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Chemical profile, antiproliferative and pro-apoptotic activities of essential oils of *Pulicaria arabica* against A549 lung cancer cell line

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ABSTRACT

Pulicaria arabica has been traditionally utilized in folk medicine for various purposes such as ulcer treatments as well as antidiarrheal agent. Herein, the chemical profiles of *Pulicaria arabica* essential oils (PAEOs) and the *in vitro* antiproliferative effect of PAEOs were investigated. Hydrodistillation was employed to prepare PAEOs which were then characterized by GC/MS, while the antiproliferative effects were investigated by MTT assay as well as flow cytometric and RT-PCR analysis. Sixty-four (99.99 %) constituents were recognized from PAEOs. Carvotanacetone (36.97 %), (-)-carvomenthone (27.20 %) and benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy- (6.92 %) were the main components. PAEOs displayed IC_{50} values ranging from 30 to 50 μg/mL. DNA content analysis revealed that A549 cells exposed to PAEOs exhibited an increase in G1 cells population. The flow cytometry analysis results also showed that the PAEOs antiproliferative effect was mediated via apoptosis induction. Furthermore, a modulation in the pro-apoptotic markers (caspase-3 and Bax) and anti-apoptotic (Bcl-2) was also observed. In conclusion, PAEOs exhibited a moderate anti-proliferative effect on A549 cells through modulating the cell cycle progression and apoptosis initiation. These findings could offer a potential therapeutic use of PAEOs in lung cancer treatment.

1. Introduction

Cancer is still a massive health issue and leading cause of death globally with nearly 10.0 million cancer deaths and around 19.3 million new cancer cases have been occurred in 2020 (Sung et al., 2021). With approximately 2 million cases and 1.8 death each year, lung cancer is the leading cause of cancer incidence and mortality worldwide among all cancers (Thandra et al., 2021). The search for novel therapeutic approaches and more potent chemotherapeutic agents is still among the main goals in cancer therapy research (Pucci et al., 2019). Over the past few decades, the use of chemically synthesized drugs has not greatly increased the overall survival rate of cancer patients. As a consequence, innovative chemoprevention techniques and new strategies are required to enhance the effectiveness of current cancer therapies (Choudhari et al., 2019). In addition, the current anticancer therapy studies focus in searching for efficient agents without any side effects. In this respect, chemical components found in plants have proven to be very promising. Since phytochemicals have pleiotropic functions and can target different pathways, they are considered as among the best candidates for the development of anticancer drugs (Singh et al., 2016). Moreover, medicinal plants are still considered a rich source of various constituents with a variety of structural arrangements and interesting biological activities (Vickers, 2002, El-Shemy et al., 2003, El-Shemy et al., 2007).

Several essential oils (EOs) have been identified to possess numerous biological bioactivities, the most important of which is its antiproliferative activity. It considered a rich source for compounds that

Abbreviations: PAEO, Pulicaria arabica essential oils; GC–MS, Gas chromatography–mass spectrometry; PI, Propidium Iodide; RT-PCR, Reverse transcriptase polymerase chain reaction.

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exert antiproliferative effect via various mechanisms including apoptosis induction and cell cycle arrest. Moreover, the use of EOs constituents along with classical chemotherapy drugs have provided a new application in the field of cancer management (Sharma et al., 2022).

Asteraceae family is one of the largest flowering plant families with almost around 1300 species belonging to 900 genera (Trease and Evans, 1983). Among them, *Pulicaria* genus encompassing 100 species distributed across Asia, Europe as well as North Africa. (Dubaie and Al-Khulaidi 1993). In Arabic Peninsula, *Pulicaria* species are recognized for their common uses including alleviating inflammations, herbal teas, and insect repellant (Dubaie and Al-Khulaidi 1993). Previously, phytochemical investigation led to the identification of numerous phytoconstituents from *Pulicaria* species including phenolics, flavonoids, steroids monoterpene, diterpenes, triterpenes, and sesquiterpenes (Liu et al., 2010). In addition, certain species of *Pulicaria* have been reported to exhibit various biological activities including cytotoxic effects (*P. orientalis* and *P. crispa)* (Alo Yahya et al., 1988, Ali et al., 2001), antibacterial activity (*P. dysenterica* and *P. undulata*) (El-Kamali et al., 1998, Basta et al., 2007), antispasmodic and antihistaminic effect of *P. glutinosa* and *P. dysenterica* (Mahfouz et al., 1973, Tanira et al., 1996).

Pulicaria arabica (L.) Cass. stands out as an aromatic species within the subfamily of Tubuliflorae. It is thriving in various regions of Saudi Arabia and its aqueous decoctions employed as traditional folk remedies in numerous localities. It has been also utilized for the controlling digestive disorders problems (Mossa et al., 1987). The nature of *P. arabica*'s oil components explains its uses to treat digestive ailments since these components promote healthy digestion, act as a gastrointestinal sanitizer, and treat gastritis issues. Meanwhile, the prevalence of sesquiterpene constituents in the oil of *P. arabica* gives it a significant standing in the flavor and perfume industry (Mossa et al., 1987). In different countries, *P. arabica* has a long history of use in traditional medicine including ulcers and diarrhea treatments. It was also utilized to cure swelling and boils (Ammar et al., 2020). Only few investigations have been reported regarding the biological activity of *P. arabica* essential oil. In their study (Djermane et al., 2016), it was found that *P. arabica* essential oil exhibited a promising antimicrobial activity and a weak antioxidant capacity. The findings of another study showed that *P. arabica* had a promising hepatic and nephritic protective effects in addition to its potent analgesic, antipyretic, and anti-inflammatory properties (Yusufoglu, 2014). The insecticidal activity against different types of insect's species was also reported for the essential oils extracted from *P. arabica* (Ammar et al., 2020).

