatography (GC) analysis

Determinations of the concentration of the major compounds of the essential oil were performed using an Angilent 7890A gas chromatography. The samples were analyzed using a quartz capillary column. The flow rate of the carrier gas (N_2) was 1.0 mL/min, 0.5 μLof sample was injected with the split ratio of 1:3. The oven temperature was programmed as follow: 90°C (4 min), 5°C/min to 130°C (5 min), 5°C/min to 190°C (2 min), 5°C/min to 250°C (3 min). The concentrations of 1,8-cineole, γ-terpinene, terpinene-4-ol, α-terpineol, and safrole of essential oil were calculated by an external reference method [12].

Light microscopy assay

A549 cells and MCF-7 cells at the logarithmic growth phase (2-3×10^5/mL) were seeded in 96-well cell culture plates (100 μL/well) and cultured overnight. After the adhesion, the drugs were added (100 μL/well) at different concentrations, whereas the control group was supplemented with RPMI1-64, and the cells were then cultured for 48 h. The cell growth and morphology were observed under the microscope.

Inhibition experiments on cancer cell growth in vitro

The effects of the different drug concentrations on cancer cell growth were examined by ATP-TCA, as described previously [13]. A549 and MCF-7 cells at the logarithmic growth phase were seeded in 96-well cell culture plates, and drugs at different concentrations were added. The RPMI1-640 medium and ATP inhibitor were utilized as the negative and positive control, respectively. After cultured for 48 h, ATP extract and luciferin-luciferase were added, and then measurements were performed immediately via the BHP9504-based microplate luminescence analyzer. The inhibition rate, median

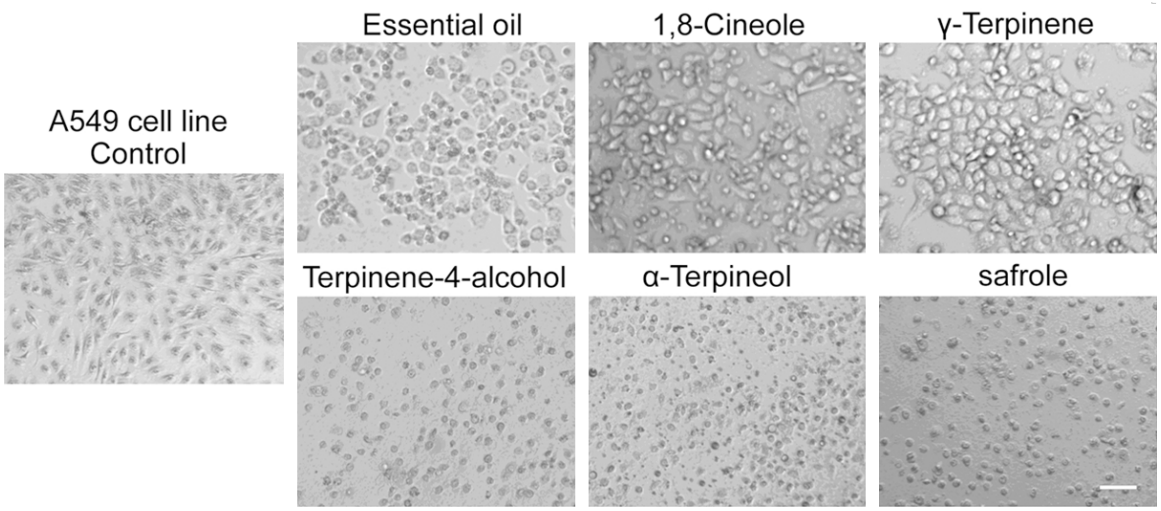

Figure 1. The effect of essential oil of *C. longepaniculatum* and its major components (50 μg/mL) on A549 cell growth. Bar = 100 μm.

