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Antibacterial, Antifungal, Antioxidant, and Anti-Proliferative Effects of *Eucalyptus camaldulensis* and *Pistacia atlantica* Ethanol Extracts

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ABSTRACT

The need to find alternatives to existing antibiotic drugs and to cure cancer increases every day. Medicinal plants contain a vast array of substances that indicate antimicrobial and anti-proliferative activity. Therefore, antibacterial, antifungal, antioxidant, and anti-proliferative effects of ethanol extracts of *Eucalyptus camaldulensis* and *Pistacia atlantica* were evaluated. Antibacterial effect was measured using agar well diffusion and microtiter plate dilution methods. Antioxidant effect was assessed using DPPH and ABTS assays. Anti-proliferative effects were assessed using the MTT assay against K562 leukemia cell line. *Eucalyptus camaldulensis* fruits and leaves extracts and *P. atlantica* leaves extract had strong antibacterial activity against *Bacillus megaterium*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus aureus*, except *P. atlantica* leaves extract did not have an antibacterial effect against *Corynebacterium diphtheria*. *Pistacia atlantica* leaves extract had strong antifungal activity against *Aspergillus alliaceus*, *Aspergillus niger*, *Rhizoupus stolonifer*, and *Cunninghamella elegans* while it was insensitive against *Aspergillus flavus*, which was the only tested fungi affected by *E. camaldulensis* fruit extract. *Eucalyptus camaldulensis* leaves extract had strong antifungal activity against *A. alliaceus*. *Pistacia atlantica* petioles extract had strong cytotoxic activity against K562 cell line while the cytotoxic effect of *E. camaldulensis* fruits and leaves extracts were moderate. All plant extracts had low cytotoxic effects against normal skin fibroblast cell line. *Pistacia atlantica* leaves extract had the highest total phenolic content followed by *E. camaldulensis* leaves extract; then, *E. camaldulensis* fruit extract had the highest total flavonoids content followed by *P. atlantica* leaves extract then *E. camaldulensis* leaves extract.

***Keywords*:** Antibacterial, Antifungal, Antioxidant, *Eucalyptus camaldulensis*, *Pistacia atlantica*

Introduction

Medicinal plants have long been used to treat pain and diseases. In modern history, medicinal plants have become an important source of novel naturally derived drugs and pharmaceuticals. Medicinal plants are diverse in their chemical composition, which is responsible for their biological activity.[1] In recent years, the large-scale indiscriminate use of common antibacterial and antifungal agents has resulted in widespread resistance among pathogenic microorganisms. This has presented the medical community and global health officials with huge challenges due to the emergence of infections caused by multidrug-resistant pathogens (MDRP).[2] In this context, plant materials have provided a continuous and sustainable source of bioactive substances that have been used effectively to treat diseases caused by MDRP. [2, 3] The antioxidant activity of natural plant supplements and medicinal plants is well recognized in medical practice and the ethno-pharmaceutical industry. Plants, which are rich in natural antioxidants such as polyphenols, carotenoids, and vitamins E and C, have a broad range of bioactivity including anti-inflammatory, anti-aging, anti-atherosclerosis, and anticancer.[4] Exogenous plant antioxidants are important to prevent oxidative damage produced by the release of free radicals. In fact, oxidative damage is well known to play a major role in the pathogenesis of several life-threatening diseases affecting the nervous and cardiovascular systems.[5]

There has been an increasing focus on the anticancer activity of many medicinal plants as a potential adjuvant for cancer chemotherapy.[6] The anticancer phytochemicals were attributed to mechanisms targeting cell apoptosis, mitosis, oncogenic enzymes, cellular oxidation, angiogenesis, reactivation of tumor suppressor genes, oncogenic suppression, and epigenetic modulation.[6, 7]

Leukemia is a common malignant tumor of hematopoietic progenitor cells.[8, 9] Despite recent developments in the treatment of this cancer, the disease still causes high mortality; therefore, the use of medicinal plants with proved anticancer activity as an adjunctive treatment of this cancer is noteworthy.[9] *Eucalyptus camaldulensis*, a common plant of the Myrtaceae family, is known to exhibit antibacterial, anti-inflammatory, insecticidal, and antioxidant activities.[10, 11] On the other hand, *Pistacia atlantica*, a tree of the Anacardiaceae family, has been used for its antioxidant, antibacterial, anti-inflammatory, antidiarrheal, antiulcer, and anticancer activities.[11] The objective of this study was to evaluate the antibacterial, antifungal, antioxidant, and anti-proliferative activities of *E. camaldulensis* and *P. atlantica* ethanol extracts *in vitro*. This type of research provides a first-step solution to the problem of finding alternatives to existing resistant antifungal and

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antibacterial agents, and a candidate source of drugs to cure leukemia. As far as the knowledge of the authors, none of these tested plants has been investigated for this purpose.

