# The Potential Role of Phytochemicals of *Juniperus procera* in the Treatment of Ovarian Cancer and the Inhibition of Human Topoisomerase II Alpha Activity

Bioinformatics and Biology Insights
Volume 18: 1–7

DOI: 10.1177/11779322241248904

Ateeq A Al-Zahrani

Chemistry Department, University College at Al-Qunfudhah, Umm Al-Qura University, Makkah, Saudi Arabia.

**ABSTRACT:** A variety of active chemicals found in medicinal plants can be used to develop new medications with few adverse effects. In vitro and in silico analyses were used to evaluate the anticancer properties of *Juniperus procera* fruit and leaf extracts. Here, we show that the methanolic extract from *J procera* fruit and leaf extracts inhibits 2 human ovarian cancer cell lines, A2780CP and SKOV-3. The leaf extract demonstrated strong cytotoxicity against A2780CP with an IC50 of 1.2 µg/mL, almost matching the IC50 of the anticancer medication doxorubicin (0.9 µg/mL). Higher antioxidant activity was observed in the fruit than leaf extract. The molecular docking results showed that the active component, podocarpusflavone A, was the best-docked chemical with the human topoisomerase II alpha enzyme. According to our knowledge, this is the first in vitro study to show the cytotoxicity of *J procera* extracts against the 2 previously described human ovarian cancer cell lines. The fact that the podocarpusflavone A molecule may have an inhibitory effect on the human topoisomerase II alpha enzyme was also revealed by this first in silico analysis. Our findings imply that the *J procera* fruit and leaf methanolic extract has anticancer characteristics that may guide future in vivo studies.

**KEYWORDS:** *Juniperus procera* (Arar), anticancer activities, ovarian cancer, in silico, protein docking, enzyme inhibition

**RECEIVED:** December 3, 2023. **ACCEPTED:** April 4, 2024.

**TYPE:** Research Article

**FUNDING:** The author received no financial support for the research, authorship, and/or publication of this article.

**DECLARATION OF CONFLICTING INTERESTS:** The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Ateeq A Al-Zahrani, Chemistry Department, University College at Al-Qunfudhah, Umm Al-Qura University, Saudi Arabia. Email: aaalzahrani@uqu.edu.sa

## Introduction

Traditional medicine has made use of a wide variety of naturally occurring plants to treat various malignancies and infectious disorders. Medicinal plants are considered a significant source for the creation of anticancer medicines.[1] Compared with ordinary synthetic chemistry, medicinal plants have a higher structural variety and potency, making them a viable source of new therapeutic medicines. In general, medicinal plants are affordable, accessible, and used extensively in many nations as a treatment for infections and cancer.[2]

Saudi Arabia contains a diverse range of flora. *Juniperus procera* Hochst (*J procera*) is one of the most valued medicinal plants in Saudi Arabia.[3] *Juniperus procera* is a member of the Cupressaceae family and is known for its antioxidant, insecticidal, antimicrobial, and anticancer properties.[4] *Juniperus procera* extracts were studied for their potential cytotoxicity. A very limited number of studies were conducted on *J procera* anticancer activity, and the first research we found through the Google Scholar web search engine was dated 2019.[5] From 2019 to the present, there has been an increased interest in *J procera*, and numerous studies on its possible medical advantages have been carried out. The SCC-9 cancer cells were effectively inhibited by bioactive substances found in *J procera*.[6] The anticancer effects of the biosynthesized silver nanoparticles (AgNPs) against lung cancer A549 and H1975 cells were also assessed in the study utilizing *J procera* fruit and leaf extracts. The findings support the medicinal and biological potential of AgNPs by demonstrating their significant toxicity against in vitro lung cancer cells.[7] More recently, a study by Alhayyani et al[8] demonstrated that *J procera* leaf extracts were cytotoxic to HepG2, MCF-7, HCT116, and JK cancer cell lines.

Etoposide, teniposide, daunorubicin, doxorubicin, and mitoxantrone are only a few examples of clinically effective anticancer medications that target the human topoisomerase II alpha enzyme (TOP2A). Anticancer drugs that target the TOP2A enzyme are useful in the treatment of patients with human ovarian cancer.[9] High levels of TOP2A, which is necessary for cell division and a biomarker for cell proliferation, are expressed during mitosis.[10] TOP2A is a target for many inhibitors as a treatment for many cancers.[11]

The objective of this study was to evaluate the fruit and leaf extracts of *J procera's* anticancer capabilities. In addition, another objective was to investigate the juniper's chemical constituents and identify particular chemicals as potential inhibitors.

