RESEARCH ARTICLE

REVISED Assessing the cytotoxicity of phenolic and terpene fractions extracted from Iraqi *Prunus arabica* on AMJ13 and SK-GT-4 human cancer cell lines

[version 2; peer review: 1 approved, 1 approved with reservations]

Matin Adil Mahmood[1,2], Abdulkareem Hameed Abd[1], Enas Jawad Kadhim[3]

[1]Department of Pharmacology, College of Medicine, Al-Nahrain University, Kadhimiya, Baghdad, Iraq
[2]Department of Pharmacology, College of Pharmacy, Al-Kitab University, Altun Kopre, Kirkuk, Iraq
[3]Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq

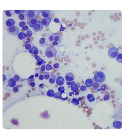
v2 **First published:** 21 Apr 2023, **12**:433
https://doi.org/10.12688/f1000research.131336.1
Second version: 24 Jul 2023, **12**:433
https://doi.org/10.12688/f1000research.131336.2
Latest published: 10 Sep 2024, **12**:433
https://doi.org/10.12688/f1000research.131336.3

Abstract

Background: Breast and esophageal cancer are the most aggressive and prominent causes of death worldwide. In addition, these cancers showed resistance to current chemotherapy regimens with limited success rates and fatal outcomes. Recently many studies reported the significant cytotoxic effects of phenolic and terpene fractions extracted from various *Prunus* species against different cancer cell lines. As a result, it has a good chance to be tested as a complement or replacement for standard chemotherapies.
Methods: The study aimed to evaluate the cytotoxicity of phenolic and terpene fractions extracted from Iraqi *Prunus arabica* on breast (AMJ13) and esophageal (SK-GT-4) cancer cell lines by using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide). Analysis using the Chou-Talalay method was performed to assess the synergistic effect between the extracted fractions and chemotherapeutic agent (docetaxel). Moreover, high-performance liquid chromatography (HPLC) analysis was conducted for the quantitative determination of different bioactive molecules of both phenolic and terpene fractions in the extract.
Results: According to the findings, the treatment modalities significantly decreased cancer cell viability of AMJ13 and SK-GT-4 and had insignificant cytotoxicity on the normal cells (normal human fibroblast cell line) (all less than 50% cytotoxicity). Analysis with Chou-Talalay showed a strong synergism with docetaxel on both cancer cell lines (higher cytotoxicity even in low concentrations) and failed to induce cytotoxicity on the normal cells. Important flavonoid glycosides and terpenoids were detected by HPLC, in particularly, ferulic acid, catechin, chlorogenic acid, β-sitosterol, and campesterol.
Conclusions: In conclusion, the extracted fractions selectively

inhibited the proliferation of both cancer cell lines and showed minimal cytotoxicity on normal cells. These fractions could be naturally derived drugs for treating breast and esophageal cancers.

Keywords
Prunus arabica, Phenolic, Terpene, Cytotoxicity assay, HPLC, Chou-Talalay



This article is included in the Cell & Molecular Biology gateway.

Corresponding author: Matin Adil Mahmood (matin.a.mahmood@uoalkitab.edu.iq)

Author roles: Mahmood MA: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Abd AH**: Conceptualization, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing; **Kadhim EJ**: Conceptualization, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2023 Mahmood MA *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Mahmood MA, Abd AH and Kadhim EJ. **Assessing the cytotoxicity of phenolic and terpene fractions extracted from Iraqi *Prunus arabica* on AMJ13 and SK-GT-4 human cancer cell lines [version 2; peer review: 1 approved, 1 approved with reservations]** F1000Research 2023, **12**:433 https://doi.org/10.12688/f1000research.131336.2

First published: 21 Apr 2023, **12**:433 https://doi.org/10.12688/f1000research.131336.1

REVISED **Amendments from Version 1**

Notable addition based on the reviewer's suggestion is the inclusion of selectivity index analysis. This addition enhances the robustness and relevance of our research.
Furthermore, Standard deviation (SD) values for the data points in Figures 1-3 (that represent the percentage of cytotoxicity) have been added to provide a better understanding of the variability in the results and improve the overall quality of the figures. Alongside the reported IC50 values, the corresponding (SD) values are included. This will give readers a more comprehensive view of the precision and reliability of the IC50 measurements.
More papers and some recent reviews are included to provide a more in-depth comparison of the results of this study to those of other studies with similar plant extracts.
The references in the results and discussion sections have been updated and revised.

Any further responses from the reviewers can be found at the end of the article

Introduction

A critical global health issue is breast cancer, the most prevalent cancer diagnosed worldwide, with 2.26 million incidents expected and it is the major cause of cancer-related deaths among women. Although breast cancer is considered a common disease in developed societies, in 2020, the world's less developed regions accounted for two-thirds of breast cancer-related deaths and more than 50% of all breast cancer diagnoses.[1]

In terms of cancer-related deaths, esophageal cancer (EC) occurs sixth on the list of the deadliest diseases (436,000 fatalities). Meanwhile, 473,000 cases have been recorded worldwide.[2] Even though chemotherapeutic regimens and radiation therapy are more effective methods for treating cancer, they are nonselective, have substantial side effects, and can harm normal healthy tissues, leading to severe unanticipated and undesirable side effects.[3] Initially, several tumors appeared amenable to treatment. However, with time, resistance might develop for a number of reasons, such as mutations in DNA and metabolic changes that cause the drug to be inhibited and degraded.[4]

The occurrence of natural compounds as anticancer agents is estimated to exceed 60% of the current anticancer drugs.[5] These drugs from natural origins can be used for both cancer prevention and treatment due to their pharmacological safety and can be used independently or in conjunction with existing chemotherapeutic treatments to improve the therapeutic efficacy or to reduce chemotherapy-induced toxic effects.[6] The constant change and injury that occurs in the human body over decades requires a development of revolutionary and highly precise "arms" capable of successfully combating malignant cells. Natural products are a powerful and endless source for identifying the finest anticancer prospects.[7]

Prunus arabica is recognized as a distinct species from the farmed almond *P. dulcis.* Both, however, are members of the *Prunus* genus and the *Rosacea* family. This species was given its scientific name based on its geographical location where it first appeared. This taxon is indigenous to mild climate Asia regions, including the Fertile Crescent Mountains, as well as Turkey, Iran, and Iraq.[8]

Prunus arabica is a thick undomesticated almond species with an unbarked stem that remains green even during dormancy.[9] A wide range of biological and pharmacological effects from different *Prunus* species show great promise for the treatment of various cancers.[10] Flavonoids, steroids, terpenoids, poly phenols, and other chemicals have all been identified from various *Prunus* species.[11] Polyphenols and terpenoids which are found plentifully in plants, show anticancer effects, via inhibiting cancer cell growth, blood vessel formation, metastasis, inflammation, and inducing cell death.[12] For this purpose, the anticancer effect of phenolic and terpene fractions from *Prunus arabica* extract was investigated using the AMJ13 and SK-GT-4 cancer cell lines and normal human fibroblasts (NHF).

Methods

Preparation and separation of plant extract

The full protocol can be found in the *Extended data.*[45] Approximately 500 gm of granular powdered plant that had been shade-dried for 12 days then defatted for 24 hours with hexane (BDH chemicals, England cat-no. BDH24575.100E) in a ratio of 1:3 W/V before being dried at room temperature. In a Soxhlet apparatus (BOECO, Germany) the defatted plant components were separated using two liters of 80% ethanol (Sigma-Aldrich, Germany cat-no1070172511) until completely depleted. A thick, dark-greenish-yellow residual (known as the crude fraction A) was obtained by drying the alcoholic extract by evaporation at low pressure and temperatures below 40°C by using IKA RV 10 Rotary Evaporator (Germany). This fraction was acidified using 300 ml of 5% HCl (Sigma-Aldrich cat-no. 1009861000) to pH 2 and then split with ethyl acetate (Sigma-Aldrich, Germany cat-no. 319902-1L) to acquire two distinct layers (the acidic aqueous layer and ethyl acetate layer-crude fraction). The crude fraction was dried out using low-pressure evaporation in an IKA RV 10 Rotary Evaporator (Germany) then basified with 300 ml of 5% NaOH (Honeywell, USA cat-no. 30620) to pH

10 and extracted by adding chloroform (Honeywell, USA cat-no. C2432) to the separatory funnel in order to obtain two layers; the aqueous basic layer and the and chloroform layer. The basic water layer evaporated to the point of dryness and acidified with 5% HCl to reach pH 2, then extracted with ethyl acetate to get another fraction designated as fraction (F-B). The chloroform layer which was separated by the same steps and partitioned with 80% methanol (Biochem Chemopharma, France cat-no. 213032500) and petroleum ether (Sigma-Aldrich, Germany cat-no. 32299) to obtain another two layers; fraction (C) the petroleum ether and methanol fraction which was considered as fraction D.[13]

Preliminary qualitative phytochemical analysis of fractionated extract of *Prunus arabica*

The full protocol can be found in the *Extended data.*[46] Standard protocols were used in chemical testing to identify the active components using ethanolic extracts from various plant fractions.[14]

I. **Alkaloids test**: Precisely 2 ml of alcoholic extract and fractions were stirred with 5 ml of 1% HCl (Sigma-Aldrich cat-no. 1009861000) on a steam bath. Mayer's reagent (prepared by dissolving 1.35 gm mercuric chloride (Sigma-Aldrich, Germany ca-no. 215465) in 60 ml water + 5 gm potassium iodide (Sigma-Aldrich, Germany cat-no. 221945) in 10 ml water) and Wagner's reagent (prepared by dissolving 1.27 gm of iodine (Sigma-Aldrich, Germany cat-no. 1047630050) and 2 gm of potassium iodide in 100 ml of water) were used. White and reddish-brown colored precipitates were considered as indications of the presence of alkaloids.

II. **Flavonoids tests**

  a. Lead acetate test: precisely 1 ml of 10% lead acetate solution (BDH limited, England cat-no. LL0093) was incorporated into 2 ml of alcoholic extract and fractions. The presence of a yellowish-white precipitate indicated the presence of flavonoids.

  b. NaOH test: 2 ml of the extract and fractions were subjected to aqueous NaOH and HCl; the development of a yellowish-orange color indicated the presence of flavonoids.

III. **Steroids tests**

  a. H_2SO_4 test: 2 ml of sulfuric acid (BDH limited, England cat-no. BDH3068-500MLP) was added to the extract, a green color was formed as an indication to the presence of steroids.

