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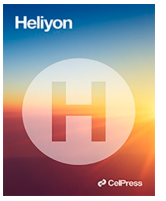

Research article

# Evaluation of the anticancer and antibacterial activities of moscatilin

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ABSTRACT

Orchids (*Dendrobium* sp.*)* have been the subject of extensive research due to their ubiquitous pharmacological, antimicrobial, and anticancer properties. Moscatilin is a bibenzyl secondary metabolite enriched in orchids that exhibits anticancer and antimicrobial properties through mechanisms that have not yet been fully elucidated. The current study aimed to assess the *in vitro* anticancer and antibacterial potential of moscatilin. The *in vitro* anti-proliferative effects of moscatilin against breast cancer-MCF-7 and liver-HepG2 cells were assessed using the dimethylthiazol-diphenyltetrazolium bromide assay. Selected six pro-apoptotic (*caspase-3, 8, 9, p53, p21 & Bax*) and two anti-apoptotic (*Bcl-xL & Bcl-2*) gene markers were assessed *via* qPCR and tested antibacterial activity against various bacterial strains using disc diffusion and broth dilution methods. Moscatilin decreased the cellular viabilities of HepG2 and MCF-7 cancer cells, with anti-proliferation rates of 66 % ($IC_{50}$ 51 ± 5.18 μM) and 58 % ($IC_{50}$ 57 ± 4.18 μM), respectively. This effect was selectively observed in cancer cells, and the impact of moscatilin on non-cancerous MCF-12 cells was marginal. Moreover, moscatilin-treated cells exhibited higher mRNA levels of *caspase-3,8, 9, Bax, p53,* and *p21*, whereas lower levels of *Bcl-2* and *Bcl-xL*, two anti-apoptotic markers, were observed. Furthermore, moscatilin exhibited varying degrees of antibacterial activity against the bacterial strains investigated. Notably, the highest antibacterial potentials were observed against *Staphylococcus epidermidis* and *Klebsiella pneumonia*, while the lowest inhibitory activity was observed in *Escherichia coli* and *Pseudomonas aeruginosa*. Overall, these findings demonstrated that moscatilin exerts potent anticancer effects *via* apoptosis and has antimicrobial properties against Gram-negative and Gram-positive bacteria that are clinically relevant. These findings highlight the potential of moscatilin as a natural therapeutic candidate for the treatment of cancer and clinically important bacterial pathogens.

## 1. Introduction

Breast, lung, liver, and prostate cancers are the most predominant types of malignancies worldwide. The incidences of breast and liver cancers have gradually increased in recent years. In 2020, around 2.3 million breast cancer cases were reported, leading to 685,000 deaths worldwide [1]. In addition, hepatocellular carcinoma (HCC) was the most common type of liver cancer, accounting for 7.5 % and 3.5 % of all cancer types in men and women, respectively. The therapeutic and preventative applications of complementary and alternative medicines that rely primarily on phytochemical-enriched medicinal herbs are increasing, particularly those possessing

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antimicrobial and anticancer activities [2]. Antimicrobial resistance (AMR) is a global threat that occurs when microbes develop mechanisms to defend themselves against drugs designed to eradicate them, culminating in difficult-to-eradicate infections and pose an increased disease transmission risk. Excessive use of antibiotics in human patients, livestock breeding, and sanitation are among the factors that contribute to the current AMR rates [3]. Because AMR has surfaced as one of the most serious challenges plaguing healthcare systems in the past few decades, researchers worldwide have begun to consider AMR. Strikingly, if no novel classes of antimicrobial drugs are discovered, approximately 10 million AMR-related deaths are projected to occur by 2050, along with significant socioeconomic burdens [3]. In recent decades, several plant-derived antibacterial and anticancer products have been synthesized *via* different signal transduction pathways [4,5]. Globally, up to 200 species are currently considered medicinal plants and approximately 25 % of contemporary medicines are of plant origin [6].

Due to their multifaceted pharmacological properties, the ethnomedicinal use of orchids (Orchidaceae) can be backdated to ancient times, more specifically in the Indo-Asian and Pacific regions, as well as through practices of Traditional Chinese Medicine (TCM). *Dendrobium* species, often referred to as Shihu or Huangcao, are the second biggest genus in the Orchidaceae family. To date, many bioactive secondary metabolites have been discovered in *Dendrobium* species that are reported to belong to various groups, including bibenzyls, alkaloids, fluorenones, coumarins, phenanthrenes, and sesquiterpenoids [7]. These phytoconstituents are characterized by a high degree of metabolite resemblance, which gives them a distinctive advantage over synthetic drugs, as they are target-specific with minimal off-target toxicity, thereby rendering their transport to intracellular sites highly efficient. Notably, most medicines authorized by the United States Food and Drug Administration (US FDA) and European Medicines Agency (EMA) are plant-derived secondary metabolites, with natural anticancer products proving to be the most effective [8].

