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Polyphenol-rich *pimenta dioica* extract induces mitochondrial apoptosis in ovarian cancer cells

Polifenolce zengin pimenta dioica ekstraktı over kanseri hücrelerinde mitokondriyal apoptozu indükler

Duygu Aygüneş Jafari

Ege University Faculty of Medicine, Department of Medical Biology, Izmir/Türkiye

ABSTRACT

Aim: Ovarian cancer is a highly lethal gynecologic malignancy, accounting for more deaths than any other female reproductive cancer. Standard chemotherapy often fails over time due to chemoresistance and toxic side effects, resulting in poor five-year survival rates under 40%. There exists an increasing scholarly interest in phytochemicals as potential anticancer agents, attributable to their multifaceted mechanisms of action and comparatively reduced toxicity profiles. *Pimenta dioica* (allspice) berries are rich in antioxidants and contain bioactive compounds such as eugenol and gallic acid with reported antiproliferative properties. However, their effects in ovarian cancer remain underexplored.

Materials and Methods: *P. dioica* extract was evaluated on human ovarian adenocarcinoma OVCAR-3 cells. Cytotoxicity was assessed by WST-1 viability assays across a 12.5-200 µg/mL dose range (48-72 h exposure) to determine IC_{50} values. Apoptosis induction was analyzed by Annexin V-FITC /PI via flow cytometry. Expression of apoptosis related genes (BAX, BCL-2, CASP3, CASP9 and TP53) was quantified by qRT-PCR.

Results: *P. dioica* extract inhibited OVCAR-3 cell viability in dose and time dependent manner ($p<0.05$). The 72 h IC_{50} was 92 µg/mL, indicating moderate cytotoxic activity. Gene expression analysis revealed upregulation of pro-apoptotic mediators (BAX, CASP3, CASP9, TP53) and concomitant downregulation of anti-apoptotic BCL-2 ($p<0.05$).

Conclusion: *P. dioica* extract exerts cytotoxic and pro-apoptotic influences on ovarian cancer cells. These findings suggest that allspice-derived phytochemicals trigger apoptosis pathways in OVCAR-3 cells, supporting their potential as natural anti-cancer agents. Subsequent in vivo investigations and the isolation of phytochemicals are imperative to advance the development of adjuvant therapies utilizing *P. dioica* or chemopreventive strategies for ovarian cancer.

Keywords: *Pimenta dioica*, ovarian cancer, apoptosis, OVCAR-3, mitochondrial pathway

ÖZ

Amaç: *Over kanseri, kadınlarda en yüksek ölüm oranına sahip jinekolojik malignitedir ve diğer tüm kadın üreme sistemi kanserlerinden daha fazla ölüme yol açmaktadır. Standart kemoterapi, zamanla gelişen kemorezistans ve toksik yan etkiler nedeniyle sıklıkla başarısız olur; bu da beş yıllık sağkalım oranlarının %40'ın altında kalmasına neden olur. Son yıllarda, fitokimyasalların çok yönlü etki mekanizmaları ve görece düşük toksisite profilleri nedeniyle potansiyel antikanser ajanlar olarak değerlendirilmelerine yönelik akademik ilgi artmaktadır. Pimenta dioica (yenibahar) meyveleri, eugenol ve gallik asit gibi biyoaktif bileşenler içeren, antioksidan açısından zengin bitkilerdir ve antiproliferatif özellikleri bildirilmiştir. Ancak, over kanseri üzerindeki etkileri büyük ölçüde araştırılmamıştır.*

Corresponding author: Duygu Aygüneş Jafari
Ege University Faculty of Medicine, Department of Medical Biology, Izmir/Türkiye
E-mail: *duygu.aygunes@ege.edu.tr*
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Gereç ve Yöntem: *Pimenta dioica ekstresi, insan over adenokarsinom hücre hattı OVCAR-3 üzerinde değerlendirildi. Sitotoksisite, 12.5-200 µg/mL doz aralığında ve 48-72 saatlik inkübasyon sürelerinde WST-1 canlılık testi ile değerlendirilerek IC_{50} değerleri belirlendi. Apoptoz indüksiyonu, Annexin V-FITC/PI boyaması kullanılarak akış sitometrisi ile analiz edildi. Apoptozla ilişkili genlerin (BAX, BCL-2, CASP3, CASP9 ve TP53) ekspresyon düzeyleri qRT-PCR yöntemiyle ölçüldü.*