The main purpose of this study was to identify the chemical components found in *P. arabica* essential oils. The possible mechanism in inhibiting A549 lung cancer cells proliferation by *P. arabica* essential oils was also explored. To the best of our knowledge, the current study is the first report that investigate the cytotoxic activity along with the potential mechanism of *P. arabica* essential oil in A549 lung cancer cells.

2. Materials and methods

2.1. Plant material and volatile oil preparation

During the spring season in April (2022), the aerial part of *P. arabica* was bought from a local farm (Riyadh city, Saudi Arabia. Prof. Mothana, Faculty of Pharmacy (Pharmacognosy Department), King Saud University, verified the identity of the *P. arabica* samples. The aerial parts were crushed (250 g of plant material in 0.75 L of water), and then hydrodistillation was performed on them for three hours in Clevenger apparatus at temps ranging from 80 °C to 100 °C. The generated volatile oil was filtered and stored at 4 °C.

2.2. GC–MS analysis and compound identification

The components of *P. arabica* essential oil (PAEO) were recognized through the use of GC–MS instrument (PerkinElmer, USA) as previously described (Hidayathulla et al., 2018). Temperature program was commenced at 40 °C, maintained for 2 min, subsequently raised to 200 °C at a rate of 5 °C per min, which was also followed by a 2 min hold. The temperature was increased from 200 °C to 300 °C in 5 °C min^{-1} and maintained for an additional 2 min. The acquired mass spectra were compared with those from the National Institute of Standard and Technology and WILEY Spectral libraries. Additionally, the mass spectra of compounds obtained from PAEO were cross-referenced with those of analogous compounds in the Adams Library (Adams and Cory, 2007) and the Wiley GC/MS Library (McLafferty and Stauffer, 1989).

2.3. MTT assay

The MTT assay was accomplished as described earlier (Nasr et al., 2020). The MTT assay is utilized to measure the metabolic activity as an indicator of cell survival and cytotoxicity. Briefly, 1×10^5 cells/mL of lung (A549), colorectal (LoVo), breast (MCF-7) cancer cells and HUVEC normal cells were plated in 96 well plates and incubated for 24 h at 37 ∘C. Thereafter, all cells were treated with various doses of PAEO (12.5, 25, 50 and 100 μg/mL), vehicle dimethyl sulfoxide (0.1 % DMSO) or standard doxorubicin. After 24 h incubation, 10 μL of 5 mg/mL MTT solution was added and the cells were further incubated for 4 h. Isopropanol was then used to solubilize formazan product and optical density was assessed at 570 nm employing an ELISA plate reader (Bio-Tek, USA).

2.4. DNA content flow cytometry analysis

Cell cycle sample preparation was completed as described previously (Nasr et al., 2020). Briefly, A549 cells were incubated 0.1 % DMSO (vehicle) or PAEOs at 15 and 30 μg/mL. After 24 h of treatment, A549 cells were harvested by centrifugation, washed by phosphate buffer saline (PBS,1X) and fixed with cold ethanol (−20 °C) for 4 h. Thereafter, cells were resuspended in 100 μL of RNase solution and 100 μL of propidium iodide in dark. Subsequently, samples were analyzed by flow cytometer.

2.5. Flow cytometry apoptosis detection

Cell death mode was quantified using a dual staining FITC Annexin V/PI apoptosis detection Kit (BioLegend, CA, USA) according to the manufacturer's instructions. In brief, A549 cells were seeded for 24 h and treated accordingly at 15 and 30 μg/mL. Following a wash with cold PBS, the cell pellet was resuspended in 1 × Binding buffer (100 μL). Subsequently, 5 μL of FITC Annexin V and 5 μL of propidium iodide (PI) were introduced, and the mixture was incubated at room temperature in the dark for 15 min. Upon staining, 400 μL of the binding solution was added and the stained cells were immediately analyzed using a FACScan flow cytometer.

2.6. RT-PCR apoptotic gene investigation

A549 cells were exposed to 15 and 30 μg/mL doses for 24 h, while untreated cells served as the control. Total RNA was prepared from treated and untreated samples using Trizol reagent (Invitrogen, USA) as previously described (Rio et al., 2010). To quantify RNA, the Nano-drop was employed, and 1 μg of RNA was used to synthesize the cDNA according to manufacturer's guidelines of cDNA synthesis kit (Invitrogen, USA). Gene expression levels of, Bax, Bcl2and caspase 7 genes were examined by RT-PCR employing specific primers sets of each gene, with β-actin serving as an internal control. The 1.2 % agarose gel with ethidium bromide was used to electrophorese the RT-PCR products, and the gel was then photographed on gel documentation machine.

Table 1
Chemical composition of *P. arabica* essential oils obtained by hydrodistillation.