## Materials and Methods

### *Plant collection and identification*

The fruit and leaf samples of *J procera* obtained from Raghadan forest, located in Al Bahah Region, Kingdom of Saudi Arabia. The plant samples were identified by Ahmed M. Abd-ElGawad, Department of Plant Production, College of Food

& Agric. Sciences, King Saud University P.O. Box 2460, Riyadh, Saudi Arabia 11451; aibrahim2@ksu.edu.sa.

### *Extraction of phytochemicals*

The fruit and leaf samples were washed using distilled water to remove unwanted materials and dust. Then, the plant samples were dried in a shaded area at room temperature. 250 g of shade-dried leaves and fruits were ground into a fine powder and extracted 3 times in 96% ethanol. The plant samples were then filtered through the Whatman No. 1 filter paper. The filtrates were evaporated to dryness using a rotary evaporator. The homogenate samples were then resuspended in methanol (2 mg/mL), stored at −20°C.

### *Preparation of cell lines*

The 2 human ovarian cancer cell lines (A2780CP and SKOV-3) and the normal human skin fibroblast (HSF) cells were obtained from Nawah Scientific Company, Egypt. Human skin fibroblast cells were grown in DMEM medium (BioWhittaker). SKOV-3 and A2780C cells were maintained in RPMI-1640 medium. Doxorubicin was obtained from Sigma-Aldrich and dissolved in dimethyl sulfoxide (DMSO, Cat. No. 20385.02, Serva, Heidelberg, Germany) and stored at −20°C. The cells were incubated at 37°C in a 5% $CO_2$ atmosphere. The extracts were prepared in DMSO (20 mg/mL stock) and kept at −20°C.

### *MTT assay for cell viability*

Cell viability was checked by following the method of El-Senduny et al.[12] MTT formazan dye was dissolved into DMSO and measured through a plate reader (BioTek, Gen5) at λ = 570 to 630 nm. In brief, 100 µL of cells were seeded in a 96-well plate and left overnight in an incubator at 37°C and 5% $CO_2$. Under the same condition, 200 µL of the fruit and leaf extracts (100 µg/mL each) or DMSO (0.5% V/V) were added to each well and incubated for 48 hours. Then, 20 µL of MTT (3-[4,5-dimethylthiazoyl]-2,5-diphenyl-tetrazolium bromide, 5 mg was dissolved in 1 mL phosphate-buffered saline) was added to each well. The plates then were incubated as mentioned before for 4 hours. Formazan crystals were solubilized using 200 µL of 10% acidified sodium dodecyl sulfate solution (prepared in 0.01 N HCl and 1× phosphate-buffered saline [PBS]). Four hours later, the absorbance was determined at λ = 570 to 630 nm with a plate reader (BioTek, Gen5). The IC50 of fruit or leaf extracts was determined by preparing serial dilutions of the cells (range from 0.10% to 1.00%.) and left for 48 hours. Finally, the cell viability was detected by using MTT as described above. All measurements were performed in triplicate, and the mean ± standard deviation (SD) of the 3 measurements was expressed as µg/mL.

### *Screening for selective anticancer activity of the 2 extracts*

The normal HSF cells were used to screen the selectivity of the Juniperus fruits and leaves extracts against cancerous cell lines. Cells were seeded in a 96-well plate and incubated overnight at 37°C and 5% $CO_2$. Later, serial dilutions of the fruit and leaf extracts 100, 50, 25, 12.5, 6.25, or 3.125 µg/ml were added to the cells, and the plates were incubated as mentioned for 48 hours. The cells viability was checked using MTT assay. The selectivity index (SI) was determined using the following equation[13]

$$\text{Selectivity index (SI)} = \frac{\text{IC50 normal cells}}{\text{IC50 cancer cells}}$$

### *Scavenging activity of fruits and leaf extracts*

The antioxidant activity of fruits and leaf methanolic extracts was studied using DPPH (2,2-diphenyl-1-1picrylhydrazyl) assay (Cat. No. D9132-1G, Sigma-Aldrich) as reported by Musa et al.[14] 200 µL of each fruit, leaf extracts and ascorbic acid were added to 1000 µL of DPPH, and then all the 3 mixtures were incubated in the dark for 30 minutes at room temperature. The reading of the developed color was measured at 517 nm using spectrophotometer (BioTek Instruments, Inc, Winooski, VT, USA). The percentage of the free-radical scavenging activities of the examined extracts, relative to ascorbic acid, were estimated using the following equation

$$\text{Percentage of scavenging radical} = \left(\frac{A_B - A_S}{A_B}\right) \times 100$$

where $A_B$ is the absorbance of the blank and $A_S$ is the absorbance of the sample.