Bulgular: *Pimenta dioica ekstresi, OVCAR-3 hücre canlılığını doza ve zamana bağımlı şekilde anlamlı olarak inhibe etti ($p<0.05$). 72 saatlik IC_{50} değeri 92 µg/mL olarak belirlendi ve bu, orta düzeyde sitotoksik aktiviteye işaret etti. Gen ekspresyon analizleri, pro-apoptotik aracılar (BAX, CASP3, CASP9, TP53) düzeylerinde artış ve anti-apoptotik BCL-2 düzeyinde azalma gösterdi ($p<0.05$).*

Sonuç: *Pimenta dioica ekstresi, over kanseri hücreleri üzerinde sitotoksik ve pro-apoptotik etkiler göstermektedir. Bu bulgular, yenibahar kaynaklı fitokimyasalların OVCAR-3 hücrelerinde apoptoz yolaklarını aktive ettiğini ve doğal antikanser ajanlar olarak potansiyel taşıdıklarını göstermektedir. P. dioica'nın adjuvan tedavilerde veya kemopreventif stratejilerde kullanılabilirliğini ilerletmek için in vivo çalışmalar ve fitokimyasalların izolasyonu gereklidir*

Anahtar Sözcükler: *Pimenta dioica, over kanseri, apoptoz, OVCAR-3, mitokonriyal yolak*

INTRODUCTION

Ovarian cancer is still a pressing worldwide health challenge because of its late diagnosis, aggressive behavior and limited treatment success. It ranks as the second most prevalent malignancy within the realm of gynecologic oncology; however, it constitutes the predominant factor contributing to mortality associated with gynecologic cancers (1). Most patients present with advanced-stage disease and despite initial responsiveness to cytoreductive surgery and platinum/taxane chemotherapy, the majority experience relapse with platinum-resistant disease. As a result, long-term outcomes are poor, overall five-year survival rates are only around 45-50% and under 40% in some estimates. Recurrent ovarian carcinoma is notoriously difficult to treat, owing to the development of multidrug resistance and cumulative toxicities (e.g. nephrotoxicity) from standard chemotherapeutics (2). These limitations underscore the urgent need for novel therapeutic approaches that can overcome chemoresistance and improve patient prognosis (3).

In recent years, naturally derived compounds and phytochemicals have gained attention as potential anti-cancer agents in ovarian and other cancers. Such compounds often exert antioxidant, anti-inflammatory and cytotoxic effects via multiple mechanisms, which may help target heterogeneous tumor cell populations. Importantly, many phytochemicals have long histories of human use (e.g. in diet or traditional medicine) and are perceived to have fewer adverse effects on normal tissues. Researchers are increasingly exploring plant-derived products as adjuncts or alternatives to conventional chemotherapy to enhance efficacy and reduce systemic toxicity (4, 5). Notable examples include curcumin, resveratrol and thymoquinone, which have shown anti-proliferative and apoptosis-inducing effects in preclinical ovarian cancer models (6). For instance, thymoquinone (from *Nigella sativa*) reduced OVCAR-3 cell count in time and dose dependent manner and upregulated expression of p53 and CASP3 genes, highlighting promise of phytochemicals in reactivating apoptotic pathways in ovarian carcinoma (7).

Pimenta dioica (allspice, also known as Jamaican pepper) is an aromatic medicinal plant whose berries are widely used as a culinary spice and folk remedy. All parts of the plant, especially the unripe dried berries, are rich in polyphenols and essential oils (9-11). Phytochemical investigations have identified numerous bioactive constituents, including eugenol (a phenolic constituent comprising a major fraction of allspice essential oil) and gallic acid derivatives, among others. Recent studies indicate that eugenol and gallic acid exhibit selective antiproliferative and proapoptotic impacts on various human cancer cells (12, 13). Eugenol, has demonstrated broad anticancer activity across leukemia, breast, colon, prostate and other cancer types, often inducing apoptosis by reactive oxygen species (ROS) generation and mitochondrial dysfunction. *In vitro* and *in vivo* work by Zhang and Lokeshwar (2012) further revealed that an aqueous allspice berry extract contains novel polyphenols (e.g., ericifolin) with potent anti-tumor effects in prostate and breast cancer models. These findings suggest that *P. dioica* harbors compounds capable of modulating key pathways such as apoptosis, cell cycle arrest and oxidative stress in cancer cells. Moreover, studies using human cancer cell models have shown that allspice extracts possess

potent antioxidant and growth-inhibitory properties, suggesting potential utility against ovarian carcinoma (9-11).

