


In Vitro and In Vivo Efficacy Studies of Lavender *angustifolia* Essential Oil and Its Active Constituents on the Proliferation of Human Prostate Cancer

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Abstract

Lavandula angustifolia is the most widely cultivated *Lavandula* species. The extraction of its flower and leaves has been used as herbal medicine. In this study, the *in vitro* antitumor activities were tested on human prostate cancer PC-3 and DU145 cell lines. Flow cytometry technology was applied to study apoptosis induction and cell cycle arrest. The PC-3 cell line was used to establish subcutaneous xenograft tumors in nude mice. Paraffin sections from xenograft tumor specimens were used in the TUNEL (terminal deoxynucleotide transferase dUTP nick end labeling) assay and an immunohistochemistry assay to detect cell proliferation markers Ki67 and PCNA. Lavender essential oil, linalool, and linalyl acetate showed stronger inhibitory effect on PC-3 cells than on DU145 cells. The apoptotic cell populations observed in PC-3 cells treated with lavender essential oil, linalool, and linalyl acetate were 74.76%, 67.11%, and 56.14%, respectively. The PC-3 cells were mainly arrested in the G₂/M phase. In the xenograft model with PC-3 cell transplantation, essential oil and linalool significantly suppressed tumor growth. The immunosignals of Ki67 and PCNA in the essential oil, linalool, and linalyl acetate treatment groups were significantly lower than that of the control group in xenograft tumor sections. The TUNEL assay indicated that each of the 3 phytochemicals significantly induced apoptosis compared to the control group. This study provides novel insight and evidence on the antiproliferative effect of *L. angustifolia* essential oil and its major constituents on human prostate cancer. The antitumor effect was associated with cell proliferation inhibition and apoptosis induction in xenograft tumors.

Keywords

Lavandula angustifolia, linalool, linalyl acetate, prostate cancer, PC-3

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Introduction

Prostate cancer is by far the most commonly diagnosed cancer among American men, and it is the second leading cause of death by cancer.¹ Conventional prostate cancer treatments include prostatectomy, radiotherapy, hormone therapy, chemotherapy, and biological therapy. Side effects such as sexual function disorder, impotence, diabetes, and cardiovascular disease have been observed.² Recent surveys reveal that complementary medications, such as natural products, can improve the treatment in many cancer patients with minimal side effects.^{3,4}

Androgens are the major growth factors for the normal prostate. Recent studies suggest that the androgen receptor and its signaling pathway significantly contribute to the

metastatic prostate cancer progression.⁵ Although many patients initially respond to androgen depletion therapy,

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most of them will eventually progress and develop castration-resistant disease.⁶ Due to the complexity of the androgen receptor-targeted therapy for prostate cancer, newer agents must be discovered.⁷ Meanwhile, oncogenic fusion proteins have been discovered in prostate cancer tissues. It adds one more layer of complexity to the protein targeting network in prostate cancer cells.⁸

Transmembrane protease, serine 2 (TMPRSS2) is an androgen-responsive gene. The overexpression of ERG (ETS-related gene) proto-oncogene is one of the distinct characteristics of prostate cancer. The fusion of TMPRSS2 with ERG is a frequent event in prostate cancers, especially in hormone refractory prostate cancer.^{9,10} TMPRSS2-ERG contributes to androgen-independence in prostate cancer cells by disrupting androgen receptor signaling pathways.¹¹ Overexpression of ERG may contribute to the development of castrate-resistant prostate cancer. In addition, androgen treatment can activate ataxia telangiectasia mutated (ATM) in normal prostate epithelial cells and the knockdown of ATM can induce TMPRSS2-ERG fusion transcription.¹² Many studies have shown that microRNA (miRNA) act as tumor suppressors. For personalizing prostate cancer therapy development, miRNA signature identification and validation, particularly in TMPRSS2-ERG-positive prostate cancer cells, has been emerging as a promising trend.^{13,14}

The cytokine tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has attracted considerable attention as a novel anticancer agent owing to its selectivity toward tumor cells. However, prostate cancer cells such as DU145 and PC-3 are resistant to 100 ng/mL TRAIL-induced apoptosis.^{15,16} TRAIL sensitizers from natural sources are considered attractive with less toxicity and resistance in normal cells.^{17,18} TRAIL binds to 2 death receptors, DR4 and DR5, and 3 decoy receptors, DcR1, DcR2, and osteoprotegerin.¹⁹ Phytochemicals that increase DR4 and DR5 expression levels can augment the TRAIL-induced apoptosis.²⁰

Lavandula angustifolia Mill. (Lamiaceae) belongs to the mint family, which is native to the western Mediterranean.^{21,22} In the form of oil extraction, the flower and leaves of *L. angustifolia* are used as herbal medicine.²³ Over the past decade, substantial scientific evidences have been accumulated to suggest that the extracts of *L. angustifolia* have antibacterial, antioxidant, and anticancer activities.^{23,24} It has been used in the treatment of gastrointestinal disorders, irritability, and rheumatism. Studies have showed that the distillation extract of *L. angustifolia* had cytostatic and apoptotic effects on human lung cancer (A-549), breast cancer (MCF-7), cervical cancer (HeLa), and prostate cancer (PC-3).²⁵

The main components of *L. angustifolia* distillation products are linalool (26% to 49%) and linalyl acetate (17.6% to 53%), and they are responsible for the characteristic flavor and the therapeutic properties.^{22,26} Linalool and linalyl acetate are naturally occurring phytochemicals showing anti-inflammatory activity in carrageenan-induced edema rats.²⁷

Cell line-based studies suggested that linalool had antiproliferative activity against SW 620, Hep G2, A-549, and T-47D cells via inducing apoptosis and activating antitumor immunity.²⁸ An *in vivo* sarcoma-180 solid tumor model study stated that the antitumor potential of linalool is achieved by oxidative stress modulation.²⁹ Linalool also exhibited very high antimicrobial activity against *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Candida albicans*.³⁰ Linalyl acetate is the acetate form of linalool. It has a typical prodrug behavior.²⁷ Linalyl acetate can alter the melanogenesis of melanoma cells through oxidative stress and the JNK and ERK signaling pathways.³¹

In this study, the antiproliferative effects of the *L. angustifolia* essential oil and its main components, linalool and linalyl acetate, on human prostate cancer cells were investigated. Efficacies of the compounds were evaluated and compared in both *in vitro* cell culture assays and xenograft tumor model.

