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Chemical composition, cytotoxic effects, antimicrobial and antibiofilm activity of *Artemisia arborescens* (Vaill.) L. growing wild in the province of Agrigento, Sicily, Italy

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ABSTRACT

Artemisia arborescens (Vaill.) L. is a perennial shrubby plant growing along the coastal strips of the Mediterranean region. It is used in traditional medicine. Its essential oil and solvent extracts exhibit a very interesting chemotherapeutic potential, which makes this plant useful in maintaining human health. The goal of this study was to determine the phytochemical composition of the petroleum ether and methanol extracts, as well as to evaluate anticancer

activities and antimicrobial and biofilm formation reduction. Thirty-nine phytochemical compounds in negative ion mode, and 25 in positive ion mode were identified by HPLC-ESI-QTOF-MS. All four extracts reduced the viability of human MDA-MB231 and HCT116 cancer cells suggesting a similar cytotoxic efficacy of the different extracts in MDA-MB231 and more pronounced antiproliferative effects on HCT116 cells treated with HPEE and CPEE.

Antimicrobial activity was exhibited by the hot petroleum ether extract against all tested bacteria (MIC 0.15-2.5 mg/mL), except for *S. agalactiae*.

Both methanol extracts showed activity against *C. albicans* with a MIC value of 2.5 mg/mL, and a lack of antibacterial activity. To whom concern the anti-biofilm activity, hot methanol extract shows the highest activity in inhibiting biofilm formation among all extract.

GRAPHICAL ABSTRACT

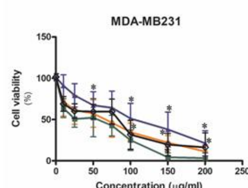
Artemisia arborescens

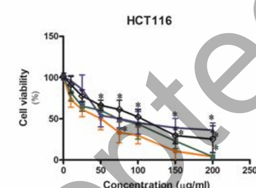
HPLC-ESI-QTOF-MS → 39 phytochemical compounds in negative ion mode
25 phytochemical compounds in positive ion mode

EXTRACTS:
CPEE = cold petroleum ether extract
HPEE = hot petroleum ether extract
CME = cold methanol extract
HME = hot methanol extract

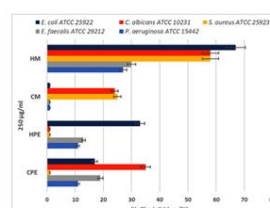
	Minimum inhibitory concentration (MIC) in mg/mL			
	CM	HM	CPE	HPE
S. aureus ATCC 25923	>2.5	>2.5	>2.5	2.5
P. aeruginosa ATCC 15442	>2.5	>2.5	2.5	1.25
E. coli ATCC 25922	>2.5	>2.5	>2.5	2.5
E. faecalis ATCC 29212	>2.5	>2.5	2.5	2.5
S. epidermidis ATCC 12228	>2.5	>2.5	>2.5	0.15
S. hominis ATCC 27844	>2.5	>2.5	>2.5	2.5
P. acnes ATCC11827	>2.5	>2.5	>2.5	2.5
S. agalactiae ATCC 13813	>2.5	>2.5	>2.5	>2.5
C. albicans ATCC 10231	2.5	2.5	1.25	1.25

ANTIMICROBIAL ACTIVITY





CYTOTOXIC ACTIVITY



INHIBITION OF BIOFILM FORMATION

KEYWORDS: *Artemisia arborescens* (Vaill.) L., HPLC-ESI-QTOF-MS, antitumor effects, antimicrobial activity, antibiofilm activity

Introduction

Artemisia arborescens (Vaill.) L. is an aromatic perennial shrubby plant of the Asteraceae family that has a bushy habit, with erect white-tomentose stems, very branched and lignified at the base (Figure 1). It has grey-green to silver very jagged leaves. The inflorescence is an erect spike, composed of many small light-yellow flowers. The fruits are achenes.

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[FIGURE 1 NEAR HERE]

It grows on arid and uncultivated soils, mainly rocky near the sea, limestone cliffs, roadsides, tuffs, garrigue (Figure 2) (Pignatti 1982; Garcia et al. 2006; Fascella et al. 2021).

[FIGURE 2 NEAR HERE]

A. arborescens is found along the Mediterranean coastal strips from 0 to 1000 m asl where it develops into shrubs even more than one meter high. It inhabits the whole Mediterranean region growing naturally along the coasts of Portugal, Spain, and Greece, and from Algeria to Libya and Turkey; in France, Balearics and Cyprus, it is a naturalized alien (Garcia et al. 2006). In Italy it is possible to find it from north to south along on most of the Tyrrhenian coast and along the southern Adriatic coast (Figure 3) (IPFI 2018).

[FIGURE 3 NEAR HERE]

A. arborescens is also known with several common popular names coming from the regions of origin some of which are: tree wormwood (England), baumwermut (Germany), armoise arborescente (France), ajenjo moruno (Spain), drvenasti pelin (Croatia), losna arbustiva do algarve (Portugal) (Greuter and von Raab-Straube, 2006). In Italy, some vernacular names are artemisia arborescente, assenzio arboreo, assenzio arbustivo, arcemesa, assenzu, assinziu arburio, attentu, dunzel, erva janca, nascienza, nasacenzio, senzu (PFI, 2018).

A. arborescens can be considered an important medicinal species useful as remedies for several health problems. Particularly, a decoction of the leaves can be used externally as vaginal antiseptic while the infusion of leaves is a useful drink to treat common colds, vertigo and helminthiasis (Merzouki et al. 2000). In North Africa, it is also considered a spice which is added to green tea or coffee to aromatize them (Trendafilova et al. 2021). *A. arborescens* is one of the most cited plants, in Sicily and Sardinia, used for gastrointestinal disorder, dermatologic problems, fever, respiratory complaints, and skeleto-muscular disorders (Leonti et al. 2009). The wine infusion of flowers of *A. arborescens* was used for toothache, as

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digestive and for worm in Italy, in the Campania hinterland (De Natale and Pollio 2007). Finally, *A. arborescens* is used in Sicily to make a decoction by crushing and soaking their aerial parts in cold water and untreated wine, to relieve the symptoms of skin disease (Bader et al. 2015).

Essential oils from *A. arborescens* were also studied in depth, revealing a broad range of bioactivity due to the presence of several constituents. Particularly, antimicrobial activity (Benbelaïd et al. 2014; Said et al. 2016), cytotoxic activity (Ali et al. 2014), chemopreventive and antioxidant activity (Ornano et al. 2013), antiherpevirus activity (Saddi et al. 2007), were reported from the *A. arborescens* essential oils. Solvent extracts of *A. arborescens* were also studied albeit to a lesser extent than essential oils. Antihypertensive activity and lipid-lowering effect on rats were shown by the aqueous extracts of *A. arborescens* (Belmokhtar et al. 2020) as well as for weed control in durum wheat (Carrubba et al. 2020). Dichloromethane extract of aerial part of *A. arborescens* was also analyzed showing inhibitory effects in two in vivo anti-angiogenic assays, zebrafish embryos and chick chorioallantois membrane (Costa et al. 2016). Antioxidant activity of bioactive polyphenolic phytochemicals extracted by a step-by-step optimization process, was described for *A. arborescens* (Shehata et al. 2015).