Despite its phytochemical richness and culinary prevalence, *P. dioica* remains under-investigated in the context of ovarian cancer. To date, there is a paucity of mechanistic studies examining allspice's anti-cancer effects in ovarian tumor models. Most research on *P. dioica* has focused on other systems, with report demonstrating that allspice extract suppressed androgen receptor signaling and induced intrinsic apoptosis in prostate cancer cells and another showing growth inhibition via autophagy in breast cancer. No published studies have specifically evaluated allspice's efficacy against human ovarian carcinoma cells, representing a critical knowledge gap. Considering the ongoing need for novel therapeutic strategies in ovarian cancer and the documented bioactivity of *Pimenta dioica* constituents in other malignancies, it was hypothesized that *P. dioica* extract may exhibit cytotoxic and pro-apoptotic effects in ovarian cancer cells, potentially involving molecular mechanisms such as mitochondrial-mediated apoptosis, as previously reported in other cancer models.

This study investigates the anti-cancer properties of *Pimenta dioica* extract in the OVCAR-3 human ovarian adenocarcinoma cell line. The analysis focuses on its effects on cell viability cytotoxicity, its potential to induce apoptosis and the associated molecular mechanisms, particularly the expression patterns of key apoptotic genes. These evaluations aim to elucidate therapeutic potence of *P. dioica* in ovarian cancer and support rationale for further preclinical development of allspice-derived compounds as anticancer agents.

MATERIALS and METHODS

Cell line and culture conditions

OVCAR-3 (human ovarian adenocarcinoma) cells were commercially obtained (ATCC, USA) and used as the in vitro model of ovarian cancer. RPMI-1640 medium (ATCC, USA) with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin was used for the cells. Cells were incubated at 37 °C with 5% CO_2 and passaged at 80% confluence using trypsin-EDTA and seeded as needed for assays. All experiments were performed on cells in the logarithmic growth phase and each assay included untreated control cells grown in parallel.

Preparation of *Pimenta dioica* extract

Pimenta dioica berry extract powder was commercially obtained from Greenext (UAE). The supplier extracted and standardized the material using an ethanol/water (v/v) solvent system to ensure a high polyphenol content. Upon receipt, a laboratory stock solution was prepared by dissolving the lyophilized extract powder in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. The stock was aliquoted and stored at 4 °C. Working concentrations were freshly prepared by diluting the stock into culture medium immediately before each experiment. The final DMSO concentration did not exceed 0.1% (v/v) in any treatment or control well to prevent solvent-related effects and maintain experimental reproducibility

Experimental design and treatment

Cells were plated in appropriate culture plates and they were allowed to attach for 24 h before application. To evaluate dose-response effects, OVCAR-3 cells were exposed to a range of *P. dioica* extract doses of 12.5, 25, 50, 100 and 200 µg/mL. These concentrations were selected based on our prior studies. Treatment durations of 48 and 72 hours were used to assess time-dependent effects. Untreated control cells (0 µg/mL) were included for each time point. Each condition was set up in triplicate wells.

Cell viability (WST-1) assay

Cytotoxic effects of *P. dioica* extract were quantified using the WST-1 colorimetric assay (Roche, Basel, Switzerland), which measures the metabolic activity of viable cells. Following the completion of treatment for the designated durations, 10 µL of the WST-1 reagent was administered into each well of a 96-well plate, which contained 100 µL of culture medium per well, and subsequently incubated for a period ranging from 1 to 3 hours at a temperature of 37 °C, according to the guideline established by the manufacturer. The assessment of cell viability was determined as a percentage relative to the untreated control, which is regarded as representing 100% viability. Dose-response curves were generated plotting % viability against extract dose. IC_{50} values at 48 and 72 h were determined by Graphpad Prism.