Materials and Methods

Plant extract preparation

Eucalyptus camaldulensis fruits and leaves and *P. atlantica* leaves and petioles were collected in April 2015 from Jerash city in northern Jordan and were identified by Abu-Irmaileh. Voucher specimens were deposited for each plant used in this study at the Herbarium of Biological Sciences Department at the University of Jordan (Voucher numbers: *Eucalyptus camaldulensis*: 31764 and *Pistacia atlantica*: 32531. Plant materials were left to dry in the dark at room temperature. After drying, the plant parts were ground using an electric grinder (Thomas Scientific, USA). Extraction was performed by adding ultrapure (99.7%) ethanol to the milled plant material (1:3 ratio w/v). The extract/ethanol mixture was incubated for 72 h at 23°C in the dark with continuous stirring. Then, the extracts were passed through Whatman filter paper (No. 1). The filtered solution then was evaporated under negative pressure (12 mbar) and continuous rotation using a rotary evaporator apparatus with a water bath (Stuart, UK) at 50°C until complete dryness. Plant extracts were kept at 4°C for further experiments. For antibacterial, antifungal, and anti-proliferative activities, plant extracts were dissolved in dimethyl sulfoxide (DMSO) to a final stock concentration of 100 mg/mL.

Determination of total phenolic content

The total phenolic content (mg GAE/g) was determined following the Folin–Ciocalteu method.[12] Gallic acid was used as the standard phenolic compound. Briefly, 0.1 mL of the plant extract was left to react with 2.5 mL of 0.2 N Folin–Ciocalteu phenol reagent for 5 min. Then, 2 mL of 7.5% sodium carbonate was added to stop the reaction and the absorbance of the solution was determined at 765 nm following incubation at room temperature for 2 h.

Determination of total flavonoids content

The total flavonoids content (expressed as milligram rutin equivalents (RE) per gram dried plant powder) was determined using previously published methods with minor modifications.[13] Briefly, a mixture containing 0.5 mL of plant extract and 0.3 mL of 0.5% $NaNO_2$ and 0.3 mL of 0.1% $AlCl_3$ was prepared and left to react for 5 min. Then, 2.0 mL of 1.0 M NaOH was added, and the volume of the solution was made up to 10 mL with distilled water and mixed thoroughly. The flavonoids content was determined by measuring the absorbance of the solution at 510 nm.

Determination of total tannins content

The total tannin content (mg/g of dry material) was determined by the casein precipitation method with some modifications.[14] Briefly, a mixture containing 1 g casein, 6 mL plant extract, and 12 mL of distilled water was made. Then, the mixture was left to shake gently for 3 h at 25°C. The mixture was then passed through Whatman filter paper. The resultant filtrate was then diluted to 25 mL with distilled water. Total tannin content was determined by measuring the absorbance at 500 nm.

Determination of antioxidant effect

The free radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays was used to determine the plant extracts' antioxidant capacity.[15, 16] For the DPPH assay, 50 µL of the ethanol plant extracts were mixed with 2.5 mL of 0.12 mM ethanol DPPH. Fifty microliter (50 µL) of ethanol was used as a control. The amount of reduction of DPPH was measured by absorbance at 517 nm (Thermo Fisher Scientific, USA) after 20 min incubation at 24°C. The percentage of growth inhibition was calculated according to the following equation:

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

The ABTS radical cation assay was performed by adding 7.0 mM ABTS and 2.45 mM potassium persulfate, and incubation at 23–24°C for 15–16 h. The ABTS solution was diluted using 20 µL of the standard. Then, 20 µL of plant extract was added to 2.0 mL of the diluted ABTS solution and incubated at 23–24°C for 6 min. The absorbance was then measured at 734 nm (Thermo Fisher Scientific, USA).

Antibacterial activity assay

The determination of antibacterial activity of plant extracts was performed by agar well diffusion on Mueller–Hinton agar plates (MHA) (Mast Group Ltd, UK).[17] Briefly, 100 µL of bacterial suspension containing approximately 10^7 CFU/mL was seeded on MHA plates. Wells of diameter 4 mm were punched into the agar plates and 10.0 µL of plant extract (1 mg/mL) loaded into each well. Positive and negative control wells were loaded with 10.0 µL chloramphenicol (1 mg/mL) and 10.0 µL DMS, respectively. The plates were incubated at 37°C for 24 h. Three replicates of each plant extract were conducted.