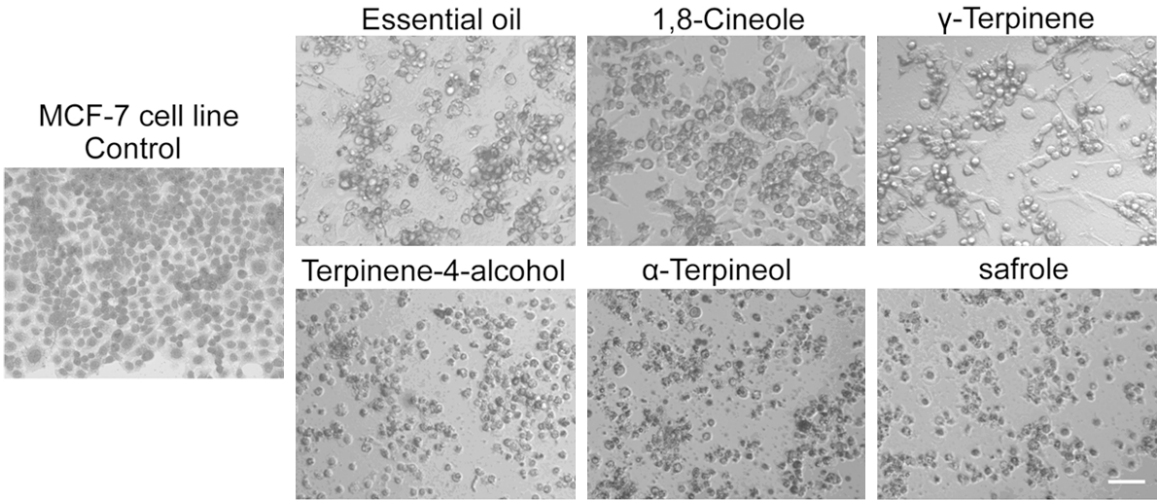

Figure 2. The effect of essential oil of *C. longepaniculatum* and its major components (50 μg/mL) on MCF-7 cell growth. Bar = 100 μm.

inhibitory concentration (IC_{50}), and IC_{90} were analyzed by the BHP software [13].

Cell cycle measurement

Tumor cells were treated with or without 50 μg/ml *C. longepaniculatum* essential oil or its major compounds at 37°C for 24 h and then harvested. FACScan flow cytometry was performed as previously described [14]. The percentages of the cells at different cell cycle phases, or those that were undergoing apoptosis, were evaluated by Calibur FACScan flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA).

Western blot analysis

After treatments, tumor cells were collected and the cell pellets were resuspended in lysis buffer and lysed at 4°C for 1 h. The lysis buffer consisted of 50 mM Hepes, pH 7.4, 1% Triton X-100, 2 mM sodium orthovanadate, 100 mM sodium fluoride, 1 mM acetic acid, 1 mM phenylmethanesulfonyl fluoride (PMSF), 10 mg/L aprotinin, and 10 mg/L leupeptin (Sigma-Aldrich, St. Louis, MO, USA). After centrifugation at 12,000 *g* for 15 min, the supernatant protein content was determined using the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of total pro-

Table 1. Inhibitory effects of essential oil of *C. longepaniculatum* and its major components on A549 cell growth (mean ± standard deviation, $n = 3$)

Drug	Inhibitory effects (%)						Correlation coefficient
	200 (μg/mL)	100 (μg/mL)	50 (μg/mL)	25 (μg/mL)	12.5 (μg/mL)	6.25 (μg/mL)	(R^2)
Essential oil	37.70±0.20	18.48±0.32	19.49±0.59	19.96±1.02	16.94±0.46	13.94±0.56	0.55
1,8-cineole	14.68±0.52	11.09±0.38	6.97±0.40	5.17±0.32	1.43±0.23	-5.32±0.38	0.22
γ-terpinene	45.98±1.72	23.70±0.20	17.61±0.09	11.46±0.33	8.34±0.22	7.92±1.78	0.78
Terpinene-4-alcohol	99.69±0.80	60.29±2.02	21.23±1.22	21.69±1.60	12.7±0.48	12.64±0.30	0.78
α-terpineol	99.9±1.00	94.69±2.10	33.63±0.62	30.07±2.02	23.91±1.36	20.59±0.94	0.80
Safrole	99.82±1.88	100.04±2.51	77.22±1.10	72.02±1.92	65.47±2.52	17.14±0.14	0.84

tein were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes, and the membranes were soaked in blocking buffer (5% skim milk). Antibodies against p21, p53 and Actin B were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). Proteins were visualized using horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse IgG and 3,3-diaminobenzidine tetrahydrochloride (DAB) as the HRP substrate [15].

Statistical analysis

Experiments were conducted thrice in triplicate. Statistical analysis of data was carried out by using SPSS 16.0 software. Data were represented as mean ± standard deviation by one-way analysis of variance (ANOVA).

Results

GC analysis

The essential oil extracted from *C. Longepaniculatum* leave was a colourless transparent liquid with an extraction yield of 2.98%. The concentrations of 1,8-cineole, γ-terpinene, terpinene-4-ol, α-terpineol, and safrole in the essential oil were obtained by GC analysis with the values of 56.15%, 1.78%, 3.87%, 10.12% and 0.03%, respectively.