Apoptosis analysis

Apoptosis induction by *P. dioica* extract was carried out staining with Annexin V-FITC and propidium iodide (PI) using flow cytometry. After 72 h of treatment of IC50 dose, OVCAR-3 cells

were trypsinized and washed with serum free medium or cold phosphate buffered saline (PBS). 5×10^5 cells per sample were reconstituted in 100 µL of binding buffer and subsequently subjected to incubation with 5 µL of Annexin V-FITC (BD Pharmingen kit) and 5 µL PI (50 µg/mL) for a duration of 15 minutes in a controlled dark environment, following kit instructions. A total of 10,000 events were collected per sample. Data were compensated and quadrant gates set based on single-stained and unstained controls. The ratio of cells in each quadrant (viable, early apoptosis, late apoptosis, necrosis) was quantified using flow cytometry analysis software.

Gene expression analysis (qRT-PCR)

To investigate the underlying molecular mechanisms, the mRNA expression levels of key apoptotic regulators were analyzed in OVCAR-3 cells following treatment. Cells were exposed to *Pimenta dioica* extract at the 72-hour IC_{50} concentration and subsequently harvested for RNA isolation. Total RNA was isolated using a commercial purification kit (Qiagen, Germany) in accordance with the protocol of the manufacturer. One microgram of isolated RNA from each sample was subsequently converted into complementary DNA (cDNA) through the utilization of a reverse transcription kit (Qiagen, Germany) following standard procedures.

Quantitative real time PCR was carried out to evaluate the expression of apoptosis related genes in OVCAR-3 cells following treatment. Reactions were conducted using the Qiagen SYBR Green PCR Kit (Qiagen, Germany) on a Roche LightCycler 480 system. Total RNA was reverse-transcribed to cDNA and gene expression levels were quantified using the comparative 2^ΔΔCt method. GAPDH served as the internal housekeeping gene for normalization. All reactions were run in triplicate and a no-template control was included to monitor for potential contamination. Results are expressed as fold-changes in mRNA expression relative to untreated controls.

Statistical analysis

Data are represented as mean ± standard deviation (SD) collected from three different experimental trials. Statistical evaluations were conducted employing GraphPad Prism 10.6. A one-way analysis of variance (ANOVA), succeeded by Tukey's post-hoc analysis, was utilized for comparisons among multiple groups, whereas a two-way ANOVA was implemented for datasets exhibiting time- and dose-dependence. The half-maximal inhibitory concentration (IC_{50}) values were determined along with their corresponding 95% confidence intervals. Statistical significance was set at $p < 0.05$.

RESULTS

Cytotoxic effects on cell viability

The extract of *Pimenta dioica* demonstrated a reduction in the viability of OVCAR-3 ovarian cancer cells in a manner that is dependent on both concentration and duration of exposure. Figure-1 illustrates the dose-response relationships observed after 48 and 72 hours of treatment. At 48 h, concentrations ≥100 µg/mL reduced cell viability by approximately 50%, with an estimated IC_{50} of 109 µg/mL. After 72 h, the cytotoxic effect was more pronounced, yielding an IC_{50} of 92 µg/mL and near-complete growth inhibition at 200 µg/mL. According to NCI botanical criteria, this corresponds to moderate cytotoxicity (IC_{50}: 20–100 µg/mL). The 72-hour IC_{50} value was selected for downstream mechanistic studies.

Induction of apoptosis

The induction of apoptosis subsequent to treatment with *P. dioica* was scrutinized at the IC_{50} concentration (92 µg/mL) employing flow cytometry and utilizing the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, USA), in accordance with the protocols established by the manufacturer. The OVCAR-3 cell line was subjected to the extract for a duration of 72 hours and both untreated control and treated groups were stained with Annexin V-FITC and propidium iodide (PI) to distinguish viable, apoptotic and necrotic populations. As shown in Figure-2, cells treated with *P. dioica* extract exhibited a pronounced increase in Annexin V–positive fractions compared with the negative (untreated) control, indicating significant induction of apoptosis ($p < 0.001$). The shift in cell distribution from the viable quadrant toward early and late apoptotic regions demonstrates that the extract-induced reduction in cell viability predominantly results from apoptotic rather than necrotic mechanisms.