Materials and Methods

Materials

The plant *Lavandula angustifolia* was harvested from Malong, Yunnan, China (N25°23', E103°37'). The extract (essential oil) was kindly provided by Yunnan Nuoye Biotech, Inc. Human prostate cancer cell lines, PC-3 and DU145, were obtained from the Chinese Academy of Sciences Cell Bank. Linalool, linalyl acetate, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), and the Annexin V-FITC Apoptosis Detection Kit were obtained from Sigma Aldrich (St Louis, MO). The DNA content quantitation assay kit was purchased from KeyGEN BioTECH (Nanjing, Jiangsu, China).

Liquid Chromatography/Mass Spectrometry (LC/MS)

The presence of linalool and linalyl acetate in the essential oil was assessed by LC (Ekspert Ultra LC 100, Eksigent part of AB SCIEX)/MS (3200 Q TRAP LC/MS/MS system, AB SCIEX) with a reverse-phase column (Thermo Accucore C18). The chromatography conditions were a mobile phase A of ddH₂O with 0.1% formic acid and B of acetonitrile with 0.1% formic acid. The gradient program was (a) 10% solvent B for 0.5 minutes; (b) a linear gradient from 10% to 90% solvent B over 4 minutes; (c) 0.5 minutes with 90% solvent B; and (d) 3 minutes with 10% solvent B. EMS+ was used as the scan type.

Cytotoxicity Assay

Human prostate cancer cell lines, PC-3 and DU145, were maintained in F-12 and Dulbecco's modified Eagle medium

(DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, Life Technologies). Cells were plated in a clear 96-well flat-bottomed plate at the density of 2500 cells/well in 90 μ L of growth medium. After 12 hours, *L. angustifolia* essential oil, linalool, or linalyl acetate solution was added at different concentrations. One percent dimethyl sulfoxide (DMSO) in phosphate-buffered saline (PBS) and 10% trichloroacetic acid solution was used as negative and positive controls, respectively. After the treatment for 48 hours, MTT was added and incubated at 37°C for 4 hours. After the incubation, the medium was carefully removed and 50 μ L DMSO was added to each well. After 10-minute incubation at 37°C, the plate was then read at 540 nm on a plate reader.

Wound Healing Assay

Human prostate cancer cells, PC-3 and DU145, were cultured to >90% confluence in 6-well plates. The cells were rinsed with PBS and starved in low serum medium (0.5% FBS in F-12 or DMEM) overnight. On the bottom of the dish, a line was drawn with a marker. A sterile 200 μ L pipet tip was used to scratch through the cells to create wounds perpendicular to the marker line. The cells were gently washed with PBS and incubated with 1.5 mL medium or medium with added drug. Pictures were taken by Nikon Eclipse 80i microscope system using phase-contrast objective (10 \times). All the images were analyzed by the TScratch program (www.cse-lab.ethz.ch).

Apoptosis

The induction of apoptosis was assessed using a Sigma Annexin V-FITC Apoptosis Detection Kit. According to the manufacturer's instructions, 1×10^6 cells/mL human prostate cancer cells, PC-3 and DU145, were treated with lavender essential oil (0.05%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) for 24 hours. Both adherent and floating cells were collected and suspended in $1 \times$ binding buffer. Cells were stained with Annexin V-FITC conjugate and propidium iodide (PI). After 10-minute incubation at ambient temperature in dark, the samples were quantified by flow cytometry (PARTEC CyFlow Space). The experiments were repeated thrice.

Cell Cycle Arrest

The cell cycle analysis was performed using a KeyGEN BioTECH DNA content quantitation assay kit. Human prostate cancer cells, PC-3 or DU145, were treated with lavender essential oil (0.05%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) for 24 hours. After the treatment, cells were collected and fixed with 70% ethanol at 4°C for 2 hours. DNA was stained with PI in the presence of 1% DNase-free RNase A at 37°C for 30 minutes before flow cytometry

analysis (PARTEC CyFlow Space). The distribution of cells in distinct cell cycle phases was determined using FloMax Ver. 2.82 (QA GmbH, Munster, Germany) cell cycle analysis software. The experiments were repeated 3 times.

Western Blot

DU145 and PC-3 cells were seeded in 6-well plates. Cells were treated with *L. angustifolia* essential oil (0.05%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) for 24 hours. After the treatment, cells were washed with PBS and collected. Cells were lysed with RIPA buffer on ice for 30 minutes. The protein concentration was determined by BCA kit (Beyotime Biotechnology, Shanghai, China). The proteins were separated on 10% SDS-PAGE and electrotransferred onto polyvinylidene fluoride membrane. The membrane was blocked with 5% bovine serum albumin (BSA) and immunoblotted with antibodies against DR4, DR5, and β -actin (Abcam Plc, Cambridge, MA).

Maximum Tolerable Dose

Male Balb/c nude mice (SPF, 6 weeks) were purchased from HFK Bioscience Co, Ltd (Certificate No. 11401300024642; Beijing, China). The animals were fed with standard commercial diet purchased from Keaoxieli Feed Co, Ltd (Certificate No. 11002900015471; Beijing, China). Nude mice were housed in SPF animal experiment center that is specific pathogen-free with a 12-hour light-dark cycle. All animal usage and operation procedures were approved by Kunming Medical University Experimental Animal Ethics Committee (Approval No. KMMU 2015008).

Five groups of nude mice ($n = 5$) received subcutaneous injection of lavender essential oil, linalool, or linalyl acetate at 5 different doses (10, 25, 50, 100, and 200 mg/kg body weight). One additional group of animals received the drug vehicle in PBS as control. Animals were monitored for 7 days for any sign of toxicity. For necropsy, major organs, such as heart, liver, spleen, kidney, and intestine, were collected. Tissue and organ damage, bleeding, or necrosis was evaluated by hematoxylin and eosin (HE) stain. Briefly, tissue samples were fixed in 10% neutral formalin and embedded in paraffin. Alum hematoxylin stain solution was used to stain the nuclei after the sections were deparaffinized and rehydrated. The samples were differentiated in 1% HCl-MeOH solution and washed separately with 70%, 85%, 95%, and 100% methanol for 5 minutes. Then, the samples were stained with eosin for 2 minutes. Sections were washed, dehydrated, cleared, and mounted for microscopic observation.

