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RESEARCH ARTICLE

Evaluation of the Antimicrobial and Cytotoxic Activity of Cultivated *Valeriana officinalis*

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Abstract

Drug resistance refers to the reduction in the effectiveness of a drug in treating a disease or improving the stability of symptoms. It can occur in various types of pathogens, including bacteria, parasites, viruses, fungi, and cancer cells. This experimental study was conducted between 2018 and 2019 in an area with an annual mean rainfall of 130mm. The sowing date was September 10th, and 2-3 seeds were planted per cell. MTT assays 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) were used to determine the percentage of viability in adenocarcinomic human alveolar basal epithelial cells (A549) and Medical Research Council cell strain 5 (MRC5) cell lines incubated with methanolic extract and valerenic acid for 48 hr. The methanol extract was prepared by adding 1000 mg of rhizomes to 100 mL of methanol, followed by sonication for 30 minutes, stirring, and centrifugation at 4000 rpm for 10 minutes. Minimum inhibitory concentration (MIC) and agar gel diffusion were used to assess the antimicrobial activity of the methanol extract of valerian against two important pathogenic microorganisms, *Staphylococcus aureus* and *Candida albicans*. However, valerenic acid did not reveal antimicrobial activity at doses of 200, 100, 50, 25, 12.5, and 6.25 µg/mL. The methanolic extract of *V. officinalis* contains high quantities of sesquiterpenes, specifically valerenic acid, which did not show cytotoxic effects on A549 and MRC5 cell lines as assessed by the MTT assay. *In vivo* evaluation of the extract in mice and guinea pigs did not reveal any toxic effects based on histopathological and clinical symptom assessments. Our study confirms that *Valeriana officinalis* has dose-dependent potential to improve existing treatment approaches for *Staphylococcus aureus* and *Candida albicans* infections.

Keywords

Antimicrobial; Cytotoxicity; Medicinal plants

Introduction

In recent decades, the pharmaceutical industry has developed several novel antibiotics. However, there has been a concerning rise in the spread of multidrug-resistant strains of pathogenic bacteria. Notably, a significant public health concern arises from the increasing number of immunity-suppressed patients in hospitals, which has led to the emergence of new multi-resistant bacterial strains (1, 2). Natural antimicrobial agents are essential in reducing the global burden of infectious diseases. Medicinal plants have proven to be valuable sources of natural products for pharmaceutical purposes, particularly in response to the re-emerging of pathogens. Valerian belongs to the

Valerianaceae family, which comprises approximately 350 herbaceous perennial species. These species include, but are not limited to, *Valeriana officinalis*, *Valeriana jatamansi, Valeriana longiflora, Valeriana wallichii, Valeriana microphylla, Valeriana hardwickii, Valeriana quadrangularis*, etc (1, 2).

Valeriana officinalis var. *latifolia* is a perennial herb with a short rhizome, reaching a height of 1.5-2 m (3). This cultivated plant is native to Asia, Africa, and North America and is widely grown in the northwestern regions of Iran, which is located in the southwest of Asia (4). *V. officinalis* primarily originates from the mountainous areas of Iran, specifically the provinces of Azerbaijan, Kermanshah, and Hamadan. Due to its ornamental, minor culinary, and medicinal uses, it has been extensively cultivated in these mentioned provinces of Iran (5).

Active components are primarily found in the roots and rhizome's periphery (4). *V. officinalis* essential oil derived from the rhizome contains volatile compounds such as valerenic acid, alkaloids, and various free amino acids (3).The structural diversity of terpenes is primarily determined by the number of isoprene units. Terpenes are classified into hemiterpenes (1 isoprene unit, C5), monoterpenes (2 isoprene units, C10), sesquiterpenes (3 isoprene units, C15), diterpenes (4 isoprene units, C20), triterpenes (6 isoprene units, C30), and tetraterpenes (8 isoprene units, C40). Monoterpenes make up the majority of essential oil components (approximately 90%), followed by sesquiterpenes (6).

V. officinalis is a well-known medicinal plant that contains various chemical constituents in its essential oil extract, including monoterpenoids, sesquiterpenoids, iridoid derivatives, and alkaloids. It is known that the C15 sesquiterpenoid valerenic acid (VA) is a pharmacologically active ingredient responsible for the sedative properties of *V. officinalis* (7).

The percentage of valerenic acid varies among different species of *V. officinalis*. Additionally, environmental factors such as the use of fertilizers, the timing of harvesting, and the drying methods employed may affect the valerenic acid content of crops (4, 5). Significant amounts of valerenic acid have been reported in valerian roots when direct seed sowing and transplanting seedlings in autumn were compared with spring transplantation in the same multi-cell trays and under similar transplantation conditions (8).

According to ancient Iranian medicine, Valerian root possesses various medicinal properties, including an anticonvulsant effect, relief from nervous disorders, antispasmodic and sedative effects, treatment of insomnia, antihistamine properties, anxiety reduction, treatment of melancholy, relief from sciatica pain, diabetes treatment, and wound healing (8, 9).

The antimicrobial activity of *Valeriana jatamansi* extract containing valerenic acid in its rhizomes and rootlets was tested against *Pseudomonas aeruginosa* and *Candida albicans,* demonstrating activity against these microorganisms (9, 10).

It is essential to emphasize the need for more studies focusing on the use of plants as therapeutic agents, particularly those related to the control of common microorganisms associated with respiratory diseases. Until now, research similar to the present study, which investigates the cytotoxic effect of the methanolic extract of *V. officinalis* and its active components on human lung cells, assesses antimicrobial activities against common human pathogens, and examines the extract's impact on vital organs, has been limited. This study was conducted to address the gaps.