Alterations in apoptotic gene expression

The molecular mechanisms that govern the allspice-induced cellular apoptosis were elucidated through the examination of the expression levels of pivotal apoptosis related genes in both treated and untreated cellular

populations. The findings from the quantitative RT-PCR analyses are presented in Figure-3.

OVCAR-3 cells treated with *Pimenta dioica* extract (92 µg/mL, 72 h) exhibited a gene expression profile consistent with activation of the intrinsic apoptotic pathway.The pro-apoptotic gene BAX (a member of the Bcl-2 family) was upregulated approximately 2-fold compared to untreated controls ($p < 0.05$), while the anti-apoptotic gene BCL2 was significantly downregulated to approximately 0.5-fold of control levels ($p < 0.05$), resulting in an increased BAX/BCL2 ratio; an established molecular signature of mitochondrial-mediated apoptosis. Moreover; mRNA expression of CASP3 (executioner caspase) and CASP9 (initiator caspase of the intrinsic pathway) increased by approximately 1.8-fold and approximately 1.5-fold, respectively ($p < 0.05$). A significant upregulation (approximately 2.5-fold) was also observed in TP53, ($p < 0.05$). Altogether, these transcriptional changes, characterized by elevated TP53, BAX, CASP9 and CASP3 expression with BCL2 suppression, corroborate the phenotypic evidence of apoptosis and provide empirical evidence for the engagement of the mitochondrial pathway in *P. dioica*-induced cytotoxicity.

While protein-level confirmation was not performed in this preliminary study, the mRNA data strongly indicate that *P. dioica* extract tilts the balance toward pro-apoptotic signaling in OVCAR-3 cells. Taken together, the results show that treatment with allspice extract not only reduces ovarian cancer cell viability but actively induces programmed cell death via upregulation of apoptotic genes and downregulation of survival genes.

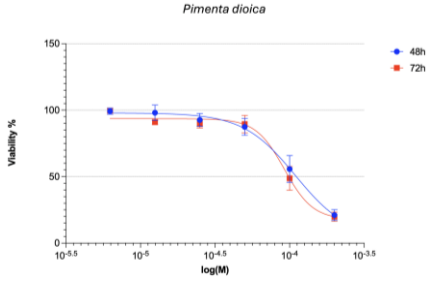

Figure-1. *Pimenta dioica* extract reduced OVCAR-3 cell viability in a dose- and time-dependent manner. Cells were treated for 48 h and 72 h and viability was measured by WST-1 assay. IC_{50} values were 109 µg/mL (48 h) and 92 µg/mL (72 h). Data are mean ± SD of three independent experiments.

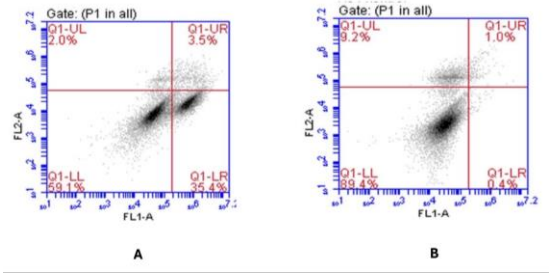

Figure-2. *Pimenta dioica* extract induces apoptosis in OVCAR-3 cells. (A) Cells treated with P. dioica extract (72 h) show increased early and late apoptotic populations, as assessed by Annexin V/PI staining. (B) Untreated control cells show minimal apoptosis. Quantification indicates a significant shift toward apoptosis in the treated group.

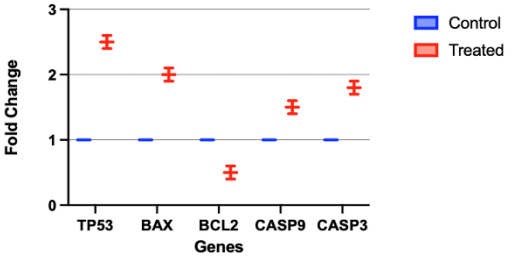

Figure-3. Relative expression of apoptosis-related genes in OVCAR-3 cells treated with *Pimenta dioica* extract (92 µg/mL, 72 h). qRT-PCR analysis demonstrates fold alterations relative to the untreated control, with normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The data are expressed as mean ± standard deviation (SD) derived from three independent experimental trials ($p < 0.05$ compared to control).