Moscatilin, also known as "Dendrophenol" ($C_{17}H_20O_5$, Mol. wt. = 304.34 g $mol^{-1}$), is classified as the most prominent bibenzyl derivative that was originally isolated from the threatened *Dendrobium moscatum (*wild orchid) species [9]. Moscatilin has been shown to exert multifaceted pharmacological activities such as anti-inflammatory, antioxidant, antiplatelet aggregation, anti-proliferative, antimutagenic, and anti-angiogenesis effects [10,11]. In 2003, the first report demonstrated that moscatilin, which is derived from the orchid *Dendrobrium loddigesii*, exhibits *in vitro* anticancer properties against three different cancer cell types, namely placenta, stomach, and lung cancers [12]. Moscatilin also possesses many antimetastatic functions. Moscatilin's association with APC10/DOC1 suggested that the medication is engaged in post-replicative inhibition, while its relationship with PKM2 demonstrated inhibition towards the glycolytic pathway in cancer cells. Moscatilin has been also shown to function as an effective inhibitor of cell cycle [13].

Numerous studies on the cytotoxicity of moscatilin have revealed its broad spectrum activity against other types of cancerous cell lines derived from various tissues, including head and neck, esophagus, pancreas, and skin, *via* apoptosis- and reactive oxygen species (ROS)-dependent and anti-metastatic mechanisms [14,15].

The antibacterial and anti-lipid peroxidation potencies of crude extracts derived from various parts of *Dendrobium nobile* have also been reported. Devi and co-woerkers reported the antitumor activities of extracts of *D. nobile* in a Dalton's lymphoma-induced mouse model, with a statistically significant prolongation of survival time [16]. However, the molecular mechanism by which the extracts of various orchids or pure moscatilin exert their biological activities remains elusive and not fully understood. Therefore, the primary objectives of the current work were threefold: *i*) to assess the antimicrobial activities of moscatilin against three Gram-positive and three Gram-negative bacteria using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) techniques; *ii*) to investigate the *in vitro* anticancer potency of moscatilin in MCF-7 (breast) and HepG2 (liver) cells; and *iii*) to dissect the underlying mechanisms using the MTT proliferation assay and qPCR-based mRNA expression profiling of selected pro- and anti-apoptosis marker genes.

## 2. Methods

### 2.1. Cells and reagents

Breast cancer (MCF-7; American Type Culture Collection (ATCC) HTB-22) and HCC (HepG2; ATCC HB-8065) cell lines were procured from the American Type Culture Collection (ATCC; Manassas, VA, USA). The antibiotic penicillin/streptomycin and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Gibco (Grand Island, NY). Fetal bovine serum (FBS), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), RNeasy Micro, $RT^2$ SYBR® Green/ROX™ qPCR Mastermix and QuantiTect Reverse Transcription Kits were all purchased from Qiagen (Maryland, USA). Moscatilin was obtained from EMMX Biotechnology (EN10271; CA, USA).

### 2.2. Cytotoxicity assays

To assess the cytotoxicity of moscatilin, the MTT assay was conducted on MCF-7 and HepG2. This method relies on the reduction of tetrazolium to insoluble formazan crystals by mitochondrial dehydrogenases in viable cells [17]. Briefly, MCF-7 and HepG2 cells were separately cultured as monolayers in complete DMEM augmented with FBS at 10 % and grown in humidified conditions in an incubator with $CO_2$ at 5 %. After 2–3 sub-cultures, both cell lines (100 μL/well, $5 \times 10^3$ cells/well) were separately plated onto 96-well plates with complete DMEM and cultured until reaching 70–80 % confluency. Thereafter, cells were exposed to moscatilin (25, 50, 100, 200 or 300 μM) for 24 h. Following exposure, each well received 10 μL of 5 mg/mL MTT in phosphate buffered saline (PBS) and was subsequently cultivated at 37 °C for 4 h. Each well also received 100 μL DMSO to solubilize the formazan crystals. After 10 min of incubation, the optical density (OD) of each well was measured at 590 nm by use of ELX-808 microplate reader (Biotek, USA). Values representing the half-maximal inhibitory concentration ($IC_{50}$) resulting in 50 % growth inhibition were calculated from the

concentration versus viability plot using Probit analysis through an Excel macro.