Efficacy Study in Nude Mice

Human prostate cancer cell line PC-3 was used to establish the synergetic subcutaneous tumor model. Cell suspension

(2×10^6 cells in 0.1 mL PBS) was inoculated subcutaneously into the rear flanks of the male nude mice. Once xenograft tumors were palpable ($\sim 30 \text{ mm}^3$), animals were randomly divided into 4 groups and were treated with solvent control, lavender essential oil, linalool, or linalyl acetate. Mice received drugs at the dose of 200 mg/kg body weight twice a week for 4 weeks via subcutaneous injection. Tumor growth was measured twice a week with a caliper. Tumor volume was calculated by the following formula: $L \times W \times H \times 0.5236$. A tumor growth curve was then generated by plotting the relative fold value of tumor volume at each time point compared to its initial volume by the following formula: $(V_t - V_0)/V_0 \times 100\%$.

Immunohistochemistry

All the mice were euthanized after the last dose of the treatments on the 28th day. Tumor tissues were collected, washed with PBS, fixed in 10% neutral formalin, and embedded in paraffin. Immunohistochemistry for PCNA and Ki67 was performed with antigen retrieval. Briefly, paraffin-embedded tumor sections were deparaffinized and rehydrated. Antigen recovery was achieved by boiling the tissues in citrate acid buffer (10 mM, pH 6.0). Nonspecific binding sites were blocked using 1% BSA before incubation with antibodies at 4°C overnight. After the treatment of primary and secondary antibodies, the sections were stained with DAB solution. The samples were then counterstained with HE, as described above. The sections were visualized using a DAKO LSAB Detection System (Catalog #K0679, DAKO USA). The immunosignal index was calculated by the following formula: (positive cells number/total cell number) $\times 100\%$.

Apoptosis in Xenograft Tumor

The terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay was performed to detect apoptotic cell death in xenograft tumors using the in situ TUNEL Cell Apoptosis Detection Kit-Biotin-POD (KGA702). The slide was prepared according to the manufacturer's instructions. Briefly, paraffin-embedded tumor sections were washed in PBS 3 times after being deparaffinized and rehydrated. Proteinase K (100 μL) was added to the sections and incubated at 37°C for 30 minutes. After the treatment, the sections were washed 3 times in PBS and incubated in 3% H_2O_2 for 10 minutes at ambient temperature. DNase I (100 μL) was added to the samples and incubated at 37°C for 10 minutes. Samples were washed and dried, 50 μL TdT enzyme mix was added, and reacted at 37°C for 60 minutes. Then, 50 μL streptavidin-HRP working solution was added, and the samples were incubated at 37°C for 30 minutes in dark. After the reaction, 50 μL DAB was added and reacted at ambient temperature for 1 minute. The samples were counterstained with HE, as described above. The sections

were visualized and the immunosignal index was calculated.

Statistical Analysis

GraphPad Prism 5 software was used for statistical analysis. A *t* test was used for a statistical comparison of the 2 means. One-way ANOVA was used to compare differences between groups. In all comparisons, statistical significance was set at $P \leq .05$.

Results

Linalool and Linalyl Acetate in the *L. angustifolia* Essential Oil

LC/MS/MS was used to examine if linalool and linalyl acetate are present in the essential oil. The retention times of extractions of 177-178 (linalool + Na^+) and 219-220 (linalyl acetate + Na^+) were 3.09 minutes and 3.71 minutes, respectively. The content of linalool and linalyl acetate found in the essential oil was $30.5 \pm 0.5\%$ and $15.8 \pm 0.5\%$, respectively.

In Vitro Cytotoxicity

Viability assay was performed to evaluate the inhibitory effects of *L. angustifolia* essential oil, linalool, and linalyl acetate on human prostate cancer cells. The IC_{50} of the essential oil treatment determined in DU145 and PC-3 cells was $0.199 \pm 0.026\%$ and $0.037 \pm 0.011\%$ (v/v), respectively. The IC_{50} of linalool and linalyl acetate was $7.22 \pm 0.28 \mu\text{M}$, $11.74 \pm 0.62 \mu\text{M}$, in DU145, and $3.06 \pm 0.22 \mu\text{M}$ and $4.98 \pm 0.31 \mu\text{M}$, respectively, in PC-3. These results indicate that *L. angustifolia* essential oil and its main components, linalool and linalyl acetate, displayed potent cytotoxicity against both PC-3 and DU145 cell lines.

Wound Healing Assay

The *in vitro* inhibition effect of the drugs on cell migration was studied by scratch assay. The open image areas were quantified (Figure 1). *L. angustifolia* essential oil, linalool, and linalyl acetate significantly inhibited migration of DU145 and PC-3 cells ($P < .05$) in both dose- and time-dependent manners. As shown in Figure 1, after the treatment of 0.005% (v/v) of lavender essential oil for 24 hours, the open image areas of PC-3 and DU145 cell monolayer were 10 and 4.25 times larger than that of the control group, respectively.

Apoptosis and Cell Cycle Arrest

Annexin-V/PI staining was used to examine the apoptotic induction of drugs in PC-3 and DU145 cells. Following the