### *Molecular docking*

The crystal structure of Human topoisomerase II alpha (PDB code: 5GWK) was downloaded from protein data bank (PDB). Four inhibitors, etoposide (the native ligand found in 5GWK structure), doxorubicin, daunorubicin, mitoxantrone and 124 phytochemicals of *J procera* were obtained from PubChem Search database as canonical smiles strings. Etoposide, doxorubicin, daunorubicin, and mitoxantrone were used as positive controls. All 4 drugs are known for their use as a medication of several cancers including ovarian cancer.[11,15-18] The smiles strings were converted to pdb files by a web server called CORINA.[19] All the ligands were further prepared using Chimera software tools.[20] The ligand preparation includes adding hydrogen, removing solvents, and determining the charge. Molecular docking of each compound was carried out by AutoDock software version 4.2.6.[21] Protein molecule

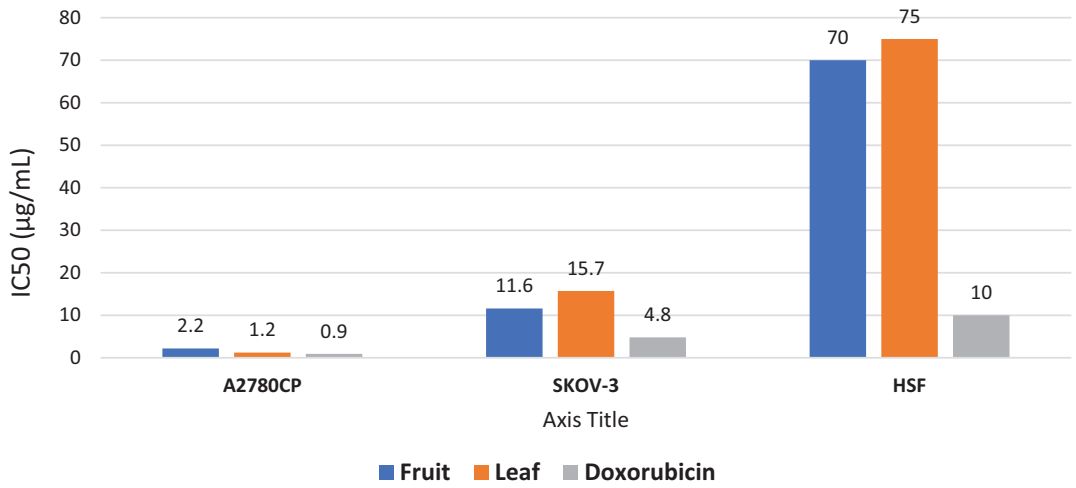


**Figure 1.** The anticancer activity of *Juniperus procera* fruit and leaf extracts against A2780CP and SKOV-3.

(5GWK) was optimized by AutoDock Tools (ADT). The optimization includes deleting water molecules, adding polar hydrogen, merging non-polar hydrogen, and computing Gasteiger charges. The grid box was set to 40*40*40 points and centered with the coordinates x: 23.350, y: −38.580, and z: −59.570. These values of grid box were selected based on the active site of DNA cleavage of topoisomerase II alpha-DNA complex.[22] The docking was performed using the genetic algorithm parameters, 150 population size, 27 000 maximum number of generations, 0.02 mutation rate, and 0.80 crossover rate. After completion of docking, conformations with the best binding affinity were analyzed using chimera software tools and PDBsum server.

### *Evaluation of absorption, distribution, metabolism, excretion, and toxicity*

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction based on the Lipinski rule of 5 (RO5)[23] was performed for all the ligands using the SwissADME web server http://www.swissadme.ch/. In addition, the bioactivity as an enzyme inhibitor for all the ligands was calculated using Molinspiration server https://molinspiration.com/cgi-bin/properties. Cytotoxicity was calculated using ProTox-II server https://tox-new.charite.de/protox_II/index.php?site=compound_input.

## Results

### *Cell viability and selective anticancer activity*

The anticancer assay was performed against 2 types of cancer (Figure 1 and Table 1). More cytotoxicity was observed against ovarian cancer (SKOV-3) using the fruit extract compared with the leaf extract. On the contrary, the leaf extract displayed a cytotoxicity against A2780CP with an IC50 of 1.2 µg/mL, almost similar to the IC50 (0.9 µg/mL) achieved by the anticancer drug, doxorubicin. There was no significant difference between IC50 of fruit and leaf extracts on A2780CP cancer cells (2.2 and 1.2 µg/mL, respectively). Also, there was no significant difference between IC50 of fruit and leaf extracts on SKOV-3 cancer cells (11.6 and 15.7 µg/mL, respectively). Furthermore, the fruit and leaf extracts had higher selectivity for cancer cells than for normal cells (SI > 2).