Materials and Methods

Study design

This research was conducted at a plant site located at Shahid Beheshti University, Tehran, Iran (35° 48'18.7 "N 51° 23'23.4" E) during the years 2018-2019, with an average annual rainfall of 130 mm. The sowing date was September 10, 2018, and 2-3 seeds were planted per cell. Organic fertilizer was used in this research to enhance the production of more valerenic acid during the extraction process. Briefly, the field was irrigated daily for 10 days after seeding in autumn (September). In the following autumn, the plants reached a height of over one meter and produced white flowers, which were cut to encourage the growth of roots and rhizomes. One week before harvesting, irrigation was discontinued to optimize the crop, and the collected rhizomes were washed with water on emergence. Finally, the collected rhizomes were air-dried for three days.

Extraction

The air-dried rhizomes were ground into a fine powder. The methanolic extract was prepared by adding 1000 mg of rhizomes to 100 mL of methanol. The mixture was then sonicated for 30 minutes using a Parsonic s11, stirred overnight with an ARE magnetic stirrer, and finally centrifuged at 4000 rpm for 10 minutes using an Eppendorf 5702 R centrifuge.

Isolation and detection by High-performance liquid chromatography (HPLC)

Subsequently, the prepared methanolic extract (20µL) was injected into a C18 column with a mobile phase consisting of water and acetonitrile (80:20) at a flow rate of 0.5 mL/min for component detection using HPLC system (Waters2695) with a UV 996 detector. The methanolic extract was concentrated using a rotary evaporator at 40°C, resulting in a final concentration of 36 mg/mL. The extract was kept in the refrigerator.

After the extraction process and HPLC analysis, a stock solution of extract was prepared using 70% methanol as a solvent, achieving a final concentration of 36 mg/mL, and it was also stored in a dark and cool place.

Human Lung Cells Culture for Cytotoxicity Screening

The normal human foetal lung fibroblast cell line MRC5 and the adenocarcinoma human alveolar basal epithelial cell line A549 were purchased from the National *Cell Bank* of the Pasteur Institute of Iran. MRC5 and A549 cells were

cultured using 10% Dulbecco's modified Eagle's medium (DMEM), 10% (v/v) foetal bovine serum (FBS), and 1% gentamicin (Sigma-Aldrich, USA) at 37°C with 5% CO_2 for optimal cell growth. The culture medium was refreshed every 72h to to maintain cell viability and productivity for further evaluations. Cell suspensions of A549 and MRC-5 were prepared at a density of 3-5 × 10^6 cells/mL for the MTT assay to assess the activity of the crude extract of *V. officinalis* on human lung cells. Monolayer cell formations were confirmed visually using an inverted phase-contrast microscope.

Cytotoxic activity by MTT Assay

Each well initially contained a consistent and effective cell population, with eighty to ninety percent confluency, equating 3×10^5 cells per microplate well for the initial MTT test (MBS257032). The subsequent preparation of the cell suspension using trypsin as a protease was acceptable. Consequently, for the *in-vitro* experiment, 11.5 mL of cell suspension containing 1×10^6cells were seeded in a ninety-six-well plate and incubated at 37 °C with 5% CO_2 for 48 h (10).

Subsequently, prepared extractions with different concentrations (3600, 1800, 900, 450, 225, 112.5, 56.25, and 28.125 µg/ mL) and valerenic acid (Sigma (CAS NO3569-10-6)), at a concentration range of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/mL, were added in triplicate. Methanol and DMEM were added as solvents in the control groups in selected cell culture wells. Cell viability was determined after 72 h of incubation. Following the commercial kit protocol, 10µL of 5 mg/mL MTT (Sigma) was added to each well and incubated for 4h at 37°C. Next, 70µL of dimethyl sulfoxide (DMSO) was added to the medium culture with MTT to dissolve formazan crystals, and the mixture was then incubated in a shaker incubator for 20min. Subsequently, the optical density was recorded at 595 nm using a microplate reader, and the $IC_{50}$was calculated. The absorbance for each well was measured at 595nm in a microtiter plate reader, and the percentage of cell viability (CV) was calculated manually using the formula:

CV= Average abs of duplicate extract wells /Average abs of control wells × 100 %

A dose-response curve was plotted to enable the calculation of the concentrations that kill 50% of the A549 and MRC-5 cells (IC_{50})(10).

Antimicrobial assay

Staphylococcus aureus (ATCC 1431), *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 5295), and *Pasteurella multocida* (ATCC 6258) were selected as common human pathogens for antimicrobial assays.

The minimum inhibitory concentration (MIC) values of herb extracts were determined using the microdilution process in ninety-six-well microliter plates with different concentrations. These values were obtained from the IC_{50} calculation based on the MTT method. Microorganism inoculums were prepared in Mueller-Hinton Broth(Himedia, India), and the turbidity was adjusted to 0.5 McFarland and further diluted to achieve a final turbidity of approximately 1×10^6 CFU/Ml. Plant extracts were prepared at a concentration of 200mg/mL in DMSO as the solvent. For the antimicrobial assays, six different concentrations of valerenic acid (200, 100, 50, 25, 12.5, and 6.25 µg/mL) and six different concentrations of crude extract (800, 400, 200, 100, 50, 25, and 12.5 µg/mL), along with positive and negative controls, were prepared and incubated at 37°C for 24 h.

Every inoculum was spread evenly over Mueller-Hinton agar plates for the agar gel diffusion test. 20 ml of glucose-supplemented Mueller-Hinton agar (BBLTM®) (2%, w/v), and methylene blue (0.5µg/mL) were combined with 1 mL of each yeast and the mentioned bacterial strain, and turbidity was adjusted to the McFarland No. 0.5 (3 ×10^8 CFU/mL) level. Sterile filter paper discs (6 mm) loaded with 10 mg/mL, 50 mg/mL, and 100 mg/mL of valerian extract and 1 mg/mL, 5 mg/mL, and 10 mg/mL of valerenic acid were placed over the solidified petri plates and left for incubation at 37°C for 16-18 h. Petri plates were used to assess the inhibitory zone around the disc, which was measured and expressed in millimeters (mm). The diameters of < 8 mm, between 8 to 12mm, and >12mm were considered resistant, moderately susceptible, and susceptible, respectively (11, 12).