### 2.3. qPCR gene expression analysis

MCF-7 and HepG2 cell at a density of $1 \times 10^5$ cells per well were separately cultured for 24 h in 24-well plates, and subsequently exposed to 50 μM moscatilin for 48 h at 37 °C. The adherent cell fraction was harvested and subjected to total RNA extraction using the RNeasy MicroKit. cDNA was synthesized using the QuantiTect Reverse Transcription Kit and then subjected to qRT-PCR using the $RT^2$ SYBR® Green/ROX™ qPCR Mastermix. Reactions were performed using a 7500 Fast qPCR Thermal Cycler (Applied Biosystems). The thermal cycling program included the following steps: initial denaturation at 95 °C for 3 min, followed by thirty-five cycles at 94 °C for 1 min and 60 °C for 1 min. The relative expression ratio was calculated using the $2^{-\Delta\Delta Cq}$ procedure following normalization to the reference *GAPDH* housekeeping gene. The oligo sequences utilized for amplification are provided in Table 1.

### 2.4. Collection of microbial strains

The bacterial strains utilized in the current study included three Gram-positive (*Bacillus subtilis*, ATCC 10400; *Staphylococcus aureus*, ATCC 29213; *Staphylococcus epidermidis,* ATCC 12228) and three Gram-negative (*Pseudomonas aeruginosa*, ATCC 27853; *Escherichia coli*, ATCC 25922; *Klebsiella pneumonia*, ATCC 13883) were obtained from Hafr Al Batin Central Hospital, Hafr Al Batin, Eastern Province (KSA).

### 2.5. Antibacterial activity of moscatilin

The antibacterial assay for moscatilin was performed using an agar well diffusion assay, as previously described [22]. Approximately 100 μL of each strain ($1.0 \times 10^7$ bacterial CFU/ml) was moistened and spread onto a plate. Six wells with 5-mm diameter were punched and sealed using a sterile cork-borer. Different concentrations (50, 100, 200 or 400 μM; 100μL/well) of moscatilin were poured into each plate and subsequently left for 30 min at 4 °C to allow proper diffusion into the agar. Thereafter, the inhibition zones were measured in millimeters (mm) following a 24-h incubation period at 37 °C. Chloramphenicol (25 μg/mL) was provided as a positive control, whereas PBS was used as a negative control. The MIC and MBC of moscatilin were assessed against *S. aureus*, *S. epidermidis*, *B. subtilis, E. coli*, *K. pneumonia,* and *P. aeruginosa* using a broth micro-dilution assay, as previously described with modifications [22]. Briefly, 100 μL of nutrient broth with moscatilin at different concentrations (*i.e.*, 1.56, 3.125 6.25, 12.5, 25, 50, 100, 200 and 400 μM) was added. Thereafter, 10 μL of fresh bacterial suspension ($5 \times 10^6$ CFU/mL) was pipetted in each well. After 24 h of incubation at 37 °C, the OD was measured at 590 nm by use of ELX-808 (Biotek, USA) microplate reader. The smallest concentration of moscatilin that halted observable bacterial growth compared to control was recorded as the MIC, whereas the lowest concentration of moscatilin that resulted in no growth was recorded as the MBC [22].

### 2.6. Statistical analysis

The obtained data were analyzed by use of version 25 of the SPSS software and expressed as the mean ± standard deviation (SD). The Student's unpaired *t*-test was used to compare the means of two independent groups. *P*-values below 0.05 were regarded as statistically significant.