**Table 1.** The values of anticancer activity of the fruit and leaf extracts.

| CELLS | FRUIT | LEAF | DOXORUBICIN |
|---|---|---|---|
| A2780CP | 2.2 | 1.2 | 0.9 |
| SKOV-3 | 11.6 | 15.7 | 4.8 |
| HSF | >50 | >50 | 10 |

IC50 (µg/mL).

### *Scavenging activity*

The DPPH free-radical scavenging activity of the fruit extracts was evaluated. The DPPH assay showed significant differences of reactive oxygen species (ROS) quenching activity in the fruit extract with 28.08 ± 1.92 µg/mL compared with the leaf extract with 64.03 ± 2.29 µg/mL; $P < .05$ (Figure 2). These results indicate that fruit extracts have higher levels of antioxidants than leaf extracts.

The cell viability and scavenging activity results showed a significant cancer activity against the cancer cells A2780CP and SKOV-3.

Based on the in vitro experiments, a docking study was performed on 124 phytochemicals from *J procera* (Arar) to identify the precise compounds that may be potential anticancer inhibitors.

### *Molecular docking analysis*

The possible inhibition influences of *J procera* phytochemicals against the human topoisomerase II alpha enzyme were investigated using docking analysis. The first step in the docking

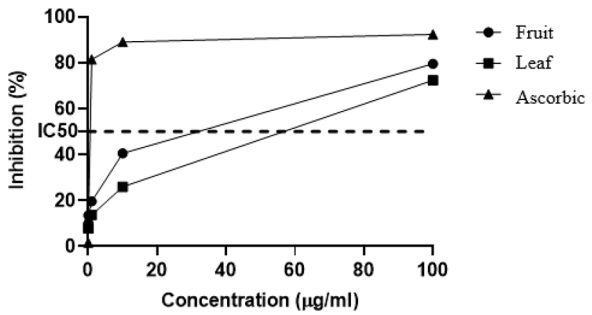


**Figure 2.** Antioxidant activity using DPPH radical scavenging assay.

analysis using AutoDock software was validating the docking protocol by redocking the native inhibitor (etoposide) into the active site of the human topoisomerase II alpha enzyme. For this, etoposide ligand was deleted from chain A in 5GWK structure and another etoposide ligand, generated as described in the methods section, was redocked into 5GWK structure using AutoDock tools. AutoDock software was able to redocked etoposide ligand into the active site of the enzyme and the docked ligand was approximately superimposed with the relevant co-crystallized etoposide indicating the accuracy of the docking protocol (Figure 3).

The docking results (Figures 3 and 4 and Table 2) showed that there was a possible strong interaction between podocarpusflavone A and the human topoisomerase II alpha enzyme. Podocarpusflavone A gave the lowest binding energy of −10.47 and the strongest interaction with the human topoisomerase II alpha enzyme among the 124 phytochemicals in Arar and the 3 controls. Podocarpusflavone A interacts with the human topoisomerase II alpha enzyme by forming hydrogen bonds with 2 amino acids: ASP463 and ARG487 (Table 3). Thirty-five non-bonded contacts were formed between podocarpusflavone A and amino acids: GLU461, GLY462, ASP463, SER464, ARG487, GLY760, and MET762. These 2 hydrogen bonds and the 35 non-bonded contacts may explain the strong interaction between podocarpusflavone A and human topoisomerase II alpha.

AutoDock tools also offer the rmsd (Root Mean Square Deviation) of the obtained poses in addition to the docking scores. The optimal position resulted in an rmsd value of 0, which showed that there was no difference in the average distance between 5GWK and the docked structure. In addition, rmsd of 0 shows that the human topoisomerase II alpha enzyme is not affected by the podocarpusflavone A ligand.

### *Absorption, distribution, metabolism, excretion, and toxicity of compounds*

Table 4 lists the physicochemical characteristics of the best-docked compound and the control molecules. Surprisingly, 3 of the anticancer medications on the market—etoposide, daunorubicin, and doxorubicin—did not adhere to the Lipinski regulations. All 3 medicines violate the Lipinski criteria more than once. According to the bioinformatics findings, each of the 7 compounds had a good chance of acting as an enzyme inhibitor. The best enzyme inhibitor scores were 0.66 for daunorubicin and 0.65 for doxorubicin, respectively. The cytotoxicity tests for the 3 natural substances in Arar, podocarpusflavone A, Sugiol, and deoxypodophyllotoxin were negative. On the contrary, daunorubicin, doxorubicin, and mitoxantrone (but not etoposide) were 3 anticancer medications that produced active cytotoxicity