In this work, our aim was to estimate and characterize the phytochemicals content of *A. arborescens* in petroleum ether and methanol extracts as well as to evaluate its antimicrobial, antibiofilm, and anti-proliferative properties. Moreover, considering that its decoction is able to reduce skin disease (Bader et al. 2015), we also investigated the antibacterial activity against some reference strains of dermatological interest: *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Propionibacterium acnes*, and *Streptococcus agalactiae*.

Materials and methods

Plant materials

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The aerial parts of *A. arborescens* was collected in the clayey and well-ventilated hilly soils of the countryside of Agrigento (Sicily). Subsequently, the aerial part of the plants was air dried in the shade at room temperature for 3 weeks and then chopped for further analysis.

Extraction procedure

A. arborescens (30 g of aerial dried plant material) was macerated in 500 mL for 72 h at room temperature under magnetic stirring with two solvents of different polarity: methanol ACS reagent 99.8% (Sigma-Aldrich) and petroleum ether 40-60°C (Macron). After this time, the mixture was filtered to separate the solution from the solid residue.
The solution was evaporated to dryness under reduced pressure at 55°C to obtain 2.56 g of brown oil methanol extract and 0.13 g of white solid petroleum ether extracts. Furthermore, the solid residue, coming from the filtration of the cold macerates, were extracted in a continuous extraction apparatus (Soxhlet) until exhaustion both with 250 mL of fresh methanol and with 250 mL fresh petroleum ether of for 48h. The solvent of each extract was completely removed to dryness under reduced pressure at 55°C to obtain g 0.77 of methanol extract and g 0.08 of petroleum ether extract. The extracts were stored at -20°C until the use.

Reversed Phase HPLC/MS experiments

Water and acetonitrile were of HPLC/MS grade. Formic acid was of analytical quality. Samples for HPLC were prepared diluting the obtained extract with H_2O (1:1, v/v). The HPLC system was an Agilent 1260 Infinity. A reversed-phase Agilent Poroshell 120 EC-C18 column (50mm×3.0mm, particle size 2.7μm) with a Phenomenex C18 security guard column (4mm×3mm) was used.
The flow-rate was 0.4 mL/min and the column temperature was set to 30°C. The eluents were formic acid-water (0.1:99.9, v/v) (phase A) and formic acid-acetonitrile (0.1:99.9, v/v) (phase B). The following gradient was employed: 0-10 min, linear gradient from 5% to 95% B; 10-12 min, reconditioning to 5% B; 12-15min, 5% B isocratic. Injection volume was 15 μL. The

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eluate was monitored through MS TIC together with the UV chromatogram at 270 nm. Mass spectra were obtained on an Agilent 6540 UHD accurate-mass Q-TOF spectrometer equipped with a Dual AJS ESI source working in negative and positive mode. N_2 was employed as desolvation gas at 320°C and a flow rate of 7 L/min. The nebulizer was set to 20 psig for negative mode and 25 psig for positive mode. The Sheat gas temperature was set at 295°C and a flow of 8 L/min. A potential of 2.6 kV was used on the capillary for negative ion mode and a potential of 3.5 kV for positive ion mode. The fragmentor was set to 175V. MS spectra were recorded in the 100-1000 m/z range.

Compounds 1, 2, 4, 5, 6, 7, 8, 18, 19, 25, 26, 30, 32 (table 1) and 1, 5, 11, 13 (table 2) were identified by comparison with standards. Other metabolites identification was performed by comparison with the Metlin database (Scripps Center for Metabolomics, https://metlin.scripps.edu) or with available literature. Compounds 6, 27, 28 from table 1 and 19, 21 from table 2 were found in both CME and HME as trace amount. Compounds 1, 14, 35 from table 1 and 15,18 from table 2 were found in both CPEE and HPEE as trace amount.

Cell lines and culture conditions

Triple negative breast cancer MDA-MB231 cells, obtained from Istituto Scientifico Tumori (Genoa, Italy), were grown as monolayers in DMEM medium. HCT116 adenocarcinoma colon cancer cells, provided by Interlab Cell Line Collection (ICLC, Genoa, Italy), were cultivated in RPMI 1640 medium. DMEM and RPMI were both supplemented with 10% (v/v) fetal bovine serum (FCS), 2 mM glutamine and 1% non-essential amino acids. The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 as previously reported (Lauricella et al. 2019). For the experiments, cells were plated on 96-well plates, then were allowed to adhere overnight in culture medium before the treatment with chemicals or vehicle only.

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Cell viability assay

For these experiments, MDA-MB231 or HCT116 cells were plated in 96-well plate (8×10^3/well) in the presence of different concentrations of the extracts. After 48 h cell viability was determined by a colorimetric assay incubating the cells with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), as previously reported (Raffa et al., 2015, 2017). MTT is yellow tetrazolium salt that can be reduced to purple formazan by mitochondrial enzymes of living cells. The absorbance of the formazan was measured by a microplate reader (OPSYS MR, Dynex Technologies, Chantilly, VA, USA) at 540 nm with a reference wavelength of 630 nm and cell viability was quantified as the percentage of the optical density (OD) values of treated cells compared with that of untreated control cells. Each experiment was performed in triplicate.

Microbial Strains

The reference strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus hominis* ATCC 27844, *Propionibacterium acnes* ATCC11827, *Streptococcus agalactiae* ATCC 10231, *Candida albicans* ATCC 10231 were used in the determination of Minimum Inhibitory Concentrations (MICs), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) and Inhibition of Biofilm Formation (IBF) tests. The bacterial strains were cultured aerobically in Muller-Hinton broth (MHB) or tryptic soy agar (TSA). Fungal *C. albicans* strain was cultured aerobically on Sabouraud broth or agar medium.