Compound Name	Chemical formula	Molecular weight (g/mol)	RT (min)	Area%	RI
alpha.-Phellandrene	$C_{10}H_{16}$	136.23	10.079	0.1	1005
(-)-Carvomenthone	$C_{10}H_{18}O$	154.25	16.648	27.2	1142
Carvotanacetone	$C_{10}H_{16}O$	152.23	17.724	36.9	1128
Carvenone	$C_{10}H_{16}O$	152.23	18.708	2.9	1136
3-Methyl-4-isopropylphenol	$C_{10}H_{14}O$	150.22	19.148	1.8	1148
Isophorone	$C_9H_{14}O$	138.21	19.383	1.03	1158
cis-Carvyl acetate	$C_{12}H_{18}O_2$	194.27	19.818	0.1	1164
Eugenol	$C_{10}H_{12}O_2$	164.20	20.333	0.3	1179
2-Allyl-4-methylphenol	$C_{10}H_{12}O$	148.20	20.819	0.1	1185
(-)-Chrysanthenone	$C_{10}H_{14}O$	150.22	21.254	1.9	1192
Benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy	$C_{12}H_{18}O_2$	194.27	22.044	6.9	1208
5-methoxy-1-methyl-1H-Benzimidazole-2-methanol	$C_{10}H_{12}N_2O_2$	192.21	22.393	0.2	1256
(+/-)-Lavandulyl acetate	$C_{12}H_{20}O_2$	196.29	23.474	2.1	1278
2-*tert*-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180.24	23.629	0.8	1294
gamma.-Muurolene	$C_{15}H_{24}$	204.35	24.287	0.5	1378
beta-Elemene	$C_{15}H_{24}$	204.35	24.407	0.3	1392
alpha-Farnesene	$C_{15}H_{24}$	204.35	25.202	0.1	1428
Neryl isobutyrate	$C_{14}H_{24}O_2$	224.34	25.477	0.7	1458
2-Methylbicyclo[3.3.1]nonane	C10H18	138.25	26.341	0.1	1465
1-(2-amino-pyrimidin-4-ylmethyl)-cyclohexanol	$C_{11}H_{17}N_3O$	207.27	26.827	0.1	1524
alpha.-Cadinol	$C_{15}H_{26}O$	222.37	27.342	0.5	1547
1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl-	$C_{15}H_{26}$	206.37	28.143	0.2	1564
Tetradecanal	$C_{14}H_{28}O$	212.37	28.487	0.3	1612
7-isopropylidene Bicyclo[3.3.0]octan-2-one	$C_{11}H_{16}O$	164.24	30.146	0.1	1625
4-Isopropyl-*cis*-bicyclo[4.3.0]-2-nonen-8-one, (4R,S)-	$C_{12}H_{18}O$	178.27	30.587	0.1	1658
6,10,14-Trimethylpentadecan-2-one	$C_{18}H_{36}O$	268.5	31.222	0.1	1785

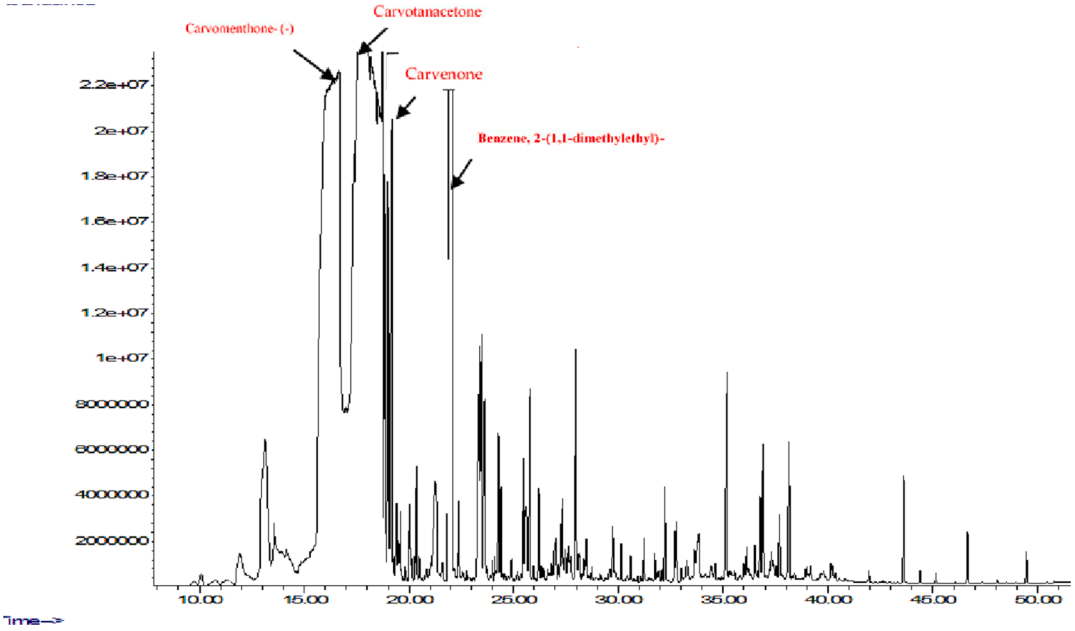

Fig. 1. GC–MS chromatogram of *P. arabica* essential oils.