IV. **Terpenoids test (Salkowski test):** 5 ml of plant extract mixed with 2 ml of chloroform (Honeywell, USA cat-no. C2432), and 3 ml of concentrated sulphuric acid (BDH limited, England cat-no. BDH3068-500MLP) was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Ultrasonic Extraction of Phenolic Compounds and terpenes

Reference standards and reagents

The reference standards (Caffeic acid cat-no C0625, (+)-Catechin hydrate cat-no. C1251, Chlorogenic acid cat-no. 00500590, Ferulic Acid cat-no. PHR1791, Gallic acid monohydrate cat-no. 27645, p-Coumaric acid cat-no. C9008, Quercetin cat-no. Q4951, Rutin hydrate cat-no. R5143, β-Sitosterol cat-no. 43623, Campesterol cat-no. C5157, Stigmasterol cat-no. 47132) were purchased from Sigma Aldrich, Germany. The pure water used in the study was distilled with a Milli-Q. (Millipore, Bedford, MA, USA). Chemicals, including methanol (cat-no. 106007), acetonitrile (cat-no. 100030), and acetic acid (cat-no. 543808) (HPLC grade), were all ordered from Merck Ltd, Mumbai, India. Before usage, the solvents were processed using 0.45 mm pore size (Millipore) membrane filters.

Instrumentation and analytical conditions

Individual phenolic components identification was conducted by reversed-phase high-performance liquid chromatography utilizing a Sykam HPLC chromatographic system (Germany) integrated with ultraviolet detection (Sykam S 3240 UV/Vis Multichannel Detector, Germany) and sample delivery system (Sykam S1122 Solvent deliver system, Germany). The column temperature was maintained at 30 °C (Sykam S 4011 column thermo controller, Germany). The gradient elution method, with eluent A and eluent B (methanol and 1% (v/v) formic acid in water respectively), was carried out as follows: initial 0–4 min, 40% B; 4–10 min, 50% B; and flow-rate of 0.7 ml/min. Approximately 100 µL of the samples were injected. An autosampler (Skynm S5200 sample injector, Germany) analyzed the standards automatically. Spectral data was recorded at a 280 nm.[15]

The following conditions were used for the terpene fraction; mobile phase acetonitrile: distilled water: acetic acid (60:25:5), column = C18-ODS (25 cm * 4.6 mm), detector = UV- 220 nm, and the flow rate was 1 ml/min.

Cell lines

AMJ13 breast cancer[16] and NHF normal cell lines (normal human derived adipose tissue)[17] were cultivated in Roswell Park Memorial Institute medium RPMI 1640 (Capricorn, Germany cat-no. RPMI-A) with 10 % fetal bovine serum (FBS) (Capricorn, Germany cat-no. FBS-22A), antibiotics (penicillin and streptomycin) (Capricorn, Germany cat-no. PS-B) then incubated at 37 °C. SK-GT-4, the esophageal cancer cell line,[18] was maintained in minimum essential medium MEM (Capricorn, Germany cat-no. MEMA-RXA). Cells were passaged using Trypsin-EDTA (Capricorn, Germany cat-no. TRY-1B), replanted at 50% confluence twice weekly, and incubated at 37 °C (Cypress Diagnostics, Belgium).[16]

AMJ13, NHF cell lines provided by the Iraqi Center for Cancer Research and Medical Genetics. SK-GT-4 were supplied by European Collection of Authenticated Cell Cultures (ECACC).

Cytotoxicity assays

The full protocol can be found in the *Extended data*.[47] The MTT assay for cell viability[19] was performed to measure the cytotoxic effect of the extracted fractions. Cell lines were planted into 96-well plates (Santa Cruz Biotechnology, USA cat-no. sc-204447) at 1×10^4 cells/well. After 24 hrs, or until a confluent monolayer was achieved, cells were treated with the tested compound. After 72 hours of exposure, cell viability was assessed; the medium was removed by aspiration and 28 μL of 2 mg/ml MTT (MTT stain obtained from Bio-World, USA cat-no. 42000092-1) was added and incubated at 37 °C for 1.5 hours.

After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 μL of dimethyl sulfoxide (DMSO) (Santacruz Biotechnology, USA cat-no. sc-202581) was used to solubilize the residual crystals in the wells, proceeded by incubation for 15 min at 37 °C with shaking.[20] Using a microplate reader (Genex-lab, USA cat-no. MR-100) the absorbance was measured at 492 nm; the experiment was carried out in triplicate. Untreated cells, MTT solution, and a solubilizing buffer (DMSO) will be placed in the wells designated as a control. Due to its proven lack of cytotoxicity in cell culture, a final concentration of 0.5% DMSO was used for this study. The following formula was applied to determine the percent of cytotoxicity[21]:

$$\%\text{Cell viability} = \left(\frac{\text{AT}}{\text{ANT}}\right) \times 100$$

$$\%\text{Cytotoxicity} = 100 - \text{cell viability}$$

AT: Absorbance of treated cells with tested compounds, ANT: absorbance of untreated cells.

Chou-Talalay analysis

Synergism or interaction of phenolic and terpene fractions with docetaxel was investigated using a non-constant ratio. Analysis of the combination was performed by the Chou-Talalay method. CompuSyn was used to derive the corresponding combination indices (CI) (CompuSyn, Inc., Paramus, NJ, USA). The combination indices were calculated using non-constant phenolic, terpene, and docetaxel ratios and mutually exclusive formulations. A CI value between 0.9 and 1.1 indicates an additive effect, a CI value below 0.9 denotes synergy, and a CI above 1.1 denotes antagonism.[22]

Selectivity Index analysis

The Selectivity Index (SI) is calculated by dividing a compound's IC_{50} value against normal cells by its IC_{50} value against cancer cells.[23,24] High-selectivity compounds have a SI value greater than 3, while low-selectivity compounds have a SI value of 3.[24]

Statistical analysis

The MTT assay results were analyzed statistically with an ANOVA test in GraphPad V 7.00 (for windows), an open-access alternative that can perform an equivalent function is R. The unpaired t-test was used to compare groups; P-values <0.05 were considered statistically significant.

Results

Qualitative phytochemical analysis

The phytochemical screening results are given in Table 1.

Table 1. **Phytochemical analysis of *Prunus arabica* crude extract and isolated fractions.**

Crude and fractions	Alkaloids	Flavonoids	Steroids	Terpenoids
Crude A	+	+	+	+
Fraction B	-	+	-	+
Fraction C	-	-	-	+
Fraction D	-	-	+	+

+, - represent the presence and absence of phytoconstituents, respectively.

Table 2. **Phenolic acid and corresponding concentration expressed in mg/gm of dry fraction extract.**

Phenolic acid	Retention time in min	Conc. mg/gm
P-coumaric acid	2.18	1.954
Ferulic acid	3.22	3.992
Gallic acid	4.79	1.618
Caffeic acid	6.08	1.925
Quercetin	7.89	2.125
Rutin	8.33	1.328
Catechin	10.2	2.451
Chlorogenic acid	12.08	2.491
Unknown	14.05	–

Table 3. **Terpenes and corresponding concentration expressed in mg/gm of dry fraction extract.**

Terpenes	Retention time in min	Conc. mg/gm
Unknown	2.69	–
B sitosterol	4	3.979
Unknown	4.79	–
Campesterol	11.5	4.358
Stigmasterol	10	2.350

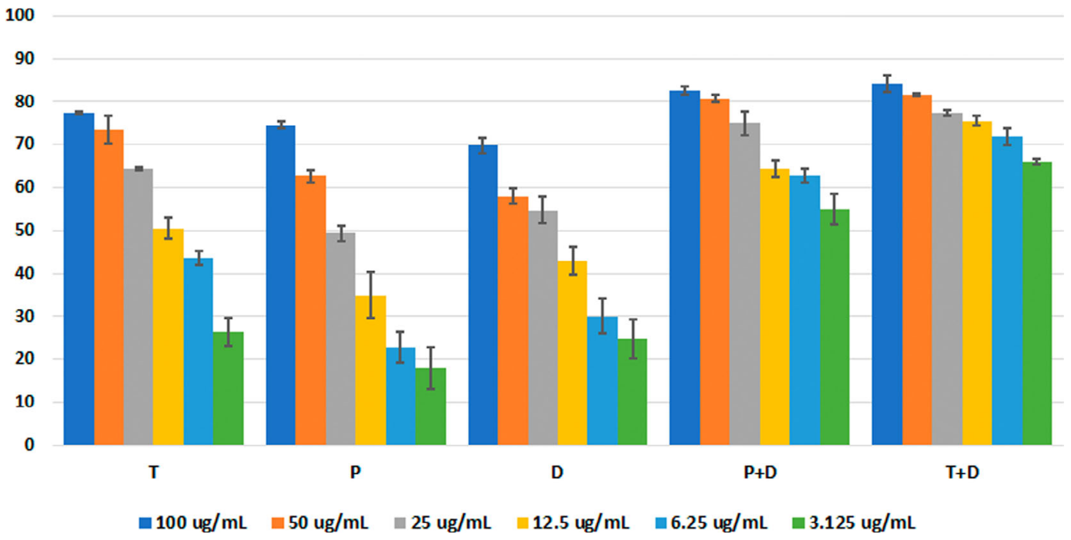


Figure 1. **The cytotoxicity of different concentrations on the AMJ13 cell line, T: terpenes, P: phenolic, D: docetaxel, P+D: phenolic and docetaxel, T+D: terpene and docetaxel.**

To discover individual compounds of extracted fractions, HPLC analysis was used. Seven phenolic acids (p-coumaric acid, ferulic acid, gallic acid, caffeic acid, quercetin, rutin, catechin) and three terpenes (B sitosterol, campesterol, stigmasterol) were detected from extracted fractions of *Prunus arabica*, as shown in Tables 2 and 3.