Determination of antibacterial minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was only performed for the plant extracts against sensitive bacterial strains using microtiter plate dilution.[18] Plant extracts were diluted two-fold using Mueller–Hinton broth medium (MHB) (Mast Group Ltd, UK). Briefly, the first well was loaded with 180 µL of MHB while the second to tenth wells were filled with 100 µL of the medium. The dried extracts were prepared for MIC by dissolving with DMSO. The first well received a plant concentration of 0.1 mg/mL. The second to tenth wells were serially diluted to create a serial dilution from 10 to 0.02 µg/mL. Then, 100 µL of approximately 1 McFarland standard of freshly grown bacteria was added to each well and the plates incubated for 24 h at 37°C. The MIC was determined visually as the lowest concentration that visually led to growth inhibition of bacteria. DMSO and chloramphenicol were used as negative and positive controls, respectively.

Antifungal activity assay and fungal culture preparation

Five pathogenic fungal spp.—*Aspergillus alliaceus*, *Aspergillus flavus*, *Aspergillus niger*, *Cunninghamella elegans*, and *Rhizoupus stolonifer*—were used in the antifungal assay. Fungi were grown and maintained in Potato Dextrose Agar media (PDA) (Mast Group Ltd, UK). The cultures were adjusted to have a density of 2 x 10^5 spore/mL for each fungal strain.

The antifungal activity of the plant extracts was assayed in triplicate using the agar well diffusion assay.[19] Briefly, 100 µL of fungal suspension was spread uniformly onto PDA plates. The plates were incubated at room temperature to dry for 5 min. Wells of diameter 5.0 mm were made in the agar plate using a sterile cork-borer. Plant extracts were dissolved in 5.0 mL DMSO and 100 µL from each plant extract was added to each well on the seeded plates. The plates were incubated for one hour for proper diffusion at room temperature before being transferred to the incubator for 48 h at 27°C. The antifungal activity of each plant extract was recorded by measuring the inhibition zone diameter in millimeters. Cycloheximide (25 µg/mL) and nystatin (2.5 µg/mL) were used as standard antifungal agents.

Determination of antifungal minimal inhibitory concentration (MIC)

Plant extracts that showed positive results using the well diffusion method were selected to measure MIC [20] according to previously described protocols with some modifications.[21] Dilution series of the plant extracts were prepared using DMSO (50 µg/mL to 1.56 µg/mL). From each concentration of the plant extract, 100 µL was added to 900 µL of fungi grown in RPMI medium (RPMI) (1640; Thermo Fisher Scientific, USA) and the plates were incubated at 27°C for 48 h. MIC was the lowest concentration that showed clear color, indicating no visible growth.

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Cytotoxic activity assay

Leukemia K562 cell line (ATCC CCL-2431) and non-cancerous (normal) skin fibroblast cell line (CCD-1064Sk; ATCC CRL-2076) were used in this study. K562 leukemia cells were cultured using RPMI 1640 medium (HyClone, USA) while fibroblast cells were cultured using DMEM (Dulbecco's Modified Eagle Medium) high-glucose medium (HyClone, USA). Both media were supplemented with 10% bovine fetal serum, 2.0 mM L-glutamine (1%), penicillin (100 U/mL), and streptomycin (100 µg/mL) (HyClone, USA). Cells were incubated at 37°C with 95% air/5% CO_2 in a cell culture incubator (NuAire, USA).The cytotoxicity of the plant extracts was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay against the cancer cell line and the fibroblasts cell line as control.[22] Briefly, 100 µL of medium containing 4 x 10^5 cells was loaded into each well of a 96-well culture plate and incubated in a humidified incubator for 23–24 h at 36–37°C under 5% CO_2 conditions. Afterwards, each well was loaded with 100 µL of vehicle control (DMSO) or plant extract in a two-fold gradient concentration ranging from 3.12 to 800 µg/mL and incubated again at 36–37°C for 72 h. Untreated cells were run as a negative control. Doxorubicin (98.0–102.0% HPLC grade; Sigma-Aldrich, USA) was used as a positive control. The absorbance was measured at 570 nm (Promega, USA). The obtained absorbance was then compared with the optical density of the untreated cells.

Statistical analysis

For determination of total phenolic content, ten concentrations (00 to 1000 µg/µL of gallic acid in 80% methanol) were used to prepare the standard calibration curve. For determination of total flavonoids content, the standard curve was prepared using different concentrations of rutin (20, 40, 60, 80, and 100 µg/mL).

For antibacterial activity and antioxidant activities, three replicates of each plant extract were conducted. For the antifungal activity assay, the averages of three replicates of the inhibition zones (mm) were measured. For the cytotoxic activity assay, IC_{50} was calculated using Prism software (version 5).

