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Acacia cyanophylla, Eucalyptus camaldulensis, and *Pistacia atlantica* Ethanol Extracts Revealed Cytotoxicity of Breast Cancer Cell Lines

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ABSTRACT

The available surgical and chemotherapeutic treatments of breast cancer are costly and associated with undesired side effects. Therefore, new alternative treatments need to be investigated using medicinal plants that have been the source of active ingredients for many drugs. The aim of this study is to evaluate the *in vitro* cytotoxicity of ethanol extracts of *Acacia cyanophylla* leaves, *Eucalyptus camaldulensis* leaves and fruits, and *Pistacia atlantica* leaves and petioles against four breast cancer cell lines and one normal skin fibroblast cell line using MTT colorimetric assay. Phytochemical analysis of the plant extracts was conducted using High Pressure Liquid Chromatography (HPLC). *Acacia cyanophylla* leaves extract showed high cytotoxicity against MDA-MB-231 and very low cytotoxicity against the normal cell line. *Eucalyptus camaldulensis* fruits extract showed high cytotoxicity against SKBR3 and ZR-75-1 cell lines, with moderate cytotoxicity against the normal cell line. *Pistacia atlantica* petioles extracts showed moderate cytotoxicity against SKBR3 cell lines and low cytotoxicity against the normal cell line. All plant extracts showed low cytotoxicity against T47D cell line. Unique chemicals were detected in each extract that could explain the difference in cytotoxicity. Chemical composition analysis of the plant extracts revealed that isorhamnetin-3-*O*-robinobioside and spathulenol are candidate compounds for *in vivo* study in order to investigate their efficacy and safety as potential drugs to treat breast cancer. In conclusion, *A. cyanophylla*, *E. camaldulensis*, and *P. atlantica* extracts have potential cytotoxic effect against breast cancer cells and could be a potential source of compounds to develop a drug to treat breast cancer.

***Keywords*:** Breast Cancer, Medicinal Plants, Cytotoxicity, *Acacia*, *Eucalyptus*, *Pistacia*.

Introduction

Breast cancer is the leading cause of cancer-related death in women in the world.[1] It has been estimated that the incidence of new breast cancer cases in the world is over two million while the related mortality is 600,000.[1] Breast cancer is a heterogeneous disease, with great variation in its genotypic and phenotypic characteristics. The most common type of breast cancer is invasive ductal carcinoma followed by invasive lobular carcinoma.[2,3] Several therapeutic options are available to treat breast cancer including surgical excision, chemotherapy, immunotherapy, radiotherapy, and combinations thereof. However, many of these treatments suffer great limitations including efficacy, availability, cost, side effects, and cytotoxicity to normal tissues. Therefore, finding an alternative treatment that is safe and effective is needed to overcome these limitations.

One of the current trends in cancer research is focused on investigating the anticancer properties of various medicinal plants with the potential to discover new drugs to treat breast cancer.

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Anticancer activity of many ingredients in medicinal plants targets cell apoptosis, mitosis, oncogenic enzymes, cellular oxidation, angiogenesis, reactivation of tumor suppressor genes, oncogene suppression, and epigenetic modulation.[4-6]

Acacia cyanophylla is an evergreen plant belonging to the Fabaceae family. *Acacia cyanophylla* has antimicrobial, hypoglycemic, and anti-inflammatory activity.[7-9] *Eucalyptus camaldulensis* belongs to the Myrtaceae family. Leaf extracts of *E. camaldulensis* exhibit antibacterial, anti-inflammatory, insecticidal, and antioxidative activity.[10-14] Traditionally, *E. camaldulensis* has been used as an antiseptic agent and in the treatment of upper respiratory tract infections.[13] *Pistacia atlantica* is a tree that belongs to the Anacardiaceae family. *Pistacia atlantica* exhibits antioxidative, antibacterial, anti-inflammatory, antidiarrheal, antiulcer, and anticancer activity.[15-18] In fact, mastic gum resin derived from the *P. atlantica* subspecies kurdica exhibits cytotoxicity against bile duct cancers, pancreatic carcinoma, gastric adenocarcinoma, colonic adenocarcinoma, and normal human colon fibroblast cell lines.[18] The methanol extract of *P. atlantica* fruits was found to exhibit significant cytotoxic effects against human colon carcinoma HT29 cells.[19,20] In fact, *P. atlantica* has been used traditionally to treat eczema, asthma, upper respiratory tract infections, and urolithiasis.[21]

The present study investigates the cytotoxicity of ethanol extracts of *A. cyanophylla*, *E. camaldulensis*, and *P. atlantica* against four different breast cancer cell lines and examines their phytochemical compositions to find likely drug candidates to treat breast cancer.