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Determination of Minimum Inhibitory Concentrations (MICs) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC)

The *A. arborescens* extracts subjected to in vitro microbiological experimentation were initially solubilized in DMSO at the following concentrations: cold methanol extract (CME) (53 mg/mL); hot methanol extract (HME) (49 mg/mL); cold petroleum ether extract (CPEE) (27 mg/mL); hot petroleum ether extract (HPEE) (78 mg/mL). MICs were determined by a microdilution method (Schillaci et al. 2010). Briefly, a series of solutions were prepared with a range of concentrations from 2.5 to 0.035 mg/mL (obtained by two-fold serial dilution). The serial dilutions were made in Mueller–Hinton broth (MH) (Sigma Aldrich) in a 96-wells plate, starting from a work solution of 5 mg/mL in MH. The antibacterial activity was tested against all bacterial strains. To each well, 10 μL of a bacterial suspension from a 24 h culture grown at 37 °C for 24 hours on Tryptic Soy Agar (TSA), containing ~10^6 cfu/mL was added. A positive and negative control, consisting respectively of bacterial strains in the medium without extract, and the medium without both extract and inoculum were also included in the 96-wells plate. To exclude an antimicrobial role of DMSO, further growth control wells with the same concentration of solvent contained in the highest tested concentration, were included. A substance control, consisting only of the substance solution without bacterial inoculum were added to evaluate the absorbance of substance. The plate was incubated at 37 ◦C for 24 h, the MICs were determined by a microplate reader (Glomax Multidetection System TM297 Promega, Milano Italy) as the lowest concentration of compound whose OD, read at 570 nm, was comparable with the negative control wells (broth only, without inoculum).

Antifungal activity against *C. albicans* ATCC 10231 were evaluated by using the micro-method described above, using Sabouraud broth (BS) (Sigma-Aldrich) as growth medium.

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To determine the MBC and MFC, we added 100 μL from each negative well and from the positive control of MIC determination onto a TSA plates for 24h at 37°C. The MBC or MFC were defined as the lowest concentration of substance allowing a microbial growth up to a maximum of three colonies.

Inhibition of Biofilm Formation (Crystal Violet Method)

A. arborescens extracts were tested for their ability to inhibit the growth as biofilms of *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 15442, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *C. albicans* ATCC 10231 using the crystal violet method (Federico et al., 2021). Bacteria and fungi were grown in Tryptic Soy Broth (TSB, Sigma) and Sabouraud broth (BS) respectively containing 2% glucose overnight at 37°C. After the incubation time, 2.5 μL of each microbial suspension was placed into each well of a sterile flat-bottom 96-well loaded with 200 μL of TSB or BS with 2% glucose, supplemented with several sub-MIC concentrations, ranging from 250 to 0.5 μg/mL of each extract. The plates were incubated at 37 ◦C for 24 h; after this incubation time, the medium was removed, the plates were washed twice with sterile NaCl 0.9%, air-dried and then each well was filled with 100 μL of crystal violet solution (0.1%) for 15 min. The plate was then washed three times with water, and the crystal violet was dissolved in 200 μL of ethanol by pipetting up and down. Each assay was performed in triplicate and repeated at least twice. The plate was read at 570 nm using a microplate reader (Glomax Multidetection System TM297 Promega, Milano, Italy). Inhibition percentages at several concentrations of extract were obtained by comparing the OD of control wells with that of the sample wells, the following formula was used:

Inhibition (%) = (OD growth control-OD sample)/OD growth control) x 100.

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BIC_{50} (the concentration at which the percentage of inhibition of biofilm formation is equal to 50%) was calculated using AAT Bioquest, Inc. Quest Graph™ IC50 Calculator (v.1), retrieved from https://www.aatbio.com/tools/ic50-calculator-v1.

Statistical analysis

Data are expressed as means ± standard error (SE). Results of cytotoxicity were analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 software (San Diego, CA, USA). $P<0.05$ was established as the threshold for statistical significance.

Results and discussion

Reversed Phase HPLC/MS profiles

Thirty-nine phytochemical compounds were determined by HPLC-ESI-QTOF-MS in negative ion mode and 25 in positive ion mode, in cold and hot petroleum ether extracts (see figures 4A-D as representative traces) and in cold and hot methanol extracts of *A. arborescens* (see figures 4E-H as representative traces).

[FIGURE 4 NEAR HERE]

According to literature, the genus *Artemisia* consists of about 500 species, occurring throughout the world. The different species of *Artemisia*, also including *A. arborescens* have a vast range of biological activities, including cytotoxic, antihepatotoxic, antibacterial, antifungal, antioxidant, antimalarial (Kumar and Kumari 2018). Organic acids, phenolic and polyphenolic acids, flavanols, flavonols, flavones and other flavonoids, terpenes and terpenoids constitute major classes of phytoconstituents identified in these *A. arborescens* extracts.

Compounds identified in these four extracts have been characterized by means of MS data.

Products detected in negative ion mode are summarized in Table 1, whereas compounds found in positive ion mode are summarized in Table 2, including molecular formula, retention

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time (min), calculated and experimental m/z. Biological activity of most relevant compounds is also reported.

[TABLE 1 NEAR HERE]

[TABLE 2 NEAR HERE]

Cytotoxic effects on tumor cells in culture

To demonstrate a possible antiproliferative effect of *A. arborescens* extracts, we focused our study on two different tumor cell lines, MDA-MB231, a triple negative breast cancer cell line and HCT116, an adenocarcinoma colon cancer cell line. To this end, both cancer cell lines were treated for 48 h with increasing concentrations (within the range of 10-200 µg/ml) of the different *A. arborescens* extracts prepared in different chemical conditions to compare the best extract with cancer cell inhibitory effect. The analysis was performed by using CME, HME, CPEE and HPEE, respectively. Then, the viability was assessed by MTT assay. As shown in Fig. 5, shows, compared to cells treated with vehicle only used as control, all the extracts reduce the viability of MDA-MB231 cells in a dose-dependent pattern. A reduction of about 50% of cell viability was obtained with 100 µg/mL of CME (-54%) and with 75 µg/mL of the other extracts (HME -56%, CPEE -48% and HPEE -54%), thus suggesting a similar cytotoxic efficacy of the different extracts. The different extracts were also effective in reducing the number of HCT116 viable cells in a dose dependent manner (Figure 5). However, in HCT116 cells the antiproliferative effects resulted more pronounced in cells treated with HPEE or CPEE (-50% with 50 µg/mL HPEE and -51% with 75 µg/mL CPEE, respectively) than those incubated with CME or HME (-51 % and -49 % with 100 µg/mL of CME and HME, respectively).

[FIGURE 5 NEAR HERE]

Antimicrobial activity.

Cold and hot petroleum and methanol ether extracts of *A. arborescens* were tested against relevant pathogens *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 15442, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, fungal strain *C. albicans* ATCC 10231 and against some reference strains of dermatological interest: *S. epidermidis* ATCC 12228, *S. hominis* ATCC

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27844, *P. acnes* and ATCC 11827, *S. agalactiae* ATCC13813. The antimicrobial activity against free-living microbiological strains is expressed in terms of MICs in mg/mL and is showed in Table 3.