In vitro cytotoxic activity

The MTT assay was used to evaluate the cytotoxicity and therapeutic efficacy of the isolated fractions in the cancer and normal cell lines (AMJ13, SK-GT-4 and NHF). As shown in Figures 1–3, the results showed that the treatments significantly decreased the viability of the cancer cells (>50% at higher concentrations) with minimal cytotoxic effects on the normal cells (<50% cytotoxicity). On the AMJ13 cell line, phenolic, terpene fractions, and docetaxel had a half maximal inhibitory concentration (IC_{50}) of 29.34±2.37, 8.455±3.02, and 14.51±0.77 μg/ml, respectively, as shown in

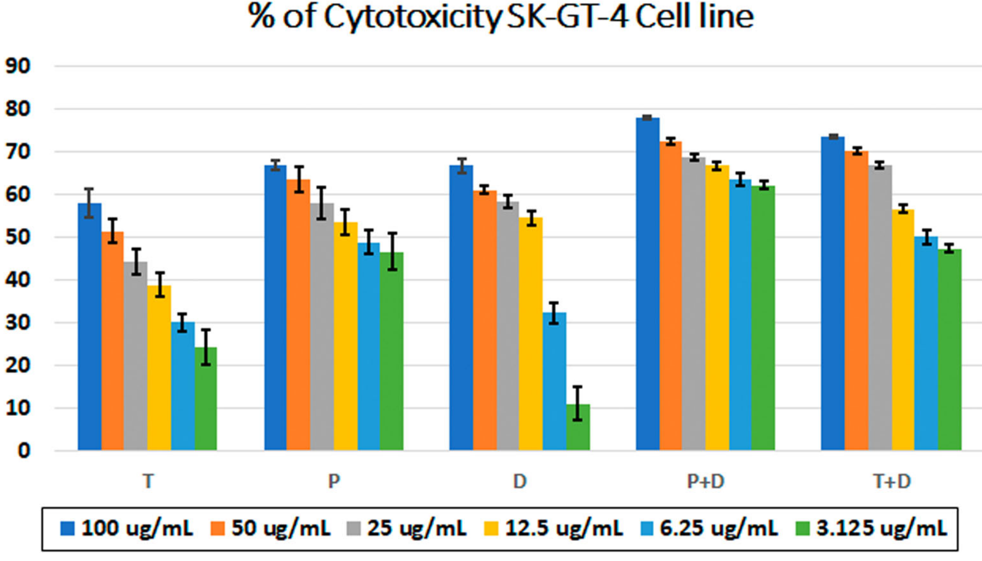


Figure 2. The cytotoxicity of different concentrations on the SK-GT-4 cell line, T: terpenes, P: phenolic, D: docetaxel, P+D: phenolic and docetaxel, T+D: terpene and docetaxel.

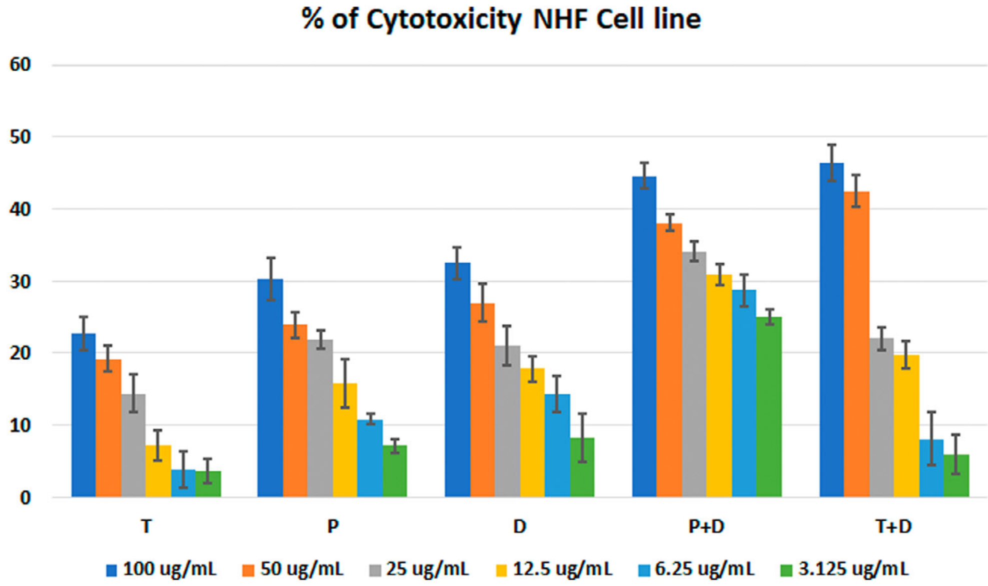


Figure 3. The cytotoxicity of different concentrations on the NHF cell line, T: terpenes, P: phenolic, D: docetaxel, P+D: phenolic and docetaxel, T+D: terpene and docetaxel.

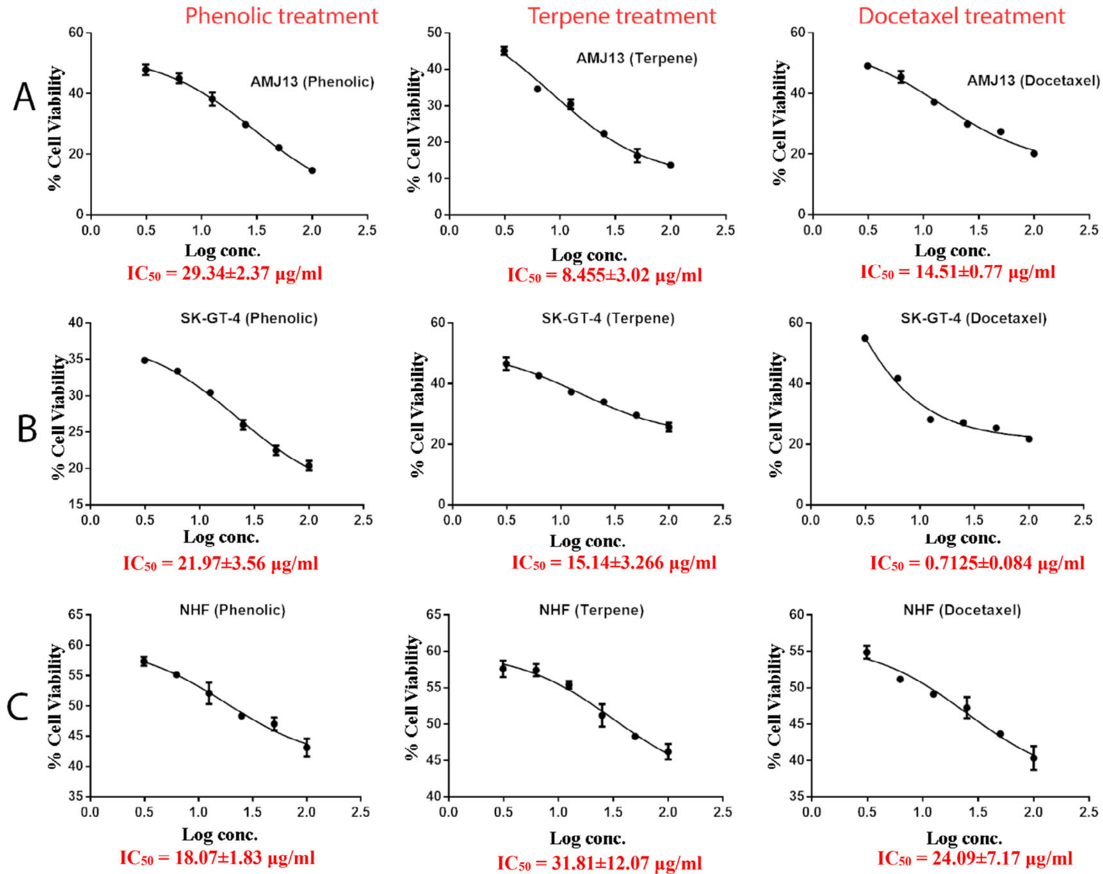


Figure 4. The half maximal inhibition concentration (IC_{50}) for phenolic, terpene, and docetaxel, respectively, on A: AMJ13, B: SK-GT-4, and C: NHF cell lines.

Figure 4. On the SK-GT-4 cell line, phenolic, terpene fractions, and docetaxel had an IC_{50} of 21.97±3.56, 15.14±3.266, and 0.7125±0.084 µg/ml, respectively, as shown in Figure 4. While on the NHF cell line, the phenolic, terpene, and docetaxel had an IC_{50} of 18.07±1.83, 31.81±12.07, and 24.9±7.17 µg/ml, respectively.

Extract-docetaxel potential interaction (Chou–Talalay analysis)

The possible interactions between extracted fractions (phenolic, terpene) and docetaxel therapy on the AMJ13 and SK-GT-4 cell lines were analyzed. The quantification of synergism or antagonism is defined as a mass-action law issue (determined by the combination index CI values) and cannot be determined by the statistical p values.[22] Chou-Talalay equations were used to calculate the combination's value. A CI value less than 0.9, the effects were assumed to be synergistic; a CI value between 0.9 and 1.1, the effects were considered as additive; while a value larger than 1.1, the effects were assumed to be antagonistic.[25] After an exposure period of 72 hours, the phenolic fraction of *P. arabica* with docetaxel produced a strong to very strong synergic cytotoxic effect against AMJ13 and SK-GT-4 cancer cell lines in comparison with a single treatment. The terpene fraction showed almost the same synergism effect when combined with docetaxel (Figure 5).

Morphological analysis (crystal violet staining)

Morphological alterations were examined with crystal violet staining of the cell lines after 72 hr. Exposure to the IC_{50} of the phenolic fraction, terpene, docetaxel, and their combinations with chemotherapy as shown in Figures 6, 7 and 8. The captured images are for cells treated with (100 µg/ml) of the tested fractions or their combinations with docetaxel on cancer or normal cell lines.

Selectivity index

The provided Table 4 illustrates the selectivity index for the tested compound in this study.