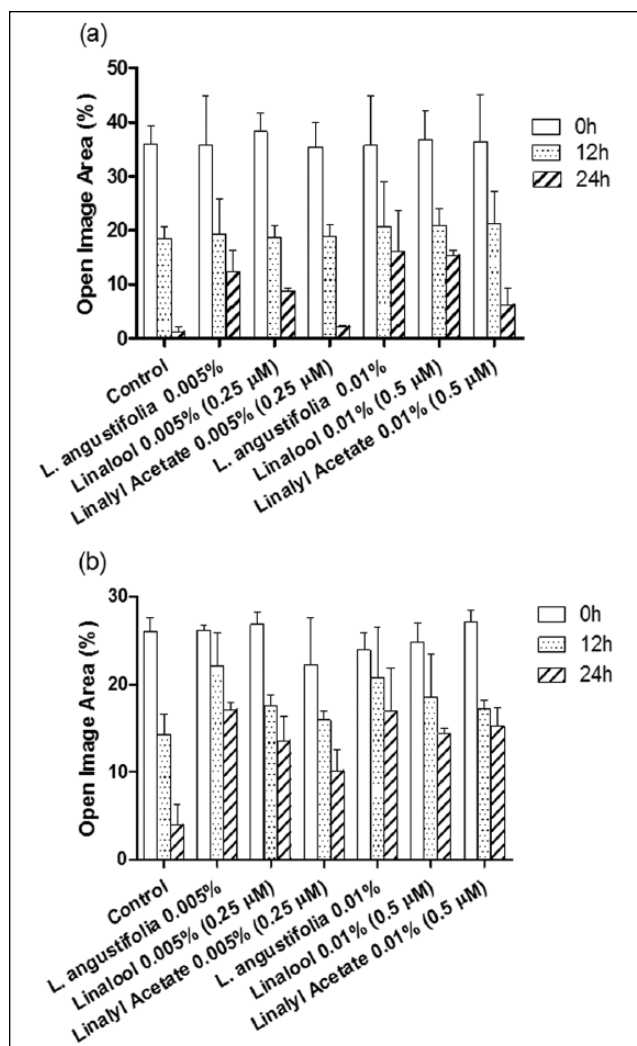


Figure 1. The quantified open image area in wound healing assay of DU145 (a) and PC-3 (b) cells treated by *L. angustifolia*, linalool, or linalyl acetate at different time points (mean \pm SD, $n = 3$).

treatment of lavender essential oil (0.5%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) for 24 hours, the proportion of apoptotic cells was significantly increased in both PC-3 and DU145 cells compared to the control group (Figure 2). Apoptotic cell population observed in PC-3 cells treated with PBS (control), lavender essential oil, linalool, and linalyl acetate was 2.11%, 74.76%, 67.11%, and 56.14%, respectively. In DU145, the apoptotic cell population of control, lavender essential oil, linalool, and linalyl acetate treatment was 1.96%, 10.64%, 21.47%, and 12.15%, respectively.

To investigate the effects of the drugs on cell cycle regulation, cell cycle distribution was studied by flow cytometry after PI staining. The distributions of PC-3 and DU145 cells with a 24-hour treatment of lavender essential oil (0.05%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) in cell

cycle are shown in Figure 3a. The percentage of cells in G_2/M phase was significantly increased after the treatment in PC-3 cells. The percentage of cells in G_2/M phase was 2.56%, 10.04%, 11.62%, and 11% in control, lavender essential oil, linalool, and linalyl acetate treatment groups, respectively. However, in DU145 cell line, cells were arrested in the S phase instead of the G_2/M phase (Figure 3b). The S phase cell population of control, lavender essential oil, linalool, or linalyl acetate treatment was 25%, 36.93%, 39.74%, and 29.79%, respectively.

DR4 and DR5 Regulation

The expression levels of DR4 and DR5 in DU145 and PC-3 prostate cancer cells subjected to treatments were assessed by Western blotting (Figure 4). The normalized expression of DR4/ β -actin and DR5/ β -actin are shown in Figure 4b and c. In DU145 cell line, both DR4 and DR5 were significantly upregulated in the essential oil and linalool treatment groups. However, in PC-3 cell line, there was no significant difference in the expressions of DR4 and DR5 in any of the treatment groups compared to the control group.

Xenograft Tumor Growth in Nude Mice

Before the *in vivo* efficacy studies, the maximum tolerable dose was determined in nude mice bearing xenograft tumors established from prostate cancer PC-3 cell line (Figure 5). At the maximum dose of 200 mg/kg body weight, mild edema in heart was observed in animal treated with *L. angustifolia* essential oil. However, there was no significant differences of histological finding between the control group and the treatment groups (Table 1). These results suggest the treatments to the heart, liver, spleen, intestine and kidney of the tested animals do not cause obvious toxic effect. Therefore, the dose of 200 mg/kg body weight was safe to animals and used for the subsequent experiment.

The antitumor effects of lavender essential oil, linalool, and linalyl acetate were examined in mouse xenograft model. Human PC-3 cell line was used to establish subcutaneous xenograft tumors in nude mice. As shown in Figure 6, at the end of the treatment, compared to the control group, lavender essential oil ($P < .001$), linalool ($P < .001$), and linalyl acetate ($P = .016$) significantly suppressed xenograft tumor growth.

Cell Proliferation and Apoptosis in Xenograft Tumor

Tumor cell proliferation indexes were assessed by detecting PCNA and Ki67 immunosignals in xenograft tissues. As shown in Figure 7a, the expressions of PCNA and Ki67 were dramatically reduced in xenograft tumors from mice