[TABLE 3 NEAR HERE]

All two methanol extracts showed activity against *C. albicans* ATCC 10231 with a MIC value of 2.5 mg/mL, and a lack of antibacterial activity at the highest concentration against all bacterial pathogens tested. HPEE showed an interesting activity against all tested Gram-negative and Gram-positive bacteria, except *S. agalactiae*, with MIC values ranging from 0.15 to 2.5 mg/mL. To underline the activity of HPEE against the relevant pathogens *S. aureus* and *P. aeruginosa* well known for their antibiotic resistance and against which new source of active antimicrobial molecules are urgently needed. The activity against the dermatological pathogen *P. acnes* is also very interesting and might support the use of the plant as a source of plant-based (plant-derived) antibiotics in the treatment of dermatological problems. CPEE was active at 2.5 mg/mL against *P. aeruginosa,* and at 1.25 mg/mL against fungal *C .albicans* ATCC 10231. No bactericidal or fungicidal activities were detected at the maximum tested concentration of 2.5 mg/mL.

Anti-biofilm activity of *A.arborescens* extracts.

The four extracts were tested for their ability to inhibit biofilm formation of *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 15442, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and fungal strain *C. albicans* ATCC 10231. Inhibition of biofilm formation at the maximum tested concentration (250µg/mL) is reported in Figure 6. HME exhibited the highest activity in inhibiting biofilm formation of *E. coli*, *S. aureus*, and *C. albicans* with inhibition percentages ranging from 67 and 58%. The concentration at which the percentage of inhibition of biofilm formation is equal to 50% (BIC_{50}) was also consequently calculated. BIC_{50} at 104, 136, 68 µg/mL of HME were obtained in inhibiting biofilm formation of *E. coli*, *S.aureus* and *C. albicans*, respectively. Biofilms are highly resistant to conventional antibiotics and the search for new antimicrobials capable of interfering with growth as a community by pathogens is a desired feature.

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[FIGURE 6 NEAR HERE]

Conclusions

In this work, the phytochemicals content as well as the anticancer and antimicrobial activities of *A. arborescens* in both petroleum ether and methanol extracts were determined.

HPLC-TOF/MS analysis of both cold petroleum ether and cold and hot methanol extracts of *A. arborescens* suggested the presence of 39 phytochemical compounds in negative ion mode, and twenty-five phytochemicals in positive ion mode divided into the following categories: phenolic and polyphenolic acids, flavonols and derivatives, and other compounds such as ginnalin B, artabsinolide B, fargesin, which were present in different proportion in each extract. All the extracts of *A. arborescens* reduce the viability both of MDA-MB231 cells and HCT116 cells in a dose-dependent manner. In particular, the antiproliferative effects were more pronounced in HCT116 cells treated with HPEE and CPEE than CME and HME.

As regards the antimicrobial activity of *A. arborescens,* PEE, especially hot PEE, was more active in inhibiting free-living cells. Alternatively, hot methanol extract showed good activity in inhibiting biofilm formation. Among all strains tested, the fungal strain of *C. albicans* was the most susceptible to *A. arborescens* extracts.

ABBREVIATION: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMEM, Dulbecco's modified Eagle medium; MH, Mueller–Hinton broth; BS, Sabouraud broth; TSB, Tryptic Soy Broth; MIC, minimum inhibitory concentrations; MBC, minimum bactericidal and concentrations; MFC, minimum fungicidal concentrations; CPEE, cold petroleum ether extract; HPEE, hot petroleum ether extract; CME, cold methanol extract; HME, hot methanol extract.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Ali NAA, Wurster M, Denkert A, Al-Sokari SS, Lindequist U, Wessjohann L, 2014. Cytotoxic and antiphytofungal activity of the essential oils from two artemisia species. World J. Pharm. Res., 3(Suppl.5), 1350-1354.

Alves ACS, Mainardes RM, Khalil NM, 2016. Nanoencapsulation of gallic acid and evaluation of its cytotoxicity and antioxidant activity. Mater. Sci. Eng. C 60, 126-134.

Bader A, Martini F, Schinella GR, Rios JL, Prieto JM, 2015. Modulation of Cox-1, 5-, 12- and 15-Lox by popular herbal remedies used in southern Italy against Psoriasis and other skin diseases. Phytother. Res. 29: 108-113.

Belmokhtar M, Kharoubi O, Dida N, Bouakline HE, Benglia A, Benyettou I, Benahmed F, Aoues A, 2020. Antihypertensive activity and lipid-lowering effect of tree wormwood (*Artemisia arborescens*) on rats intoxicated with aluminium chloride. South Asian J. Exp. Biol., 10, 58-69.

Benbelaïd F, Khadir A, Abdoune MA, Bendahou M, Muselli A, Costa J, 2014. Antimicrobial activity of some essential oils against oral multidrug resistant *Enterococcus faecalis* in both planktonic and biofilm state. Asian Pac. J. Trop. Biomed., 4, 463-472.

Accepted Manuscript

Carrubba A, Labruzzo A, Comparato A, Muccilli S, Spina A, 2020. Use of plant water extracts for weed control in durum wheat (*Triticum turgidum* L. subsp. durum desf.). Agronomy (Basel, Switzerland) (2020), 10, 364.

Costa R, Ragusa S, Russo M, Certo G, Franchina FA, Zanotto A, Grasso E, Mondello L, Germanò MP, 2016. Phytochemical screening of *Artemisia arborescens* L. by means of advanced chromatographic techniques for identification of health-promoting compounds. J. Pharm. Biomed. Anal. 117, 499-509.

De Natale A, Pollio A, 2007. Plants species in the folk medicine of Montecorvino Rovella (inland Campania, Italy). J. Ethnopharmacol., 109, 295-303.

Devia KP, Malar DS, Nabavi SF, Sureda A, Xiao J, Nabavi SM, Daglia M, 2015. Kaempferol and inflammation: from chemistry to medicine. Pharmacol. Res., 99, 1-10

Fascella G, Militello M, Carrubba A, 2021. Propagation of *artemisia arborescens* L. by stem-cutting: adventitious root formation under different conditions. Propag. Ornam. Plants 12, 171-177.

Federico S, Pitarresi G, Palumbo FS, Fiorica C, Catania V, Schillaci D, Giammona G, 2021. An asymmetric electrospun membrane for the controlled release of ciprofloxacin and FGF-2: evaluation of antimicrobial and chemoattractant properties. Mater. Sci. Eng. C. Mater. Biol. Appl. 123, 112021.

Garcia S, Garnatje T, Twibell JD, Vallès J, 2006. Genome size variation in the *Artemisia arborescens* complex (Asteraceae, Anthemideae) and its cultivars. Genome 49, 244–253.