General Test/ Abnormal toxicity in Mice and Guinea pig

In the present study, a total of 30 female outbred mice (Swiss Webster) and 12 outbred Guinea pigs (Dunkin Hartley) were selected, with five mice and two Guinea pigs assigned to each of the three sample groups for extraction, along with three control groups treated with PBS. The animals were kept individually in appropriate cages at the Pasteur Institute of Iran in a noise-free, air-conditioned house with a mean temperature of 21°C and subjected to a 10:14 hour light-dark cycle. The animals were fed with commercially balanced diet pellets and ozonated drinking water.

An abnormal toxicity test was conducted by intraperitoneal injections of 60 mg/kg of valerian total extract into each of the prepared samples. This includes 5 healthy mice (17-18 g) and 2 healthy guinea pigs (250-400 g), over a 7-day period. The main criteria for accepting the samples were general health symptoms, without weight loss or mortality (13).

At the end of the study, mice and Guinea pigs were euthanized using an approved protocol in an O_2+CO_2 chamber based on an approved protocol. The lung, liver, heart, and kidneys were isolated for further histopathological evaluations.

The fixed lung, heart, liver, and kidney tissue samples were dehydrated in graded alcohols, cleared in xylene (Sigma Aldrich, Cat No. # 534056), and embedded in paraffin. These samples were then serially sliced into 4-6 µm sections. The prepared slides were stained with Hematoxylin- Eosin (code no: ab245880) and oriented horizontally to obtain the largest cut surface. Images were captured using a light microscope with 4X, 10X, and 40X magnification (Zeiss, Germany).

Results

Plant collection and characterization

The weight of the raw roots and rhizomes was approximately 20 grams. They were rinsed and left to air dry for 7 days, following the plant cultivation in September 2018 (Fig 1).

Plant Extraction and HPLC Analysis

As seen in Figure 2, the analysis using Millennium 32 software revealed that the amount of valerenic acid in each gram of crude extract was approximately 0.3 mg/g of the total plant extraction.

Fig1. *V. officinalis* plant before flowering (**A**), isolated rhizomes (**B**), white flowers (**C**), collected rhizomes dried in fresh air conditions (**D**).

MTT analysis

The results of MTT assays, presented in Figures 3 and 4, depict the percentage of cell viability values for two different cell lines incubated for 48 hours with methanol extract and valerenic acid. Based on the obtained results, the percentage of cell viability decreased with increasing concentrations of both methanol extract and valerenic acid samples. In contrast, in the control wells (cells + DMEM), cell viability remained unchanged after 48 hours of incubation.

In MRC-5 cells, with an ascending concentration of valerenic acid ranging from10µg/mL– 100µg/mL, the cell viability rate decreased from 98% to 10% (Fig. 3A). In A549 cells, the viability percentage decreased from 98% to 5% with rising concentration of valerenic acid ranging from 3µg/mL-50µg/mL (Fig. 3 B). In both evaluated cells, this reduction in viability was statistically significant (p = 0.023), indicating the cytotoxicity of valerenic acid.

In A549 cells treated with extracts at various concentrations (ranging from 10–500µg/mL), cell viability decreased from 99 to 20%, demonstrating a significant decrease in viability as the extract concentration increased (p = 0.029). Furthermore, MRC-5 cells incubated with the extract at four different concentrations (230µg/mL, 120µg/mL, 50µg/mL, and 15µg/ml) exhibited viability percentages of 2%, 95%, 97%, and 99%, respectively (Fig 3).

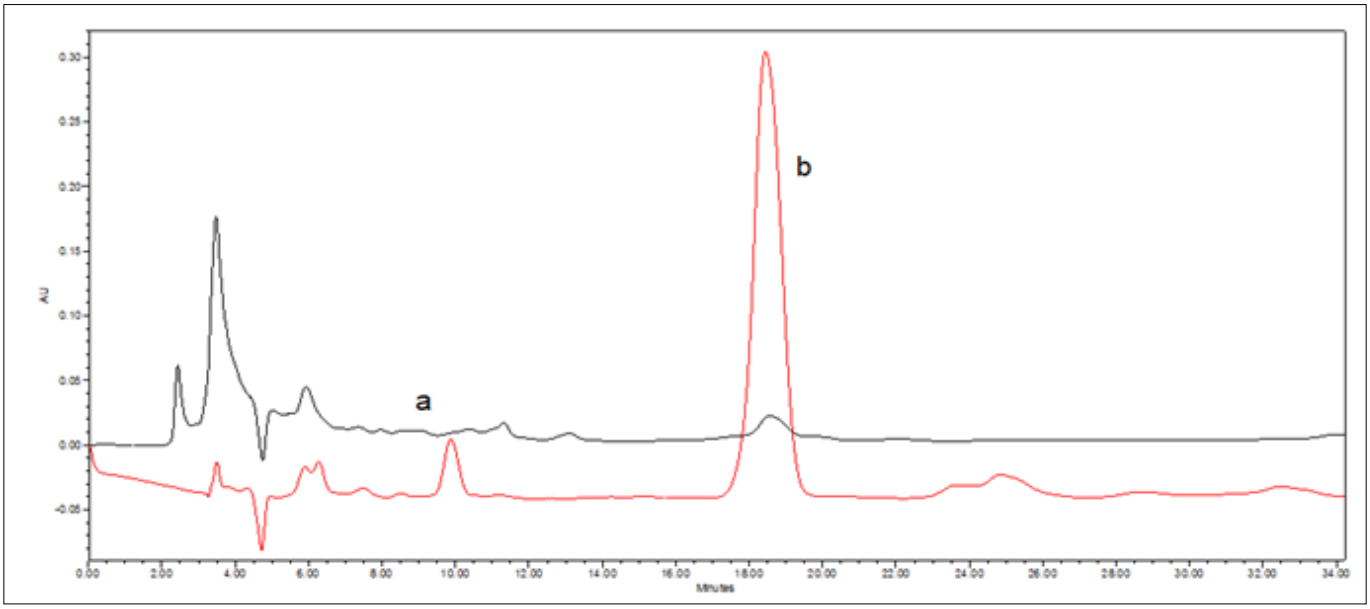


Fig 2. Chromatogram of methanolic extract of *V. officinalis* by HPLC. Black graph (**a**) relates to the prepared sample and red graph (**b**) relates to the standard of valerenic acid