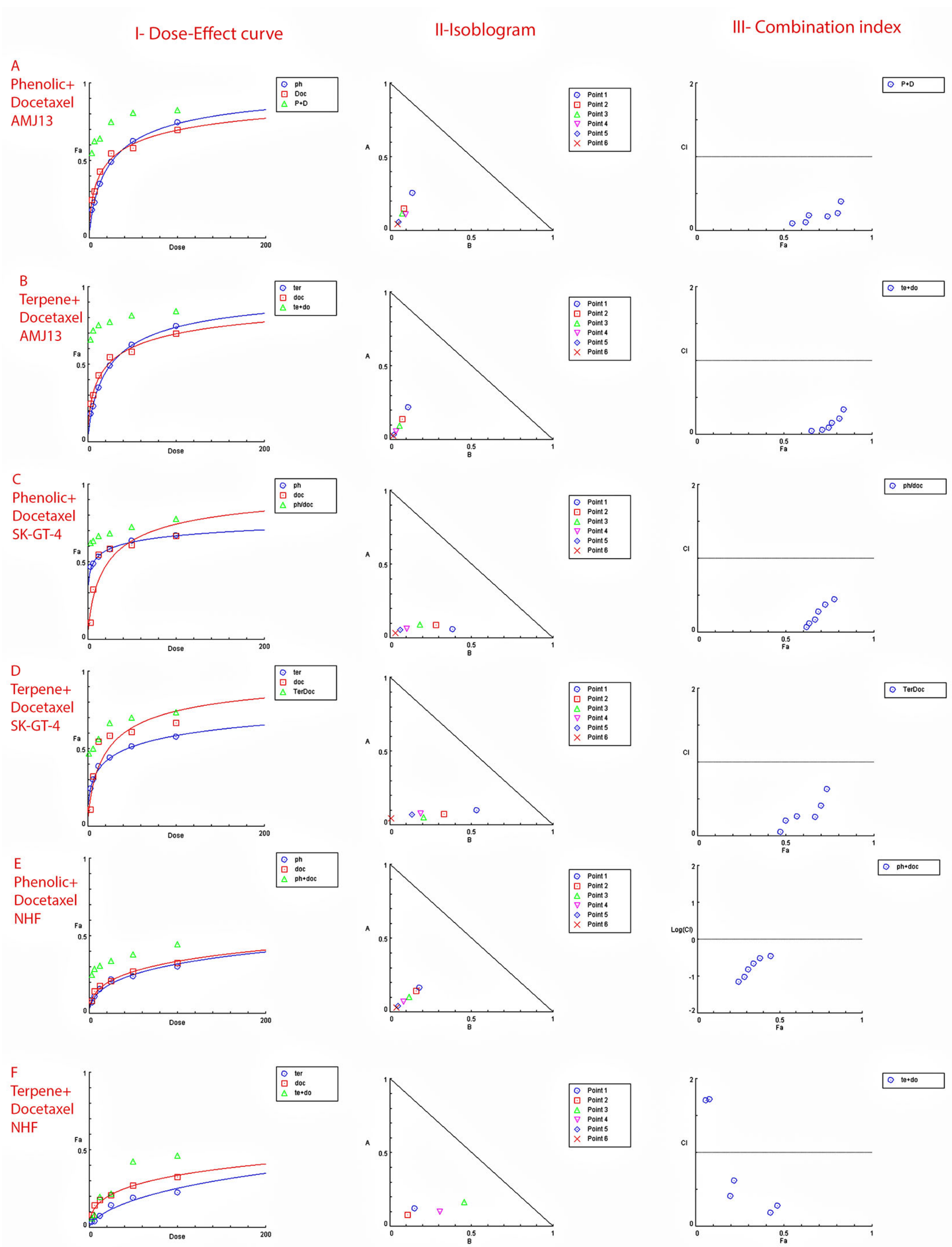


Figure 5. The dose–response curves, the normalized isobologram and combination index with non-constant ratios. Data is expressed as fraction affected (fa) against combination index plots. Combination index (CI) value less than 0.9 indicates synergy, CI values between 0.9 and 1.1 indicates additive, and CI value larger than 1.1 indicates an antagonism. (A and B) Represents the effect on AMJ13 cell line by phenolic-docetaxel and terpene-docetaxel combinations respectively, all points are showing synergy to very strong synergy; (C and D) represents the effect on SK-GT-4 cell line by phenolic-docetaxel and terpene-docetaxel combinations respectively, all of the points are showing synergy to very strong. (E and F) Show the effect on the NHF cell line by phenolic-docetaxel and terpene-docetaxel combinations respectively, tested points showed inconsiderable cytotoxicity as all concentrations failed to emerge 50% cytotoxicity. The data represents six separate experiments.

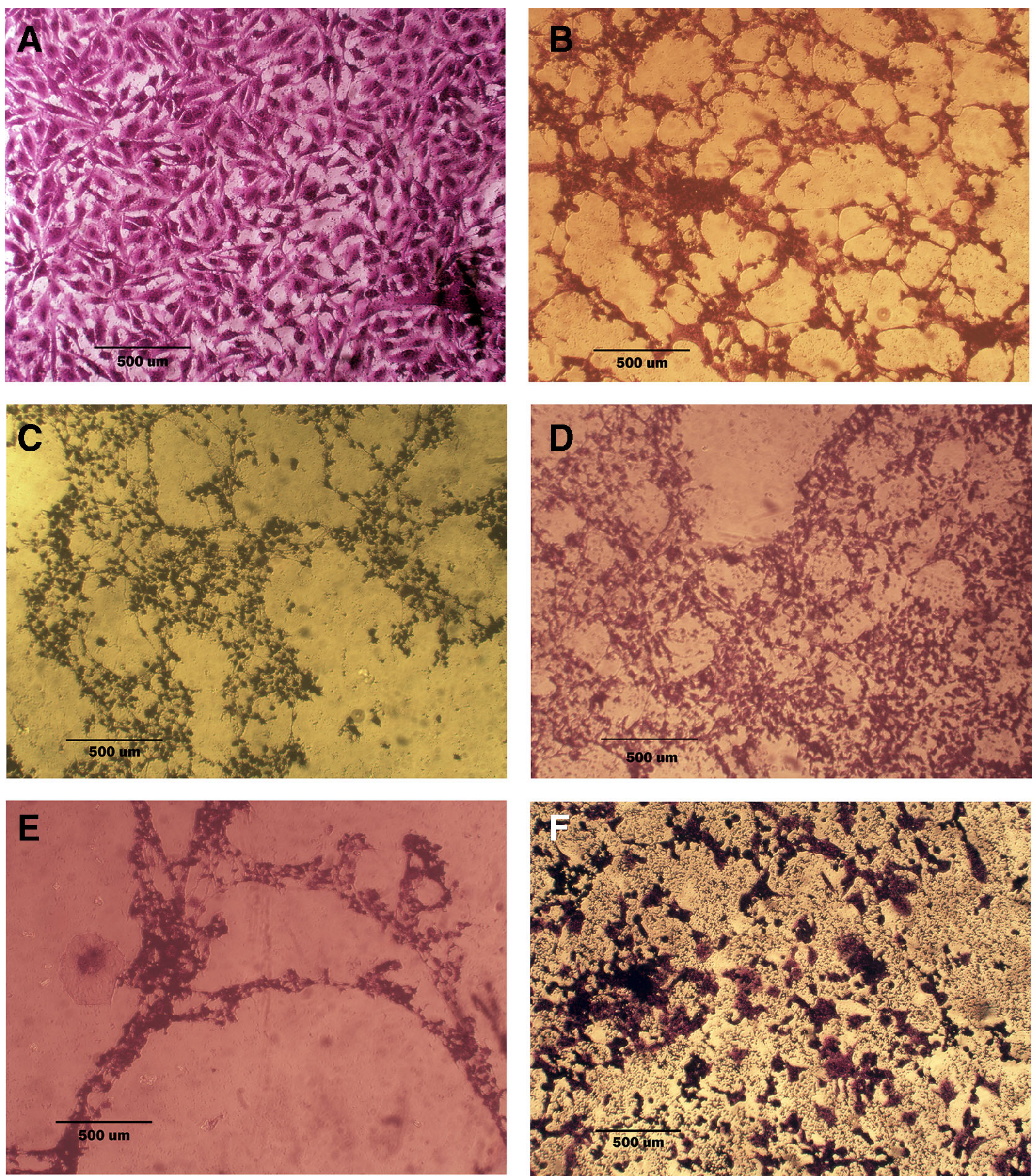


Figure 6. Morphology of AMJ13 cell line. A: untreated cells show multipolar elongated epithelial-like shape, with multiple nuclei in most of the cells and nuclear polymorphism, many cells showed mitotic figures B, C, and D cell treated with terpene fraction phenolic fraction and docetaxel respectively; the treated cells showed a shrinkage, cytoplasm and cell membrane disappearance, stromal edema, nucleus shrinkage and marked decrease in the number of cells. E and F cells were treated with a combination therapy of phenolic plus docetaxel and terpene plus docetaxel, respectively; the treated cells showed more prominent cytotoxic effects than single treatment with a dense nucleus. The microscopic images were captured at 10× by an inverted microscope (IXplore Standard Olympus, Japan).

Discussion

Polyphenols and terpenes are the most abundant and widely distributed compound in the plant kingdoms and groups.[26] *Prunus* species have been found to be a potential dietary supplement and a good source of phenolic and terpene bioactive chemicals.[27]

In the current study, lead acetate and NaOH tests for polyphenols gave a positive result, meaning the presence of phenolic compounds in *P. arabica* extract. The dark color might indicate the presence of large quantities of polyphenols and flavonoids.[28] The H_2SO_4 test gave a dark pink or red color and greenish color, respectively, as an indication of the

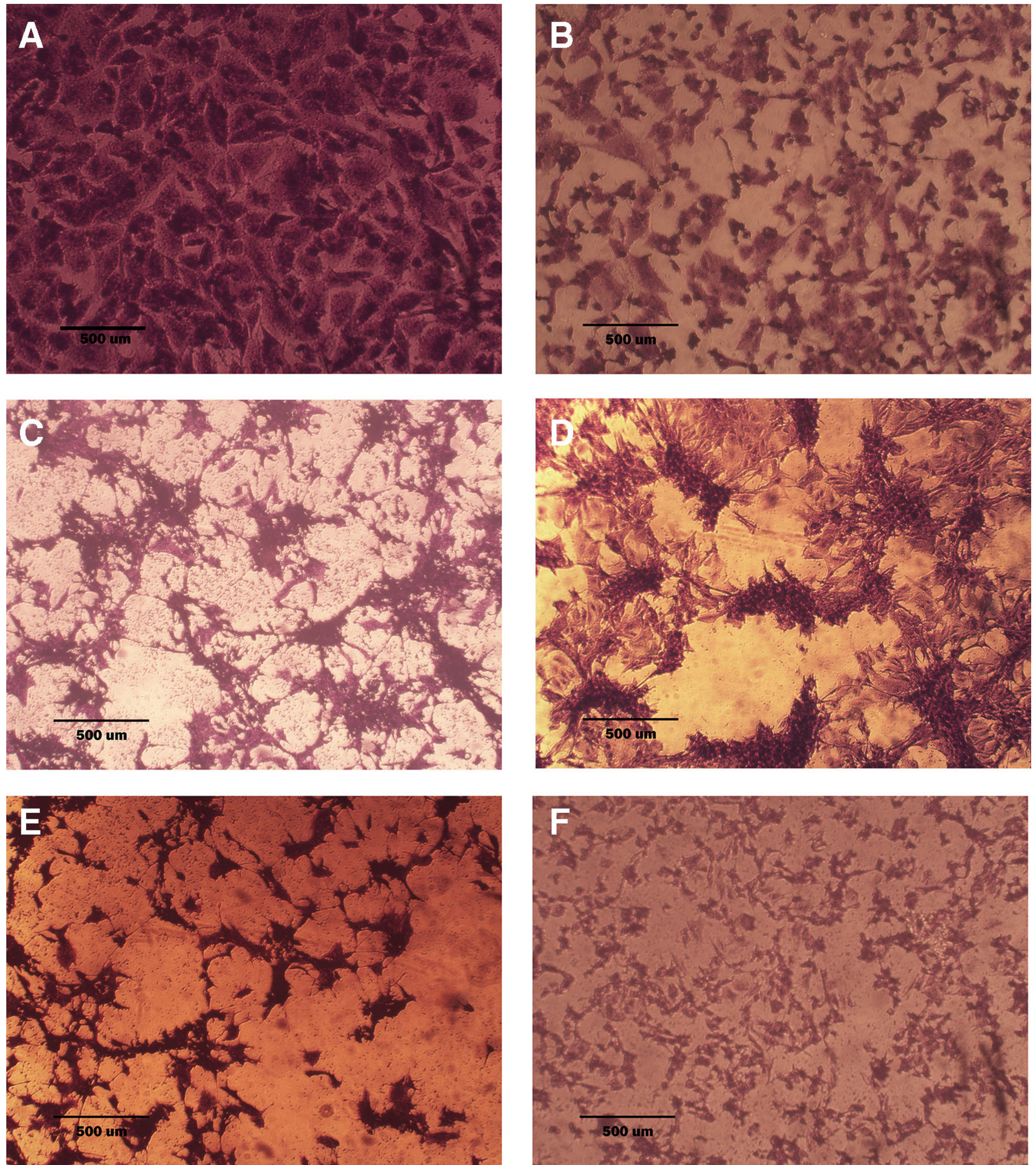


Figure 7. The morphology of SK-GT-4 cell line. A: untreated cells are squamous or poorly differentiated and irregular in shape. B: cells treated with terpene fraction showed shrinkage; the squamous cell border remained intact, while other cells (no bordered cells) showed stromal edema. C: cells treated with phenolic fraction showed an increment of the stromal edema and disappearance of squamous cells with no ductal nuclear aggregation. D: docetaxel-treated cells showed focal aggregation and cellular shrinkage. E and F cells were treated with combination therapy phenolic plus docetaxel and terpene plus docetaxel, respectively; the treated cells showed more shrinkage than single treatment (very small sized cells), the squamous cell border is intact, destruction of ductal/basal cell membranes with no focal aggregation. The microscopic images were captured at 10× by an inverted microscope (IXplore Standard Olympus, Japan).