DISCUSSION

This study evaluated the anti-cancer activity of *Pimenta dioica* berry extract against human ovarian cancer cells and demonstrated that it significantly reduced cell viability and induced apoptosis in the OVCAR-3 cell line. The cytotoxic activity of the *P. dioica* extract was clearly demonstrated by a marked reduction in OVCAR-3 cell viability after 72 h of treatment, yielding an IC_{50} value of 92 µg/mL. This potency is consistent with previous studies reporting comparable IC_{50} values (~100 µg/mL) for allspice extracts in other cancer cell models, such as androgen-dependent prostate cancer cells (8) and breast cancer models (14; 15). The viability data also align with earlier reports classifying allspice extracts as exhibiting moderate anticancer activity in vitro, a profile that is typical for crude plant-derived preparations.

Collectively, these results indicate that the bioactive constituents of *P. dioica* effectively compromise cellular survival mechanisms within a 72-hour exposure window (8, 14, 15).

It is worth noting that the essential oil of *P. dioica*, which contains a high concentration of eugenol (60-75%), has demonstrated much lower IC_{50} values in several cancer cell lines (16). This discrepancy suggests that eugenol is likely one of the principal cytotoxic constituents and that the potency of the crude extract could be enhanced by enrichment of such active compounds. Importantly, the crude extract's selective toxicity toward ovarian cancer cells and presumably less towards normal cells (given allspice's common dietary use) is a desirable attribute for a potential therapeutic agent.

Crucially, our data indicate that *P. dioica* extract not only inhibits proliferation but actively induces apoptosis in ovarian cancer cells. Flow cytometry showed a clear increase in both early and late apoptotic populations upon treatment, with minimal necrosis, pointing to activation of programmed cell death pathways. This finding is consistent with the mechanism of action reported for many plant-derived anti-cancer agents, which often trigger apoptosis as the main route of tumor cell killing. In the context of allspice, our results echo those from prior studies on different cancers (8, 14).

In a study, it was observed that an allspice berry extract induced apoptosis in prostate cancer cells (8). Similarly, a recent study on gastric cancer cells found that allspice essential oil caused apoptotic cell death associated with sphingosine kinase inhibition and subsequent caspase activation (17). Although the molecular targets may vary, a unifying theme is emerging: *P. dioica*'s phytochemicals can initiate apoptosis in cancer cells, which is a favorable outcome for anti-cancer therapy.

Gene expression analysis revealed downregulation of BCL2 and upregulation of BAX in *Pimenta dioica* treated OVCAR-3 cells, resulting in a shift of the BAX/BCL2 ratio toward a pro-apoptotic profile. This is significant for intrinsic (mitochondria-mediated) apoptosis, as an increased Bax/Bcl-2 ratio promotes outer mitochondrial membrane permeabilization and release of pro-apoptotic factors. The concurrent rise in caspase-9 and caspase-3 mRNA levels in this study suggests that the extract triggers the activation of initiator caspase-9 (associated with intrinsic pathway) and the executioner caspase-3, culminating in apoptotic DNA fragmentation and cell dismantling. These molecular events align well with literature reports on eugenol, the major phenolic in allspice. Eugenol has been shown to trigger apoptosis in HeLa cells by increasing the expression of Bax and caspases while suppressing anti-apoptotic and survival signals. A particular investigation indicated that administration of eugenol resulted in increased concentrations of cytochrome c, Bax, caspase-3, caspase-9 and alongside a reduction in Bcl-2 and inflammatory mediators like COX-2 and IL-1β, indicating that eugenol primarily causes apoptotic cell death (20). The results obtained from this investigation are consistent with these previous observations and provide compelling evidence that intrinsic pathway-mediated apoptosis serves as the predominant mechanism of cellular demise elicited by *Pimenta dioica* extract in ovarian carcinoma cells. The tumor suppressor p53 was another important molecule influenced by the allspice extract. It is noted that a significant upregulation of *TP53* gene expression in OVCAR-3 cells after treatment. Although OVCAR-3 cells harbor mutations in the *TP53* gene (as do most high-grade ovarian carcinomas), the increase in p53 transcript might reflect a cellular stress response or activation of any remaining wild-type p53 function. Interestingly, other phytochemicals like thymoquinone have also been shown to activate p53 pathways in ovarian cancer cells, leading to apoptosis (7). Restoration or amplification of p53-mediated apoptotic signaling in ovarian cancer is particularly relevant, since p53 dysfunction is a key factor in chemoresistance. If allspice-derived compounds can enhance p53 activity or mimic its downstream effects, they could help to overcome the apoptosis resistance seen in p53-mutant ovarian tumors.