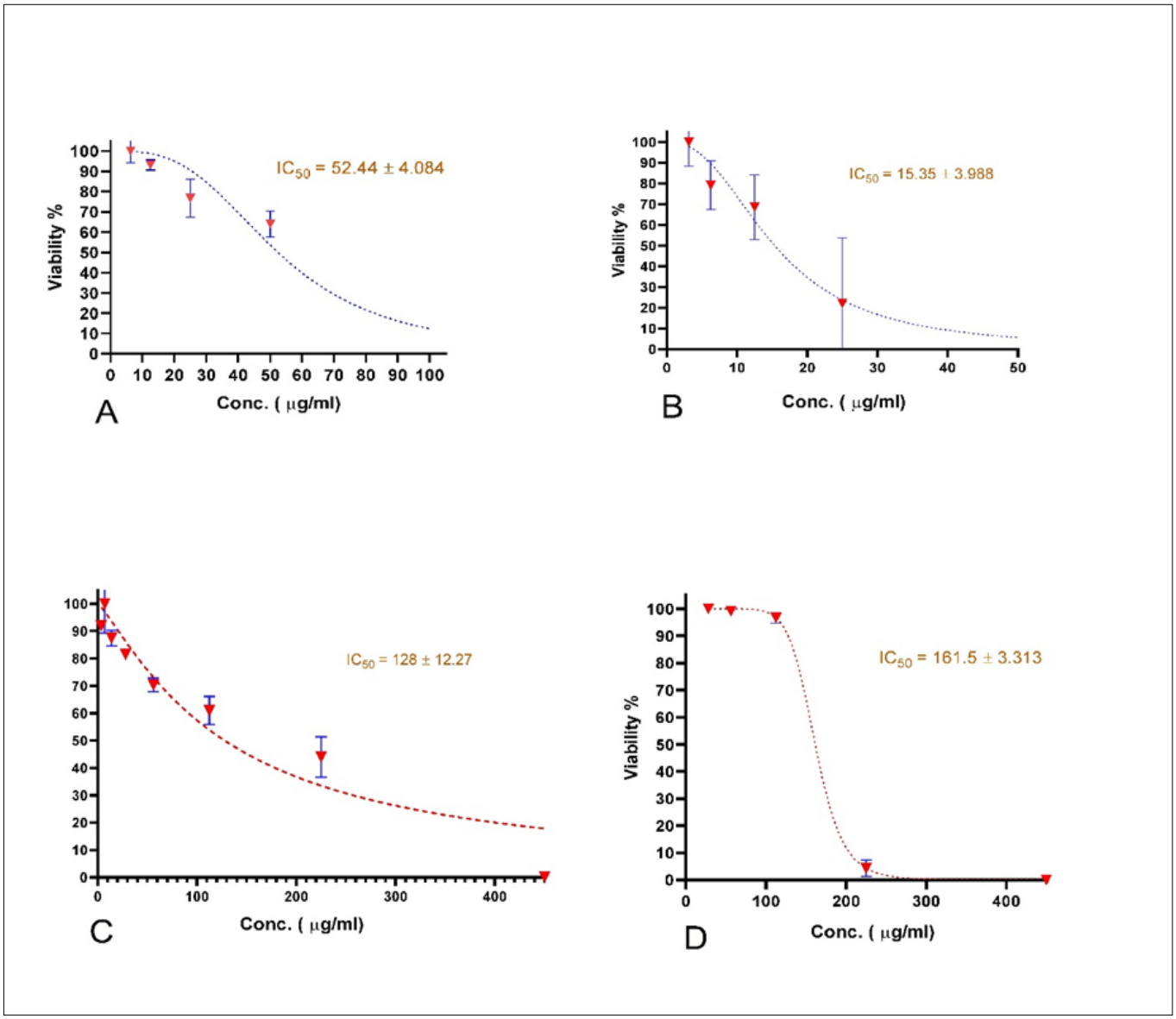
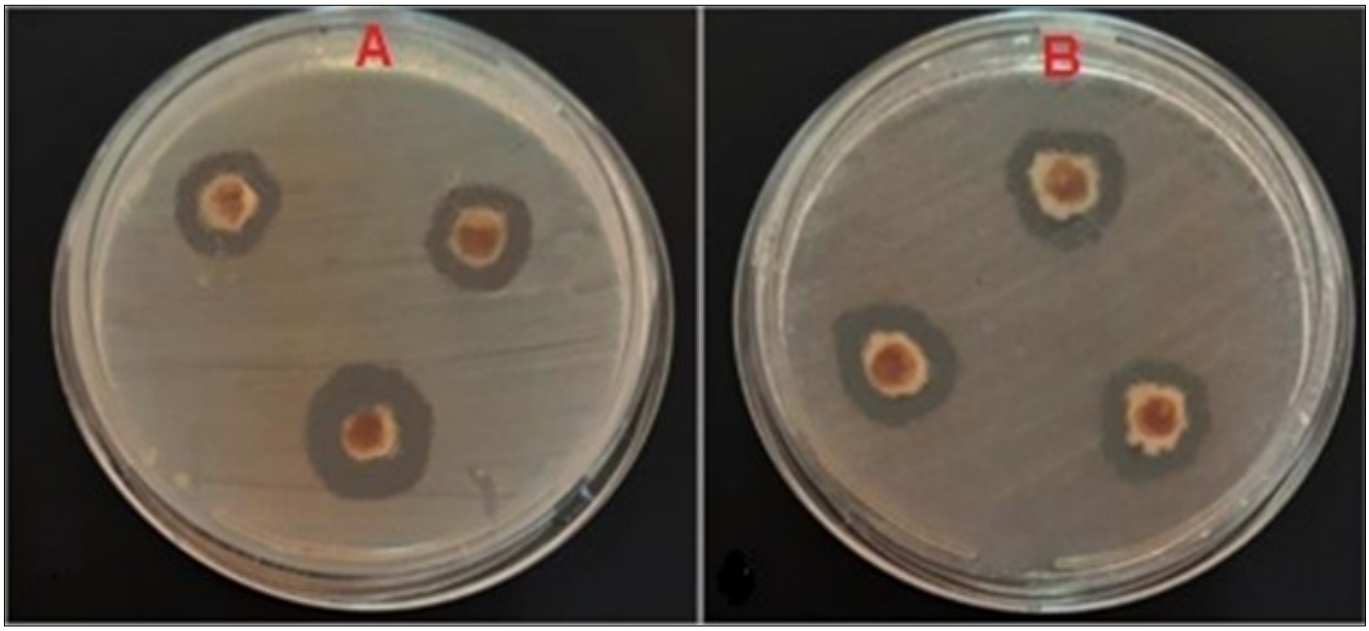