presence of steroids, while the chloroform and sulphuric acid test produced a greyish color which was considered an indication of the presence of terpenes.

The data for the HPLC analysis showed that the phenolic fraction of the extracted *P. arabica* contains eight phenolic compounds (p-Coumaric acid, ferulic acid, gallic acid, caffeic acid, quercetin, rutin, catechin, and chlorogenic acid), the terpene fraction contained three major terpenes (β-sitosterol, stigmasterol, and campesterol) as well as some non-phenolic organic and inorganic components at deceptive values.

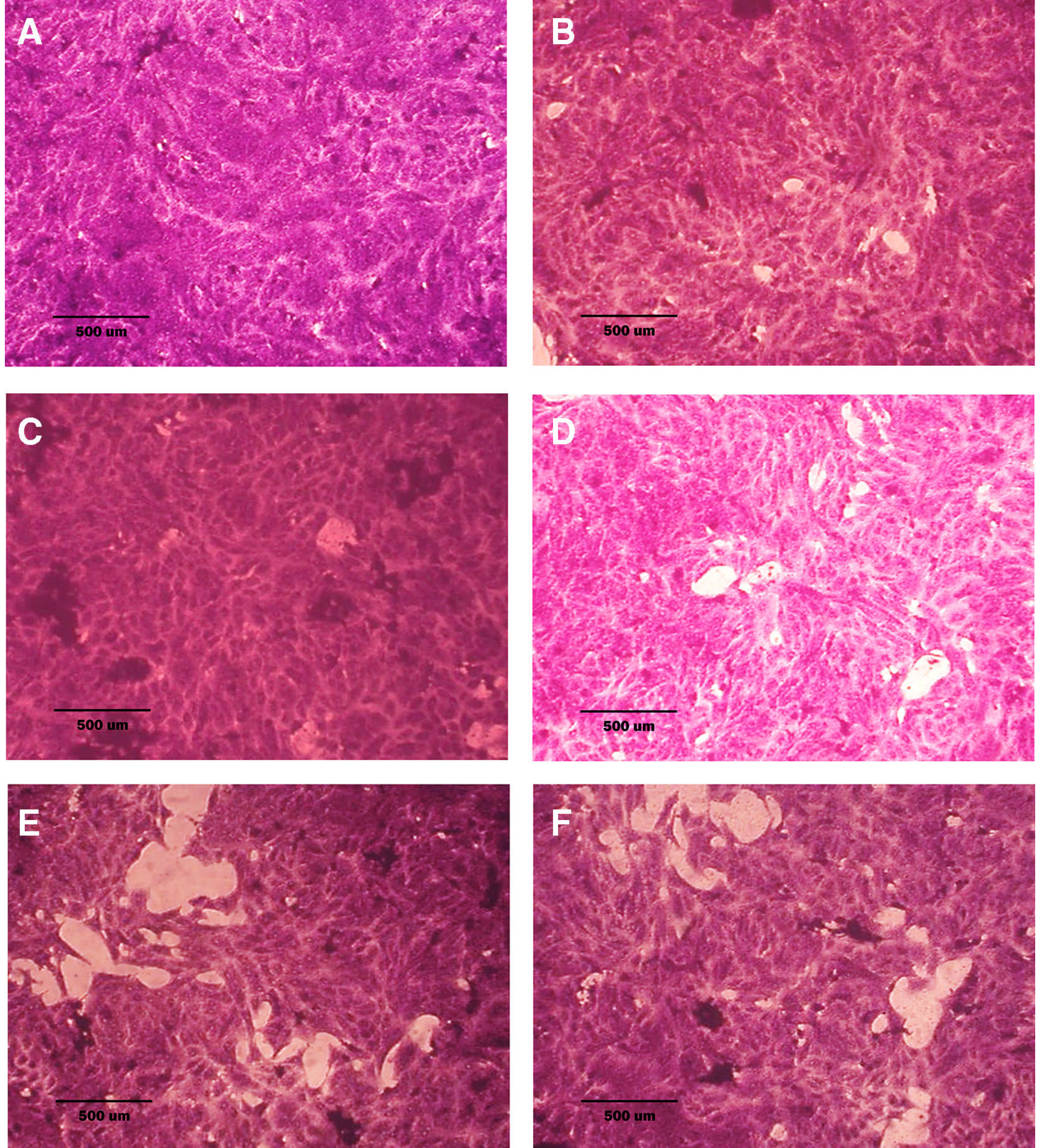


Figure 8. Morphology of NHF cell line. A: untreated cells; cells appear as plump spindle-shaped or stellate-shaped cells with centrally placed oval or round nuclei. B: cells treated with terpene fraction showed mild apoptosis. C: cells treated with phenolic fraction showed condensation of cells with no edema. D: docetaxel-treated cells showed focal apoptosis with mild stromal edema, and the cells remained the same size. E and F cells were treated with a combination therapy of phenolic plus docetaxel and terpene plus docetaxel, respectively; the treated cells showed prominent apoptosis compared to the single treatment. The microscopic images were captured at 10× by an inverted microscope (IXplore Standard Olympus, Japan).

Table 4. IC_{50} values and SI of phenolic, terpene and docetaxel treatments on cancer cell lines.

Compound	Cell line	IC_{50} µg/ml	SI
Phenolic fraction	AMJ13	29.34±2.37	0.61
	SK-GT-4	21.97±3.56	0.82
	NHF	18.07±1.83	

Table 4. *Continued*

Compound	Cell line	IC_{50} µg/ml	SI
Terpene fraction	AMJ13	8.455±3.02	3.76
	SK-GT-4	15.14±3.26	2.10
	NHF	31.81±12.07	
Docetaxel	AMJ13	14.51±0.77	1.66
	SK-GT-4	0.7125±0.084	33.81
	NHF	24.09±7.17	

Data are means ± SD of the IC_{50} in µg/ml for each treatment, against the evaluated cell lines. The selectivity index (SI) represents IC_{50} for the normal cell line divided by the IC_{50} for the cancerous cell line after 72 h. Docetaxel was used as a positive control.

From the eight phenolic compounds found in our phenolic fraction of the *P. arabica* extract, the highest component concentration was ferulic acid (3.992 mg/gm) with a retention time of 3.22 min, which may contribute to the high efficacy of the phenolic extract as a cytotoxic biological chemical on different cancer cell lines.[29] Research has indicated that ferulic acid induces cell death by decreasing the Bcl-2 and increasing the *BAX* gene expression or by upregulation of caspase-3 and cleaved caspase-9.[30]

The highest component of the terpene fraction was campesterol (4.358 mg/gm), with a retention time of 11.5 min. A study by Hyocheol B. *et al.* confirmed that campesterol could inhibit both cellular growth and cell cycle progression by regulating the PCNA (proliferating cell nuclear antigen) and PI3K/MAPK (phosphatidylinositol-3-kinase/mitogen-activated protein kinase) signal pathways. Moreover, their results also showed that campesterol could prevent the clustering of ovarian cancer cells.[30] Some undiscovered phenolic and terpene compounds may be presented by peaks on the chromatograms, hydroperoxides or peroxides produced from terpenes are likely responsible for these found but unidentified peaks.[31]

The present work studied the cytotoxic effects of the extracted fractions (phenolic and terpene) of *P. arabica* alone and in combination with docetaxel and compared their novel effects with the single chemotherapeutic agent (docetaxel) on AMJ13, SK-GT-4, and NHF cell lines. Breast cancer and esophageal carcinoma are considered highly malignant tumors, which lead to poor prognoses.[32] The low efficacy of currently available breast and esophageal cancer chemotherapeutics and radiation moreover these therapies are associated with severe adverse effects and patients can develop resistance to these agents.[33,34]

In vitro, the results of this study appear that the treatment with the phenolic and terpene extract of *P. arabica* significantly reduced cell viability and triggered apoptosis when compared to the control group in both AMJ13 and SK-GT-4 cell lines (Figures 1–2). During this study against the AMJ13 cell line, the terpene fraction showed comparable cytotoxic effects to docetaxel even in concentrations as low as 25 µg/ml, the IC_{50} for phenolic terpene and docetaxel against AMJ13 cell line was 29.34±2.37, 8.455±3.02 and 14.51±0.77 µg/ml respectively (Figure 4).

The phenolic fraction showed almost an equal cytotoxicity to docetaxel against the SK-GT-4 cell line, while terpene showed less significant cytotoxicity in comparison with other tested treatments; the IC_{50} for phenolic terpene and docetaxel against SK-GT-4 was 21.97±3.56, 15.14±3.26, 0.7125±0.084 µg/ml respectively as shown in Figure 4. All treatment concentrations for phenolic and terpene fractions failed to show significant cytotoxicity on the NHF cell line (less than 50%).