Results and Discussion

In vitro antibacterial activity was assayed by measuring the inhibition zone using the agar diffusion method. The antibacterial activity of *E. camaldulensis* fruits and leaves and *P. atlantica* leaves ethanol extracts against the selected pathogenic Gram-positive and Gram-negative bacteria is reported in Table 1. *Eucalyptus camaldulensis* fruits and leaves and *P. atlantica* leaves extracts showed a larger inhibition zone against *P. mirabilis* than that of chloramphenicol, followed by *C. diphtheria* (except for *P. atlantica* leaves extract that had no activity against *C. diphtheria*), whereas the extracts had similar inhibition zone size against *P. aeruginosa* ATCC 27853 and smaller inhibition zone against *B. megaterium*, *K. pneumonia*, *S. aureus* ATCC 43300, and *S. aureus* ATCC 33591. MIC values (µg/mL) of *E. camaldulensis* fruits and leaves and *P. atlantica* leaves ethanol extracts against selected pathogenic Gram-positive and Gram-negative bacteria are shown in Table 2. *Eucalyptus camaldulensis* fruits and leaves extracts had lower MIC values against *B. megaterium* and *P. mirabilis* than that of chloramphenicol, whereas *P. atlantica* leaves extract had the same MIC value against *B. megaterium* and *P. mirabilis* as that of chloramphenicol. Interestingly, the three plant extracts had lower MIC values against *P. aeruginosa* ATCC 27853 and *C. diphtheria* than that of chloramphenicol except for *P. atlantica* leaves extract that had no activity against *C. diphtheria*. However, the three extracts had higher MIC values against *K. pneumonia*, *S. aureus* ATCC 33591, and *S. aureus* ATCC 43300.

Table 1: *In vitro* antibacterial effect of *Eucalyptus camaldulensis* and *Pistacia atlantica* ethanol extracts against selected pathogenic bacteria (inhibition zone in mm). Standard deviation (SD) was calculated for three replicates.

	Bacterium						
Tested Materials	***B. megaterium***	***C. diphtheriae***	***K. pneumoniae***	***P. mirabilis***	***P. aeruginosa* ATCC 27853**	***S. aureus* ATCC 33591**	***S. aureus* ATCC 43300**
Eucalyptus camaldulesnsis (fruits)	12.3 ± 0.5	19.6 ± 0.6	17.3 ± 0.6	18.7 ± 0.6	22.7 ± 0.6	17.7 ± 0.5	15.7 ± 0.6
Eucalyptus camaldulensis (leaves)	13.7 ± 1.2	18.0 ± 0.0	16.3 ± 0.6	17.0 ± 0.0	21.0 ± 1.0	15.0 ± 0.0	14.7 ± 0.6
Pistacia atlantica (leaves)	12.3 ± 0.6	0.0	17.3 ± 0.6	16.3 ± 0.6	20.3 ± 0.6	17.0 ± 1.0	8.0 ± 0.0
Chloramphenicol	29.7 ± 0.6	15.0 ± 0.0	22.5 ± 0.5	10.0 ± 0.0	20.0 ± 0.0	34.7 ± 0.6	23.0 ± 0.0

Table 2: MIC (µg/mL) of *Eucalyptus camaldulensis* and *Pistacia atlantica* ethanol extracts against selected bacteria.

	Bacterium						
Tested Materials	***B. megaterium***	***C. diphtheriae***	***K. pneumoniae***	***P. mirabilis***	***P. aeruginosa* ATCC 27853**	***S. aureus* ATCC 33591**	***S. aureus* ATCC 43300**
Eucalyptus camaldulensis (fruits)	5	1.25	2.5	0.31	1.25	2.5	5
Eucalyptus camaldulesnsis (leaves)	5	1.25	2.5	0.63	2.5	5	5
Pistacia atlantica (leaves)	10	ND*	1.25	1.25	2.5	1.25	10
Chloramphenicol	10	5	0.63	1.25	10	0.08	0.31