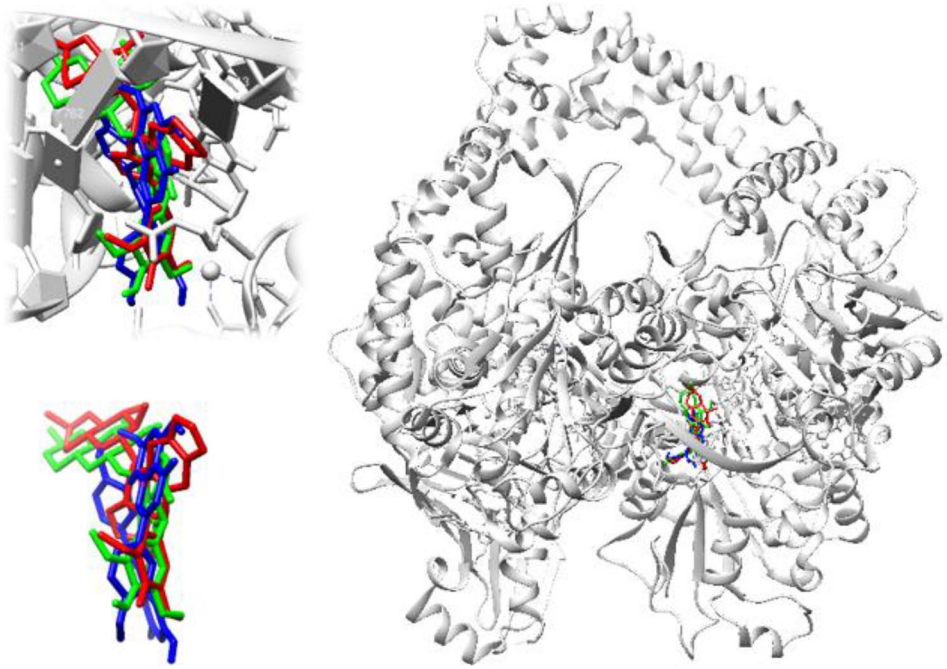

**Figure 3.** Comparison of docked ligands. The figure on the right presents 5GWK structure (gray) with 3 ligands, the native etoposide (red), the generated etoposide (green), podocarpusflavone A (blue). The figures on the top and bottom left present the 3 ligands in closer view.

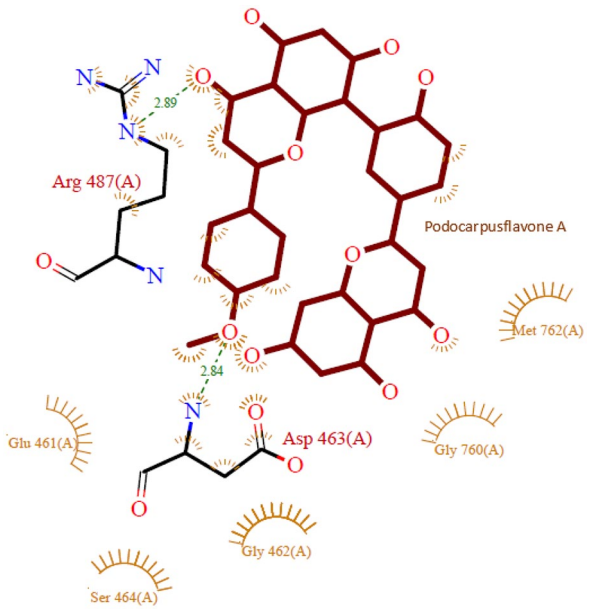


**Figure 4.** Podocarpusflavone A-human topoisomerase II alpha interaction contacts.

ratings. As for TPSA (Topological Polar Surface Area), molecules that have a polar area greater than 140 Å$^2$ tend to have the lowest ability to penetrate cell membranes. Sugiol and deoxypodophyllotoxin showed the best TPSA scores with 37.30 and 72.45, respectively. The rest of the 7 compounds gave scores greater than 140 Å$^2$ indicating that they have less ability as drugs to permeate cell membranes. In terms of GI absorption (gastrointestinal absorption), the highest scores were recorded by sugiol and deoxypodophyllotoxin (Table 4).