Light microscopy assay

Following the treatment with essential oil and its major components of *C. Longepaniculatum* for 48 h, the morphology of A549 and MCF-7 cell was changed at different degrees (**Figures 1** and **2**). In the control group, the cells were adherent and exhibited as round, oval, and polygon, or fusiform. They also displayed good refraction and clear cell boundaries. However, in the treatment groups, the cells were crumpled. The cell size and quantity were reduced, the cells were gradually rounded-up, and the nuclei became pyknotic in a dose-dependent manner. α-terpineol and safrole at a dose of 6.25 μg/mL induced significant morphological changes of A549 cells, whereas 50.00 μg/mL of terpinene-4-ol were necessary to achieve this effect. It was found that γ-terpinene, terpinene-4-ol, α-terpineol, and safrole at a dose of 25 μg/mL caused considerable changes in the morphology of MCF-7 cells. There were no obvious differences between the influence of the control group and the treatments with the essential oil of *C. longepaniculatum*, 1,8-cineole, or γ-terpinene on A549 cell morphology, as well as between the action of the control group and the treatments with essential oil or 1,8-cineole on MCF-7 cell morphological characteristics.

Inhibition experiments on A549 cell growth in vitro

The inhibitory effect of essential oil and its major components on A549 cells were determined by ATP-TCA at a concentration range of 6.25-200 μg/mL. Terpinene-4-ol, α-terpineol, and safrole exerted relatively higher anti-proliferative effect on A549 cells, in which 99.69%, 99.90%, 99.82% growth inhibitions were observed, respectively, at the concentration of 200 μg/mL (**Table 1**). These three compounds inhibited the A549 cells proliferation in a dose-dependent manner with a high correlation coefficient ($R^2 \geq 0.78$). At 200 μg/mL, γ-terpinene and the essential oil of *C. longepaniculatum* displayed a moderate suppressive effect on A549 with 45.98% and 37.70% inhibitions, respectively. The correlation coefficient R^2 of them were 0.55 and 0.78, respectively. 1,8-cin-

Table 2. Inhibitory effects of essential oil of *C. longepaniculatum* and its major components on MCF-7 cell growth (mean ± standard deviation, *n* = 3)

Drug	Inhibitory effects (%)						Correlation coefficient
	200 (μg/mL)	100 (μg/mL)	50 (μg/mL)	25 (μg/mL)	12.5 (μg/mL)	6.25 (μg/mL)	(R^2)
Essential oil	15.25±0.26	14.82±0.30	8.28±0.21	2.62±0.11	1.08±0.12	0.45±0.09	0.35
1,8-cineole	9.85±0.82	6.03±0.54	4.87±0.42	3.38±0.18	1.74±0.12	-4.84±0.06	0.14
γ-terpinene	56.87±1.74	35.22±2.08	22.31±1.96	17.07±0.94	8.45±2.88	-2.05±2.07	0.27
Terpinene-4-alcohol	99.17±2.75	50.06±2.18	48.04±2.04	37.25±1.20	27.19±2.02	12.94±0.71	0.85
α-terpineol	99.61±1.70	45.50±2.45	44.68±2.80	27.3±0.85	23.56±0.90	20.36±1.20	0.74
Safrole	99.79±0.86	100.02±0.96	58.80±1.80	57.05±1.98	27.49±1.80	25.62±1.06	0.91

Table 3. Effect of 50 μg/ml essential oil and its major components on the cell cycle of various tumor lines

Cell line	A549 cells			MCF-7 cells		
Cell cycle	G_0/G_1	G_2	S	G_0/G_1	G_2	S
Control	50.8±7.0[a]	24.0±3.6[b]	25.2±3.4[d]	52.3±6.8[a]	23.7±3.8[a]	24.0±3.0[e]
Essential oil	55.7±6.2[b]	23.5±3.3[a]	20.8±2.9[c]	53.8±6.9[b]	23.5±3.4[a]	22.7±3.0[d]
1,8-cineole	51.2±7.0[a]	24.2±3.6[b]	24.6±3.4[d]	52.5±6.2[a]	23.2±3.4[a]	24.3±3.0[e]
γ-terpinene	54.9±6.0[b]	24.2±3.7[b]	20.9±2.3[c]	56.1±6.3[c]	24.0±3.6[a]	19.9±2.6[c]
Terpinene-4-alcohol	61.1±7.4[c]	26.0±3.2[c]	12.9±1.5[b]	62.3±7.2[d]	26.1±4.1[c]	11.6±1.9[b]
α-terpineol	60.5±7.1[c]	25.8±3.0[c]	13.7±1.9[b]	62.8±7.5[d]	25.4±4.1[b]	11.8±2.2[b]
Safrole	62.9±6.8[d]	26.3±3.9[c]	10.8±1.7[a]	64.3±7.9[e]	26.8±4.0[c]	8.9±1.6[a]