*ND: non-determinant

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In vitro antifungal activity measured by the inhibition zone of *E. camaldulensis* fruits and leaves and *P. atlantica* leaves extracts is reported in Table 3. The inhibition zone in (mm) produced by *P. atlantica* leaves extract was the largest against *A. niger* followed by *C. elegans*, *A. alliaceus*, and *R. stolonifera* while it was insensitive against *A. flavus*. For *E. camaldulensis* leaves extract, the inhibition zones were the largest against *A. alliaceus* and then against *A. flavus* while it was insensitive against all other tested organisms. A fungal strain was considered antifungal agent-resistant when the inhibition zone diameter was equal to or smaller than 8 mm.[23] MIC values (µg/mL) of *E. camaldulensis* fruits and leaves and *P. atlantica* leaves extracts are shown in Table 4. *Pistacia atlantica* leaves extract had low MIC against *A. alliaceus* and *C. elegans* followed by *A. niger* and *R. stolonifer* while *E. camaldulensis* fruit extract had a low MIC value against *A. flavus*, *E. camaldulensis* leaves extract had the same MIC value against *A. alliaceus* as that of *P. atlantica*. In fact, *P. atlantica* and *E. camaldulensis* extracts had low MIC values and not much higher than the MIC values of nystatin (positive control) against *A. alliaceus* and against *C. elegans*. This indicates that *P. atlantica* and *E. camaldulensis* extracts have compounds that are good candidates to be antifungal agents against *A. alliaceus* and *C. elegans.* The antioxidant capacity of *E. camaldulensis* fruits and leaves and *P. atlantica* leaves ethanol extracts is reported in Table 5. The antioxidant capacity as determined by the DPPH assay showed strong activity produced by *E. camaldulensis* fruits and leaves and *P. atlantica* leaves extracts. In regard to the ABTS assay, the antioxidant activity produced by *P. atlantica* leaves extract was the highest followed by *E. camaldulensis* fruits and leaves extracts. The three extracts were shown to have a high total phenolic content followed by total flavonoids content and total tannins content. The anti-proliferative activity of *E. camaldulensis* fruits and leaves and *P. atlantica* leaves and petioles extracts against K562 leukemia and fibroblast cell lines is reported in Table 6. *Pistacia atlantica* petioles extract had strong anti-proliferative activity against K562 cell line and a four-fold higher cytotoxic activity against K562 cell line than that against the fibroblast cell line. The anti-proliferative activity of *P. atlantica* leaves extract against K562 cell line was similar to that of *E. camaldulensis* fruits and leaves extract. However, *E. camaldulensis* leaves and *P. atlantica* leaves extracts had almost three-fold and more than two-fold higher cytotoxicity against K562 cell line than that against the fibroblast cell line, respectively. *Eucalyptus camaldulensis* (fruits) extract has very similar toxicity against the K562 and fibroblast cell lines. The cytotoxicity of the tested plant extracts is much higher than that of doxorubicin (positive control). This is due to the nature of the plant extracts that are considered as crude extracts, whereas doxorubicin is a pure compound.

The crude ethanol extracts of *E. camaldulensis* were shown to produce strong antibacterial activity against most of the tested pathogenic bacterial. In fact, *E. camaldulensis* fruits and leaves extracts had stronger antibacterial activity against *B. megaterium, C. diphtheria, P. mirabilis*, and *P. aeruginosa* ATCC 27853 than the drug chloramphenicol.

Table 3: *In vitro* antifungal effect of *Eucalyptus camaldulensis* and *Pistacia atlantica* ethanol extracts (inhibition zone in mm). Standard deviation (SD) was calculated for three replicates.

	Fungus				
Test Materials	***A. alliaceus***	***A. flavus***	***A. niger***	***R. stolonifer***	***C. elegans***
Eucalyptus camaldulensis (fruits)	0.0	16.5 ± 1.50	0.0	0.0	0.0
Eucalyptus camaldulensis (leaves)	25.0 ± 3.61	20.0 ± 2.00	0.0	0.0	0.0
Pistacia atlantica (leaves)	21.0 ± 2.65	0.0	28.0 ± 2.00	20.5 ± 3.04	26.7 ± 3.06
Cycloheximide	0.0	23.0 ± 2.00	0.0	22.2 ± 2.02	0.0
Nystatin	0.0	18.3 ± 1.53	0.0	18.2 ± 1.04	0.0

Table 4: MIC (µg/mL) of *Eucalyptus camaldulensis* and *Pistacia atlantica* ethanol extracts against selected fungi

	Fungus				
Tested Materials	***A. alliaceus***	***A. flavus***	***A. niger***	***R. stolonifer***	***C. elegans***
Eucalyptus camaldulensis (fruits)	ND*****	12.5	ND	ND	ND
Eucalyptus camaldulensis (leaves)	3.125	25	ND	ND	ND
Pistacia atlantica (leaves)	3.125	ND	6.25	6.25	3.125
Cycloheximide	ND	25	ND	25	ND
Nystatin	ND	2.5	ND	2.5	ND

***** ND: Not-Determinant

This matches the previously reported study of the essential oil of *E. camaldulensis* that was found to exhibit strong and synergistic antimicrobial effects against multidrug-resistant *Acinetobacter baumannii* when used alone or in combination with traditional antibiotics such as ciprofloxacin, gentamicin, and polymyxin B.[24]