Many unique chemical components, including polyphenols, flavonoids, alkaloids, and terpenes, have been identified from *Prunus* species. The great structural variety of these compounds underlies their unique biological properties, which include bioavailability, antioxidant activity, and specific interactions with cell receptors and enzymes.[35]

Researchers have found that flavonoids have a wide range of biological effects in mammals, including antimicrobial, antiviral, analgesic, anti-allergic, hepatoprotective, cytostatic, and apoptotic properties.[36]

The current study confirms the findings of previous studies which showed that phytosterols, such as quercetin and β-sitosterol, protect against a wide variety of diseases and exhibit selective cytotoxicity towards cancer cells, as evidenced by high apoptosis indices in cells exposed to quercetin's anticancer activity.[37]

Many studies have demonstrated that flavonoids have cytotoxic effects, including modulating ROS-scavenging enzyme activities, cell cycle arrest, induction of apoptosis and autophagy, and suppression of cancer cell proliferation and invasiveness. In healthy cells, flavonoids function as antioxidants, but in cancer cells, they become strong pro-oxidants that induce apoptotic pathways and downregulate pro-inflammatory signals.[38]

Stigmasterol's cytotoxic actions come from its ability to induce autophagy in tumor cells, decrease their proliferation and spread, and promote their apoptosis.[39]

The increased sensitivity of cancer cells to cytotoxicity was another goal of this investigation by using phenolic or terpene fractions with docetaxel and the cumulative effects of many dosages. The MTT assay performed with docetaxel in the presence of varying amounts of phenolic or terpene fractions. According to the findings, the phenolic, terpene, and docetaxel combination substantially decreased cancer cell viability without causing appreciable damage to normal cells. The Chou-Talalay equation was used to evaluate the combinations.

The degree of synergy or antagonism cannot be assessed by p value in a statistical manner but can be quantified using CI values (combination index values).[40] Nearly all of the doses examined showed synergistic cytotoxicity against the cancer cell lines. To demonstrate their safety, testing on a normal human fibroblast cell line showed no effect at any dose of the combination of phenolic and terpene fractions with docetaxel. There have been a number of studies suggesting that phenolic acids and terpenes may boost the effect of other chemotherapies on breast cancer.[41] However, this is the first study to provide empirical evidence of synergy between phenolic and terpene fractions with docetaxel on AMJ13 and SK-GT-4 cell line (Figure 5 and Table 5).

Table 5. AMJ13, SK-GT-4, and NHF cells were treated with phenolic fractions or terpene fractions in combination with docetaxel and after 72 hours at the indicated doses, an MTT assay was conducted.

A		AMJ13	Phenolic with Docetaxel		
Points	**Ph. Conc ug/ml**	**Doc. Conc. ug/ml**	**Cytotoxicity**	**CI**	**Effect**
1	50	50	82.6%	0.396	Synergism
2	25	25	80.7%	0.238	Strong Synergism
3	12.5	12.5	75.0%	0.194	Strong Synergism
4	6.25	6.25	64.3%	0.209	Strong Synergism
5	3.125	3.125	62.7%	0.116	Strong Synergism
6	1.5625	1.5625	54.9%	0.095	Very Strong Synergism
B		**AMJ13**	**Terpene with Docetaxel**		
Points	**Ter Conc ug/ml**	**Doc. Conc. ug/ml**	**Cytotoxicity**	**CI**	**Effect**
1	50	50	84.2%	0.334	Synergism
2	25	25	81.7%	0.216	Strong Synergism
3	12.5	12.5	77.5%	0.158	Strong Synergism
4	6.25	6.25	75.5%	0.093	Very Strong Synergism
5	3.125	3.125	71.9%	0.062	Very Strong Synergism
6	1.5625	1.5625	66.0%	0.047	Very Strong Synergism
C		**SK-GT-4**	**Phenolic plus Docetaxel**		
Points	**Ph. Conc ug/ml**	**Doc. Conc. ug/ml**	**Cytotoxicity**	**CI**	**Effect**
1	50	50	77.8%	0.443	Synergism
2	25	25	72.5%	0.373	Synergism
3	12.5	12.5	68.6%	0.276	Strong Synergism
4	6.25	6.25	66.7%	0.168	Strong Synergism
5	3.125	3.125	63.5%	0.119	Strong Synergism
6	1.5625	1.5625	62.1%	0.069	Very Strong Synergism

Table 5. *Continued*

D		SK-GT-4	Terpene plus Docetaxel		
Points	**Ter Conc ug/ml**	**Doc. Conc. ug/ml**	**Cytotoxicity**	**CI**	**Effect**
1	50	50	73.5%	0.632	Synergism
2	25	25	70.2%	0.407	Synergism
3	12.5	12.5	66.8%	0.261	Strong Synergism
4	6.25	6.25	56.5%	0.265	Strong Synergism
5	3.125	3.125	50.0%	0.206	Strong Synergism
6	1.5625	1.5625	47.2%	0.054	Very Strong Synergism
E		**NHF**	**Phenolic plus Docetaxel**		
Points	**Ph. Conc ug/ml**	**Doc. Conc. ug/ml**	**Cytotoxicity**	**CI**	**Effect**
1	50	50	44.6%	0.346	Synergism
2	25	25	38.1%	0.307	Strong Synergism
3	12.5	12.5	34.1%	0.222	Strong Synergism
4	6.25	6.25	30.9%	0.152	Strong Synergism
5	3.125	3.125	28.7%	0.095	Very Strong Synergism
6	1.5625	1.5625	25.0%	0.071	Very Strong Synergism
F		**NHF**	**Terpene plus Docetaxel**		
Points	**Ter Conc ug/ml**	**Doc. Conc. ug/ml**	**Cytotoxicity**	**CI**	**Effect**
1	50	50	46.4%	0.277	Strong Synergism
2	25	25	42.5%	0.187	Strong Synergism
3	12.5	12.5	22.0%	0.622	Synergism
4	6.25	6.25	19.8%	0.407	Synergism
5	3.125	3.125	8.1%	1.724	Antagonism
6	1.5625	1.5625	6.0%	1.708	Antagonism

CompuSyn® was used to determine combination index. (A, B) show combination index (CI) values on the AMJ13 cancer cell line of the combined phenolic fraction with docetaxel and the terpene fraction with docetaxel respectively. (C, D) Represent the CI values of the phenolic fraction with docetaxel and the terpene fraction with docetaxel respectively, on the SK-GT-4 cancer cell line. (E, F) CI values of the phenolic fraction with docetaxel and the terpene fraction with docetaxel respectively, on the NHF non-cancer cell line. The effects have been described according to Hernández *et al.*, 2013.[42]

As seen in the current study and after crystal violet staining, the AMJ13, SK-GT-4, and NHF cells that were exposed to 72h of extracted fractions and docetaxel and the combination therapy revealed cell shrinkage, cytoplasm and cell membrane disappearance, stromal edema, nucleus shrinkage and a marked decrease in the number of cells compared with control (untreated) cells. The effect of combination therapy was more prominent than single therapy as the cells showed more shrinkage, extensive cell damage, and necrosis.

As a result of their specificity, chemopreventive medicines only target cancer cells. A compound's ability to selectively destroy cancer cells while having a minimal effect on healthy cells is measured by its "selectivity index."

The compound's low selectivity index suggests that it is less hazardous to healthy cells than cancerous ones. Compounds having high SI values may provide a safer and more effective cancer treatment option.[43]

Based on the test results the phenolic fraction is found to be less selective for all tested cells (SI<3). Meanwhile, terpene fraction is selective for AMJ13 cancer cells with (SI>3) and less selective for SK-GT-4 cancer cells. The Docetaxel treatment (positive control) showed an excellent SI toward SK-GT-4 cell line (SI>3). The results of this study need a further evaluation to determine the potential cytotoxicity in animal models.

Conclusion

It was observed from the results that the *P. arabica* phenolic and terpene extracts have significant cytotoxic activity on breast cancer and esophageal cancer cell lines with minimal effect on normal cells, due to the presence of effective compounds in this extract. Moreover, these active compounds increased the cytotoxic activity of docetaxel on cancer cell

lines without increasing the toxicity on normal cells. Previous studies proved the cytotoxic effect of phenolic and phytosterol fractions extracted from other *Prunus* species.

Data availability

Underlying data

Zenodo: Terpene Fractions Extracted from Iraqi Prunus arabica on AMJ13 and SK-GT-4 Human Cancer Cell Lines, https://doi.org/10.5281/zenodo.7618326.[44]

The project contains the following underlying data:

- Figure 1 - percent of cytotoxicity AMJ13 cell line.csv (the table illustrates the percent of cytotoxicity of serial concentrations of each fraction alone and combined with docetaxel for AMJ13 cancer cell line)
- Figure 2 - percent of cytotoxicity SK-GT-4 cell line.csv
- Figure 3 - percent of cytotoxicity - NHF cell line.csv (the table illustrates the percent of cytotoxicity of serial concentrations of each fraction alone and combined with docetaxel for NHF cell line)
- Figure 4 - A - IC 50 Docetaxel- AMJ13 cell line.csv (Cell viability values for serial concentrations of docetaxel in triplicate. Docetaxel exhibited cytotoxicity against AMJ13 cancer cell line with IC50 values of 14.51±0.77 μg/ml)
- Figure 4 - A - IC 50 Phenolic fraction-AMJ13 cell line.csv (Cell viability values for serial concentrations of phenolic fraction in triplicate. Phenolic fraction exhibited cytotoxicity against AMJ13 cancer cell line with IC50 values of 29.34±2.37 μg/ml)
- Figure 4 - A - IC 50 Terpene fraction-AMJ13 cell line.csv (Cell viability values for serial concentrations of terpene fraction in triplicate. Terpene fraction exhibited cytotoxicity against AMJ13 cancer cell line with IC50 values of 8.455±3.022 μg/ml)
- Figure 4 - B - IC50 Docetaxel- SK-GT-4 cell line.csv (Cell viability values for serial concentrations of docetaxel in triplicate. Docetaxel exhibited cytotoxicity against SK-GT-4 cancer cell line with IC50 values of 0.7125±0.084 μg/ml)
- Figure 4 - B - IC50 Phenolic fraction-SK-GT-4 cell line.csv (Cell viability values for serial concentrations of phenolic fraction in triplicate. Phenolic fraction exhibited cytotoxicity against SK-GT-4 cancer cell line with IC50 values of 21.97±3.56 μg/ml)
- Figure 4 - B - IC50 Terpene fraction-SK-GT-4 cell line.csv (Cell viability values for serial concentrations of terpene fraction in triplicate. Terpene fraction exhibited cytotoxicity against SK-GT-4 cancer cell line with IC50 values of 15.14±3.266 μg/ml)
- Figure 4 - C - Docetaxel- IC50 NHF cell line.csv (Cell viability values for serial concentrations of docetaxel in triplicate. Docetaxel exhibited cytotoxicity against NHF cell line with IC50 values of 24.9±7.17 μg/ml)
- Figure 4 - C - phenolic fraction- IC50 NHF cell line.csv (Cell viability values for serial concentrations of phenolic fraction in triplicate. Phenolic fraction exhibited cytotoxicity against NHF cell line with IC50 values of 18.07±1.83 μg/ml)
- Figure 4 - C - Terpene fraction- IC50 NHF cell line.csv (Cell viability values for serial concentrations of terpene fraction in triplicate. Terpene fraction exhibited cytotoxicity against NHF cell line with IC50 values of 31.81±12.07 μg/ml)
- Figure 5 - A - Phenolic plus docetaxel- AMJ13.html (Cytotoxicity of *Prunus arabica* phenolic extract combination with docetaxel on AMJ13 cancer cell line. I-Dose-Effect Curve at 50% cytotoxicity, II-Isobologram analysis displays synergism rate between phenolic fraction and docetaxel at all points of the combination as they located at the lower left of the hypotenuse, demonstrating the effect is synergistic at a 50% cytotoxicity dose, III-showing the combination index data location for each dose.)