Percentages of each phase of the cell cycle are shown as mean ± SD. [a-e]Means within a single column followed by the same letter were not significantly different according to Ducan's multiplication range test at the 5% level.

eole showed very weak inhibitory effects on A549 cells growth.

Inhibition experiments on MCF-7 cell growth in vitro

MCF-7 cells were incubated with essential oil and its major components for 48 h over a range of 6.25-200 μg/mL. Terpinene-4-ol, α-terpineol and safrole were found to be an dose-dependent inhibition effect (R^2> 0.7) on MCF-7 cells with the growth inhibition rates of 99.17%, 99.61% and 99.79% at 200 μg/mL, respectively (**Table 2**). Essential oil and γ-terpinene displayed a weak inhibitory effect against MCF-7 cells without dose-dependent manner (R^2< 0.4). 1,8-cineole did not show any inhibitory effects on MCF-7 cells growth.

Essential oil arrested tumor cell cycles

As demonstrated in **Table 3**, terpinene-4-ol, α-terpineol and safrole markedly increased the proportion of the sub-G_1 phase in A549 cells and MCF-7 cells, indicating that the apoptosis indeed occurred in these tumor cells. While Essential oil and γ-terpinene only showed a weak inhibitory effect to the sub-G_1 phase in both cell lines. And 1,8-cineole did not show any impact on cell cycles in both cell lines. Moreover, as evident from **Table 3**, terpinene-4-ol, α-terpineol and safrole also triggered G_2/M phase cell-cycle arrest.

Analyses of cell cycle related proteins

Because of the essential-oil-induced arrest of the sub-G_1 and G_2/M phase, respectively, the levels of cell cycle-related proteins were investigated. Western blot data demonstrated that treatment of A549 and MCF-7 cells with the essential oil or its major components resulted in up-regulation of p53 (**Figure 3**). Considering that p53 is an important regulator of p21 [15], p21 levels were also assessed. As seen in **Figure 3**, p21 was increased in parallel to p53. Essential oil and γ-terpinene induced a little increase of p53 and p21, less than Terpinene-4-ol, α-terpineol or safrole did. Likewise, almost no changes in the levels of p53 and p21 were observed for 1,8-cineole, indicating its ineffectiveness (**Figure 3**).

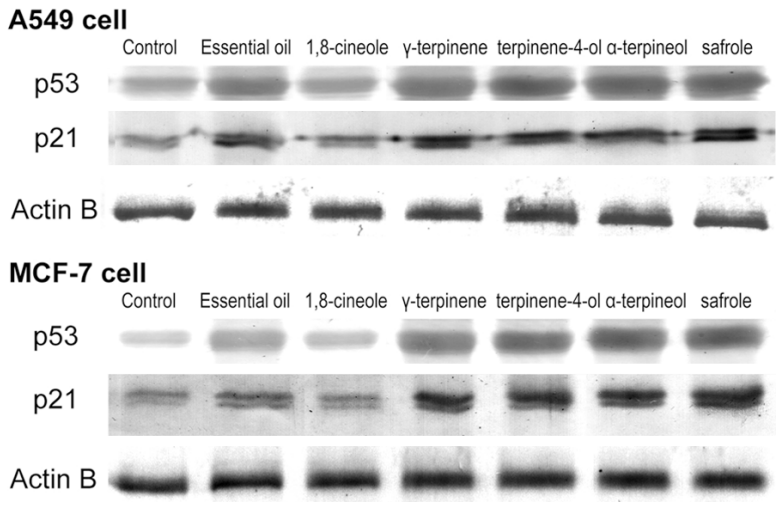

Figure 3. p53 and p21 protein levels of human tumor cells treated with or without 50 μg/ml *C. longepaniculatum* essential oil or its major components. Western blots of Actin B were used as the loading control.