Greuter W, von Raab-Straube, E, 2006 (ed.): Compositae. Euro+Med Plantbase. [updated: January 2011; accessed date: 20.11.2021]. http://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameCache=Artemisia%20arborescens&PTRefFk=7000000.

Accepted Manuscript

Gülçin I, 2006. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology, 217, 213-220.

IPFI. *Artemisia arborescens* (Vaill.) L. - Scheda IPFI, Acta Plantarum ". [updated: 2018; accessed date: 20/11/2021): https://www.actaplantarum.org/flora/flora_info.php?id=929&nnn=Artemisia%20arborescens

Ji N, Pan S, Shao C, Chen Y, Zhang Z, Wang R, Qiu Y, Jin M, Kong D, 2018. Spinacetin suppresses the mast cell activation and passive cutaneous anaphylaxis in mouse model. Front. Pharmacol., 9, 824.

Kato A, Koyama J, Shinzawa K, Imaeda S, Adachi I, Nash RJ, Fleet GWJ, Shintani M, Takeuchi C, Ishikawa I, 2019. Ginnalin B induces differentiation markers and modulates the proliferation/differentiation balance via the upregulation of NOTCH1 in human epidermal keratinocytes. Bioorg. .Med. Chem, 27, 2172-2180.

Kumar S, Kumari R, 2018. Artemisia: a medicinally important genus. J. Altern. Complement. Med. Alt. Healthcare, 7, 555723.

Lauricella M, Lo Galbo V, Cernigliaro C, Maggio A, Palumbo Piccionello A, Calvaruso G, Carlisi D, Emanuele S, Giuliano M, D'Anneo A, 2019. The Anti-Cancer Effect of *Mangifera indica* L. Peel Extract is Associated to γH2AX-mediated Apoptosis in Colon Cancer Cells. Antioxidants (Basel), 8, 422

Leonti m, Casu L, Sanna F, Bonsignore L, 2009. A comparison of medicinal plant use in Sardinia and Sicily - De MateriaMedica revisited?. J. Ethnopharmacol., 121, 255-267.

Liao S-G, J-M Yue, 2016. Dimeric sesquiterpenoid. Prog. Chem. Org. Nat. Prod., 101, 1-112.

Mancuso C, Santangelo R, 2014. Ferulic acid: Pharmacological and toxicological aspects. Food Chem. Toxicol., 65, 185-195.

Accepted Manuscript

Merzoukia A, Ed-derfoufic F, Molero Mesab J, 2000. Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). Fitoterapia, 71, 278-307.

Ornano L, Venditti A, Ballero M, Sanna C, Quassinti L, Bramucci M, Lupidi G, Papa F, Vittori S, Maggi F, Bianco A, 2013. Chemopreventive and antioxidant activity of the chamazulene-rich essential oil obtained from *Artemisia arborescens* L. growing on the isle of La Maddalena, Sardinia, Italy. Chem. Biodivers., 10, 1464-1474.

Pignatti S. 1982. Flora d'Italia, I-III. Edagricole, Bologna.

PFI. *Artemisia arborescens* (Vaill.) L. – Portal to the Flora of Italy. [updated 2018; accessed date: 20/11/2021]. http://dryades.units.it/floritaly/index.php?procedure=taxon_page&tipo=all&id=5707.

Raffa D, Maggio B, Plescia F, Cascioferro S, Raimondi MV, Cancemi G, D'Anneo A, Lauricella M, Cusimano MG, Bai R, Hamel E, Daidone G, 2015. Synthesis, antiproliferative activity and possible mechanism of action of novel 2-acetamidobenzamides bearing the 2-phenoxy functionality. Bioorg. Med. Chem. 23, 6305-6316.

Raffa D, Plescia F, Maggio B, Raimondi MV, D'Anneo A, Lauricella M, Daidone G, 2017. Anthranilamide-based 2-phenylcyclopropane-1-carboxamides, 1,1'-biphenyl-4-carboxamides and 1,1'-biphenyl-2-carboxamides: Synthesis biological evaluation and mechanism of action. Eur. J. Med. Chem., 132, 262-273.

Saddi M, Sanna A, Cottiglia F, Chisu L, Casu L, Bonsignore L, De Logu A, 2007. Antiherpevirus activity of *Artemisia arborescens* essential oil and inhibition of lateral diffusion in Vero cells. Ann. Clin. Microbiol. Antimicrob., 6, 10.

Said ME-A; Militello M, Saia S, Settanni L, Aleo A, Mammina C, Bombarda I, Vanloot P, Roussel C, Dupuy N, 2016. *Artemisia arborescens* essential oil composition, enantiomeric

Accepted Manuscript

distribution, and antimicrobial activity from different wild populations from the Mediterranean area. Chem. Biodiversity, 13, 1095-1102.

Santos MD, Almeida MC, Lopes NP, Souza GEP, 2006. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol. Pharm. Bull., 29, 2236-2240.

Shehata E, Grigorakis S, Loupassaki S, Makris DP, , 2015. Extraction optimization using water/glycerol for the efficient recovery of polyphenolic antioxidants from two Artemisia species. Sep. Purif. Technol., 149, 462-469.

Schillaci D, Petruso S, Raimondi MV, Cusimano MG, Cascioferro S, Scalisi M, La Giglia MA, Vitale M, 2010. Pyrrolomycins as potential anti-staphylococcal biofilms agents. Biofouling., 26, 433-8.

Souza P, Gasparotto Jr. A, Crestani S, Stefanello MEA, Marques MCA, Silva-Santos JE, Kassuya CAL, 2011. Hypotensive mechanism of the extracts and artemetin isolated from *Achillea millefolium* L. (Asteraceae) in rats. Phytomedicine, 18, 819-825.

Trendafilova A, Moujir LM, Sousa PMC, Seca AML, 2021. Research advances on health effects of edible artemisia species and some sesquiterpene lactones constituents. Foods, 10, 65.

Valentová K, Vrba J, Bancířová M, Ulrichová J, Křen V, 2014. Isoquercitrin: pharmacology, toxicology, and metabolism. Food Chem. Toxicol, 68, 267-282.

Uppugundla N, Engelberth A, Ravindranath SV, Clausen E, Lay JO, Gidden J, Carrier DJ, 2009. Switchgrass water extracts: extraction, separation and biological activity of rutin and quercitrin. J. Agric. Food Chem., 57,7763-7770.

Wang G, Gao J-H, He L-H, Yu X-H, Zhao Z-W, Zou J, Wen F-J, Zhou L, Wan X-J, Tang C-K, 2020. Fargesin alleviates atherosclerosis by promoting reverse cholesterol transport and reducing inflammatory response. BBA-Mol. Cell. Biol. L., 1865, 158633.