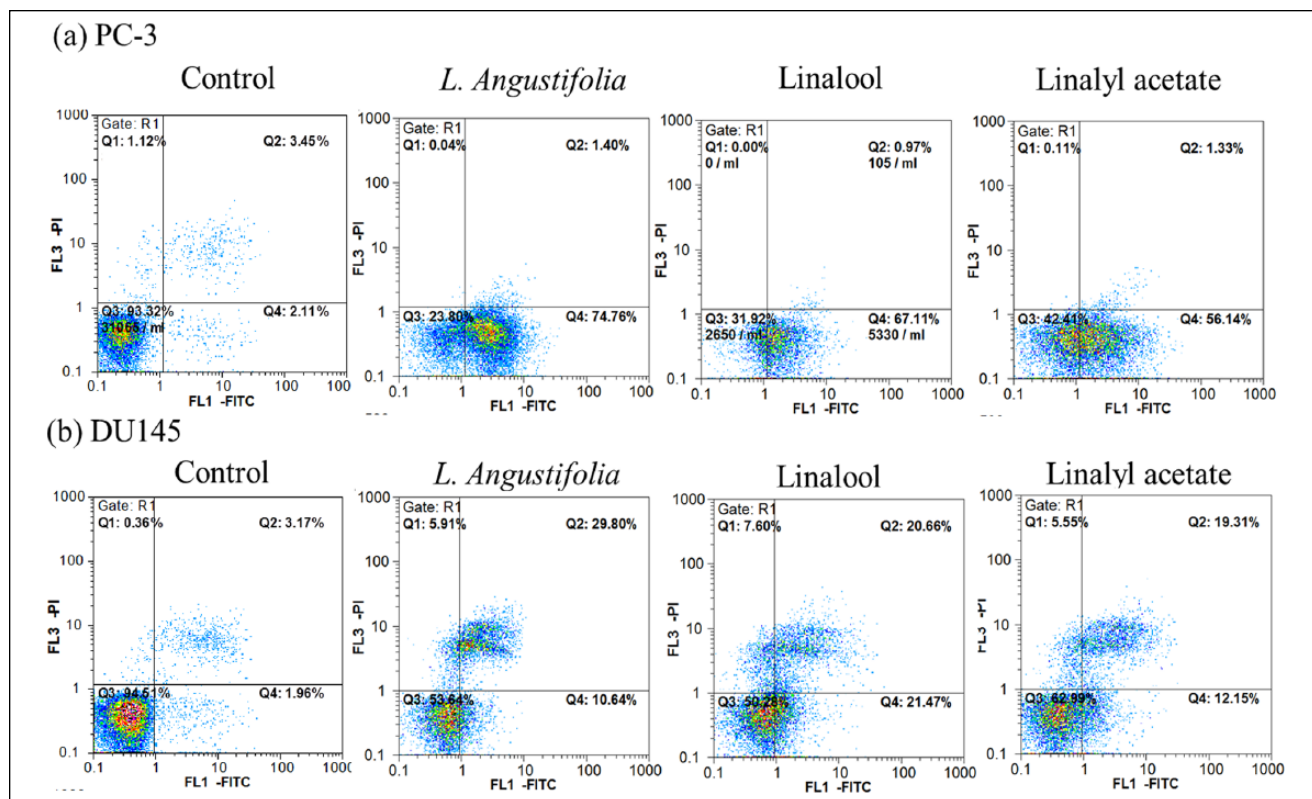


Figure 2. The effects of lavender essential oil (0.05%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) treatment on apoptosis induction in PC-3 (a) and DU145 (b) cells.

that received treatment of lavender essential oil, linalool, or linalyl acetate compared to that of the control group. These data are consistent with the tumor growth curve as measured by tumor size, indicating that lavender essential oil and linalool suppressed tumor growth *in vivo* by inhibiting tumor cell proliferation.

Finally, a TUNEL assay was performed to evaluate if the drug induced apoptotic cell death in xenograft tumor. Positive immunosignals were observed on xenograft tumor sections of mice treated by essential oil, linalool, and linalyl acetate. Quantitative analysis of the images (Figure 7b) indicated a moderate apoptosis rate in lavender essential oil and linalool treatment groups, but a relative low level in linalyl acetate-treated tumor specimens.

Discussion

Cancer is the second leading cause of death, and prostate cancer is the most commonly diagnosed cancer in men. Due to improved early detection and chemotherapy, cancer is no longer considered an incurable disease. Since natural products played an important role in drug discovery^{32,33} and studies of natural products have led to the development of many potent anticancer agents, such as vincristine, paclitaxel, and

silibinin,³⁴⁻³⁶ it is desirable to continue the efforts aiming at discovering new anticancer agents based on naturally occurring compounds. In the past decade, much effort and progress has been made in prostate cancer research.^{1,37,38} The effects of green tea polyphenols for prostate cancer prevention and treatment have been studied in epidemiological, preclinical, and early clinical investigations.³⁹

Owing to the possession of antibacterial and anti-cancer activities, plant essential oil has become the focus of phyto-medicine research^{40,41} and drug delivery system formulation development.⁴² In recent times, lavender is widely cultivated around the world, and *L. angustifolia* is the most common species. Steam distillation yields lavender essential oil, which has enjoyed a long history of folk and traditional therapeutic use for a wide range of modalities including aromatherapy, cosmetics, massage oils, and perfumes. Since the component of *L. angustifolia* essential oil may display some variation,^{22,43} the major content of the essential oil was examined by LC/MS/MS. The content of linalool and linalyl acetate in the essential oil used in this study is similar to previous reports.^{44,45} In this research, we investigated the therapeutic effect of *L. angustifolia* essential oil and its major constituents (linalool and linalyl acetate) on human prostate cancer cells.