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Materials and Methods

Extract Preparation

Leaves of *A. cyanophylla*, leaves and fruits of *E. camaldulensis*, and leaves and petioles of *P. atlantica* were collected and identified in April 2015 from Jerash city in northern Jordan. Voucher specimens were deposited for each plant used in this study at the Herbarium of Biological Sciences Department at the University of Jordan (Voucher number; *Acacia cyanophylla*: 31566, *Eucalyptus camaldulensis*: 31764, and *Pistacia atlantica*: 32531). Plant materials were left to air-dry at room temperature in a dark place for 5–7 days. Selected plant parts were ground in a grinder (Thomas Scientific, USA) to obtain a fine powder. Extraction was achieved by adding ultrapure ethanol (99.7%) to the ground plant material with a ratio of plant extract/ethanol of 1:3 (w/v). The mixture was placed under continuous stirring at room temperature (23°C) for 72 hours in the dark. The extracts were filtered through Whatman filter paper (No. 1). Ethanol was evaporated at 50°C for 45–60 minutes under negative pressure (12 mbar) and continuous rotation using a rotary evaporator (Stuart, UK) until complete dryness. Plant extracts were dissolved in DMSO (0.01%) to final stock concentration of 100 mg/mL and then filtered using a 0.22 µm filter (Thomas Scientific, USA).[22]

Cell Culture Conditions

Cells were washed three times with sterile phosphate buffer saline (PBS, pH 7.4). Then, the PBS was decanted and cells detached with 0.025% trypsin-EDTA (PAA). Media was added to a volume of 10 mL. The cell suspension was centrifuged at 1,000 rpm for 10 minutes and the pellet resuspended in 10 mL of media to make a single cell suspension. Viability of the cells was determined by Trypan Blue exclusion, which exceeded 90% as counted in a hemocytometer. The cell suspension was then diluted to give the optimal seeding density.[23] Breast mammary gland adenocarcinoma cell line (MDA-MB-231) (ATCC # HTB-26), mammary gland adenocarcinoma cell line (SKBR3) (ATCC # HTB-30), breast ductal carcinoma cell line (T47D) (ATCC # HTB-133), breast mammary gland/duct histiocytic lymphoma cell line (ZR-75-1) (ATCC # CRL-1500), and non-cancerous (normal) skin fibroblast cell line (CCD-1064Sk) (ATCC # CRL-2076) were used in this study. MDA-MB-231, SKBR3, and the fibroblast cell lines were cultured in DMEM high-glucose medium (10.3892/br.2014.306) (HyClone, USA). T47D and ZR-75-1 cells were grown in RPMI 1640 medium (HyClone, USA). Both media were supplemented with 10% fetal bovine serum, 1% of 2.0 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 µg/mL) (HyClone, USA). Prior to experimentation, cells were grown to 80% confluence in 75 cm^2 cell culture flasks (Membrane Solutions, USA) and incubated at 37°C for 72 hours under an atmosphere containing 5% CO_2 and 95% air in a cell culture incubator (NuAire, USA).[23, 29]

MTT Cell Viability Assay

The cytotoxicity of the plant extracts was determined by MTT colorimetric assay[24] against the cancer and normal fibroblast cell lines. Approximately 4 x 10^5 cells were seeded into 96-well culture plates to a final volume of 100 µL of medium per well. Culture plates were then incubated in a humidified incubator for 24 h at 37°C under 5% CO_2 to allow the cells to adhere to the plate surface before being treated with the 100 µL of plant extracts or vehicle control (DMSO 0.01%). Cells were treated with a two-fold gradient concentration of plant extracts ranging from 3.12 to 800 µg/mL and incubated at 37°C for 72 h. 10 µg/mL of doxorubicin (98.0–102.0% HPLC grade; Sigma-Aldrich, USA) was run as a positive control against the cancer and fibroblast cell lines. For a negative control, each plate had three wells without extract and three wells without cells. Cell viability was determined using the Cell Titer Non-Radioactive Cell Proliferation Assay[25] Kit (Promega, USA) according to the manufacturer's instructions. A microplate reader (BioteK, USA) was used to measure the optical density at 570 nm, and this was compared with the optical density of the control (untreated cells).