**Table 1**
Oligo sequences for the determination of apoptosis and anti-apoptotic genes.

| Gene name | Oligo sequences | References |
|---|---|---|
| *Caspase-3* | F: 5′-GCTGGATGCCGTCTAGAGTC-3′<br>R: 5′-ATGTGTGGATGATGCTGCCA-3′ | [18] |
| *Caspase-8* | F: 5′-AGAAGAGGGTCATCCTGGGAGA-3′<br>R: 5′- TCAGGACTTCCTTCAAGGCTGC-3′ | [19] |
| *Caspase-9* | F: 5′- ATTGCACAGCACGTTCACAC-3′<br>R: 5′-TATCCCATCCCAGGAAGGCA-3′ | [18] |
| *Bax* | F: 5′-GAGCTAGGGTCAGAGGGTCA-3′<br>R: 5′-CCCCGATTCATCTACCCTGC-3′ | [18] |
| *Bcl-2* | F: 5′-ACCTACCCAGCCTCCGTTAT-3′<br>R: 5′-GAACTGGGGGAGGATTGTGG-3′ | [18] |
| *Bcl-XL* | F: 5′-CAGAGCTTTGAACAGGTAG-3′<br>R: 5′-GCTCTCGGGTGCTGTATTG-3′ | [20] |
| *p53* | F: 5′-GCTCTGACTGTACCACCATCC-3′<br>F: 5′-CTCTCGGAACATCTCGAAGCG-3′ | [21] |
| *p21* | F: 5′- CTCAGAGGAGGCGCCATG-3′<br>R: 5′- GGGCGGATTAGGGCTTCC-3′ | [21] |
| *GAPDH* | F: 5′- CGGAGTCAACGGATTTGGTC -3′<br>R: 5′- AGCCTTCTCCATGGTCGTGA -3′ | [21] |

## 3. Results

### 3.1. Cytotoxic activity of moscatilin against MCF-7 and HepG2

The effect of moscatilin on MCF-7 and HepG2 cells was examined for 24 h using the MTT test at various doses ranging from 0 to 300 μg/mL, which could be inferred from the significant dose-dependent anti-proliferation activity after treatment for 24 h (Fig. 1). Notably, in comparison to the untreated control cells, the moscatilin exhibited robust cytotoxic activity to MCF-7 and HepG2 cells. After 50 μg/mL of moscatilin, they were able to significantly ($p < 0.05$) inhibit cellular growth of MCF-7 and HepG2 lines as compared to the untreated control cells. Higher concentrations of moscatilin up to 200 μg/mL exerted gradual cytotoxic activity with increasing concentration; notably, viability began to drop significantly ($p < 0.05$) at 200 and 300 μg/mL. Importantly, HepG2 cells were significantly more sensitive to moscatilin (~66 % with $IC_{50}$ of 51 ± 5.18 μM) than MCF-7 cells (~58 % with $IC_{50}$ of 57 ± 4.18 μM) ($p < 0.05$).

### 3.2. Moscatilin significantly induced gene expression of apoptosis markers in MCF-7 and HepG2 cells

The exposure of both MCF-7 and HepG2 cells to a single moscatilin dose of 50 μM for 48 h led to significantly increased mRNA expression levels of pro-apoptotic marker genes implicated in the cascade of caspases, namely *caspase-3, 8, and 9*, relative to the untreated control ($P < 0.05$) (Fig. 2A). Similarly, the mRNA levels of *Bax* in MCF-7 and HepG2 cells were significantly upregulated ($P < 0.05$), along with a concurrent significant ($P < 0.05$) downregulation in the mRNA transcriptional levels of the *Bcl-2* and *Bcl-xL* anti-apoptotic genes as compared to the control (Fig. 2B). As depicted in Fig. 2C, there was a significant overexpression of *p21* and *p53* in both cancer cell types in comparison to that in the control ($P < 0.05$).

### 3.3. Antibacterial activity of moscatilin

Moscatilin exhibited varying degrees of antibacterial effects against the tested bacteria. The antibacterial activity results of moscatilin are summarized according to the disc and agar-well diffusion methods as presented in Fig. 3 as well as Tables 2 and 3. Strong antibacterial effects were observed for moscatilin against *S. epidermidis* (ATCC 12228; MIC = 3.6 ± 0.32 μM, MBC = 6.8 ± 0.38 μM) and *K. pneumonia* (ATCC 13883; MIC = 3.5 ± 0.26 μM, MBC = 6.8 ± 0.76 μM). Moderate antibacterial activities were recorded against *S. aureus* (ATCC 29213; MIC = 7.2 ± 1.36 μM, MBC = 13.1 ± 0.16 μM) and *B. subtilis* (ATCC 10400; MIC = 6.7 ± 0.45 μM, MBC = 13.5 ± 1.27 μM). Weak antibacterial effects were observed against *E. coli* (ATCC 25922; MIC = 26 ± 0.39 μM, MBC = 52 ± 2.35 μM) and *P. aeruginosa* (ATCC 27853; MIC = 24 ± 0.76 μM, MBC = 51 ± 2.24 μM).