The pro-apoptotic activity of *P. dioica* may be partly attributed to its major bioactive constituent, eugenol, which is known to influence cellular redox homeostasis. Depending on the cellular context, eugenol can act as an antioxidant or exert pro-oxidant stress strong enough to trigger apoptotic signaling. Previous studies have shown that eugenol-containing extracts promote mitochondrial dysfunction and caspase activation in cancer cells (16), consistent with the apoptotic phenotype observed in this study. Although ROS levels were not directly measured in this study, the presence of eugenol likely contributes to the

cytotoxic and apoptosis-inducing effects of *P. dioica* in OVCAR-3 cells.

Importantly, the data presented here underscore the translational potential of *Pimenta dioica* (allspice) extract in ovarian cancer. The selective induction of apoptosis in ovarian cancer cells implies that allspice-derived compounds may serve as complementary therapeutic agents. One conceivable strategy is to combine such compounds with standard chemotherapeutic regimens to sensitize tumor cells toward treatment-induced apoptosis, thereby potentially reducing required chemotherapy doses and mitigating adverse effects. Indeed, the promise of allspice extracts in adjunctive cancer therapies has been discussed previously (14, 18). Moreover, preclinical investigations demonstrated that a polyphenol-rich extract of allspice delayed tumor growth and improved survival in a prostate cancer xenograft model without obvious toxicity to normal tissues (19). These in vivo findings reinforce the concept that allspice constituents may selectively target malignant cells while sparing healthy cells; a highly desirable characteristic for anticancer agents. The widespread culinary use of allspice-bearing berries also suggests a favorable safety and patient-acceptability profile. Importantly, rigorous toxicological evaluations and detailed pharmacokinetic/ pharmacodynamic studies are required before clinical translation.

Despite the promising findings, several limitations should be acknowledged. This study was conducted using an *in vitro* model based on a single ovarian cancer cell line (OVCAR-3), which provides a controlled system to explore cellular responses but does not adequately represent the multifaceted dynamics of tumor biology in a living organism. The crude *P. dioica* extract contained multiple phytochemicals, making it difficult to identify the specific compound responsible for the observed cytotoxic and pro-apoptotic effects. Bioactive constituents such as eugenol, gallic acid, quercetin and ericifolin are known to exhibit anticancer properties, yet their relative contributions remain unclear. Future studies will be designed to address these limitations and to give a wider understanding of extract safety and underlying mechanisms.

In summary, the present findings add to the growing evidence that plant-derived compounds possess therapeutic potential against ovarian cancer. The demonstration that *P. dioica* extract induces apoptosis and modulates apoptosis-related gene expression in OVCAR-3 cells provides a preliminary basis for its further exploration as a potential anticancer agent. Continued research addressing the identified gaps will help determine its translational relevance and therapeutic feasibility.

CONCLUSION

This study provides novel evidence that *Pimenta dioica* (allspice) berry extract exerts significant antitumor activity against human ovarian cancer cells. The extract reduced OVCAR-3 cell viability and induced apoptosis, as confirmed by Annexin V-FITC/PI flow cytometry and modulation of apoptosis-related genes (upregulation of *BAX*, *CASP3*, *CASP9*, *TP53* and downregulation of *BCL2*). These findings indicate that *P. dioica* phytochemicals can activate intrinsic apoptotic pathways in ovarian carcinoma cells. Overall, the results support the potential of *P. dioica* as a promising natural source for anticancer agents. Further preclinical research focusing on compound isolation, mechanistic characterization and in vivo validation will be critical to assess its therapeutic applicability and safety.

Conflict of interest

Author declares no conflict of interest.

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