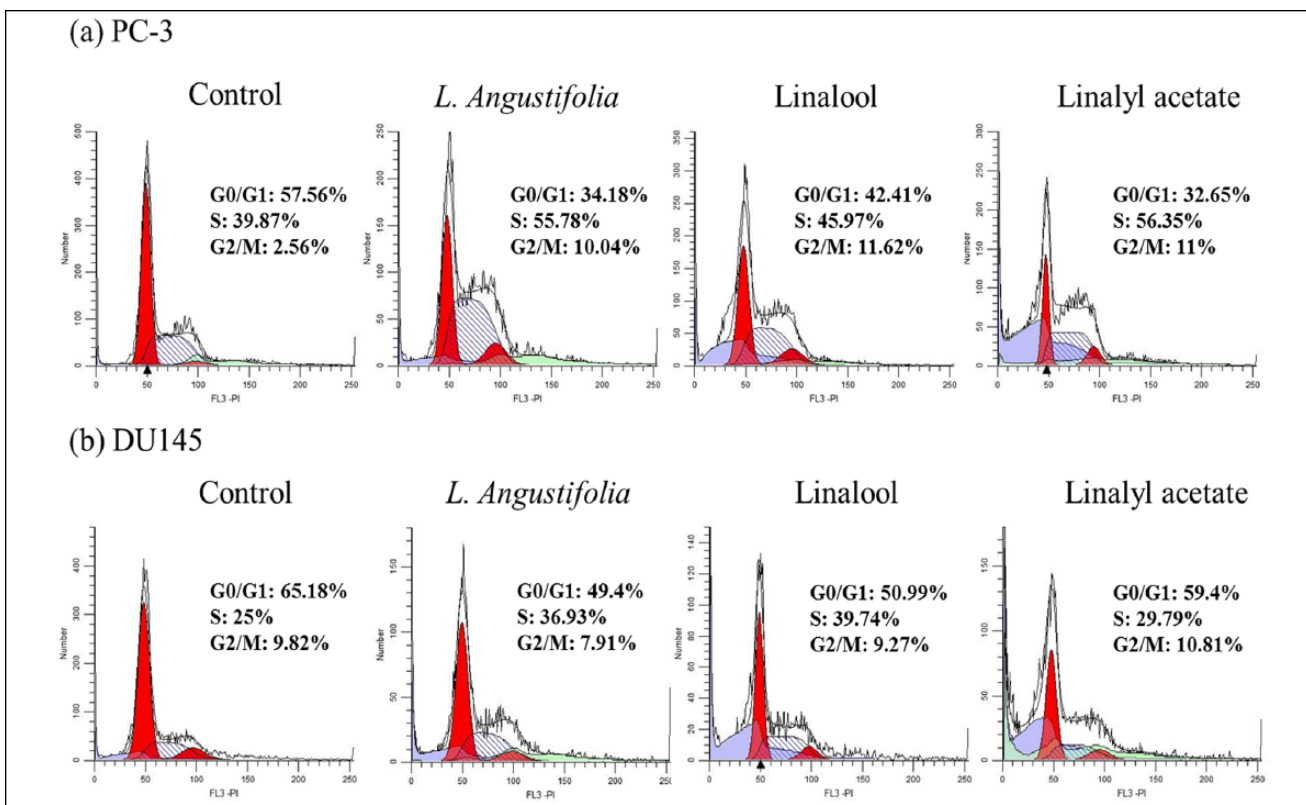


Figure 3. The effects of lavender essential oil (0.05%, v/v), linalool (2.5 μ M), or linalyl acetate (2.5 μ M) treatment on cell cycle progression in PC-3 (a) and DU145 (b) cells.

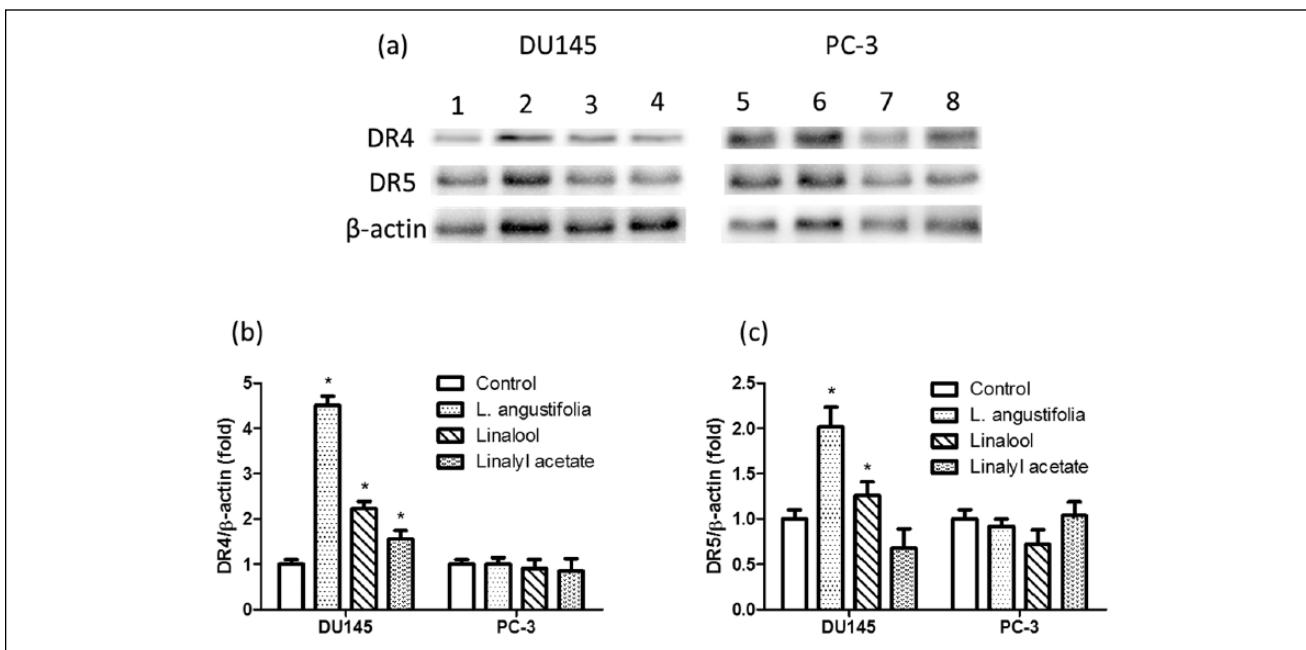


Figure 4. The expression of DR4 and DR5 in DU145 and PC3 cells was assessed by Western blot. Lanes 1 and 5: control; lanes 2 and 6: essential oil; lanes 3 and 7: linalool; lanes 4 and 8: linalyl acetate.