## Discussion

As the best solvent in which a significant amount of active plant components could be produced, methanol was used to extract the active compounds from *J procera*.[8,24] The fruit and leaf extracts in our investigation demonstrated high cytotoxicity against the ovarian cancer cells SKOV-3 and A2780CP, with an IC50 value of less than 20 µg/mL for all tested samples. The US National Cancer Institute's guidelines for in vitro cytotoxicity after 72 hours of exposure state that crude extracts with an IC50 less than 20 µg/mL are strongly antiproliferative.[25] These 2 extracts are therefore considered to be interesting candidates for further investigation against diverse tumor types. No previous data were reported regarding the use of *J procera* extracts for cytotoxicity against the 2 mentioned cancer cells. There have been previous studies on other species of juniper. Leaf extract of *Juniperus excelsa* showed moderate anticancer activity with an IC50 value of 17.7 µg/mL against the A2780 ovarian cell line.[26] In a study similar to this study, but on another type of cancer cells, fruit extract of *J procera* was more cytotoxic (IC50 = 8.80 µg/mL) than leaves (IC50 = 11.44 µg/mL) against Carcinoma colon (Caco2) cell lines.[5] Among 27 medicinal plants, extracts of *J excelsa* showed more effective anticancer activity against MCF-7 cell line.[27] In recent studies, the results obtained by Alhayyani et al[8] demonstrated that *J procera* leaves' methanolic extract was effective in suppressing cancer cells in colon (HCT116), liver (HepG2), breast (MCF-7), and erythroid (JK-1) cell lines.

The relationship between antioxidant properties and tumor treatment is complicated, considering the numerous studies supporting the effectiveness of antioxidants in treating cancer. On the contrary, there are also research findings that do not support this conclusion. Singh et al[28] have reported that flavones function as antioxidants and also have the ability to suppress cell growth. Furthermore, these antioxidant compounds actively engage in cellular molecular processes associated with the onset and progression of cancer. As a result, they have the potential to serve not only as preventive measures against cancer but also as effective anticancer agents.[29] This complex relationship between antioxidants and tumor growth prevention

**Table 2.** Docking score of *Juniperus procera* phytochemicals, the native inhibitor, and controls against the human topoisomerase II alpha enzyme.

| COMPOUND | NAME | PUBCHEM CID | AUTODOCK SCORE | INHIBITION CONSTANT NM (NANOMOLAR) |
|---|---|---|---|---|
| Control 1 | Etoposide | 36462 | −10.87 | 10.72 |
| Control 2 | PARPi | 130443213 | −10.73 | 13.58 |
| Control 3 | Daunorubicin | 30323 | −10.31 | 27.79 |
| Control 4 | Doxorubicin | 31703 | −10.13 | 37.5 |
| Control 5 | Mitoxantrone | 4212 | −6.91 | 8750 |
| Control 6 | Paclitaxel | 36314 | −2.91 | 7360000 |
| *J procera* phytochemicals that gave best binding energy | | | | |
| 1 | Podocarpusflavone A | 5320644 | −10.47 | 21 |
| 2 | Sugiol | 94162 | −9.18 | 186.53 |
| 3 | Deoxypodophyllotoxin | 345501 | −8.95 | 277.07 |

**Table 3.** Hydrogen bonds involved in the interactions between etoposide-TOP2A and podocarpusflavone A-TOP2A.

| COMPOUND | HYDROGEN BONDS | | | | | | |
|---|---|---|---|---|---|---|---|
| | RESIDUE | AA | DISTANCE H-A* | DISTANCE D-A* | DONOR ANGLE | PROTEIN DONOR? | SIDE CHAIN |
| Etoposide | 463 | ASP | 2.61 | 3.53 | 149.84 | √ | X |
| | 463 | ASP | 1.64 | 2.25 | 119.05 | √ | √ |
| Podocarpusflavone A | 463 | ASP | 1.90 | 2.84 | 157.50 | √ | X |
| | 463 | ASP | 2.27 | 3.18 | 163.60 | √ | √ |
| | 487 | ARG | 3.12 | 3.80 | 127.50 | √ | √ |

Abbreviations: Distance D-A*, Distance between donor and acceptor atoms; Distance H-A*, Distance between hydrogen and acceptor atoms.