Strong antioxidant activity of the ethanol extract of *P. atlantica* leaves was also detected in this study. These results are similar to previously reported findings where positive correlations were found between the DPPH radical-scavenging activity of *P. atlantica* extracts and total phenolic content.[33] Two properties are required for a good medication to cure cancer. The first property is the safety of the medication on normal cells and the second is being an effective inhibitor of cancer cells. In this study, the plant extracts showed safety and efficacy. For instance, *P. atlantica* petioles extract had almost four-fold higher cytotoxicity against K562 cell line than that against fibroblast cell line. This makes *P. atlantica* petioles an excellent source of candidate medications for leukemia. *Pistacia atlantica* leaves extract had more than two-fold higher cytotoxicity against K562 cell line than that against fibroblast cell line. *Eucalyptus camaldulensis* leaves extract had almost three-fold higher cytotoxicity against K562 cell line than that against the fibroblast cell. *Eucalyptus camaldulensis* fruits extract had very similar anti-proliferative activity against K562 cell line and toxicity against the fibroblast cell line.

Pistacia atlantica anti-proliferative activity was different between the leaves and the petioles extracts. This variation is rationally attributed to the difference in their chemical compositions. An IC_{50} less than 20 µg/mL of the crude extract was considered to indicate high cytotoxic activity.[34]

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Table 5: Antioxidant capacity of *Eucalyptus camaldulensis* and *Pistacia atlantica* ethanol extracts and total phenolic, total flavonoids, and total tannins contents

Plant Extract	DPPH (%)	ABTS (%)	Total Phenolic (mg GAE/g)	Total Flavonoids (mg RE/g)	Total Tannins (mg/g)
Eucalyptus camaldulensis (fruits)	94	59	329	268	84
Eucalyptus camaldulensis (leaves)	89	55	446	158	79
Pistacia atlantica (leaves)	89	87	450	197	88

Table 6: *In vitro* cytotoxic effects of *Eucalyptus camaldulensis* and *Pistacia atlantica* ethanol extracts against K562 leukemia cancer cell line

Test Materials	IC_{50} (µg/mL)		
	K562 Leukemia	Fibroblast (normal skin) CCD-1064SK	No. of fold difference in cytotoxicity
Eucalyptus camaldulensis (fruits)	31.0	38.4	1.2
Eucalyptus camaldulensis (leaves)	26.9	76.9	2.9
Pistacia atlantica (leaves)	32.9	68.1	2.1
Pistacia atlantica (petioles)	16.5	61.4	3.7
Doxorubicin	0.38	0.80	2.1

It was found that the main phytochemicals in *E. camaldulensis* essential oil were spatulenol, cryptone, p-cimene, 1,8-cineole, terpinen-4-ol, and β-pinene.[24] The antibacterial effects were most likely attributed to the polar terpene compounds found in this plant extract.[24] The fruits and leaves extracts had very similar results. This could be due to the similar phytochemical compositions of the two parts. Moreover, the phenolic compounds known to be responsible for antibacterial activity were identified in significant amounts of both *E. camaldulensis* leaves and fruits extracts.[25]

The crude extract of *P. atlantica* leaves also showed antibacterial effects against *K. pneumonia*, *P. aeruginosa* ATCC 27853, *P. mirabilis*, and *S. aureus* ATCC 33591. In fact, *P. atlantica* leaves extract had stronger antibacterial activity against *P. aeruginosa* ATCC 27853 than chloramphenicol. Similar results were also reported previously using aqueous leaf extract of *P. atlantica* against resident bacteria of the mouth and saliva including *S. mutans* and *S. mitis*.[26, 27] This plant was also found to be rich in polyphenolic and flavonoid compounds that are well-known for their antibacterial activity.[25]

This study showed strong antifungal activities of the crude ethanol extracts of *E. camaldulensis* fruits and leaves against the fungi *A. alliaceus* and *A. flavus*. Previous research has also shown similar results against five different *Fusarium* spp.[28] Similar to our results, the phytochemical composition of this extract that could be responsible for its antifungal activities was reported to contain 1,8-cineole, α-pinene, α-phellandrene, and p-cymene.[28] *Pistacia atlantica* leaves extract had strong antifungal activity against *A. alliaceus* and *C. elegans* followed by *A. niger* and *R. stolonifer*. Strong fungistatic and fungicidal activities have been reported for both *P. atlantica* fruits and leaves extract against *Candida albicans*, *Candida glabrata*, and *Saccharomyces* cerevisiae.[29] The main phytochemicals of *P. atlantica* extract were β-myrcene, α-pinene, limonene, trans-caryophyllene, α-amorphene, and neo-allo-ocimene.[29] Moreover, phenolic compounds are known for their antifungal activity.[30] In fact, the total phenolic content measured in *P. atlantica* leaves extract was the highest among the tested extracts.