The Percentage of growth inhibition was calculated according to the following equation:

$$\text{Growth inhibition (\%)} = \frac{\text{Absorbance of a blank} - \text{Absorbance of a sample}}{\text{Absorbance of a blank}} \times 100$$

Sample Preparation for HPLC

50 mg of each plant extract was inserted into a 5 mL glass amber tube and 1.5 mL of HPLC-grade methanol was added. The sample was vortexed for 3 minutes and then centrifuged at 1,500 g for 5 minutes. The supernatant was filtered by 0.45 µm PTFE syringe filter (Merck, Germany).

HPLC Methodology

Chromatographic separation was performed using a Shimadzu HPLC (Japan) equipped with an LC-8030 Tandem mass, SIL-30C autosampler with cooler, CTO-30 column oven, LC-30A pump, and CBM-20A system controller. The PrimeSIL (Wesley Technologies, Inc., USA) C18 column (10 cm x 0.21 µm x 2.1 mm) with guard column used an isocratic mobile phase of water (0.1% formic acid): acetonitrile (0.1% formic acid) 10:90 at a flow rate of 0.2 mL/min for 11 minutes and column temperature of 40°C. The autosampler temperature was maintained at 4°C and the injection volume was 20.0 µL. For mass spectrometry, an electrospray ionization (ESI) interface was used with positive and negative screening modes at 3 kV capillary voltage, 120°C source block temperature, and 45°C desolation gas temperature.[26]

Statistical Analysis

Inhibitory dose–response curves were drawn after normalization (the highest value was defined as 100 and the lowest value was defined as 0). The plant extract concentrations were transformed into logarithmic values. IC_{50} values were calculated from the inhibitory dose-dependent curves using GraphPad Prism software (version 8.0.2).

Results and Discussion

Concentrations of 50 µg/mL and 100 µg/mL of each plant extract were evaluated by MTT assay to screen for extracts with a considerable degree of cytotoxicity. IC_{50} was calculated for those plant extracts that had less than 50% survival of the cancer cell line after 72 hours exposure time. IC_{50} was defined as the concentration required to inhibit the growth of 50% of cells. According to the United States National Cancer Institute (NCI) plant screening program, IC_{50} less than 20 µg/mL of the crude extract is considered high cytotoxicity.[27] In this study, plant extracts with IC_{50} values of 21–40 µg/mL and IC_{50} > 40 µg/mL were considered to have moderate and low cytotoxicity, respectively.

In vitro cytotoxicity of the ethanol plant extracts of *A. cyanophylla* (leaves), *E. camaldulensis* (fruits and leaves), and *P. atlantica* (leaves and petioles) against breast cancer and fibroblast cell lines was determined (Table 1). Dose–response curves of the treated and the normal cell lines were drawn as shown in Figure 1. *Acacia cyanophylla* leaves extract showed high cytotoxicity against MDA-MB-231 cell line and very low cytotoxicity against the normal fibroblast skin cell line (CCD-1064SK).

Eucalyptus camaldulensis fruits extract showed high and similar cytotoxicity against SKBR3 and ZR-75-1 cell lines. However, *E. camaldulensis* fruits extract showed low cytotoxicity against MDA-MB231 and T47D, and moderate cytotoxicity against the normal cell line. *Eucalyptus camaldulensis* leaves extract showed low cytotoxicity against T47D and the normal cell line. *Pistacia atlantica* leaves extract showed moderate cytotoxicity against MDA-MB-231 cell line and low cytotoxicity against T47D and the normal cell line. *Pistacia atlantica* petioles extract showed moderate cytotoxicity against SKBR3 cell line and low cytotoxicity against MDA-MB-231, T47D, and the normal cell line.

Phytochemical composition analysis results for the ethanol extracts of *A. cyanophylla* leaves, *E. camaldulensis* leaves and fruits, and *P. atlantica* leaves and petioles are reported in Table 2. Further analysis revealed the unique composition of each plant extract (Table 3).

MTT assay is one of the most used viability assays in biomedical research. In fact, although the assay has several limitations, it is still

widely used for determining cytotoxicity against cancer cell lines. To improve its reliability, the test was performed under highly consistent conditions that considered interfering factors such as variations of the incubation time of the samples and solubilization of the formazan crystals.