**Table 4.** Physicochemical properties of compounds.

| COMPOUND | LIPINSKI | GI ABSORPTION | TPSA (Å²) | ENZYME INHIBITOR | CYTOTOXICITY |
|---|---|---|---|---|---|
| Etoposide | No | Low | 160.83 | 0.30 | Inactive |
| Daunorubicin | No | Low | 185.84 | 0.66 | Active |
| Doxorubicin | No | Low | 206.07 | 0.66 | Active |
| Mitoxantrone | Yes; 1 violation | Low | 163.18 | 0.20 | Active |
| Podocarpusflavone A | Yes; 1 violation | Low | 170.80 | 0.02 | Inactive |
| Sugiol | Yes; 0 violation | High | 37.30 | 0.38 | Inactive |
| Deoxypodophyllotoxin | Yes; 0 violation | High | 72.45 | 0.12 | Inactive |

has been reviewed in depth by Didier et al.[30] They concluded that antioxidants have an effective role in preventing the development of cancer cells, but there are studies that contradict this conclusion. This confirms the need to conduct more experiments to determine the effect of antioxidants on the growth of cancer cells. In our recent study, fruit extracts displayed a greater activity as antioxidants compared with leaf extracts. One of these antioxidants, Podocarpusflavone A, showed a strong inhibition level against the human topoisomerase II alpha enzyme. This possible inhibition highlights the possibility of using Podocarpusflavone A as a natural anticancer drug.

The Docking results showed that podocarpusflavone A compound displayed the highest inhibition score against the human topoisomerase II alpha enzyme. Podocarpusflavone A compound belongs to bioflavonoids class which has been linked to anticancer activities.[31] As far as we are aware, no research has suggested that the chemical podocarpusflavone A might act as a natural inhibitor of the human topoisomerase II alpha enzyme. Numerous organic substances have undergone in vitro TOP2A inhibitor testing for the treatment of cancer.[32-34]

## Conclusions

The fruit and leaf extracts in the current investigation showed a significant amount of cytotoxicity against the ovarian cancer cell lines SKOV-3 and A2780CP. A possible inhibitory role of podocarpusflavone A against the human topoisomerase II alpha enzyme was investigated for the first time. The fact that podocarpusflavone A has the highest binding affinity for TOP2A of all anticancer medications and chemicals from *J procera* makes it a promising natural inhibitor for TOP2A and may allow for the development of new anticancer treatments. To support our in silico findings, additional in vitro and in vivo research is still required.

## Author Contributions

A.A. contributed to conceptualization. A.A. contributed to methodology. A.A. contributed to software. A.A. contributed to formal analysis. A.A. contributed to resources. A.A.-Z contributed to writing review and editing. A.A. contributed to visualization.

## Ethical Approval

Not applicable for this article.

## Human and Animal Rights

It does not contain any studies with human or animal subjects.

## Informed Consent

There are no human subjects in this article and informed consent is not applicable.

## ORCID iD

Ateeq A Al-Zahrani https://orcid.org/0000-0002-3359-7187

## Data Availability

No data sets were generated or analyzed during this study.