- Figure 5 - C - Phenolic plus docetaxel SK-GT-4.html (Cytotoxicity of *Prunus arabica* phenolic extract combination with docetaxel on SK-GT-4 cancer cell line. I-Dose-Effect Curve at 50% cytotoxicity, II-Isobologram analysis displays synergism rate between phenolic fraction and docetaxel at all points of the combination as they located at the lower left of the hypotenuse, demonstrating the effect is synergistic at a 50% cytotoxicity dose, III-showing the combination index data location for each dose.)

- Figure 5 - F - Terpene plus docetaxel NHF.html (Checking the possible combination cytotoxicity on NHF cell line. There is no cytotoxic effect for terpene fraction on NHF cell line in combination with docetaxel on cell viability. I-Dose–response curve at shows response less than 50%, II-Isobologram analysis showing no synergism between terpene fraction and docetaxel against NHF at all doses tested, III-The figure of combination index showed the absence of any synergistic points.)

- Figure 5 - E - Phenolic plus docetaxel NHF.html (Checking the possible combination cytotoxicity on NHF cell line. There is no cytotoxic effect for phenolic fraction on NHF cell line in combination with docetaxel on cell viability. I-Dose–response curve at shows response less than 50%, II-Isobologram analysis showing no synergism between terpene fraction and docetaxel against NHF at all doses tested, III-The figure of combination index showed the absence of any synergistic points.)

- Figure 5 - B - Terpene plus docetaxel AMJ13.html (Cytotoxicity of *Prunus arabica* terpene fraction combination with docetaxel on AMJ13 cancer cell line. I-Dose-Effect Curve at 50% cytotoxicity, II-Isobologram analysis displays synergism rate between terpene fraction and docetaxel at all points of the combination as they located at the lower left of the hypotenuse, demonstrating the effect is synergistic at a 50% cytotoxicity dose, III-showing the combination index data location for each dose.)

- Figure 5 - D - Terpene plus docetaxel SK-GT-4.html (Cytotoxicity of *Prunus arabica* terpene fraction combination with docetaxel on SK-GT-4 cancer cell line. I-Dose-Effect Curve at 50% cytotoxicity, II-Isobologram analysis displays synergism rate between terpene fraction and docetaxel at all points of the combination as they located at the lower left of the hypotenuse, demonstrating the effect is synergistic at a 50% cytotoxicity dose, III-showing the combination index data location for each dose.)

- Table 2 - HPLC phenolic fraction.pdf (The table illustrates the composition of phenolic fraction, retention time and their concentrations)

- Table 3 - HPLC Sterol fraction.pdf (The table illustrates the composition of terpene fraction, retention time and their concentrations)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Extended data

2- protocols.io: A full step by step assay protocols

Plant Extraction and Fractionation, https://dx.doi.org/10.17504/protocols.io.q26g7y6bqgwz/v1.[45]

Preliminary qualitative phytochemical analysis, https://dx.doi.org/10.17504/protocols.io.e6nvwj27wlmk/v1.[46]

MTT (Assay protocol), https://dx.doi.org/10.17504/protocols.io.eq2ly72emlx9/v1.[47]

References

1. Sung H, Ferlay J, Siegel RL, *et al.*: **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.** *CA Cancer J. Clin.* 2021 May 1 [cited 2022 Sep 10]; **71**(3): 209–249.
PubMed Abstract | Publisher Full Text

2. Fitzmaurice C, Abate D, Abbasi N, *et al.*: **Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study.** *JAMA Oncol.* 2019 Dec 1 [cited 2022 Sep 10]; **5**(12): 1749–1768.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Senapati S, Mahanta AK, Kumar S, *et al.*: **Controlled drug delivery vehicles for cancer treatment and their performance.** *Signal Transduct. Target. Ther.* 2018 Dec 1 [cited 2022 Sep 8]; **3**(1): 7.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Housman G, Byler S, Heerboth S, *et al.*: **Drug Resistance in Cancer: An Overview.** *Cancers (Basel).* 2014 Sep 5 [cited 2022 Sep 9]; **6**(3): 1769–1792.
PubMed Abstract | Publisher Full Text | Free Full Text

5. Bhanot A, Sharma R, Noolvi MN: *Natural Sources as Potential Anti-Cancer Agents A Review. Phytomedicine, 3, 9-26. - References.* Scientific Research Publishing; 2011 [cited 2022 Sep 10].
Reference Source

6. Tavakoli J, Miar S, Zadehzare MM, *et al.*: **Evaluation of Effectiveness of Herbal Medication in Cancer Care: A Review Study.** *Iran J. Cancer Prev.* 2012 [cited 2022 Sep 9]; **5**(3): 144–156.
PubMed Abstract | Free Full Text

7. Ekins S, Mestres J, Testa B: **In silico pharmacology for drug discovery: applications to targets and beyond.** *Br. J. Pharmacol.* 2007 Sep [cited 2022 Sep 11]; **152**(1): 21–37.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Roskov Y, Ower G, Orrell T, *et al.*: **Annual Checklist. Species 2000 & ITIS catalogue of life 2019 Annual Checklist. 2019.** 2000; 2405–884.
Reference Source

9. Brukental H, Doron-Faigenboim A, Bar-Ya'akov I, *et al.*: **Revealing the Genetic Components Responsible for the Unique Photosynthetic Stem Capability of the Wild Almond *Prunus arabica* (Olivier) Meikle.** *Front. Plant Sci.* 2021 Nov 25; **12**: 2583.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Kitic D, Miladinovic B, Randjelovic M, *et al.*: **Anticancer Potential and Other Pharmacological Properties of *Prunus armeniaca* L.: An Updated Overview.** *Plants (Basel).* 2022 Jul 1 [cited 2022 Sep 20]; **11**(14).
Publisher Full Text | Reference Source

11. Poonam V, Raunak, Kumar G, *et al.*: **Chemical Constituents of the Genus Prunus and their Medicinal Properties.** *Curr. Med. Chem.* 2011 Oct 16; **18**(25): 3758–3824.
PubMed Abstract | Publisher Full Text

12. Tomko AM, Whynot EG, O'Leary LF, *et al.*: **Anti-cancer potential of cannabis terpenes in a Taxol-resistant model of breast cancer.** *Can. J. Physiol. Pharmacol.* 2022 Aug 1 [cited 2022 Sep 21]; **100**(8): 806–817.
PubMed Abstract | Publisher Full Text

13. Harborne JB: *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 1st ed. London: Chapman and Hall; New York. Dordrecht: Springer Netherlands. 1991 [cited 2022 Aug 20]; pp. 278–278.
Publisher Full Text

14. Kokate CK, Gokhale SB, Purohit AP: **A Textbook of Pharmacognosy. 29 st. Nirali Prakashan.** 2009; 635 p.

15. Radovanović B, Mladenović J, Radovanovic A, *et al.*: **Phenolic Composition, Antioxidant, Antimicrobial and Cytotoxic Activites of *Allium porrum* L. (Serbia) Extracts. undefined.** 2015.

16. Majeed Al-Shammari A, Alshami MA, Abbas Umran M, *et al.*: **Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient.** *Breast Cancer.* 2015; **7**: 223–230.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Safi IN, Mohammed Ali Hussein B, Al-Shammari AM: **In vitro periodontal ligament cell expansion by co-culture method and formation of multi-layered periodontal ligament-derived cell sheets.** *Regen. Ther.* 2019 Dec 1; **11**: 225–239.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Contino G, Eldridge MD, Secrier M, *et al.*: **Whole-genome sequencing of nine esophageal adenocarcinoma cell lines [version 1; referees: 3 approved].** *F1000Res.* 2016 [cited 2022 Aug 21]; **5**: 1336.
PubMed Abstract | Free Full Text

19. Adil BH, Al-Shammari AM, Murbat HH: **Breast cancer treatment using cold atmospheric plasma generated by the FE-DBD scheme.** *Clin. Plasma Med.* 2020 Sep 1; **19-20**: 100103–100120.
Publisher Full Text

20. Abdullah SA, Al-Shammari AM, Lateef SA: **Attenuated measles vaccine strain have potent oncolytic activity against Iraqi patient derived breast cancer cell line.** *Saudi J. Biol. Sci.* 2020 Mar 1 [cited 2022 Aug 21]; **27**(3): 865–872.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Al-Ziaydi AG, Al-Shammari AM, Hamzah MI, *et al.*: **Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death.** *Virusdisease.* 2020 Sep 1 [cited 2022 Aug 21]; **31**(3): 341–348.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Chou TC: **Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method.** *Cancer Res.* 2010 [cited 2022 Oct 9]; **70**(2): 44–46.
Reference Source

23. Al-Jewari H, AHM AL-F, Nader M: **In vitro Cytotoxic Activity of the L-asparaginase Extracted and Purified from Pathogenic Escherichia coli Against four Leukemic cell lines**. *In: 2nd Annual International Conference of Northest Pharmacy Research, Faculty of Pharmacy. Mahasarkham University, Thailand.* 2010. pp. 21–23.