In this study, the ethanol extracts of *E. camaldulensis* fruits and leaves showed strong antioxidant activity. This antioxidant activity of *E. camaldulensis* was also reported previously.[31] It was found that the major phytochemical composition of the essential oil of *E. camaldulensis* includes p-cymene, 1,8-cineole, 1-(S)-pinene, and limonene.[31] Moreover, phenolic compounds cause antioxidant activity.[32]

In this study, the cytotoxicity of the ethanol extract of *P. atlantica* petioles was high against K562 cell line while the cytotoxic effect produced by *E. camaldulensis* fruits and leaves was moderate. At the same time, results showed low cytotoxic effects against the normal skin fibroblast cell line. Previously, strong cytotoxic effects of the ethanol extract of *P. atlantica* fruits on KB cells and human gingival fibroblast cell lines (HGF) have been reported.[35] In another study, the anti-proliferative efficacy of the mastic gum resin derived from *P. atlantica* was positively correlated with its polyphenolic contents.[36] It was suggested that a possible link exists between the phenolic acid and flavonoids contents of the extracts and its anticancer activity.[36]

Conclusion

This study showed that the ethanol extracts of *Eucalyptus camaldulensis* and *Pistacia atlantica* have strong antibacterial, antifungal, antioxidant, and anti-proliferative activity. All the extracts had strong antioxidant activity. *Pistacia atlantica* leaves extract had strong antifungal activity against *A. alliaceus*, *A. niger*, *R. stolonifer*, and *C. elegans* while it was insensitive against *A. flavus*, which was the only tested fungi affected by *E. camaldulensis* fruit extract. *Eucalyptus camaldulensis* leaves extract had strong antifungal activity against *A. alliaceus*. *Eucalyptus camaldulensis* extracts had stronger antibacterial activity against *B. megaterium*, *C. diphtheriae*, and *P. mirabilis* than the antibiotic chloramphenicol. *Eucalyptus camaldulensis* and *P. atlantica* extracts had stronger antibacterial activity against *P. aeruginosa* than the antibiotic chloramphenicol. *Pistacia atlantica* petioles extract had strong anti-proliferative activity against leukemia cell line with an almost four-fold higher cytotoxicity than the normal cell line. This makes *P. atlantica* petioles ethanol extract a very good source for compounds that can be isolated and purified to develop anti- 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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References