## 4. Discussion

The arsenal of current synthetic antimicrobial and anticancer drugs is being challenged by the increasing rate of development of multidrug-resistant (MDR) microbes and chemoresistance/insensitivity to anticancer drugs. Bacterial resistance can be established through several strategies, including horizontal gene transfer, gene mutations, efflux pumps, cell wall impermeability, and the production of antibiotic hydrolytic enzymes. Bacteria exhibiting an MDR phenotype against a broad spectrum of antibiotics develop as a consequence of the excessive use or abuse of antimicrobial agents and disrupted treatment regimens both in animals and humans, which is presently recognized as a global health crisis [3]. Chemotherapy resistance develops through alterations within tumor cells,

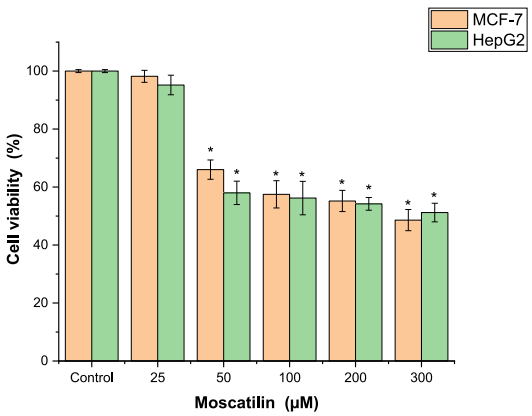


**Fig. 1.** Dose-dependent cytotoxicity of moscatilin (25, 50, 100, 200 or 300 μM) on human cancer cells MCF-7 and HepG2 cells for 24 h by use of MTT assay. The values represent the mean ± SD of three independent experiments. (*) = Indicating significantly different from the untreated control cells at $p < 0.05$.

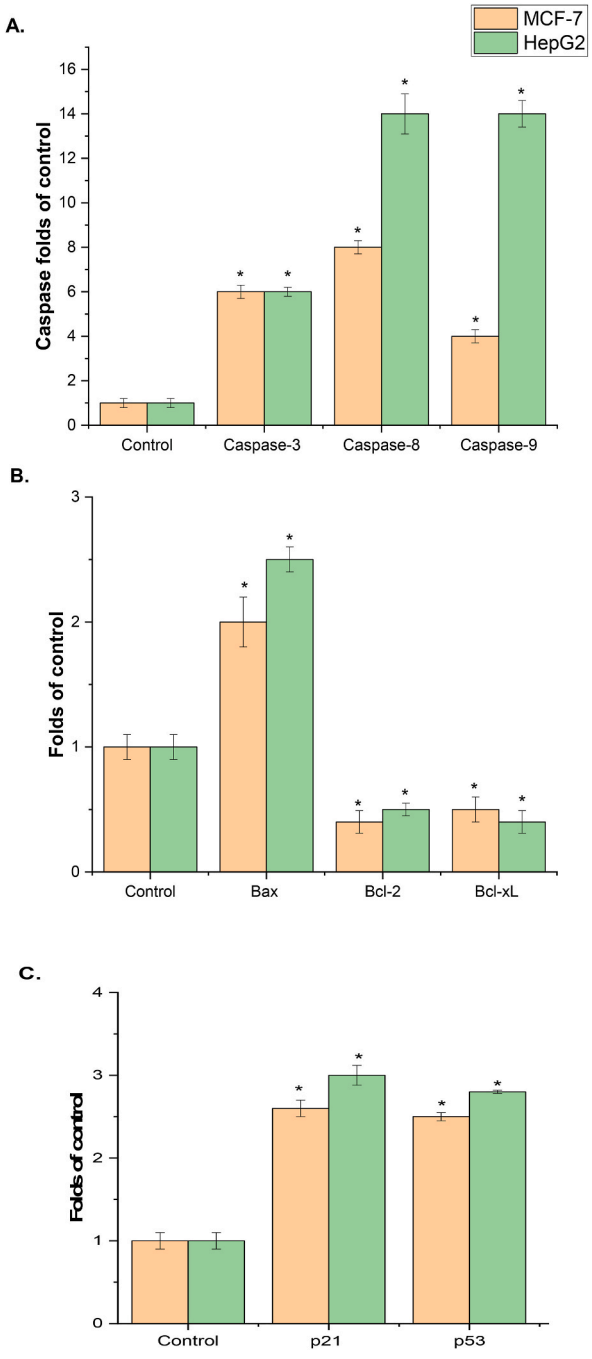


**Fig. 2.** The mRNA transcriptional levels of selected pro- and anti-apoptotic gene markers in MCF-7 and HepG2 cells. **(A)** *caspase-3, 8* and *9*, **(B)** *Bax, Bcl-2* and *Bcl-Xl*, and **(C)** *p53* and *p21*. The values represent the mean ± SD of three independent experiments. (*) = Indicating significantly different from the untreated control cells at $p < 0.05$.