Fig. 3. Percent viability of MRC-5 cell lines treated with various concentrations of valerenic acid (**A**). A549 cells viability treated with different concentrations of valerenic acid (**B**). Treated cells (**C**: MRC-5cells and **D**: A549 cell lines) with selected crude extract concentrations. All four diagrams evaluate the half maximal inhibitory concentration (IC50) of crude extract and velerenic acid.

Finally, the results obtained from the MTT assay indicated that incubation with the extract and valerenic acid for 48 hours in the lung cells can lead to a dose-dependent toxic effect.



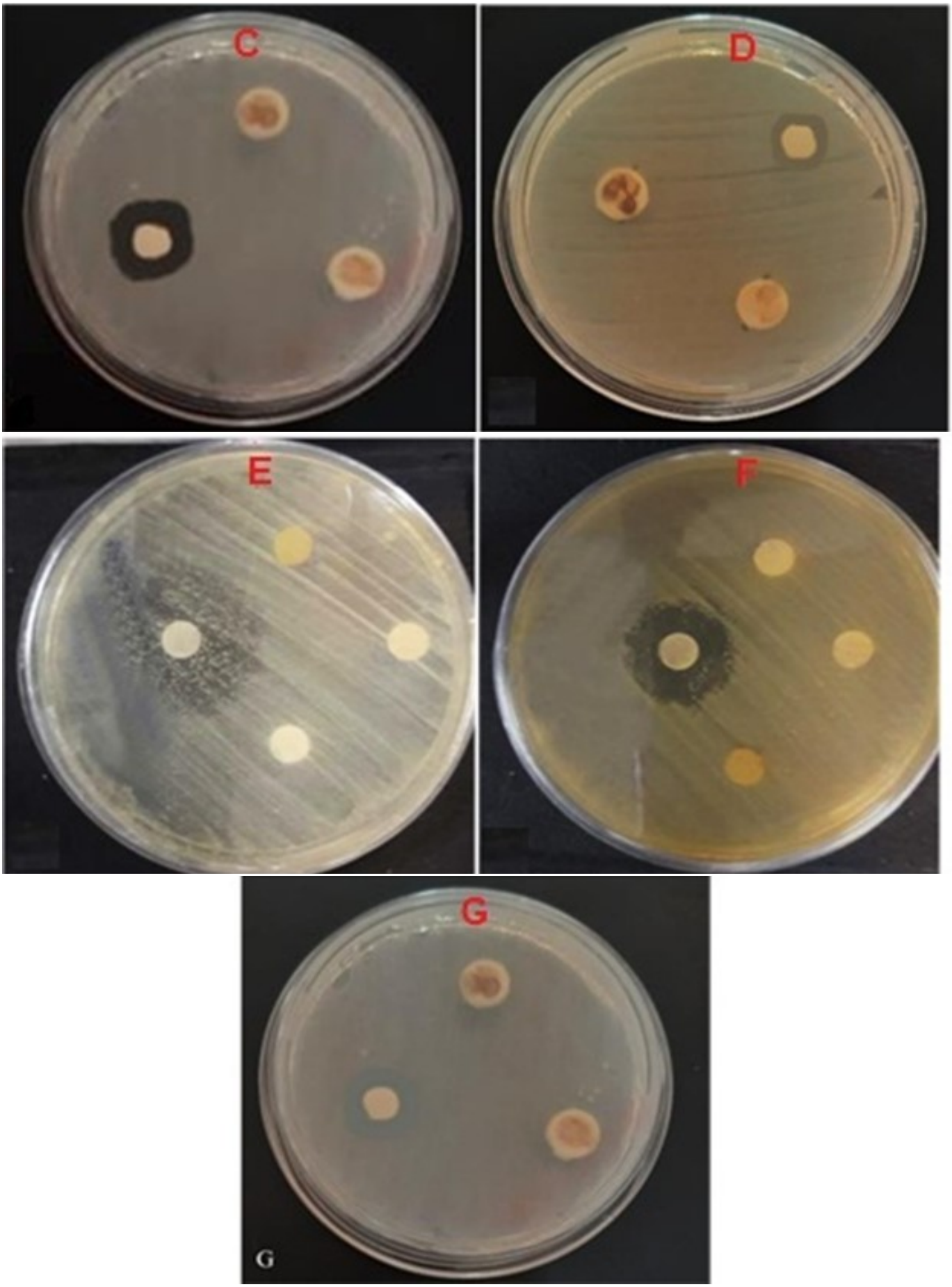


Fig 4. Agar gel diffusion assay of *S.aureus*100 mg/mL (duplicated) (**A**), *C. albicans* 100 mg/mL (duplicated) (**B**), *C. albicans* 10 mg/mL and 50 mg/mL (**C**), *S.aureus*10 mg/mL and 50 mg/mL (**D**), *P. multocida* 10 mg/mL and 50 mg/mL (duplicate) (**E**); *P. aeruginosa* 10 mg/mL and 50 mg/mL (duplicate) (**F**); *E. coli* 10 mg/mL and 50 mg/mL (**G**) with gentamycin as a positive control for gram negative bacteria, penicillin as a positive control for gram positive bacteria and fluconazole for *C. albicans*.