2.7. Statistical analysis

The average and SD were calculated using Excel. Statistical analysis of differences was carried out by student *t*-test. A p-value less than 0.05 was considered to indicate significance.

3. Results

3.1. Composition of the essential oil

The essential oils obtained by hydrodistillation (1.5 mL) of the dried aerial part of the *P. arabica* species have been analyzed by gas chromatography coupled with mass spectrometry (GC–MS). The analytical results revealed the presence of sixty-four compounds representing 99.99 % of the hydrodistillation. These major compounds identified in PAEOs were carvotanacetone (36.97 %), (-)-carvomenthone (27.20 %), benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy- (6.92 %), carvenone (2.92 %), bicyclo [3.2.0] hept-2-ene, 2-methyl- (2.46 %) and the (+/-)-Lavandulyl acetate (2.09 %) (Table 1). The chromatogram of PAEOs components is illustrated in (Fig. 1), while (Fig. 2) display the structure of the major compounds.

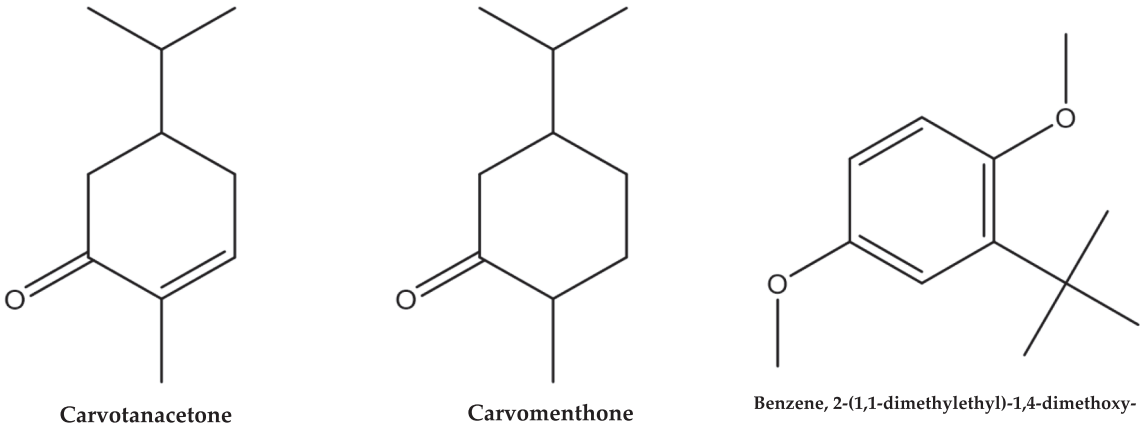

Fig. 2. Compounds with the highest abundances found in *P. arabica* essential oils.

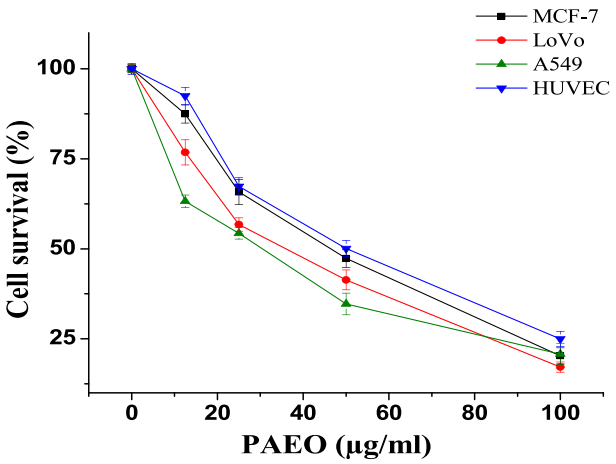

Fig. 3. *In vitro* cytotoxic effect of *P. arabica* essential oils (PAEOs) against various cancer cell line obtained from MTT assay. Cells were treated with PAEOs for 24 h and the results are presented as survival ratio in compare to the vehicle control (DMSO treated). The results are shown as mean ± SD of three independent experiments.

Table 2
Antiproliferative effects of PAEOs (IC_{50}) on growth of various cell lines as determined by MTT assay.

Sample	Cell Lines and IC_{50} (µg/mL)			
	A549	LoVo	MCF-7	HUVEC
PAEO	30.17 ± 1.3	35.44 ± 1.5	46.16 ± 2.1	49.83 ± 0.4
Doxorubicin	1.7 ± 0.11	0.95 ± 0.05	0.65 ± 0.08	2.53 ± 0.1

3.2. Determination of cytotoxic activity

The antiproliferative effects of the PAEOs was assessed in various cell lines using MTT assay. Results showed that PAEOs decreased the tested cells proliferation in a dose-dependent manner (Fig. 3). Our data revealed that PAEOs exhibited an IC_{50} values for cancer cells ranging from 30.1 µg/mL (A549) to 35.44 µg/mL for LoVo and 46.16 µg/mL with MCF-7 (Table 2). The cytotoxic effects of PAEOs were considerably lower in the non-cancerous HUVEC cells in comparison to other tested cells (IC_{50} = 49.83). It seems from our results that the human lung cancer (A549) cell line was more sensitive to PAEOs than MCF-7 and LoVo, hence, it was selected for other experiments.