24. Sutejo IR, Putri H, Meiyanto E: **The Selectivity of Ethanolic Extract of Buah Makassar (Brucea javanica) on Metastatic Breast Cancer Cells.** *J. Agromedicine Med. Sci.* 2016; **2**(1): 1.
Publisher Full Text

25. Chou TC: **Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies.** *Pharmacol. Rev.* 2006 [cited 2022 Oct 9]; **58**(3): 621–681.
PubMed Abstract | Publisher Full Text

26. Ninkuu V, Zhang L, Yan J, *et al.*: **Biochemistry of Terpenes and Recent Advances in Plant Protection.** *Int. J. Mol. Sci.* 2021 Jun 1 [cited 2022 Oct 16]; **22**(11).
PubMed Abstract | Publisher Full Text | Free Full Text

27. Martini S, Conte A, Tagliazucchi D: **Phenolic compounds profile and antioxidant properties of six sweet cherry (*Prunus avium*) cultivars.** *Food Res. Int.* 2017 Jul 1; **97**: 15–26.
PubMed Abstract | Publisher Full Text

28. Galili S, Hovav R: **Determination of Polyphenols, Flavonoids, and Antioxidant Capacity in Dry Seeds.** *Polyphenols Plants.* 2014; 305–323.
Publisher Full Text

29. Marcato DC, Spagnol CM, Salgado HRN, *et al.*: **New and potential properties, characteristics, and analytical methods of ferulic acid: A review.** *Braz. J. Pharm. Sci.* 2022 Apr 22 [cited 2023 Jun 5]; **58**: e18747.
Publisher Full Text | Reference Source

30. Bae H, Park S, Yang C, *et al.*: **Disruption of Endoplasmic Reticulum and ROS Production in Human Ovarian Cancer by Campesterol.** *Antioxidants.* 2021 Mar 1 [cited 2022 Nov 27]; **10**(3): 1–18.
Publisher Full Text | Free Full Text

31. Calandra MJ, Impellizzeri J, Wang Y: **An HPLC method for hydroperoxides derived from limonene and linalool in citrus oils, using post-column luminol-mediated chemiluminescence detection.** *Flavour Fragr. J.* 2015 Mar 1; **30**(2): 121–130.
Publisher Full Text

32. Tustumi F, Kimura CMS, Takeda FR, *et al.*: **PROGNOSTIC FACTORS AND SURVIVAL ANALYSIS IN ESOPHAGEAL CARCINOMA.** *Arq. Bras. Cir. Dig.* 2016 Jul 1 [cited 2022 Oct 20]; **29**(3): 138–141.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Burguin A, Diorio C, Durocher F: **Breast cancer treatments: Updates and new challenges.** *J. Pers. Med.* 2021; **11**(8).
PubMed Abstract | Publisher Full Text | Free Full Text

34. Yang YM, Hong P, Xu WW, *et al.*: **Advances in targeted therapy for esophageal cancer.** *Signal Transduct. Target. Ther.* 2020 Oct 7 [cited 2023 Jun 5]; **5**(1): 1–11.
Reference Source

35. Liu W, Nan G, Nisar MF, *et al.*: **Chemical Constituents and Health Benefits of Four Chinese Plum Species.** *J. Food Qual.* 2020; **2020**.
Publisher Full Text

36. Ullah A, Munir S, Badshah SL, *et al.*: **Important flavonoids and their role as a therapeutic agent.** *Molecules.* 2020; **25**.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Maiyo F, Moodley R, Singh M: **Phytochemistry, cytotoxicity and apoptosis studies of B-sitosterol-3-oglucoside and B -amyrin from prunus Africana.** *Afr. J. Tradit. Complement. Altern. Med.* 2016; **13**(4): 105–112.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Kopustinskiene DM, Jakstas V, Savickas A, *et al.*: **Flavonoids as Anticancer Agents.** *Nutrients.* 2020 Feb 1 [cited 2023 Jun 5]; **12**(2): 457.
PubMed Abstract | Publisher Full Text | Free Full Text

39. Zhang X, Wang J, Zhu L, *et al.*: **Advances in Stigmasterol on its anti-tumor effect and mechanism of action.** *Front. Oncol.* 2022; **12**: 1101289.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Chou T-C, Martin N: *CompuSyn for drug combinations: PC software and user's guide: a computer program for quantitation of synergism and antagonism in drug combinations, and the determination of IC50 and ED50 and LD50 values.* Paramus, NJ: ComboSyn; 2005 [cited 2022 Oct 23].
Reference Source

41. Gautam M, Thapa RK, Gupta B, *et al.*: **Phytosterol-loaded CD44 receptor-targeted PEGylated nano-hybrid phyto-liposomes for synergistic chemotherapy.** *Expert Opin. Drug Deliv.* 2020 Mar 3 [cited 2022 Oct 23]; **17**(3): 423–434.
PubMed Abstract | Publisher Full Text

42. Hernández JL, Padilla L, Dakhel S, *et al.*: **Therapeutic Targeting of Tumor Growth and Angiogenesis with a Novel Anti-S100A4 Monoclonal Antibody.** *PLoS One.* 2013 Sep 4; **8**(9): e72480.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Segun PA, Ogbole OO, Ismail FMD, *et al.*: **Resveratrol derivatives from Commiphora africana (A. Rich.) Endl. display cytotoxicity and selectivity against several human cancer cell lines.** *Phytother. Res.* 2019; **33**(1): 159–166.
PubMed Abstract | Publisher Full Text

44. Mahmood M, Abdulkareem A, Enas K: **Assessing the Cytotoxicity of Phenolic and Terpene Fractions Extracted from Iraqi Prunus arabica on AMJ-13 and SK-GT-4 Human Cancer Cell Lines.** 2023 Feb 8 [cited 2023 Feb 18].
Reference Source

45. Mahmood M, Kadhim EJ, Abd AH: **Plant Extraction and Fractionation. protocols.io.** 2023.
Publisher Full Text

46. Mahmood M, Abd AH, Kadhim EJ: **Preliminary qualitative phytochemical analysis. protocols.io.** 2023.
Publisher Full Text

47. Abd AH, Kadhim EJ, Mahmood M: **MTT (Assay protocol). protocols.io.** 2023.
Publisher Full Text

Open Peer Review

Current Peer Review Status:

Version 2

Reviewer Report 04 June 2024

https://doi.org/10.5256/f1000research.150979.r281243

© 2024 El-Nashar H. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Heba A. S. El-Nashar
Ain Shams University, Cairo, Egypt

This article describes the cytotoxicity of phenolic and terpene fractions extracted from Iraqi Prunus arabica on AMJ13 and SK-GT-4 human cancer cell lines. The article is well-written, some points need to be thoroughly revised to be considered for publication. The following are some comments to be considered:

1. Title... please change “on” to “against”.
2. Abstract....Please, Remove the following word: According to the findings, as conclusion
3. The following statement” The study aimed to evaluate the cytotoxicity of phenolic and terpene fractions extracted from Iraqi Prunus arabica on breast (AMJ13) and esophageal (SK-GT-4) cancer cell lines by using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide).” Should be transferred to the background.
4. Prunus arabica should be written in italic, Please Check throughout the whole manuscript.
5. Keyword should be written in alphabetical order.
6. Introduction--Some sentences lack citations, please Check them thoroughly.
7. Introduction & Discussion--Please consider the recent citations in the field of cytotoxic plant extracts as follows; https://doi.org/10.1080/14786419.2020.1765343; https://doi.org/10.1186/s12906-023-04164-9; https://doi.org/10.1016/j.jpba.2024.116121; https://doi.org/10.1002/cbdv.202300847.
8. Materials and methods...Please, define which part of the plant did you use?
9. Materials and methods- Please, Acknowledge the taxonomist who identify this plant and his affiliation.
10. Please, Mention the voucher sample of the plant and its code.
11. Discussion....You should correlate the cytotoxic activity with the identified compounds of the extract.
12. Conclusion part... It is important to mention the limitations and the future perspectives regarding this study.
13. Some grammatical errors should be corrected throughout the whole manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant science and phytochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 29 August 2023

https://doi.org/10.5256/f1000research.150979.r190235

© 2023 Sahin-Bolukbasi S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Serap Sahin-Bolukbasi
Department of Biochemistry, Faculty of Pharmacy, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

The authors have revised the manuscript and the overall quality has been improved. Hence, I recommend the acceptance of the article without further changes.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Lipidomics, Cell culture, Cytotoxic activity tests, Biotechnological drug design,

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 23 May 2023

https://doi.org/10.5256/f1000research.144170.r171852

© **2023 Sahin-Bolukbasi S.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Serap Sahin-Bolukbasi
Department of Biochemistry, Faculty of Pharmacy, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

This manuscript presents high-performance liquid chromatography (HPLC) analysis of quantitative determination of different bioactive molecules of both phenolic and terpen fractions in the Iraqi *Prunus arabica* extract. The researchers evaluated the synergistic effect between the extracted fractions and chemotherapeutic agent (docetaxel). The cytotoxic activity of phenolic and terpene fractions extracted from Iraqi *Prunus arabica* against breast (AMJ13) and esophageal (SK-GT-4) cancer cell and also normal human fibroblasts (NHF) cell.

However, the manuscript needs to be improved in order to meet the qualifications that are required for indexing. There are some issues that should be checked and corrected before indexing:

1. Please use subscript for IC_{50} (whole manuscript).

2. Please check whole manuscript for gm (it should be g?).

3. Please write which solvent was used to control wells and what was the concentration of solvent in control wells?

4. Please add ±SD values of samples in each concentration in Figure 1-3.

5. Please add ±SD values of samples in each IC_{50} concentration in manuscript.

6. Morphological analysis (crystal violet staining) section, I would like to see images of IC_{50} of the phenolic fraction, terpene, docetaxel, and their combinations with chemotherapy.

7. Please write the source of AMJ13, SK-GT-4 and NHF cell line (ATCC, ECACC, Sigma-Aldrich or other…), even if it's a gift.

8. Page 7; '…while a CI value larger than 1.1, the effects were assumed to be antagonistic…' and Figure 5 legends page 9; '…CI value less than 1 indicates an antagonism…" CI value less

than or larger tahan 1 assumed antagonistic? Please correct.

9. Please add ‘half’ in Figure 4 legend; ‘Figure 4. The **half** maximal inhibition concentration (IC_{50}) for phenolic, terpene, and docetaxel, respectively, on A: AMJ13, B: SK-GT-4, and C: NHF cell lines.’

10. Please write axis name in Figure 4.

11. The selectivity index is an important parameter for indicates the selectivity of a given samples between normal and cancer cells. Samples are expected less cytotoxic towards the healthy cells according to the cancer cells. Please calculate the selectivity indexes of samples.

12. Authors should compare the results of this study some other similar plant extracts with more details. References should be improved and updated in the results and discussion section. Indeed, the field of similar samples with regard to both their chemistry and biological relevance, include a significant number of articles and some recent reviews

13. Please use only one of the terms antiproliferative activity, cytotoxic activity, or anticancer activity in all manuscripts.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Lipidomics, Cell culture, Cytotoxic activity tests, Biotechnological drug design,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Jun 2023

matin mahmood

Dear Assoc. Prof. SERAP ŞAHİN BÖLÜKBAŞI,

Thank you for your thoughtful review of our manuscript. We greatly appreciate your feedback and suggestions for improvement. In our experiments, we used untreated cells, MTT solution, and a solubilizing buffer (DMSO) as controls. To ensure the integrity of our experimental results and minimize any potential confounding effects, we carefully selected a final concentration of 0.5% DMSO for our study. This concentration has been widely reported in the literature as non-toxic and compatible with various cell types. Regarding the morphological analysis section of this study, it is worth noting that the analysis was carried out using only a concentration of 100 ug/ml, and it is important to clarify that no analysis was conducted specifically to IC_{50} values.

Sincerely, Matin A. Mahmood

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com