The IC50 values were calculated using Graph Pad

Prism statistic software based on the mentioned concentrations. As a result, the IC50 was determined to be 52.44 µg/mL for valerenic acid and 161.5 µg/mL for total plant extraction indicating a 50% viability of MRC-5 fibroblast cells (Fig. 4 and 5). Additionally, for A549 cells, the IC50 was found to be 15.35 µg/mL of valerenic acid and 128 µg/mL of total plant extraction (Fig 3).

the growth of *E. coli,* a gram negative bacterium. However, it did inhibit the growth of *S. aureus*, a gram positive bacterium, at a concentration of 250µg/mL. Additionally, the MIC assessment of the valerian extract at a concentration of 300µg/mL inhibited the growth of *C. albicans*.

The antibacterial activity of the plant extracts was observed to be concentration-dependent based on the observed zones of inhibition. Specifically, at concentra-

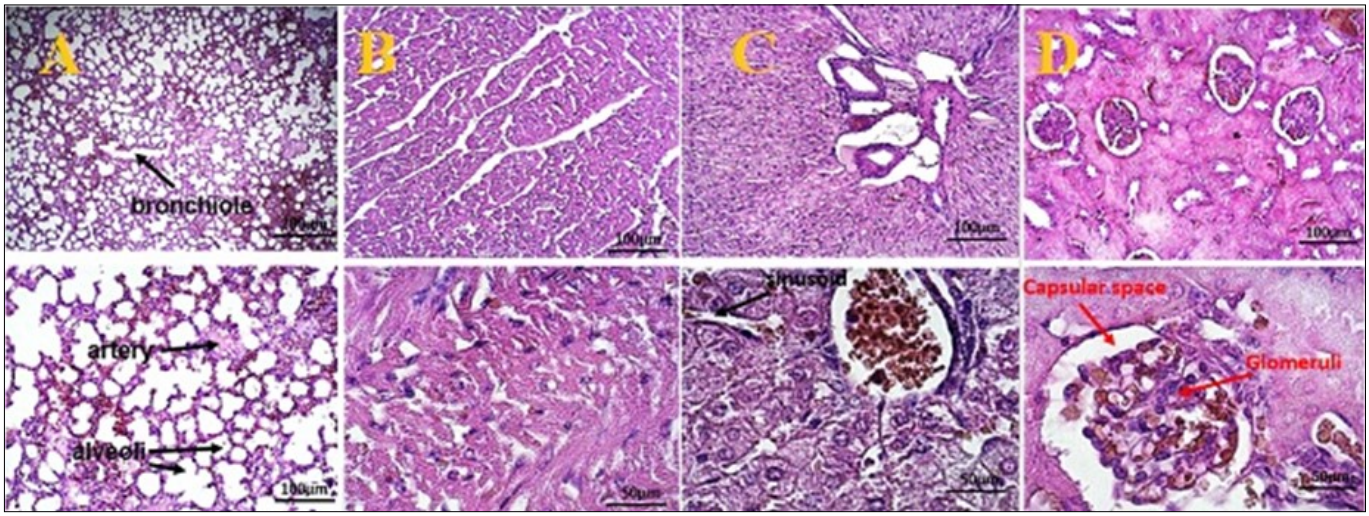


Fig. 5. Guineapig's evaluated organs after 7 days' injection of the total extract to evaluate the histopathological effects showed, as seen in figures, that no abnormal lesions were found in the isolated organs. In the lung tissue (**A**) the alveoli and arteries were normal without collapse, atelectasis and hemorrhage. The heart tissue (**B**), the liver tissue (**C**) and the kidney tissue (**D**) were normal after microscopic evaluations.

Since valerenic acid and the total raw extract did not exhibit cytotoxic effects on viable human lung cells, it was deemed worthwhile to evaluate their antimicrobial effects based on the measured IC_{50} values against the listed pathogens alongside the total raw extract for further research and potential applications.

Antimicrobial activity

The MIC assay, as an appropriate method for assessing antimicrobial activity, revealed that the methanol extract of valerian exhibited antimicrobial activity against two pathogenic microorganisms, *S. aureus* and *C. albicans.* In contrast, valerenic acid did not demonstrate antimicrobial activity at the selected doses. Specifically, the MIC assay indicated that the valerian extract was unable to inhibit

tions of 10 mg/mL and 50 mg/mL of the plant extract, no inhibition zones were observed for *S. aureus* and *C. albicans,* whereas at 100 mg/mL, the inhibition zone was the same (19.2 mm) as that of the used standards (Fig. 4; Table 1).

The mean assessments of the zone of inhibition indicated that the antibacterial and antifungal activities of the plant extracts were influenced by the concentrations of the extracts. At a concentration of 100 mg/mL of plant extract, an inhibition zone of 16.8 mm was observed for *S. aureus*, while other bacteria did not exhibit an inhibition zone. The inhibition zones at 100 mg/mL were found to be significantly different when compared to those at 10mg/mL and 50 mg/mL ($p = 0.036$) for *S. aureus* and *C. albicans*.

Table 1. Zone of inhibition against some bacteria strains by methanol extract of *Valeriana officinalis* L.