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016; 21(5):559.
2. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. Plants. 2017; 6(2):16.
3. Bush K. Antibacterial drug discovery in the 21st century. Clin Microbiol Infect. 2004; 10(s4):10-17.
4. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci. 2017; 18(1):96-127.
5. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 2015; 11(8):982-991.
6. Greenwell M and Rahman PKSM. Medicinal plants: their use in anticancer treatment. Int J Pharm Sci Res. 2015; 6(10):4103-4112.
7. Pfeffer CM and Singh ATK. Apoptosis: a target for anticancer therapy. Int J Mol Sci. 2018; 19(2):448-457.
8. Valibeigi B, Amirghofran Z, Golmoghaddam H, Hajihosseini R, Kamazani FM. Fas gene variants in childhood acute lymphoblastic leukemia and association with prognosis. Pathol Oncol Res. 2014; 20(2):367-374.
9. Esmaeilbeig M, Kouhpayeh SA, Amirghofran Z. An investigation of the growth inhibitory capacity of several medicinal plants from Iran on tumor cell lines. Iran J Cancer Prev. 2015; 8(5):4032-4032.
10. Adeniyi B, Lawal T, Olaleye S. Antimicrobial and gastroprotective activities of Eucalyptus camaldulensis crude extracts. J Biol Sci. 2006; 6(6):1141-1145.
11. Bozorgi M, Memariani Z, Mobli M, Salehi Surmaghi MH, Shams-Ardekani MR, Rahimi R. Five Pistacia species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, *and P. lentiscus*): A review of their traditional uses, phytochemistry, and pharmacology. Sci World J. 2013; 2013:33.
12. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16(3):144-158.
13. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64(4):555-559.
14. Monteiro JM, Albuquerque UPd, Lins-Neto EMdF, Araújo ELd, Amorim ELCd. Use patterns and knowledge of medicinal species among two rural communities in Brazil's semi-arid northeastern region. J Ethnopharmacol. 2006; 105(1):173-186.
15. Olugbami JO, Gbadegesin MA, Odunola OA. In vitro free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*. Pharmacogn Res. 2015; 7(1):49-56.
16. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J. 2013; 21(2):143-152.
17. Manandhar S, Luitel S, Dahal RK. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med. 2019; 2019. Article ID 1895340.
18. Ginovyan M, Petrosyan M, Trchounian A. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Compl Altern Med. 2017; 17(1):50.
19. Gabr S, Nikles S, Wenzig EM, Ardjomand-Woelkart K, Hathout RM, El-Ahmady S, Motaal AA, Singab A, Bauer R. Characterization and optimization of phenolics extracts from Acacia species in relevance to their anti-inflammatory activity. Biochem Syst Ecol. 2018; 78:21-30.
20. Ghannoum MA, Hajjeh RA, Scher R, Konnikov N, Gupta AK, Summerbell R, Sullivan S, Daniel R, Krusinski P, Fleckman P, Rich P.A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. J Am Acad Dermatol. 2000; 43(4):641-648.
21. Dulger G and Aki C. Antimicrobial activity of the leaves of endemic *Stachys pseudopinardii* in Turkey. Trop J Pharm Res. 2009; 8(4):371-375.
22. Nemudzivhadi V and Masoko P. *In vitro* assessment of cytotoxicity, antioxidant, and anti-inflammatory activities of *Ricinus communis* (Euphorbiaceae) leaf extracts. Evid-Based Compl Altern Med. 2014; 2014. Article ID 625961.
23. Oskay M, Oskay D, Kalyoncu F. Activity of some plant extracts against multi-drug resistant human pathogens. Iran J Pharm Sci. 2009; 8(4):293-300.
24. Knezevic P, Aleksic V, Simin N, Svircev E, Petrovic A, Mimica-Dukic N. Antimicrobial activity of Eucalyptus camaldulensis essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant Acinetobacter baumannii .J Ethnopharmacol. 2016; 178:125-136.
25. Maddox CE, Laur LM, Tian L. Antibacterial activity of phenolic compounds against the phytopathogen *Xylella fastidiosa*. Curr Microbiol. 2010; 60(1):53-58.
26. Hosseini F, Adlgostar A, Sharifnia F. Antibacterial activity of *Pistacia atlantica* extracts on Streptococcus mutans biofilm. Int Res J Biological Sci. 2013; 2(2):1-7.
27. Roozegar MA, Jalilian FA, Havasian MR, Panahi J, Pakzad I. Antimicrobial effect of *Pistacia atlantica* leaf extract. Bioinf. 2016; 12(1):19-21.
28. Gakuubi MM, Maina AW, Wagacha JM. Antifungal activity of essential oil of *Eucalyptus camaldulensis* dehnh. against selected *Fusarium* spp. Int J Microbiol. 2017; 2017. 8761610.
29. Falahati M, Sepahvand A, Mahmoudvand H, Baharvand P, Jabbarnia S, Ghojoghi A, Yarahmadi M. Evaluation of the antifungal activities of various extracts from *Pistacia atlantica* Desf. Curr Med Mycol. 2015; 1(3):25-32.
30. Simonetti G, Brasili E, Pasqua G. Antifungal activity of phenolic and polyphenolic compounds from different matrices of *Vitis vinifera* L. against human pathogens. Molecules. 2020; 25(16):3748
31. Sahin Basak S and Candan F. Chemical composition and In vitro antioxidant and antidiabetic activities of *Eucalyptus Camaldulensis* Dehnh. essential oil. J Iran Chem Soc. 2010; 7(1):216-226.
32. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999; 47(10):3954-3962.
33. Rigane G, Ghazghazi H, Aouadhi C, Ben Salem R, Nasr Z. Phenolic content, antioxidant capacity and antimicrobial activity of leaf extracts from *Pistacia atlantica*. Nat Prod Res. 2017; 31(6):696-699.
34. Nordin ML, Abdul Kadir A, Zakaria ZA, Othman F, Abdullah R, Abdullah MNH. Cytotoxicity and apoptosis induction of Ardisia crispa and its solvent partitions against Mus musculus mammary carcinoma cell line (4T1). Evid-Based Compl Altern Med. 2017; 2017. Article ID 9368079.
35. Jaafari-Ashkvandi Z, Shirazi S, Rezaeifard S, Hamedi A, Erfani N. Cytotoxic effects of *Pistacia Atlantica* (Baneh) fruit extract on human KB cancer cell line. Acta Med (Hradec Kral). 2019; 62(1):30-34.
36. Rahman HS. Phytochemical analysis and antioxidant and anticancer activities of mastic gum resin from *Pistacia atlantica* subspecies kurdica. OncoTargets Ther. 2018; 11:45.