affecting a variety of pathways, including genetic, epigenetic, microenvironmental, and pathophysiological pathways [23]. Despite the enormous efforts made over the past decade, cancer remains a major health concern worldwide, which necessitates urgent action focusing on the discovery of novel diagnostic and therapeutic approaches for malignant tumors. The constraints of conventional cancer therapies have prompted the identification of alternative strategies to enhance the effectiveness of cancer chemotherapy [2]. Plant-derived extracts, which are rich in natural products, are the primary repertoire of antimicrobial and anticancer agents and have been proven successful in combating several diseases and pathological conditions [4,5].

Over the past few decades, scientists worldwide have been exploring the anticancer effects of natural compounds as they can be

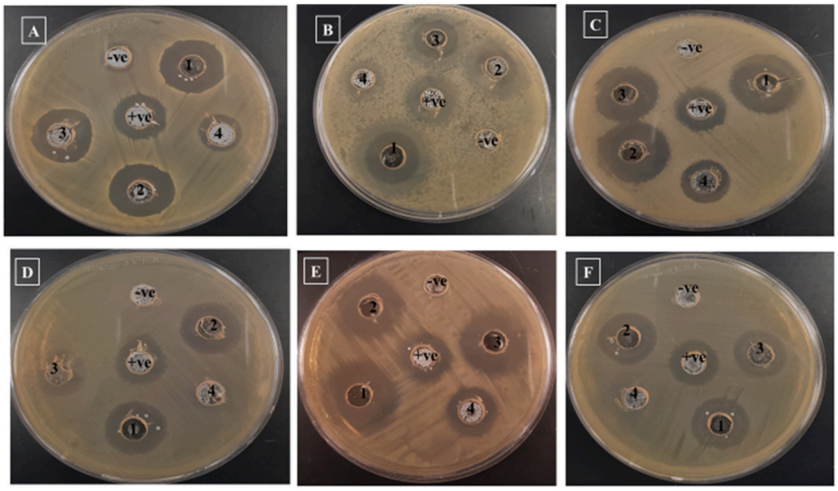


**Fig. 3.** Antibiogram representing the antibacterial activity of moscatilin toward the Gram-positive and Gram-negative bacterial pathogens under investigation. (**A**) *S. aureus*; (**B**) *S. epidermidis*; (**C**) *B. subtilis*; (**D**) *E. coli*; (**E**) *K. pneumonia*; (**F**) *P. aeruginosa.* Numbers and abbreviations inside each Petri dish denote: (**1**) 400 μM; (**2**) 200 μM; (**3**) 100 μM; (**4**) 50 μM; (**-ve**) negative control, PBS; (+**ve**) chloramphenicol (25 μg/mL) positive control.

**Table 2**
The inhibition zone (mm) of moscatilin against the Gram-positive and Gram-negative bacterial pathogens under investigation.

| Bacteria/Dilution | Positive control (25 μg/mL) | 400 μM | 200 μM | 100 μM | 50 μM |
|---|---|---|---|---|---|
| *S. aureus* (ATCC 29213) | 15 ± 0.00 | 22 ± 1.50[a] | 20 ± 1.00[a] | 18 ± 0.00[a] | 12 ± 0.00 |
| *S. epidermidis* (ATCC 12228) | 15 ± 0.00 | 20 ± 0.00[a] | 15 ± 0.00 | 12 ± 0.00[a] | 8 ± 0.00[a] |
| *B. subtilis* (ATCC 10400) | 12 ± 0.00 | 25 ± 1.00[a] | 23 ± 0.00[a] | 20 ± 1.50[a] | 15 ± 0.00[a] |
| *E. coli* (ATCC 25922) | 16 ± 0.00 | 20 ± 0.00[a] | 18 ± 0.00[a] | 15 ± 0.00 | 7 ± 0.00[a] |
| *K. pneumonia* (ATCC 13883) | 15 ± 0.00 | 25 ± 1.00[a] | 23 ± 1.00[a] | 21 ± 0.00[a] | 16 ± 1.00[a] |
| *P. aeruginosa* (ATCC 27853) | 12 ± 0.00 | 19 ± 2.00[a] | 16 ± 0.00[a] | 12 ± 0.00 | 6 ± 1.00[a] |

Mean values (±SD, $n = 3$).
[a] in the same column is significantly different from positive control according to Student's t-test with $p < 0.05$ values considered significant.