Pathogens	Zone of inhibition (mm)				
	Conc. of extract (mg/mL)			Conc. of Valerenic acid (mg/mL)	
	10	50	100	5	10
S. aureus	-	-	16.8	-	-
C. albicans	-	-	19.2	-	-
P. aeruginosa	-	-	-	-	-
P. multocida	-	-	-	-	-
E. coli	-	-	-	-	-
	Zone of inhibition (mm)				
Gentamycin (5µg/disc)	20.4				
Penicillin (10µg/disc)	18.2				
Fluconazole (15 µg/disc)	19.5				

In vivo abnormal toxicity and general safety test: Histopathological investigations

The initial weights before the extract injection and the weights of the mice and guinea pigs at the end of the study were recorded. The mean ±SD weight of mice at the beginning of the study (before injection) was 17.2±0.12g, and at the end of the study (7 days after injection), it was 22.5±0.12 g. The bodyweight of guinea pigs before the extract injection was measured at 258.23±0.25 g, and at the end of the study, it was 298.13±0.1 g. In the general test, no mortality was observed in the mice following a 24-hour intravenous injection of the extract. The animals remained in healthy conditions without any weight loss by the end of the study, and none of the animals died during the study period. Histopathological assessment of vital organs showed no anomalies (Fig. 5, 6).

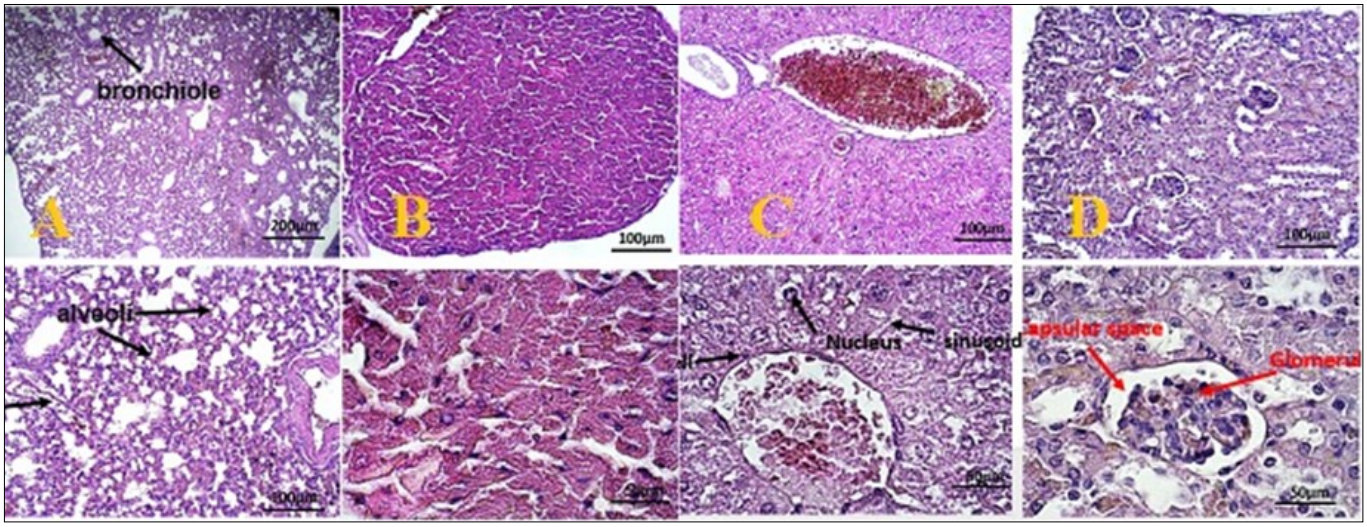


Fig 6. Mice evaluated organs after 7 days' injection of the total extract to evaluate the histopathological effects. As seen in figures, no abnormal lesions were found in the isolated organs. In the lung tissue (**A**) the alveoli and bronchioles were normal without collapse, atelectasis and hemorrhage. The heart tissue (B), the nucleus and sinusoids of liver tissue(**C**) and the tubules and glomerulus of kidney tissue (**D**) were normal after microscopic evaluations.

In both examined species, the results of the study revealed normal histological features in the treatment group. Kidney histopathological examination showed that the glomerulus epithelial cells had a normal appearance in the treatment group. Following the extraction and normal saline injection, the infiltration rate of lymphocytes remained within the normal range, and there were no signs of tissue density or hyperemia. The adjacent cortex epithelial cells did not exhibit any significant differences in all the proximal and distal tubules (Fig. 5, 6).

As shown in figure 6, the liver parenchyma, including hepatocytes, sinusoids ('lobular' region), central venules, and portal tracts, displayed normal conditions. The portal tracts, which contain a portal vein, a branch of the hepatic artery, and a bile duct, remained intact after extract injection. The hepatic cell patterns and infiltration appeared normal (Fig. 5, 6).

Discussion

The side effects of chemical drugs and antibiotic resistance have prompted researchers to explore suitable alternatives for improving human health with minimal side effects. Given Iran's resources of medicinal plants, especially in mountainous and rainy regions, we have chosen to study one of the most common and beneficial herbs in Iran. Our aim is to cultivate this herb under controlled conditions to enhance the yield of its extract.

In the present study, we aimed to evaluate the antimicrobial activity of the methanolic extract of *V. officinalis*. Additionally, we compared the antimicrobial effects of the methanolic extract of valerian root with valerenic acid. Based on the agar gel diffusion test, we observed that the average inhibitory regions induced by the plant extract against gram-positive bacteria and fungi depended on its concentration.

The timing of planting is a critical factor for achieving both high quantity and quality of aromatic medicinal plants, particularly when considering planting density. In 2010, Morteza *et al.* (14) conducted a study on the planting date of *V. officinalis*, determining that the optimal combination of planting density and date was 8 plants per square meter and September 20, respectively (8). Furthermore, compounds from plants grown in Qamsar were evaluated using GC-MS, revealing that the essential oil contained 0.6% valerenic acid. In 2009, Ghaffari *et al*. (15) detected valerenic acid in methanolic extract by TLC and HPLC (14). Valerenic acid level in some German valerian products (Futuran Baldrian tablet, Baldrian Hopfen tablet, Euvegal caplet) ranged from 0.03-0.16%, while in certain Iranian products (Neurogol tablet, Valerian capsule, and Antimigrain Herbal drop), it varied from 0.05-2.8%. A rapid, simple and practical high performance liquid chromatography (HPLC) method was used to determine the valerenic acid content of these valerian products. All brands met the acceptable range of valerenic acid content, not less than 0.04% (14, 15). In line with previous studies, our present study identifies valerenic acid in the methanolic extract using HPLC at a concentration of 0.03 mg/mL. The findings align with the results reported in earlier studies.