Population "doubling time" is the time required for a cell culture population to double in number and is calculated during the logarithmic phase of growth.[28] In this study, the incubation time of the plant extracts with the cell lines was 72 h. This incubation time exceeded the published population doubling times for most of the studied cell lines (T47D: 32 h; MDA-MB-231: 38 h; SKBR3: 30 h; and ZR-75-1: 80 h).[29] All plant extracts showed low cytotoxicity against the normal cell line except *E. camaldulensis* leaves extract showed moderate cytotoxicity. The cancer cell lines used in this study responded differently to the plant extracts. This could be due to the unique phytochemical composition of each plant extract. Moreover, the difference in response could be due to variations in the genetic makeup of each cell line.[30] Therefore, the breast cancer cell lines used in this study represent distinct subtypes that respond differently to the same potential therapeutic drug.

Analysis of the phytochemical composition of the plant extracts in this study revealed a diverse number of potentially bioactive compounds. The main compounds detected here belong to the flavonoids, monoterpenes, and flavonols. In fact, flavonoids were the most frequently detected phytochemical class in the studied plant extracts. This agrees with [31] who used six different solvents for extraction and found that ethanol achieved the maximum extraction of flavonoids and phenols.

Quercetin was detected in all plant extracts. Saponin 2 compound was also detected in *E. camaldulensis* fruits extract. Both quercetin and saponin 2 exhibit *in vitro* and *in vivo* anticancer activity including antiproliferation, antimetastasis, antiangiogenesis, antioxidant, and induce apoptosis.[32, 33] Recent research indicated that these compounds are effective in depressing cell growth of several cancer types such as breast, colorectal, stomach, head and neck, lung, ovarian, melanoma as well as leukemia.[30, 31] Similar to the findings in this study, [34] detected α-pinene, morolic acid, and terpinolene in *P. atlantica* leaves. Moreover,[35] reported quercetin in the aqueous extract of *P. atlantica* leaves, which matches our results that detected quercetin in the ethanol extract of *P. atlantica* leaves. Apigenin, ß-sitosterol, chrysophanol, dillenetin, hexadecanoic acid, and isorhamnetin-3-O-beta-D-glucoside were detected in *A. cyanophylla* and *E. camaldulensis* leaves extracts. [36] reported that apigenin inhibits cell growth, metastasis, and invasion in breast cancer cells by acting on PI3K/Akt signaling and beta 4 integrin in MDA-MB-231. In fact, ß-sitosterol was reported to play a major role in the treatment of many diseases like breast cancer.[37] Moreover, it has been reported that chrysophanol has anticancer effects against the MCF7 breast cancer cell line through mitochondrial apoptosis and ER stress induction.[44] Others reported that chrysophanol effectively suppresses the proliferation of MCF7 while dillenetin, isolated from *Dillena indica*, has anticancer activity against lung cancer (U937) and promyelocytic leukemia (HL60, K562).[45,38]

Acacia cyanophylla extract had the highest cytotoxicity among all the extracts against MDA-MB-232 cancer cell line and the lowest toxicity among all the plant extracts against the normal cell line. In fact, *A. cyanophylla* extract had more than sixteen-fold more cytotoxicity of MDA-MB-231 cancer cell line than against the normal cell line. This makes *A. cyanophylla* ethanol extract the best candidate for *in vivo* testing to develop a breast cancer drug because it meets the two conditions for any drug: efficacy and safety. Chemical composition analysis of *A. cyanophylla* extract revealed a unique compound, isorhamnetin-3-*O*-robinobioside, which is a glycosyloxyflavone. This makes isorhamnetin-3-*O*-robinobioside a candidate for future studies *in vivo* to discover a drug to treat breast cancer. It was shown in a previous study that isorhamnetin-3-O-robinobioside induces its strong antiproliferative effect by enhancing apoptosis in cancer cells.[39] *In vivo* and *in vitro* studies have indicated that *Acacia* spp. extract exhibits protective antioxidant activity against oxidative-induced damage. This could be explained by the presence of isorhamnetin-3-O-robinobioside, a flavonoid with strong antioxidant and antigenotoxic activity against lymphoblastoid cells and several human cancers including myelogenous leukemia.[39, 40]