**Table 3**
The MIC and MBC values of moscatilin toward the Gram-positive and Gram-negative bacteria pathogens under investigation.

| Bacteria/Dilution | MIC (μM) | MBC (μM) |
|---|---|---|
| *S. aureus* (ATCC 29213) | 7.2 ± 1.36 | 13.1 ± 0.16 |
| *S. epidermidis* (ATCC 12228) | 3.6 ± 0.32 | 6.8 ± 0.38 |
| *B. subtilis* (ATCC 10400) | 6.7 ± 0.45 | 13.5 ± 1.27 |
| *E. coli* (ATCC 25922) | 26 ± 0.39 | 52 ± 2.35 |
| *K. pneumonia* (ATCC 13883) | 3.5 ± 0.26 | 6.8 ± 0.76 |
| *P. aeruginosa* (ATCC 27853) | 24 ± 0.76 | 51 ± 2.24 |

Mean values (±SD, $n = 3$).

used to lower the risk of cancer with minimal or practically no side effects [4,5]. Chemotherapy is a systemic therapeutic strategy currently employed for breast cancer management, but is not fully effective [24]. The current study presented evidence that moscatilin exhibits potent anti-proliferative activity against the triple-positive breast cancer cells, MCF-7, with an observed growth inhibition of 58 % and $IC_{50}$ of 57 ± 4.18 μM (Fig. 1). Current findings are corroborated by those of an earlier study [25] that demonstrated the anti-metastatic potential of moscatilin toward MDA-MB-231 cells. MTT results also demonstrated the potent cytotoxic effect of moscatilin against the HepG2 cell line, with an observed proliferation inhibition of 66 % and an $IC_{50}$ of 51 ± 5.18 μM (Fig. 1). These findings are supported by another report by Yu et al. (2021) revealing that moscatilin exhibited potent anti-metastatic effect against SK-Hep-1 HCC cells through an Akt/NF-κB-dependent mechanism [26].

The results of qPCR gene expression profiling revealed that the *caspase-3*, *8, 9,* and *Bax* mRNA levels were significantly elevated in moscatilin-treated cells relative to those in the control. These observations are congruent with those reported in previously published work revealing that treatment with moscatilin (25 μM) induced an apoptotic pathway in cancerous cells derived from placenta, stomach and lung tissues after 48 h [12]. Furthermore, exposure to 5 μM of moscatilin enhanced the expression of apoptosis-related proteins (cleaved caspase-8, cleaved caspase-7, cytochrome *c*, cleaved caspase-9, cleaved caspase-3, and poly (ADP-ribose) polymerase

(PARP) [15]. Apoptosis is a genetically controlled type of programmed cell death (PCD) that leads to the induction of distinct morphological features, such as membrane blebbing and apoptotic body formation through proteolytic caspase cascade activation. Therefore, apoptosis is a promising target for anticancer therapy. PCD occurs *via* three primary routes, eventually leading to apoptosis: extrinsic (death receptor-mediated), intrinsic (mitochondrial-mediated), and endoplasmic reticulum stress-dependent (ERS) signal transduction pathways. These routes are triggered by both extracellular and intracellular stimuli and eventually converge at executioner caspases, leading to the proteolytic activation of hundreds of target proteins. The extrinsic pathway, also referred to as the death receptor pathway, begins outside of the cells. A pivotal mediator of the extrinsic pathway is caspase-8, whereby activated caspase-8 initiates apoptosis by inducing the executioner caspases-3, 6, and 7. Caspase-9 is an important upstream mediator of the intrinsic pathway through caspase 3 and 7 activation, resulting in apoptosis. Caspase-3 shares typical features with both exogenous and endogenous apoptotic cues due to its interactions with caspase-8 and 9 [27]. The qPCR results reported in the current study indicated a substantial moscatilin-mediated downregulation in the mRNA transcriptional activities of the *Bcl-2* and *Bcl-xL* anti-apoptotic genes, accompanied by the upregulation of apoptosis-promoting genes (*Bax*, *p53* and *p21*). These findings are consistent with an earlier study that revealed that the moscatilin-dependent engagement of apoptosis takes place *via* a reduction in *Bcl-2* and an increase in *Bax* mRNA levels in pancreatic cancer cells in a ROS-dependent manner [28]. Anti-apoptotic genes, such as *Bcl2*, blocks apoptosis by preserving mitochondrial membrane integrity. Therefore, the Bax/Bcl2 ratio determines the sensitivity to apoptotic stimuli [29]. The role of tumor suppressor genes, such as *p53*, is primarily centered on blocking the cell cycle and engagement of apoptosis *via* the regulation of the activity of Bcl-2 family proteins. Notably, data from MTT assay demonstrated that moscatilin exhibits no toxicity towards normal non-cancerous MCF-12 cells. MT-6, a derivative of moscatilin-treated SKOV3 cells, exhibited considerable cell cycle arrest in G2/M phase, followed by a rise in the number of cells in sub-G1 phase. Furthermore, MT-6 increased mitotic kinases, mitotic markers, cell cycle regulators of the G2/M transition, and apoptosis-related markers in ovarian cancer cells in a concentration-dependent manner. MT-6 therapy also reduced mitochondrial membrane potential, activated JNK, and increased DR5 expression [30]. MT-4 (2-Methoxy-5-[2-(3,4,5-trimethoxy-phenyl)-ethyl]-phenol), a moscatilin derivative, has been shown to suppress both sensitive A2780 and multidrug-resistant NCI-ADR/res cellular growth and survival. MT-4 reduced tubulin polymerization, causing G2/M arrest followed by caspase-mediated death [31]. Furthermore, moscatilin has been reported to inhibit cellular growth of breast cancer at *in vitro* and in *vivo* levels through the down-regulation of HDAC3 expression, thereby enhancing the overall histone acetylation of H3 (H3K9Ac) and H4 (H4K16Ac) [11]. This finding indicates the high selectivity of moscatilin, which is an important characteristic of natural products. The selectivity of moscatilin for cancer cells and its wide safety towards healthy cells have been previously reported [32].