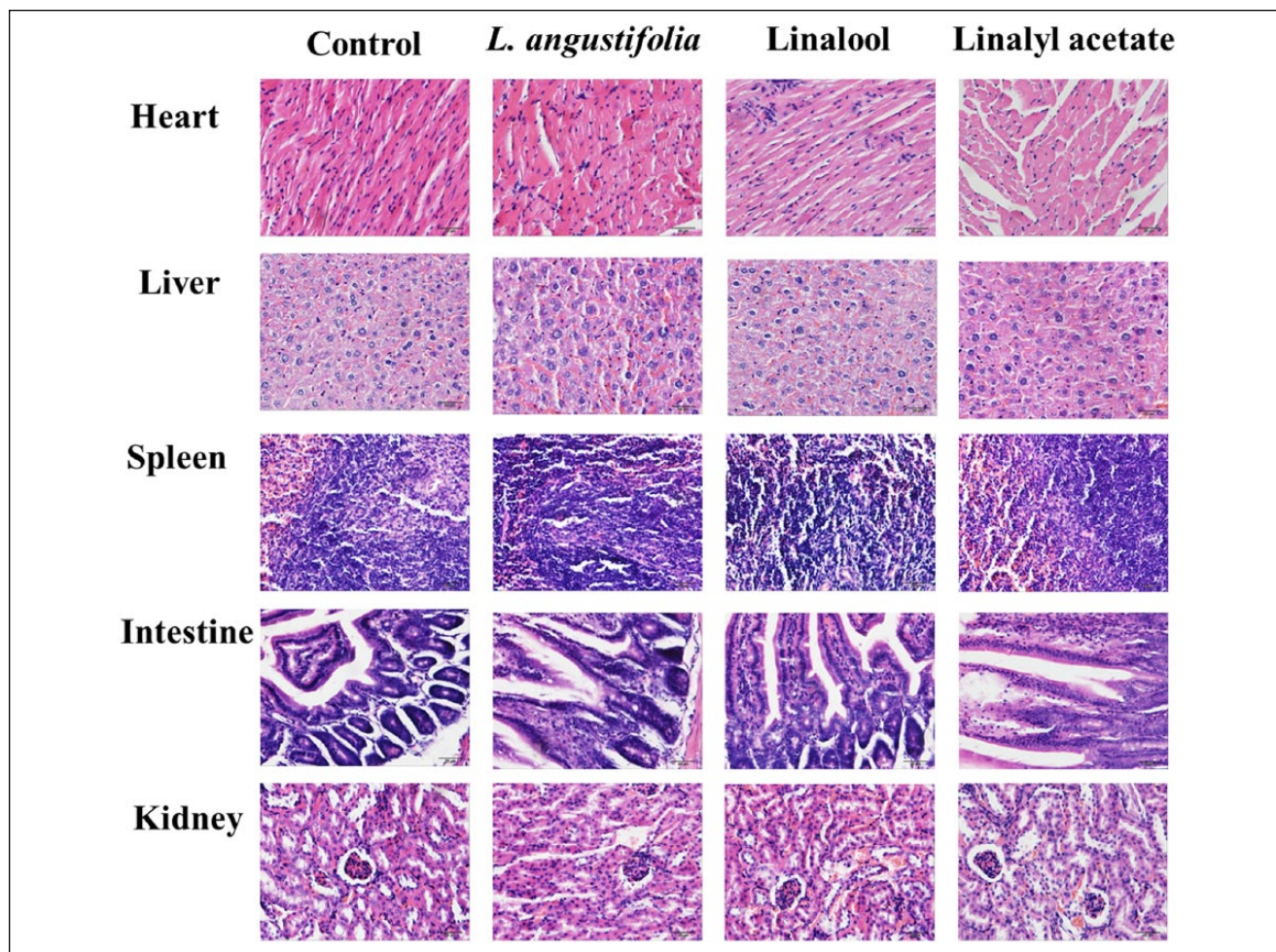


Figure 5. Microscopic observations of major mice organs after the treatment of lavender essential oil, linalool, or linalyl acetate at a concentration of 200 mg/kg.

Invasion and metastasis are the most significant biological characteristics of advanced metastatic prostate cancer. Invasion and metastasis of cancer cells involve many factors. Deriving from different origins, with DU145 cells from brain metastasis and PC-3 cells from bone metastasis,⁴⁶ DU145 and PC-3 often exhibit different metastatic potentials. Studies have suggested that DU145, but not PC-3, depends on cathepsin L + B activity for invasion.⁴⁷ In addition, DU145 cell line was found to be more resistant to the inhibition of *S100A3* expression than PC-3 cells. In PC-3 cell line, MMP-2 and MMP-9 were downregulated, whereas in DU145 cells, only MMP-9 was downregulated after the inhibition of *S100A3*.⁴⁸ In addition, TMPRSS2/ERG gene fusions showed differing effects on radiosensitivity and sensitivity to paclitaxel, a phytochemical extracted from the bark of pacific yew, in PC-3 and DU145 cell lines.⁴⁹ There are variations in ERG signaling between PC-3 and DU145 containing TMPRSS2/ERG fusions.⁴⁹ Furthermore, PC-3 is PTEN deficient, and DU145 is PTEN

functional. In conjunction with the presence of TMPRSS2/ERG fusion, loss of PTEN expression has been shown to promote prostate carcinogenesis.⁵⁰ Therefore, cell line variations and discrepancies in the molecular characteristics (ie, the fusing type of TMPRSS2/ERG) may explain why PC-3 cell line is more sensitive to the phytochemicals compared to the DU145 cells.

The suppression of tumorigenesis involves regulation of signal transduction pathways that lead to gene expression alteration, cell cycle arrest, and apoptosis. The induction of apoptosis (Type I programmed cell death) is considered as a crucial mechanism in cancer prevention and treatment. The apoptosis signaling pathway has become a promising target for novel anticancer agent development.⁵¹ Flow cytometry with FITC-Annexin V/PI staining suggested that the essential oil, linalool, and linalyl acetate could induce cell death via the apoptotic pathway. This indicates that lavender essential oil and its major constituents have the potential to be developed as chemopreventive agents. Furthermore,

Table 1. Animal Major Organ Pathological Biopsy Examination Records.

Organ	Histology	Control	<i>Lavender angustifolia</i>	Linalool	Linalyl Acetate
Heart	Karyopyknosis				
	Waviness of fibers at border				
	Neutrophil infiltration				
	Edema		+		
Liver	Necrosis				
	Infiltration of inflammatory cells				
	Cellular degeneration				
Spleen	Splenic corpuscle				
	Megakaryocyte				
Intestine	Epithelial degeneration				
	Villi edema				
	Submucosal inflammation	+	+	+	+
Kidney	Glomerulosclerosis				
	Hydropic degeneration				
	Tubular atrophy				
	Inflammation				
Overall		+	++	+	+

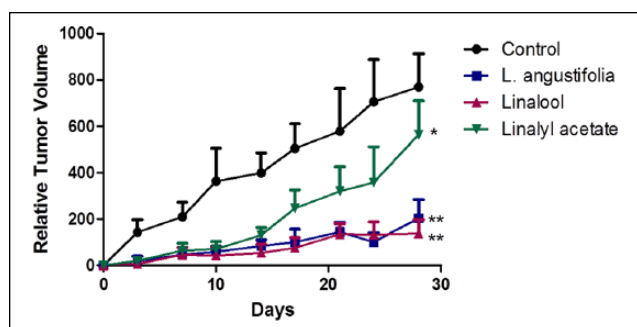


Figure 6. Lavender essential oil, linalool, and linalyl acetate significantly suppressed tumor growth in nude mice transplanted with PC-3 prostate cancer cells (mean \pm SD; n = 6; *P < .05; **P < .001).

uncontrolled cell division or proliferation of mutated cells can contribute to tumorigenesis. Both cell proliferation inhibition and apoptosis induction are highly correlated with the intracellular signaling regulations, which lead to cell cycle arrest in the G₀/G₁, G₂/M, or S phase.⁵² Blocking passage through cell cycle check points also can inhibit cancer cell growth. Lavender essential oil, linalool, and linalyl acetate induced G₂/M and S phase in PC-3 and DU145 cells, respectively, indicating that the natural products targeted differently in these cell lines.