Accepted Manuscript

Yue B , Ren I-J, Zhang J-J, Luo X-P, Yu Z-L, Ren G-Y, Sun A-N, Deng C, Wang Z-T, Dou W, 2018. Anti-inflammatory effects of Fargesin on chemically induced inflammatory bowel disease in mice. Molecules, 23, 1380.

Zhou X, Wang F, Zhou R, Song X, Xie M, 2017. Apigenin: a current review on its beneficial biological activities. J. Food Biochem., 41, e12376.

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Figure 1. *Artemisia arborescens* (Vaill.) L. (Pignatti 1982).

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Figure 2. *Artemisia arborescens* (Vaill.) L. growing in the place of collection (Garcia et al. 2006; Fascella et al. 2021).

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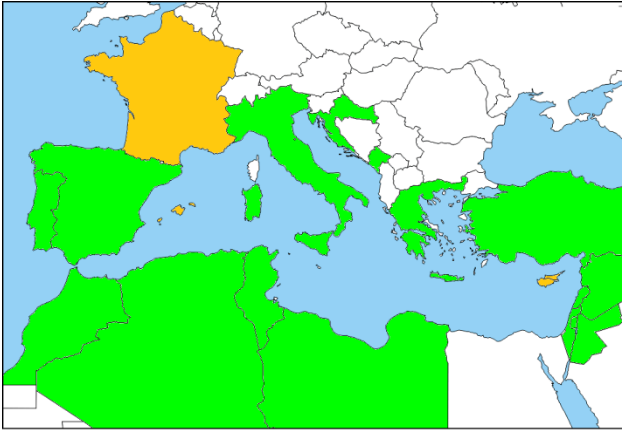

Figure 3. Distribution area of *Artemisia arborescens* (Vaill.) L.. In green the regions where it grows spontaneously, in gold those in which it grows as naturalized (Garcia et al. 2006).

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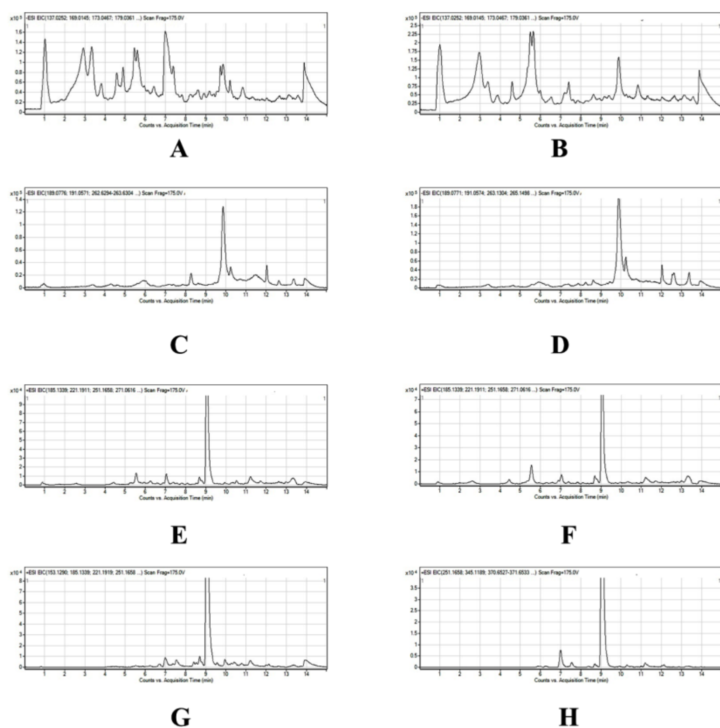

Figure 4. Representative HPLC/ESI/QTOF trace of: **A** cold methanol extract (negative mode); **B** hot methanol extract (negative mode); **C** cold petroleum ether extract (negative mode); **D** hot petroleum ether extract (negative mode); **E** cold methanol extract (positive mode); **F** hot methanol extract (positive mode); **G** cold petroleum ether extract (positive mode); **H** hot petroleum ether extract (positive mode) of *Artemisia arborescens* (Vaill.) L.

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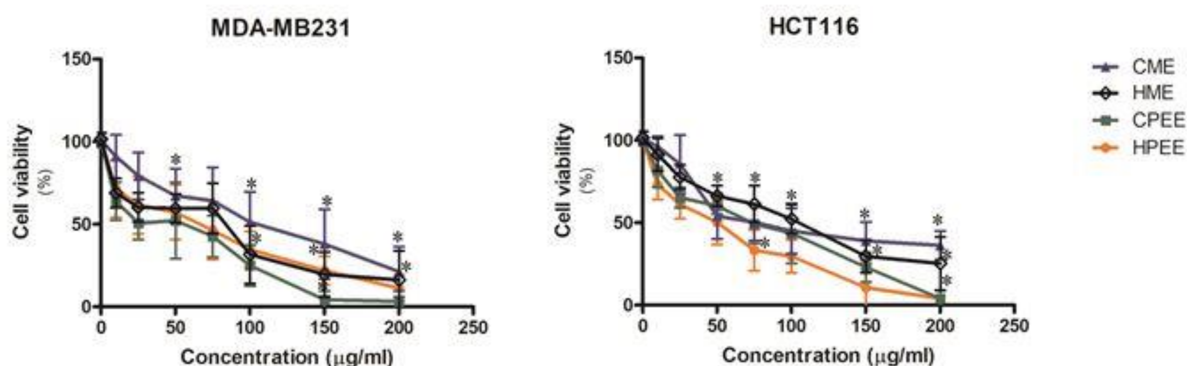

Figure 5 Analysis of cytotoxic effects exerted by *Artemisia arborescens* (Vaill.) L. extracts on breast cancer and colon cancer cells after 48 h. Extracts of *Artemisia arborescens* (Vaill.) L. were prepared in different chemical conditions (**CME**= cold methanol extract; **HME**= hot methanol extract, **CPEE**=petroleum ether extract; **HPEE**= hot petroleum ether extract) and assayed in two different tumor cell lines (breast cancer MDA-MB231 and adenocarcinoma colon cancer HCT116). The cell viability was analyzed by MTT assay, as reported in Methods. Data reported in the line chart are the mean ± SD of three independent experiments, each performed in triplicate, and expressed as percentage of the vehicle-treated control. Data were considered significant at $^{*}P < 0.05$.