3.3. Cell cycle analysis

Since the MTT results indicated that the treatment with PAEOs inhibited the cells proliferation, we further studied the influence of PAEOs treatment on cell cycle phases of A549 cells. Based on the obtained IC_{50} values from MTT assay, two different concentrations (1/2 IC_{50} = 15 µg/mL and the IC_{50} = 30 µg/mL) were chosen for flow cytometric studies to confirm the anticancer potential effects. FACS was employed to quantify the DNA content of cells in various phases following A549 treatment with PAEOs for 24 h. The G1 phase percentage of A549 cells were dose-dependently increased from 57.5 ± 0.7 % (in control) to 63.95 ± 1.21 % and 68.5 ± 0. 5 % after treatment with PAEOs at 15 and 30 µg/mL doses correspondingly (Fig. 4). As a result, cells proportions of G2/M and S phases were decreased (Fig. 4). Collectively, our data indicated that PAEOs mediated G1 cell cycle arrest in A549 cells.

3.4. Apoptosis detection

To determine the percentage of cells undergoing apoptosis and/or necrosis, cells were stained with Annexin and Propidium Iodide (PI) and subsequently analyzed using flow cytometry. As shown in (Fig. 5), A549 cells exposed to PAEOs for 24 h at 15 µg/mL led to increase apoptotic cells in both early and late stages (7.2 ± 0.28 % and 3.8 ± 0.14 % respectively), while PAEOs concentration increasing to 30 µg/mL was accompanied with a significant increase in early apoptosis and late apoptosis (increased to 28.55 ± 1.06 % and 29.55 ± 0.63 % respectively) in compare to untreated cells (3.45 ± 0.7 and 0.85 ± 0.05).

3.5. Proapoptotic and antiapoptotic gene expression determination

RT-PCR was additionally employed to evaluate alterations in the expression levels of apoptosis-regulating genes such as Bax, caspase-3, and Bcl-2. This was done to further validate the observed apoptotic effects of PAEOs on A549 cells. A549 cells treated with PAEOs exhibited a significant increase in the expression levels of Bax and Caspase-3, accompanied by a significant decrease in Bcl-2 levels. (Fig. 6).

4. Discussion

One of the most prevalent cancers in the world is lung cancer, which is also one of the cancers associated with highest incidence death (Barta et al., 2019). Natural products have greatly contributed to the development of several drugs that used in cancer chemotherapy (Newman and Cragg, 2020). Moreover, the potential for discovering a novel chemotherapeutic agent derived from medicinal plants still promising (Huang et al., 2021). Essential oils are among the natural plant products that got special consideration due to their widespread use in traditional