Eucalyptus camaldulensis fruits extract had high and similar cytotoxicity against SKBR3 and ZR-75-1 cell lines, with almost three-fold higher cytotoxicity than that against the normal cell line. This indicates *E. camaldulensis* leaves extract has unique chemical compounds from the other cytotoxic plant extracts, probably through antiproliferative activity against ZR-75-1 cell lines. Amygdalin, anthraquinone, coumarin, flavan-3-ol, kaempferide, phenol, phloroglucinol, saponin 2, and sterol class compounds were revealed by composition analysis to be unique compounds in *E. camaldulensis* leaves extract. *Eucalyptus camaldulensis* essential oil was previously reported to exhibit high cytotoxicity against the MCF7 breast cancer cell line and moderate cytotoxicity effect against normal skin fibroblasts.[41] In addition, high cytotoxic effects of *E. camaldulensis* essential oil were also reported against murine leukemic cell line (WEHI-3), colon carcinoma cell line (HT-29), and human promyelocytic leukemia cell line (HL-60).[42] This indicates that *E. camaldulensis* extract may exhibit a broad spectrum of cytotoxicity against different types of cancers.

Table 1: *In vitro* cytotoxicity of the ethanol plant extracts against breast cancer cell lines and the normal skin fibroblast cell line

Material	**IC_{50} (µg/mL)**				
	Cancer cell lines				**Normal cells**
	MDA-MB-231	**SKBR3**	**T47D**	**ZR-75-1**	**CCD-1064SK**
Acacia cyanophylla (leaves)	6.6	*	*	*	110.4
Eucalyptus camaldulensis (fruits)	48.0	14.4	76.1	13.6	38.4
Eucalyptus camaldulensis (leaves)	*	*	80.1	*	74.4
Pistacia atlantica (leaves)	38.2	*	68.7	*	68.1
Pistacia atlantica (petioles)	49.7	26.7	49.1	*	61.4
Doxorubicin	0.06	0.09	0.01	0.03	0.80

*50 µg/mL and 100 µg/mL of each plant extract were evaluated by MTT assay to screen for extracts with a considerable degree of cytotoxicity. IC_{50} was not calculated for plant extracts that had more than 50% survival of cancer cell line for 72 hours exposure time and were considered to have very low cytotoxicity.

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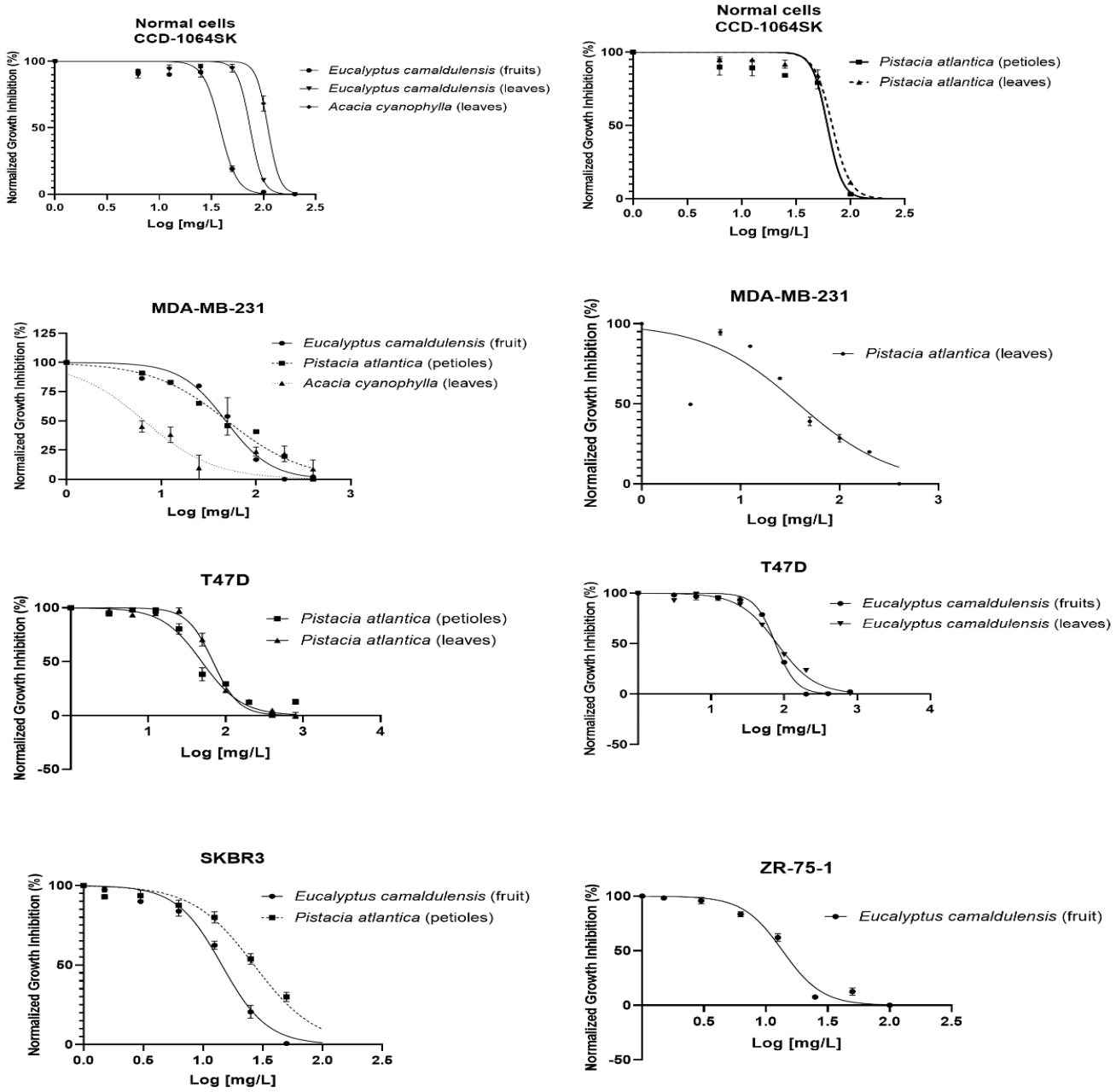