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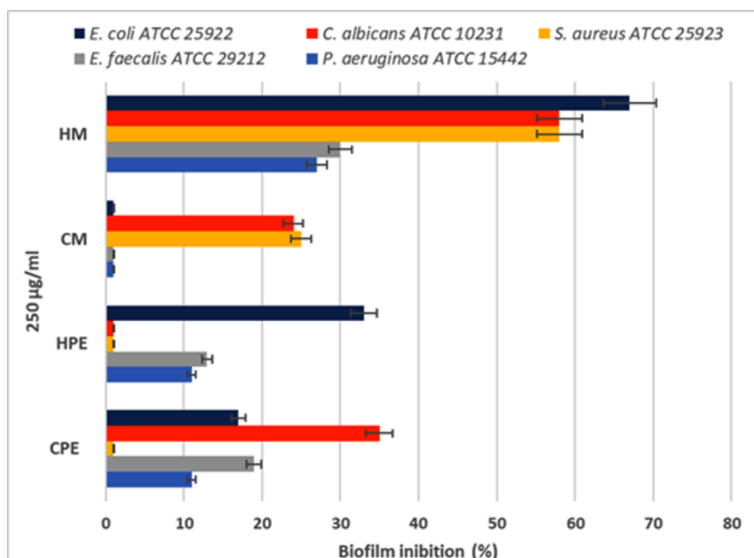

Figure 6 Inhibition of biofilm formation of bacterial and fungal pathogens by the hot and cold methanol extract (HM and CM), or the hot and cold petroleum ether extract (HPE and CPE).

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Table 1. Composition of *Artemisia arborescens* (Vaill.) L. extracts (negative ion mode).

					CME	HME	CPEE	HPEE	
	Compound	**Molecular Formula**	**RT-(min)**	**ESI$^-$ [M-H]$^-$ (m/z) (Teor.)**	**ESI$^-$ [M-H]$^-$ (m/z) (Exp.)**				**Biological activity**
1	Quinic acid	$C_7H_{12}O_6$	1.02	191.0561	191.0577	191.0578	191.0571	191.0574	
2	Gallic acid	$C_7H_6O_5$	1.27	169.0142	169.0141	169.01454			Antioxidant, neuroprotective, anticancer, anti-inflammatory, hepatoprotective, gastroprotective, cardiovascular protective activities (Alves et al., 2016).
3	Ginnalin B	$C_{13}H_{16}O_9$	1.47	315.0722	315.0733	315.0738			Acceleration of skin metabolism; arrest of proliferation at the G0/G1 phase (Kato et al., 2019).
4	Salicylic acid	$C_7H_6O_3$	2.71	137.0244	137.0251	137.0252			
5	Chlorogenic acid	$C_{16}H_{18}O_9$	2.97	353.0878	353.0896	353.0895			Antioxidant; anti-carcinogenic activities; (Santos et al., 2006).
6	Malic acid diethyl ester	$C_8H_{14}O_5$	3.39	189.0768	189.0784	189.0780	189.0776	189.0771	
7	Diethyl malonate	$C_7H_{12}O_4$	3.52	159.0663	159.0652				
8	Caffeic acid	$C_9H_8O_4$	3.83	179.0350	179.0358	179.0361			Antioxidant activity (Gülçin, 2006).
9	Gallocatechin	$C_{15}H_{14}O_7$	3.89	305.0667	305.0715	305.0707			
10	Spinacetin 3-glucoside	$C_{23}H_{24}O_{13}$	4.05	507.1144	507.1131	507.1131			
11	Luteolin 7-rutinoside	$C_{27}H_{30}O_{15}$	4.23	593.1512	593.1514	593.1521			
12	Melilotoside	$C_{15}H_{18}O_8$	4.31	325.0929			325.0953		
13	3-O-Feruloylquinic acid	$C_{17}H_{20}O_9$	4.52	367.1035	367.1062	367.1042			
14	Methylsyringin	$C_{18}H_{26}O_9$	4.58	385.1504	385.1521	385.1520	385.1514	385.1476	
15	Apigenin 7-xylosyl-glucoside	$C_{26}H_{28}O_{14}$	4.61	563.1406	563.142	563.1417			
16	3-O-Feruloylquinic acid isomer	$C_{17}H_{20}O_9$	4.83	367.1035	367.1062	367.1042			
17	Patuletin 3-glucoside	$C_{22}H_{22}O_{13}$	4.92	493.0988		493.0999			
18	Rutin	$C_{27}H_{30}O_{16}$	4.95	609.1461	609.1451	609.1451			Anticancer, antimicrobial, anti-inflammatory, antidiabetic properties; antioxidant (Uppugundla et al., 2009).
19	Isoquercitrin	$C_{21}H_{20}O_{12}$	5.14	463.0882	463.0895	463.0895			Chemoprotective; ability to scavenge ROS and RNS, (Valentová et al., 2014).
20	Quercitrin	$C_{21}H_{20}O_{11}$	5.18	447.0933	447.0961	447.0961			
21	Isorhamnetin 3-rutinoside	$C_{28}H_{32}O_{16}$	5.34	623.1618	623.1625	623.1625			
22	Di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	5.49	515.1195	515.1283	515.1283	515.1281		
23	Quercitrin isomer	$C_{21}H_{20}O_{11}$	5.58	447.0933	447.0952	447.0952			
24	Di-O-caffeoylquinic acid isomer	$C_{25}H_{24}O_{12}$	5.61	515.1195	515.1223	515.1223	515.1221		
25	Shikimic acid	$C_7H_{10}O_5$	5.64	173.0455	173.0466	173.0467			

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25	Isorhamnetin-5-glucoside	$C_{22}H_{22}O_{11}$	5.71	461.1089	461.1105	461.111	461.1011	461.1019	
26	Ferulic acid	$C_{10}H_{10}O_4$	5.96	193.0506	193.0518	193.0518			Antioxidant activity; inhibit the expression and/or activity of cytotoxic enzymes (Mancuso and Santangelo, 2014).
27	Tanacetin	$C_{15}H_{20}O_4$	6.16	263.1289	263.1304	263.1304	263.1304	263.1304	
28	Tanacetin isomer	$C_{15}H_{20}O_4$	6.39	263.1289	263.1294	263.1294	263.1294	263.1294	
29	Dihydrokaempferol	$C_{15}H_{12}O_6$	6.49	287.0561	287.0572	287.0569			
30	Kaempferol	$C_{15}H_{10}O_6$	6.57	285.0405	285.0417	285.0419			inhibiting expression of pro-inflammatory cytokines and chemokines (Devi et al., 2015).
31	Spinacetin	$C_{17}H_{14}O_8$	6.82	345.0616	345.0635	345.0635			Anti-inflammatory and anti-asthmatic activity. (Ji et al., 2018).
32	Apigenin	$C_{15}H_{10}O_5$	7.16	269.0455	269.0473	269.0473	269.0473		Cytostatic and cytotoxic effects; ability to prevent atherogenesis, hypertension, cardiac hypertrophy, ischemia/reperfusion-induced heart injury, and autoimmune myocarditis, capacity to inhibit the asthma, bleomycin-induced pulmonary fibrosis, abnormal behavior, and oxygen and glucose deprivation/reperfusion-induced neural cell apoptosis, improves the pancreatitis, type 2 diabetes, osteoporosis, and collagen-induced arthritis (Zhou et al., 2017).
33	Kaempferol 5-methyl ether	$C_{16}H_{12}O_6$	7.29	299.0561	299.0585	299.0575			
34	Diosbulbin E	$C_{19}H_{22}O_6$	7.34	345.1344	345.1362	345.1362			
35	Pinellic acid	$C_{18}H_{34}O_5$	7.40	329.2333	329.235	329.235	329.235	329.2350	
36	Eupatin	$C_{18}H_{16}O_8$	7.65	359.0772	359.078	359.0788	359.078	359.0753	
37	Cirsilineol	$C_{18}H_{16}O_7$	8.31	343.0823	343.0847	343.0847		343.0847	
38	Artemetin	$C_{20}H_{20}O_8$	9.16	387.1085			387.1088		Antioxidant and anti-inflammatory activities, ability to inhibit the cell cycle and the enzyme lipoxygenase; reduction of hypertensive response to angiotensin I. (Souza et al., 2011).
39	Arabsin	$C_{15}H_{22}O_4$	9.87	265.1445	265.1498	265.1498	265.1498	265.1498	