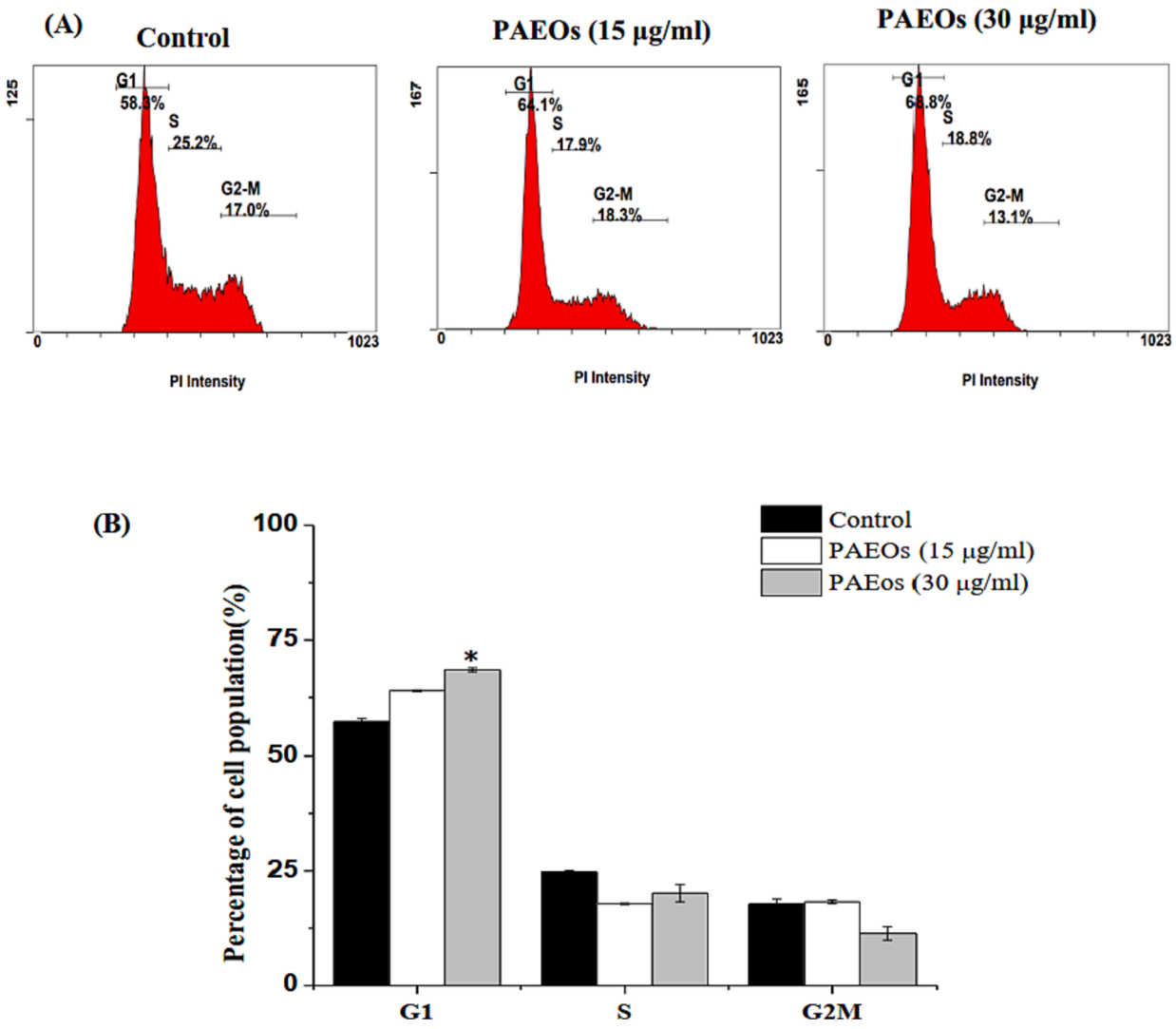

Fig. 4. Analysis of cell cycle phases in A549 cells treated with *P. arabica* essential oils (PAEO). A549 cells were treated for 24 h at 15 and 30 µg/mL for 24 h, fixed, stained with propidium iodide (PI) and analyzed by flow cytometry (A) Representative flow cytometric histogram displaying changes in the cell cycle phases. (B) Bars displaying cells percentage arrested at various stages of the cell cycle. The values are expressed as means ± S.D. of triplicates. *P < 0.05, significantly different compared with control.

therapeutic practices around the globe (Sharifi-Rad et al., 2017). The anticancer properties of different essential oils derived from diverse plant species have been documented. It has been also reported that essential oils and their components mediated their anticancer effects through activation of several cellular mechanisms (Andrade et al., 2018) (Blowman et al., 2018). In this study, the phytochemical compositions, effectiveness and action mode of *P. arabica* essential oils were investigated. Interestingly, we found that PAEOs exerted an apoptotic effect against A549 lung cancer cells which was confirmed by flow cytometry and RT-PCR analysis. The chemical investigation of Pulicaria genus demonstrated that mono-, sesqui-, and diterpenoids are the main secondary metabolites found in Pulicaria species (Assaeed et al., 2020). In this study, monoterpenoids derivatives including carvotanacetone and carvomenthone were the major compounds found in *P. Arabica* (Table 1).

The current study represents the third work regarding the *P. arabica* essential oil chemical composition. In line with this work, our data is an agreement with data reported by (Mossa et al., 1987), where sesquiterpene hydrocarbons represented the main components. Additionally, sesquiterpene hydrocarbons were among the major constituents in the EOs of *P. arabica* grown in Algeria (Ammar et al., 2020). However, there was a difference in the proportion of some compounds such as α-cadinol, which could be due to genetic and regional impact factors (Ammar et al., 2020). Previous phytochemical studies on essential oils of other *Pulicaria* species revealed some different constituents. Our findings were partially agreed with a study reported by (Mentouri 2010) who demonstrated that carvotanacetone was the major constituents in the essential oils of *P. jauberti* that grown in Yemen and essential oils of *P. crispa* that grown in Egypt (Dekinash et al., 2019). In addition, carvotanacetone was also the major constituents (87.3 %) in essential oil of *P. mouritanica* that grown in Morocco (Cristofari et al., 2011). Similarly, GC–MS analysis of *P. undulata* essential oils that grown in Egypt demonstrated that carvotanacetone was among the main compounds found *P. undulata* EOs (Mustafa et al., 2020). In contrast, the essential oil present in *P. odora* species that grown in Morocco exhibited various compounds such as thymol and isobyrate thymol (Hanbali et al., 2005), where is the main compounds found in essential oils of *P. gnaphalodes* were α-Pinene and 1.8-Cineole (Shariatifar et al., 2014). Some of the identified components such as lavandulyl acetate was also reported from the essential oil of *P. dysenterica* and *P. crispa* species growing in Serbia and Sudan respectively (Mohamed et al., 2020, Radulović et al., 2022). These differences in phytoconstituents could be due to different environmental factors including geographical location, period of vegetative cycle, drying as well as the part of the plant used or even to genetic factors