It was also observed that moscatilin possessed potent antibacterial effects against *S. epidermidis* (ATCC 12228) and *K. pneumonia* (ATCC 13883), moderate antibacterial effects against *S. aureus* (ATCC 29213) and *B. subtilis* (ATCC 10400), and weak antibacterial effects against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) (Tables 2 and 3, Fig. 3). These findings are supported by another study that revealed that extracts of *D. nobile* exhibited larger zones of bacterial inhibition against *E. coli*, *B. subtilis*, *Proteus mirabilis*, *Salmonella typhi* and *S. aureus*. However, the mechanism of action of moscatilin against bacteria is not well established [16]. Moscatilin has been shown to have a structural resemblance to resveratrol, a phytoalexin molecule synthesized by plants to combat microbial infections and transmission [32]. An early study by Mattio et al. reported that the potent resveratrol-mediated antibacterial mode of action (MOA) against *Listeria monocytogenes* was instigated by severe damage to the cell membrane, culminating in the loss of membrane potential, viability, and cultivability [33]. Therefore, it is postulated that the moscatilin-mediated antibacterial MOA proceeds primarily by targeting the plasma membrane, leading to disruption, leakage of intracellular contents, membrane disruption, and loss of viability. This postulation remains to be verified experimentally by use of transmission electron microscopy.

## 5. Conclusions

These results indicate that the viability of both selected cancerous MCF-7 and HepG2 cell lines was selectively reduced by moscatilin in a dose-dependent manner. The results demonstrated that HepG2 cells were more sensitive to moscatilin (66 % with $IC_{50}$ of $51 \pm 5.18$ μM) compared to MCF-7 cells (58 % with $IC_{50}$ of $57 \pm 4.18$ μM). Moreover, moscatilin-treated cells clearly demonstrated a significant ($P < 0.05$) upregulation in the transcription of pro-apoptosis gene markers at the mRNA level, concurrent with a significant ($P < 0.05$) downregulation of anti-apoptotic gene markers, relative to the control. It was observed that moscatilin exhibited strong antibacterial effects against *S. epidermidis* (ATCC 12228) and *K. pneumonia* (ATCC 13883), moderate antibacterial effects against *S. aureus* (ATCC 29213) and *B. subtilis* (ATCC 10400), and weak antibacterial activity against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). Apoptosis induction, which was confirmed through qPCR gene expression profiling, remains to be further verified at the protein level by western blotting. Flow cytometry should also be used to further investigate whether the observed moscatilin-mediated apoptotic engagement in cancer cells is mediated through cell cycle arrest and reactive oxygen species accumulation.

## Data availability

All data pertinent to this work will be made available upon request to the author.

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## CRediT authorship contribution statement

**Mohmmed M. Aljeldah:** Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

## Declaration of competing interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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