There are 2 typical caspase-dependent pathways involved in apoptosis: the intrinsic mitochondria-dependent apoptosis and the extrinsic death receptor-dependent apoptosis. There is accumulating evidence that upregulation of DR4 and DR5 is related to sensitization of TRAIL-induced apoptosis.⁵³ In this study, we showed that the expression levels of DR4 and

DR5 were increased by essential oil and linalool only in DU145, but not PC-3, cell line. This result is consistent with previous experiments, which indicated that the phytochemicals involve different mechanisms in the treatment of DU145 and PC-3 cells. Essential oil and linalool may augment the TRAIL-induced apoptosis by increasing the expression of DR4 and DR5 in DU145 but not PC-3 cell line. However, the study of the mechanism of TRAIL mediated signaling in TMPRSS/ERG fusion-positive prostate cancer is incomplete. Therefore, in order to understand the effect of TRAIL-mediated signaling restoration by phytochemicals, further study on the death receptors in TMPRSS/ERG fusion positive prostate cancers is necessary.

The antitumor activities of essential oils and their components have been reported. For example, the aldehyde compounds isolated from *Citrus paradisi* essential oil was found to induce apoptosis in HL-60 cells⁵⁴; jasmine essential oil has cytotoxic effects in A549 and MCF-7 cell lines²⁵; lavender essential oil showed both cytotoxic and apoptotic activities on MCF-7 and HeLa cell lines.⁵⁵ However, most of the studies were performed only in cell-based assays. In this work, the antiproliferative effects were also studied in *in vivo* model. We showed that the lavender essential oil and linalool treatment significantly suppressed the growth of xenograft mouse tumors established from PC-3 cell line. Linalyl acetate suppressed xenograft tumor growth by approximately 27% compared to the control group. Although further work is needed for further elucidating the mechanisms of the antitumor activity, these data provide preliminary evidence for the implication of lavender essential oil and its active constituents in treating human prostate cancer and other similar conditions.

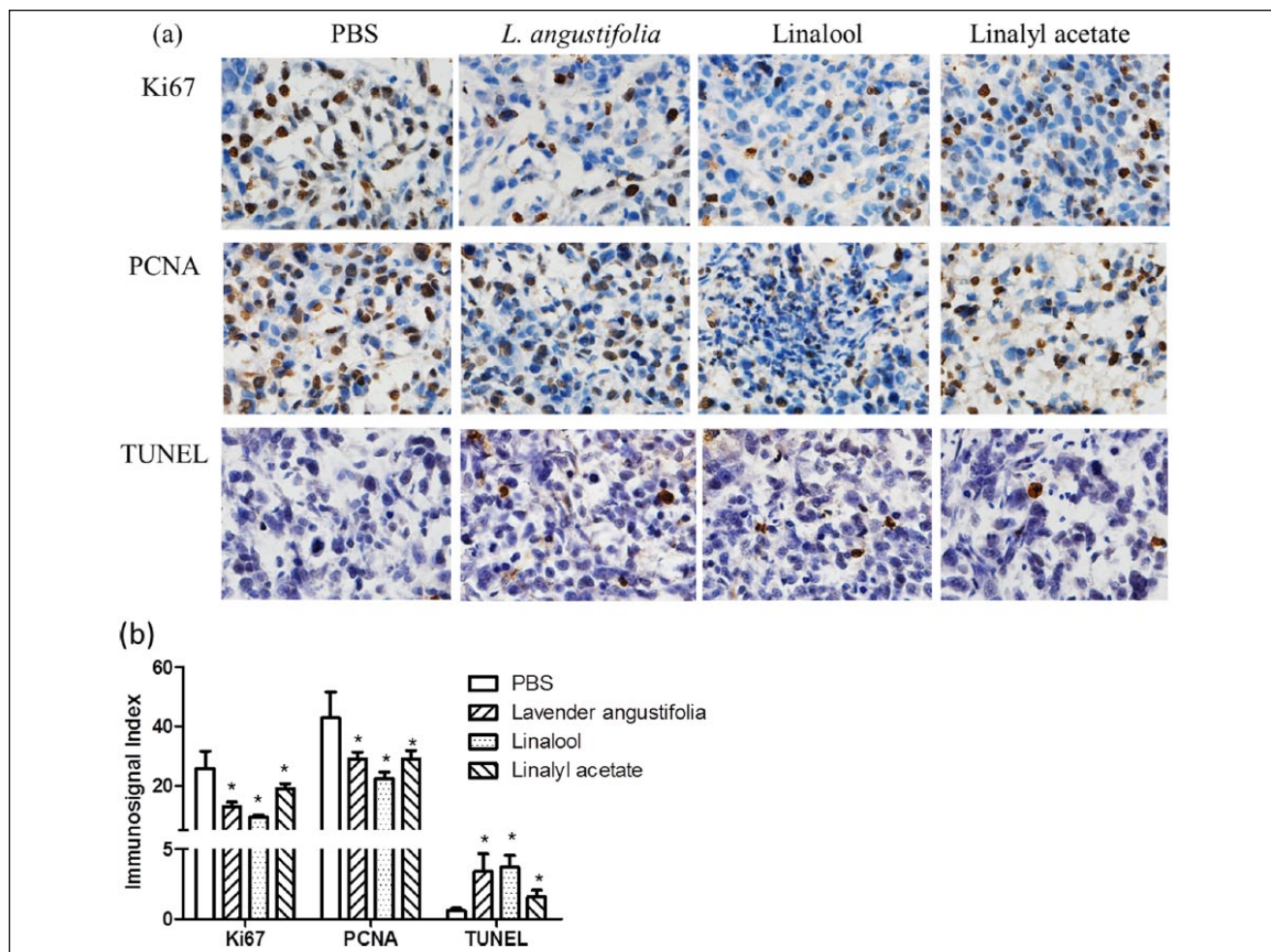


Figure 7. Lavender essential oil, linalool, and linalyl acetate significantly suppressed cell proliferation (Ki67 and PCNA) and induced apoptosis (TUNEL) (mean \pm SD; $n = 5$; $*P < .05$).

In conclusion, we demonstrated that lavender essential oil was effective in inhibiting tumor growth of human prostate cancer xenografts in nude mice. Linalool, but not linalyl acetate, mainly contributed to this effect. This antitumor effect was associated with apoptosis induction and cell proliferation inhibition. Our data suggest that the lavender essential oil and linalool have the potential to be developed as a novel therapeutic agent for prostate cancer treatment.

Declaration of Conflicting Interests

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