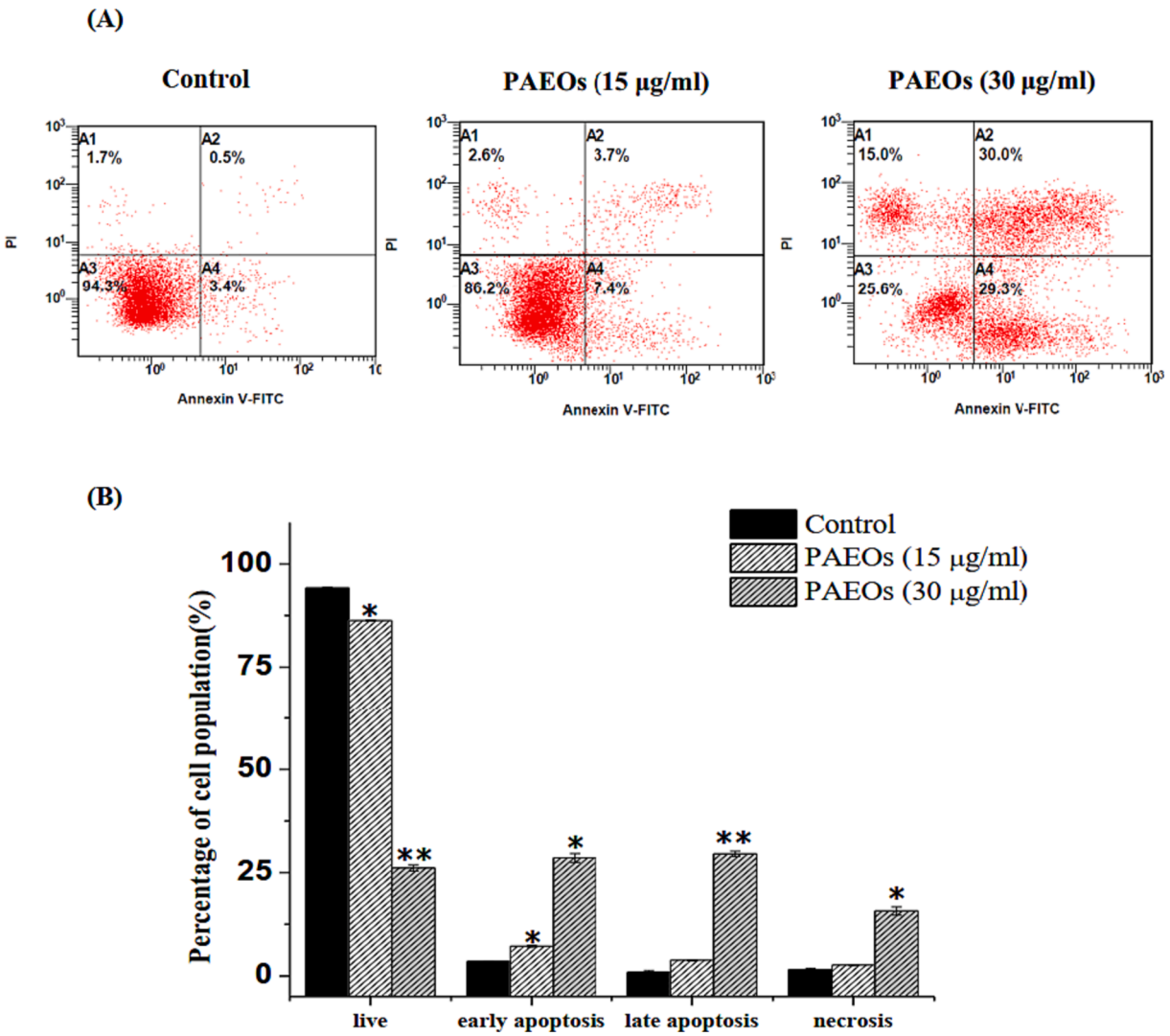

Fig. 5. Induction of apoptosis on A549 cells by *P. arabica essential oils* at 15 and 30 µg/mL assessed by flow cytometry. A549 cells were treated for 24 h with PAEOs and apoptotic cells death were quantified by flow cytometry analysis. Quadrant diagrams display cells that are necrotic (A1), late apoptotic (A2), live (A3), and early apoptotic (A4). (B) Bar graph representing the corresponding proportions of each phase, (n = 3). Values are displayed as average ± SD. Significant difference between control and treated groups (*p < 0.05, ** p < 0.01).

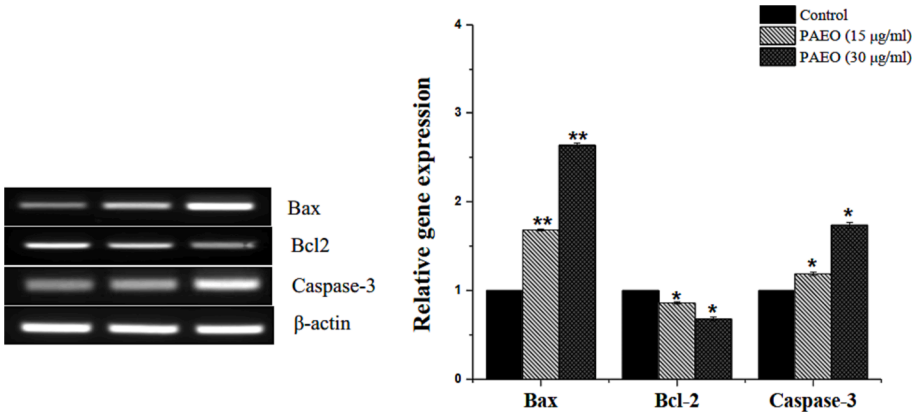

Fig. 6. Effects of PAEOs on the expression levels of Bax, Bcl-2, and Caspase-7 in A549 cells. The cells were treated with different concentrations (15 and 30 µg/mL) of PAEOs for 24 h. RNA was isolated, and the expression levels of Bax, Bcl-2, Caspase-3, expressions were analyzed by RT-PCR analysis. β-actin was used as internal control.