Figure 1: Dose response curves after treatment of four breast cancer cell lines MDA-MB-231, SKBR3, T47D, ZR-75-1 and the normal skin fibroblast cell line CCD-1064SK with ethanol extracts of Leaves of *Acacia cyanophylla*, leaves and fruits of *Eucalyptus camaldulensis*, and leaves and petioles of *Pistacia atlantica* for 72 hours, then evaluated using MTT assay.

Pistacia atlantica petioles extract had moderate cytotoxicity against the SKBR3 cancer cell line and low cytotoxicity against the normal cell line. Spathulenol, which belongs to the class sesquiterpenoid, was only detected in *P. atlantica* petioles extract. Previous studies have shown that spathulenol extracted from *Piper guineense* had antioxidant, anti-inflammatory, antiproliferative, and antimycobacterial activity.[43] Therefore, spathulenol is a candidate compound to be tested further *in vivo* as a potential drug for breast cancer.

Both leaves and petioles *P. atlantica* extracts showed different cytotoxicity effect. This difference could be attributed to the different chemical compounds that were detected in their phytochemical compositions. Previous reports have similarly indicated that IC_{50} produced by *P. atlantica* leaves extract against AGS, HeLa cancer cell lines, and HDFs fibroblast cell line were 382.3 µg/mL, 332.3 µg/mL, and 896.3 µg/mL, respectively.[17] These results are in complete agreement with reports that detected high cytotoxicity of *P. atlantica* leaves essential oil against MCF7 and T47D.[44] This antitumor activity could be due to induced apoptosis and the anti-angiogenesis effects of *P. atlantica.*[45] Moreover, *P. atlantica* leaves extract has a strong antioxidant activity, with the ability to inhibit cell proliferation and induce cancer cell death.[46, 47]

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Table 2: Phytochemical composition of the crude ethanol extracts of *Acacia cyanophylla*, *Eucalyptus camaldulensis* and *Pistacia atlantica*