## REFERENCES


1. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol*. 2005;100:72-79.
2. Latif A, Amer HM, Hamad ME, Alarifi SAR, Almajhdi FN. Medicinal plants from Saudi Arabia and Indonesia: in vitro cytotoxicity evaluation on Vero and Hep-2 cells. *J Med Plants Res*. 2014;8:1065-1073.
3. Al-Zahrani AA. Rutin as a promising inhibitor of main protease and other protein targets of Covid-19: in silico study. *Nat Prod Commun*. 2020;15:1934578 X20953951.
4. Abdelghany TM, Hassan MM, El-Naggar MA. GC/MS analysis of *Juniperus procera* extract and its activity with silver nanoparticles against *Aspergillus flavus* growth and aflatoxins production. *Biotechnol Rep (Amsterdam)*. 2020;27:e00496.
5. Magdah G. Cytogenetic toxicity of *Juniperus procera* extract with silver nanoparticles against carcinoma colon (Caco2) cell line in vitro. *Int J Pharmacol*. 2019;15:576-585.
6. AlShahrani A, AlShahrani I, Hosmani J, et al. Anticancer activity of *Juniperus procera* grown in southwestern region of Saudi Arabia on human oral squamous cell carcinoma cell lines. *Pharmacogn Mag*. 2020;16:499-509.
7. Alsubhi NS, Alharbi NS, Felimban AI. Optimized green synthesis and anticancer potential of silver nanoparticles using *Juniperus procera* extract against lung cancer cells. *J Biomed Nanotechnol*. 2022;18:2249-2263.
8. Alhayyani S, Akhdhar A, Asseri AH, et al. Potential anticancer activity of *Juniperus procera* and molecular docking models of active proteins in cancer cells. *Molecules*. 2023;28:2041.
9. Martinchick J, Rahn M, Jolles C, Holden J. Human DNA topoisomerase II-alpha. *Int J Oncol*. 1997;10:1229-1234.
10. Vanden Broeck A, Lotz C, Drillien R, Haas L, Bedez C, Lamour V. Structural basis for allosteric regulation of human topoisomerase IIα. *Nat Commun*. 2021;12:2962.
11. Skok Z, Zidar N, Kikelj D, Ilaš J. Dual inhibitors of human DNA topoisomerase II and other cancer-related targets. *J Med Chem*. 2019;63:884-904.
12. El-Senduny FF, Hegazi NM, Abd Elghani GE, Farag MA. Manuka honey, a unique mono-floral honey. A comprehensive review of its bioactives, metabolism, action mechanisms, and therapeutic merits. *Food Biosci*. 2021;42:101038.
13. Indrayanto G, Putra GS, Suhud F. Validation of in-vitro bioassay methods: application in herbal drug research. *Profiles Drug Subst Excip Relat Methodol*. 2021;46:273-307.
14. Musa KH, Abdullah A, Kuswandi B, Hidayat MA. A novel high throughput method based on the DPPH dry reagent array for determination of antioxidant activity. *Food Chem*. 2013;141:4102-4106.
15. Hoskins PJ, Swenerton KD. Oral etoposide is active against platinum-resistant epithelial ovarian cancer. *J Clin Oncol*. 1994;12:60-63.
16. Muggia FM, Hainsworth JD, Jeffers S, et al. Phase II study of liposomal doxorubicin in refractory ovarian cancer: antitumor activity and toxicity modification by liposomal encapsulation. *J Clin Oncol*. 1997;15:987-993.
17. Kongsawatvorakul C, Charakorn C, Chittithaworn S, Lertkhachonsuk AA. Effectiveness of oral etoposide in recurrent or refractory epithelial ovarian cancer, primary peritoneal cancer and fallopian tube cancer. *J Obstet Gynaecol*. 2022;42:2331-2335.
18. Foglietta F, Macrì M, Panzanelli P, et al. Ultrasound boosts doxorubicin efficacy against sensitive and resistant ovarian cancer cells. *Eur J Pharm Biopharm*. 2023;183:119-131.
19. Sadowski J, Gasteiger J, Klebe G. Comparison of automatic three-dimensional model builders using 639 X-ray structures. *J Chem Inf Comput Sci*. 1994;34 :1000-1008.
20. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem*. 2004;25:1605-1612.
21. Morris GM, Goodsell DS, Halliday RS, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J Comput Chem*. 1998;19:1639-1662.
22. Zayene M, Algethami FK, Abdelhamid HN, et al. New synthetic quinaldine conjugates: assessment of their anti-cholinesterase, anti-tyrosinase and cytotoxic activities, and molecular docking analysis. *Arab J Chem*. 2022;15:104177.
23. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2012;64:4-17.
24. Borges A, José H, Homem V, Simões M. Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*. *Antibiotics*. 2020;9:48.
25. Boik J. *Natural Compounds in Cancer Therapy*. 1st ed. Oregon Medical Press; 2001.
26. Topçu G, Gören AC, Bilsel G, et al. Cytotoxic activity and essential oil composition of leaves and berries of *Juniperus excelsa*. *Pharm Biol*. 2005;43:125-128.
27. Esmaeili S, Hamzeloo-Moghadam M, Ghaffari S, Mosaddegh M. Cytotoxic activity screening of some medicinal plants from south of Iran. *Res J Pharmacogn*. 2014;1:19-25.
28. Singh M, Kaur M, Silakari O. Flavones: an important scaffold for medicinal chemistry. *Eur J Med Chem*. 2014;84:206-239.
29. Kanadaswami C, Lee LT, Lee PPH, et al. The antitumor activities of flavonoids. *In Vivo*. 2005;19:895-909.
30. Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, Creeden JF. Antioxidant and anti-tumor effects of dietary vitamins A, C, and E. *Antioxidants*. 2023;12:632.
31. Šamec D, Karalija E, Dahija S, Hassan ST. Biflavonoids: important contributions to the health benefits of Ginkgo (*Ginkgo biloba* L.). *Plants*. 2022;11:1381.
32. Savaspun K, Boonchaisri S, Chaisuriya P, et al. Cytotoxicity and molecular docking to DNA topoisomerase II of chalcone flavokawain B isolated from *Kaempferia elegans* rhizomes. *Sci Asia*. 2023;49:421-427.
33. Lenin B, Ramasubramanyan S, Vetrivel U, Chitipothu S. Virtual screening and multilevel precision-based prioritisation of natural inhibitors targeting the ATPase domain of human DNA topoisomerase II alpha. *J Biomol Struct Dyn*. 2023;41:15177-15195.
34. Talimarada D, Sharma A, Holla H. Identification of dual binding mode of Orthodiffenes towards human topoisomerase-I and α-tubulin: exploring the potential role in anti-cancer activity via in silico study. *J Biomol Struct Dyn*. 2023;41:2789-2803.