(Amarti et al., 2010). According to National Cancer Institute (NCI) plant screening criteria, PAEOs have a moderate cytotoxic effect since the reported IC_{50} values ranged from 21 to 200 µg/ml (Anywar et al., 2022). The potential antiproliferative effects of different *Pulicaria* essential oils, have also been reported. The IC_{50} values reported for PAEOs in this study were in the same range for the essential oil of *P. incisa* grown in Egypt (Shahat et al., 2017). On the other hand, cytotoxicity results of *P. vulgaris* essential oil from Iran showed that it exhibited a potent cytotoxic activity against MCF-7 and Hep-G2 cell lines within an IC_{50} 5.36 and 7.16 µg/mL (Sharifi-Rad et al., 2014), while the essential oil of *P. vulgaris* that grown in Tunisia showed a moderate cytotoxic activity against A549 and Hela cells (Zardi-Bergaoui et al., 2020). The difference between chemical composition of these oils as well as the possible synergistic effect of these constituents may be responsible for the difference in observed results. Uncontrolled cell cycle progression is one of crucial feature of cancer cells and targeting cell cycle phases could provide promising opportunities for the cancer treatment enhancement (Alimbetov et al., 2018). In fact, several studies have demonstrated that the antiproliferative effect of different essential oils have been linked to cell cycle arrest activity (Sharma et al., 2022). Herein, we found that PAEOs inhibited the A549 cells proliferation in the G1/S phase which is frequently dysregulated in oncogenesis process (Bartek et al., 2000). Cell cycle arrest at G1-phase offers the cells the choice to either undergoing apoptotic pathway or repair mechanisms which both regulated by several genes (Bertoli et al., 2013), suggesting that PAEOs may contain constituents that regulate these factors. Cell cycle and apoptosis are tightly related, and cell cycle arrest triggers apoptosis through interfering with a number of signaling molecules (Trang et al., 2020). Additionally, apoptosis evasion is considered to distinguish hallmarks of cancer cells, therefore, apoptosis promotion can offer insights to cancer treatment (Hainaut and Plymoth, 2013). Herein, apoptosis induction by PAEOs was confirmed by flow cytometry analysis as well as by modulating Bax/Bcl-2 ratio, which considered the major mediator and controller of apoptosis (Adams and Cory, 2007). Caspases are also considered a central mediators of apoptotic cell death. One of them is caspase-3 that has incredible roles and a frequently activated during apoptosis (Porter and Jänicke, 1999). Previously, numerous essential oils from different species showed growth inhibition of cancer cells through activation of caspase-3 (Bayala et al., 2014). Interestingly, here we clearly demonstrate that PAEOs exerted its apoptotic affect against A549 lung cancer cells by significantly upregulating the expression of caspase-3. Although RT-PCR is a powerful technique that can be utilized to precisely quantify the relative gene levels, protein is typically the endpoint of gene expression. Hence, further western blot analysis is needed to confirm the presence of corresponding protein levels. Plant extracts and essential oils containing monoterpene are considered as a rich source of bioactive substances that have been investigated for treating a variety of diseases including cancer (Silva et al., 2022). The potent cytotoxic effect of PAEOs may be attributed to its high content of carvotanacetone, which was previously reported to have a potent antiproliferative and chemopreventive activities (Zheng et al., 1992, Tahri et al., 2022). lavandulyl acetate, a derivative of lavandulol, a substance that is known to be present in lavender oil may also responsible for the cytotoxic potential of PAEOs as its cytotoxic action have been previously documented (Prashar et al., 2004, Tabatabaei et al., 2018). Collectively, the several bioactive components present in PAEOs may be also acted synergistically to cause the observed cytotoxic activity.

5. Conclusion

The phytochemical profile of *P. arabica* essential oils was documented in this study. Among all ingredients, carvotanacetone, (-)-carvomenthone and benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy- (6.92 %) representing the main components. lavandulyl acetate and Bicyclo [3.2.0]hept-2-ene, 2-methyl were also present in a minor percent. Cytotoxic study demonstrated that PAEOs had a moderate antiproliferative effect on A549 cells via arrested cell cycle progression at G1 phase. Further, flow cytometry analysis revealed that apoptotic cells appeared following treatment with PAEOs. Apoptosis induction was also associated with up-regulation of Bax and caspase-3 as well as down-regulation of Bcl-2. Collectively, our results provided an *in vitro* evidence for the potential therapeutic uses of PAEOs in lung cancer treatment which should be further studied *in vivo* to elucidate the precise mechanism. The above stated results may offer a promising topic for further studies to develop a therapeutic plant extracts from *P. arabica* species.

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CRediT authorship contribution statement

Fahd A. Nasr: Methodology, Writing – original draft. **Omar M. Noman:** . **Mohammed Al-zharani:** . **Mohammad Z. Ahmed:** Methodology, Writing – review & editing. **Wajhul Qamar:** Data curation. **Syed Rizwan Ahamad:** Methodology. **Abdullah A. Al Mishari:** Resources. **Mohammed S. Aleissa:** Writing – review & editing, Resources. **Hassan A. Rudayni:** Writing – review & editing. **Ali S. Alqahtani:** Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101879.

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