Cost of essential oil and its major components at IC_{50}

Essential oil and its major components exhibited different degree of inhibition on the proliferation of A549 cells and MCF-7 cells and their costs varied with the change of IC_{50} and IC_{90} (**Table 4**). IC_{50} of essential oil, γ-terpinene, terpinene-4-ol, α-terpineol and safrole for A549 cells were decreased in turn as follow: 265.22, 217.50, 86.83, 63.40 and 10.50 μg/mL, respectively. The costs of them also reduced in the turn of IC_{50} for A549 cells. The cost of essential oil was less than that of terpinene-4-ol at IC_{50}. IC_{50} for MCF-7 were decreased in turn as follow: 1134.14, 191.81, 108.32, 98.43 and 22.21 for essential oil, γ-terpinene, α-terpineol, terpinene-4-ol and safrole, respectively. The costs of them also declined in the turn of IC_{50} for MCF-7 cells. The costs of safrole were the lowest for both A549 and MCF-7 cell lines.

Discussion

Cancer is one of the epidemic diseases, which is actually a status of uncontrolled cell growth, characterized by constant cell division and proliferation. A single cancer cell can proliferate into a colony, which is the basis of tumor incidence, metastasis, and recurrence [16, 17]. Therefore, the blockade of cell cycle, inhibition of cell proliferation, and induction of cell apoptosis are the main approaches for cancer treatment [18, 19]. Currently, in addition to the surgical method, chemotherapy and radiotherapy are the major options for cancer treatment. However, their side effects are severe, and a considerable number of patients cannot sustain these therapies, which affect seriously their life quality [20]. The identification of antitumor drugs with high efficiency and low toxicity from plants and marine organisms has become an important part of studies in developing antitumor drugs [21]. The antitumor effect of plant essential oils has gained great attention [22]. For example, the essential oils from *Pinus densiflora*, *Litsea cubeba*, and *Xanthium sibiricure*, exhibit substantial antitumor activities [23-25].

The action of drugs on cancer cells can be realized through the induction of apoptosis or necrosis, or by the cell morphological changes caused. In this study, we found that with the increase of treatment duration and drug concentration, the essential oil of *C. longepaniculatum* and its major components changed A549 and MCF-7 cell growth conditions and morphology characteristics, and exhibited different degrees of inhibitory effects on cell growth and apoptosis. Among them, α-terpineol and safrole demonstrated obvious alterations in the morphological parameters of A549 and MCF-7 cells: the cells were crumpled; the size and number of cells were reduced; they were gradually rounded-up, and the nuclei became pyknotic, the typical characteristics of cell necrosis. However, the essential oil of *C. longepaniculatum* and 1,8-cineole did not exert any influence on A549 and MCF-7 cell morphologies. Studies conducted by Ye et al. [8] showed that the essential oil of *C. longepaniculatum* and its major components are able to inhibit the growth of BEL-7402 liver cancer cells, decrease in the number of adherent cells and cell size, and the cells with cytoplasmic vacuoles and nuclei were pyknotic. Among the components, safrole exhibited the most potent effect in this process.

Table 4. Cost of essential oil and its major components at IC_{90} and IC_{50} against A549 and MCF-7

Samples	Unit price of the chemical ($/kg)	A549				MCF-7			
		IC_{90} (μg/mL)	Cost of IC_{90} ($/L)	IC_{50} (μg/mL)	Cost of IC_{50} ($/L)	IC_{90} (μg/mL)	Cost of IC_{90} ($/L)	IC_{50} (μg/mL)	Cost of IC_{50} ($/L)
Essential oil	10	477.40	0.005	265.22	0.003	2041.45	0.020	1134.14	0.011
1,8-cineole	16	1226.22	0.021	-	-	-	-	-	-
γ-terpinene	50	391.50	0.020	217.50	0.010	316.50	0.015	191.81	0.010
Terpinene-4-ol	65	175.41	0.011	86.83	0.006	181.32	0.012	98.43	0.006
α-terpineol	32	96.16	0.003	63.40	0.002	182.24	0.006	108.32	0.004
Safrole	50	78.00	0.004	10.50	0.001	87.85	0.004	22.21	0.001

"-" indicates the lack of an inhibitory effect where the IC_{50} was not calculated by statistics software.