Plant	Compound	Molecular Weight	Chemical Class
Acacia cyanophylla (leaves)	Apigenin,	270.24	Flavone
Eucalyptus camaldulensis (leaves)	ß-sitosterol	414.7	Phytosterol
	Chrysophanol	254.24	Anthraquinones
	Dillenetin	330.29	Flavonoids
	Hexadecanoic acid	256.43	Fatty acids
	Isorhamnetin-3-*O*-beta-D-glucoside	478.406	Glycosyloxyflavones
Acacia cyanophylla (leaves)	6”-*O*-(malonyl)-isoorientin	535	Acylated flavones
Pistacia atlantica (leaves)			
Pistacia atlantica (petioles)			
Acacia cyanophylla (leaves)	Myricetin 3-galactoside	480.37	Glycosyloxyflavone
Eucalyptus camaldulensis (leaves)			
Pistacia atlantica (leaves)			
Pistacia atlantica (petioles)			
Eucalyptus camaldulensis (leaves)	Genistein	270.24	Phytoestrogens
Pistacia atlantica (leaves)			
Pistacia atlantica (petiole)			
Acacia cyanophylla (leaves)	Abietadiene	272.4	Diterpenes
Eucalyptus camaldulensis (leaves)	Asphodelin A	286.23	Arylcoumarins
Pistacia atlantica (leaves)			
Eucalyptus camaldulensis (fruits)	Linalool	154.25	Terpene alcohols
Pistacia atlantica (leaves)			
Pistacia atlantica (petioles)			
Pistacia atlantica (leaves)	Morolic acid	456.7	Triterpenes
Pistacia atlantica (petioles)	Terpinolene	136.23	Monoterpenes
Acacia cyanophylla (leaves)	Quercetin	302.23	Flavonoids
Eucalyptus camaldulensis (fruits)			
Eucalyptus camaldulensis (leaves)			
Pistacia atlantica (leaves)			
Pistacia atlantica (petioles)			
Acacia cyanophylla (leaves)	Kaempferol	286.23	Flavonoids
Eucalyptus camaldulensis (fruits)			
Pistacia atlantica (leaves)			
Pistacia atlantica (petioles)			

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Eucalyptus camaldulensis (fruits) *Eucalyptus camaldulensis* (leaves) *Pistacia atlantica* (leaves) *Pistacia atlantica* (petioles)	α-Pinene	136.23	Monoterpenes
Acacia cyanophylla (leaves) *Eucalyptus camaldulensis* (leaves) *Eucalyptus camaldulensis* (fruits)	Luteolin	286.24	Flavonoids
Acacia cyanophylla (leaves) *Eucalyptus camaldulensis* (fruits)	Isorhamnetin	316.26	Flavonols
Acacia cyanophylla (leaves) *Pistacia atlantica* (leaves)	Chlorogenic acid	354.3	Cinnamic acid derivative
Eucalyptus camaldulensis (leaves)	ß – Farnesene	204.36	Sesquiterpenes
Pistacia atlantica (leaves)	Daidzein	254.241	Phytoestrogen

Table 3: Unique phytochemical composition of the crude ethanol extracts of *Acacia cyanophylla*, *Eucalyptus camaldulensis* and *Pistacia atlantica*

Plant	Compound	Molecular Weight	Chemical Class
Acacia cyanophylla (leaves)	Isorhamnetin-3-*O*-robinobioside	624.548	Glycosyloxyflavone
Eucalyptus camaldulensis (fruits)	Amygdalin	457.4	Cyanogenic glycosides
	Anthraquinone	208.216	Aroamtic hydrocarbon
	Coumarin	146.14	Benzopyron / lactone
	Flavan-3-ol	226	Flavonols
	Kaempferide	300.26	Flavanols
	Phenol	94.11	Phenol
	Phloroglucinol	126.11	Phenol derivative,
	Saponin 2	634.8	benzentriol
	Sterol (class)	248.4	Saponins Sterol
Eucalyptus camaldulensis (leaves)	P-cymene	134.21	Monoterpene
	Terpinen-4-ol	154.253	Monoterpene
	Quercetin 3,7-diglucoside	626.52	Flavonoid glycoside
Pistacia atlantica (leaves)	Cyanidine 3-o-glucoside	484.84	Anthocyanin
	Longifolene	204.3	Sesquiterpenes
	Pinocarveol	152.23	Monoterpenoid
	Tricosane	324.63	Acyclic alkane
	Verbenone	150.21	Terpene
Pistacia atlantica (petioles)	Spathulenol	220.356	Sesquiterpenoid

Conclusion

Acacia cyanophylla, *E. camaldulensis*, and *P. atlantica* ethanol extracts have the potential to treat human breast cancer. Isorhamnetin-3-O-robinobioside and spathulenol are candidate compounds for *in vivo* study to investigate their efficacy and safety as potential breast cancer drugs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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