In addition, the MTT test employed to assess the cytotoxic effects of valerenic acid and total extract of *V. officinalis*, on human lung cells. Gonzalez *et al.* (16) in their research on the cytotoxic effects of *Aldama tucumanensis, Ambrosia elatior, Baccharis artemisioides, Baccharis coridi-*

folia, Dimerostemmaas pilioides, Gaillardia megapotamica, and *Vernonanthura nudiflora* by MTT along with IC_{50} calculation, discovered that sesquiterpenes can have a promising therapeutic effect in leukaemia chemotherapy, particularly against resistant phenotypes (16).The results indicated a decrease in cell viability and inhibition of cell growth in a dose-dependent manner. Particularly, both valerenic acid and the plant extract exhibited a growth-inhibitory effect on cancerous cells in a dose-dependent manner, likely due to the antioxidant properties of this plant. Antioxidants play a significant role in suppressing oxidative stress and free radicals and contribute to the expression of apoptosis genes (16).

Subsequently, we investigated the antimicrobial activities against *Gram-negative* and positive *bacteria* and the fungi using MIC and agar gel diffusion assay. Khademian *et al.* (17) found that the methanol extract of *V. officinalis*, *S. bachtiarica,* and *T. daenensis* exhibited anti-*H. pylori* effects at a 10% concentration. However, their antibiotic properties remained uncertain at low concentrations, suggesting that the antimicrobial activity is dose-dependent (17). In traditional medicine, valerian root has been used to treat urinary tract infections. In our study, we confirmed the antimicrobial effect of *V. officinalis* on *S. aureus*. This effect can be attributed to polyphenolic compounds, antioxidant compounds, alkaloids, flavonoids, tannins, steroids, and terpenoids. These compounds have the ability to change the permeability of the cell membrane by inhibiting specific enzymes. Through these mechanisms, they exert their antimicrobial effect (16, 17).

Rondón *et al.* (18) conducted a study on the antimicrobial activity of four species of *Valeriana* and identified the presence of secondary metabolites such as alkaloids, flavonoids, tannins, steroids, and terpenoids (valepotriates and ballerina) in the extracts of *Valeriana parviflora*, *V. rosaliana*, *V. triplinervis*, and *V. phylicoides*. Inhibitory effects on *S. aureus*, a pathogen responsible for several serious infectious diseases in humans, were observed at a concentration of 100 µg/mL (18). Consequently, our research findings align with previous studies, demonstrating the inhibitory effects of *V. officinalis* on *S. aureus* and *C. albicans*. Additionally, Udgire *et al.* (19) investigated the antimicrobial activity of various solutions of *Valeriana wallichii* extract and found that the methanol extract of *V. wallichii* exhibited antimicrobial efficiency against microorganisms such as *E. coli*, *S. aureus*, *C. albicans*, and *P. aeruginosa*, in comparison to water and ethanol extracts. Therefore, our study's results with methanolic extract of *V. officinalis* also showed the ability of valerian extract to inhibit the growth of *S. aureus* and *C. albicans* (19).The presence of alkaloids, flavonoids, tannins, steroids, and terpenoids is responsible for the antimicrobial properties against the pathogens in this study. Furthermore, the active chemicals in valerian, including some histone dactylase (HDAC) inhibitors, may play a role in epigenetic gene regulation and can inhibit the growth of breast cancer cells. *V. officinalis* and valeric acid have been found to inhibit the growth of cancer cells, such as breast cancer cells, possibly by mediating epigenetic modifications (20). Additionally, de Oliveria *et al*. demonstrated the cytoprotective activities of an aqueous extract of *V. officinalis* against rotenone-induced apoptosis in human neuroblastoma SH-SY5Y cells (21). Therefore, it can be suggested that valerian has the potential for inducing apoptosis in cancer cells.

Antibiotic resistance (21-23) is an emerging challenge in the medical industry, prompting numerous studies to explore plant-based alternatives (21,22). Plant secondary metabolites are primarily responsible for their antimicrobial activity, with major groups of phytochemicals including phenolics and polyphenols such as flavonoids, quinones, tannins, coumarins, terpenoids, alkaloids, lectins and polypeptides possessing such properties (20).The increasing prevalence of drug-resistant microbial diseases represents a significant challenge to medical and pharmaceutical knowledge (23-26). Medicinal plants with antimicrobial effects warrant investigation to assess their therapeutic properties, safety, and efficacy (27-30) as they are rich in effective substances like tannin, phenol, flavonoids, anthocyanins, and antioxidant compounds (31-33).The findings of this study further confirm the antimicrobial properties of plant extracts. Currently, the methicill in *Staphylococcus aureus* resistance strains exhibits resistance to vancomycin and cefotaxime, posing a serious threat to human health. Therefore, the discovery of therapeutic agents represents an additional strategy to address antibiotics-resistance pathogens (32-34).

Conclusion

The increasing antibiotic resistance in human pathogens accentuates the importance of discovering new natural antimicrobial molecules. Current data suggests that investigating traditional medicinal plants without causing cytotoxic effects on human lung cells is valuable. Given the dose-dependent antimicrobial activity of *V. officinalis* attributed to the abundant monoterpenoids and sesquiterpenoids in the total methanolic extract, further studies on various aspects of pharmaceutical effects *in vitro* and in animal models are recommended. One of the innovative aspects of this study was the utilization of the effective compounds of *V. officinalis*, particularly sesquiterpenes, which proved effective against *Staphylococcus aureus* and *Candida albicans* infections.

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Compliance with ethical standards

Conflict of interest: The authors of this paper declare that they have no conflicts of interests associated with this paper.

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