Currently, the tetrazolium assay (MTT), as well as the sulforhodamine B (SRB), and thymidine incorporation (3H-TdR) assays are the most commonly applied approaches for anticancer drug screening *in vitro*. However, these methods have shortcomings, such as low sensitivity, complicated operation, radioactive safety concerns, and the long experimental period required [26]. ATP-TCA is a sensitive and stable approach for anticancer drug screening *in vitro*, which was developed recently. The principle of this method is that fluorescence at 562 nm can be released from fluorescein when catalyzed by luciferase under aerobic conditions. In the meanwhile, the ATP was changed to AMP, in the cases in which the released fluorescence intensity was positively correlated with ATP content. The ATP amount in the living cells is substantially constant, but in the dead cells it is rapidly hydrolyzed. Therefore, the fluorescence intensity can reflect the levels of intracellular ATP, and thus it reveals the quantity of living cells. For this reason, this approach can be utilized to evaluate the effect of drugs on cancer cell death; moreover, it has numerous advantages, such as high accuracy and sensitivity, easiness of operation, and short testing cycle [27]. In this study, we used ATP-TCA to investigate the action of the essential oil isolated from the leaves of *C. longepaniculatum* and its major components on A549 and MCF-7 cell growth. We found that the essential oil and its main compounds exhibited an inhibitory effect on the growth of both cell lines. Among the key active substances in the oil, α-terpineol and safrole manifested a potent suppressive activity on the growth of A549 (IC_{50}: 63.40 and 10.50 μg/mL, respectively) and MCF-7 (IC_{50}: 108.32 and 22.21 μg/mL, respectively) cells. However, the essential oil and 1,8-cineole displayed a weak, or even no effect on the growth of the two cell lines. Our findings are consistent with previous results indicating that safrole exerted the most powerful inhibition effect, whereas the essential oil of *C. longepaniculatum*, 1,8-cineole, γ-terpinene, and terpinene-4-ol displayed relatively weak activities on human BEL-7402 liver cancer cell growth. Their inhibition rate at the concentration of 1.28 mg/L was 90.04%, 58.63%, 42.83%, 34%, 84%, and 31.46%, respectively [8]. Studies conducted by Lina et al. [28] demonstrated that safrole can induce the apoptosis of human MG63 osteosarcoma cells. Safrole has potent antitumor effect, but it can also induces cancer at high doses [29]. α-terpineol is a flavoring substance, which is widely used in food and cosmetic products. It exhibits an antiproliferative action on MCF-7 breast cancer cells, multidrug-resistant NCI-ADR/RES breast cancer cells, NCI-H460 non-small cell lung cancer cells, PC-3 prostate cancer cells, 004FVCAR-3 ovarian cancer cells, and K-562 chronic myelogenous leukemia cells [30]. The substance can cause fatty liver disease in mice [31]. Currently, some clinical anticancer drugs, such as ifosfamide, doxorubicin, and vinblastine can also induce secondary tumors, while three harringtonine and busulfan have potential genotoxicity and carcinogenicity [32]. Therefore, attention has to be paid to both the efficacy and side effects when developing anticancer drugs. Previous reports [33] showed that the active compound Malabaricone C isolated from *Myristica fragrans* (Nutmeg) can decrease the safrole-induced risk of liver cancer.

The anti-proliferative effect of *C. longepaniculatum* essential oil on human tumor cells was determined by analyzing their cell cycle and

cell-cycle-related proteins. Plant-essential-oil-induced p53 and p21 expression is tightly correlated with apoptosis in tumor cells [24, 34]. For example, *Litsea cubeba* volatile oils induce apoptosis and causes cell cycle arrest in lung cancer cells by up-regulating p53 and p21 expression [24]. Another example of essential-oil-induced apoptosis is that bergamot essential oil and its extractive fractions inhibit human neuroblastoma cell growth through activating p38, ERK½, p53, Bcl-2 and Bax signalling pathways [34]. It has previously been found that p21 is induced by both p53-dependent and -independent mechanisms following stress, and furthermore, that p21 induction may cause cell cycle arrest [35, 36]. Safrole oxide induced a typical apoptosis in A549 human lung cancer cells by activating caspase-3, -8, and -9, as well as p53 protein [37]. Here we confirmed this essential-oil-induced apoptosis pathway and further show the ineffectiveness of 1,8-cineole on p53/p21-mediated apoptosis activation.

In summary, the essential oil of *C. longepaniculatum* and its major components were evidenced to have different degrees of inhibitory influence on the growth of A549 lung cancer cells and MCF-7 breast cancer cells. α-terpineol and safrole exhibited the most powerful suppressive action on the growth of both cell lines by activating p53/p21-mediated apoptosis and cell-cycle arresting. These findings can be the prerequisite basis for the potential development of these bioactive substances as potent antitumor drugs. However, further studies are still needed to evaluate their toxicity and safety.

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Disclosure of conflict of interest

None.

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