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Table 2. Composition of *Artemisia arborescens* (Vaill.) L. extracts (positive ion mode).

					CME	HME	CPEE	HPEE	
	Compound	**Molecular Formula**	**RT+ (min)**	**ESI$^+$ [M+H]$^+$ (m/z) (Teor.)**	**ESI$^+$ [M+H]$^+$ (m/z) (Exp.)**				**Biological activity**
1	Chlorogenic acid	$C_{16}H_{18}O_9$	2.62	355.1024	355.1044	355.1044			Antioxidant; anti-carcinogenic activities; (Santos et al., 2006).
2	Luteolin 7-rutinoside	$C_{27}H_{30}O_{18}$	4.06	595.1657	595.1690	595.1690			
3	Apigenin 7-xylosyl-glucoside	$C_{26}H_{28}O_{14}$	4.45	565.1552	565.1591	565.1591			
4	Artabsinolide	$C_{15}H_{20}O_5$	4.52	281.1384			281.1395		
5	Rutin	$C_{27}H_{30}O_{16}$	4.83	611.1607	611.1607	611.1607			Anticancer, antimicrobial, anti-inflammatory, antidiabetic properties; antioxidant (Uppugundla et al., 2009).
6	Isorhamnetin 3-rutinoside	$C_{28}H_{32}O_{16}$	5.17	625.1763	625.1798				
7	Di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	5.3	517.1341	517.1376	517.1379			
8	Di-O-caffeoylquinic acid isomer	$C_{25}H_{24}O_{12}$	5.45	517.1341	517.1377	517.1377			
9	Isorhamnetin-5-glucoside	$C_{22}H_{22}O_{11}$	5.56	463.1235	463.1266	463.1266			
10	Dihydrokaempferol	$C_{15}H_{12}O_6$	6.26	289.0707	289.0725	289.0725			
11	Kaempferol	$C_{15}H_{10}O_6$	6.37	287.055	287.058	287.058			inhibiting expression of pro-inflammatory cytokines and chemokines (Devi et al., 2015).
12	Spinacetin	$C_{17}H_{14}O_8$	6.71	347.0761			347.0821		Anti-inflammatory and anti-asthmatic activity. (Ji et al., 2018).
13	Apigenin	$C_{15}H_{10}O_5$	6.88	271.0601	271.0616	271.0616			Cytostatic and cytotoxic effects; ability to prevent atherogenesis, hypertension, cardiac hypertrophy, ischemia/reperfusion-induced heart injury, and autoimmune myocarditis, capacity to inhibit the asthma, bleomycin-induced pulmonary fibrosis, abnormal behavior, and oxygen and glucose deprivation/reperfusion-induced neural cell apoptosis, improves the pancreatitis, type 2 diabetes, osteoporosis, and collagen-induced arthritis (Zhou et al., 2017).
14	Kaempferol 5-methyl ether	$C_{16}H_{12}O_6$	7.02	301.0707	301.0717	301.0719			
15	Artabsin	$C_{15}H_{22}O_3$	7.05	251.1642	251.1658	251.1658	251.1658	251.1658	
16	Eupatin	$C_{18}H_{16}O_8$	7.36	361.0918	361.0938	361.0937			
17	Cirsilineol	$C_{18}H_{16}O_7$	8.10	345.0969	345.0995	345.0995			
18	Beta-Tujone	$C_{10}H_{16}O$	8.41	153.1274			153.1290		
19	Fargesin	$C_{21}H_{22}O_6$	8.69	371.1489	371.1528	371.1527	371.1527	371.1527	Prevention of inflammation and improvement of lipid metabolism; inhibitory effect on atherosclerosis plaque formation. (Yue et al., 2018).
20	Caryophyllene oxide	$C_{15}H_{24}O$	8.71	221.1900		221.1911	221.1919		
21	Fargesin isomer	$C_{21}H_{22}O_6$	8.8	371.1489	371.1533	371.1533	371.1532	371.1533	

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22	Artemetin	$C_{20}H_{20}O_8$	9.05	389.1231	389.1274	389.1274	389.1274	389.1275	Antioxidant and anti-inflammatory activities, ability to inhibit the cell cycle and the enzyme lipoxygenase; reduction of hypertensive response to angiotensin I. (Souza et al., 2011).
23	Chamazulene	$C_{14}H_{16}$	9.96	185.1325	185.1339	185.1339			
24	Nuciferol propionate	$C_{18}H_{26}O_2$	10.04	275.2006	275.2019	275.2019			
25	Domesticoside	$C_{15}H_{20}O_9$	10.31	345.1180	345.1189	345.1189	345.1188	345.1189	

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Table 3 Antimicrobial activity of the four extracts tested against reference bacterial and fungal free-living strains. The MIC determination is expressed in mg/mL. **CME** = cold methanol extract; **HME** = hot methanol extract; **CPEE** = cold petroleum ether extract; **HPEE** = hot petroleum ether extract.

	Minimum inhibitory concentration (MIC) in mg/mL			
	CME	**HME**	**CPEE**	**HPEE**
S. aureus ATCC 25923	>2.5	>2.5	>2.5	2.5
P. aeruginosa ATCC 15442	>2.5	>2.5	2.5	1.25
E. coli ATCC 25922	>2.5	>2.5	>2.5	2.5
E. faecalis ATCC 29212	>2.5	>2.5	2.5	2.5
S. epidermidis ATCC 12228	>2.5	>2.5	>2.5	0.15
S. hominis ATCC 27844	>2.5	>2.5	>2.5	2.5
P. acnes ATCC11827	>2.5	>2.5	>2.5	2.5
S. agalactiae ATCC 13813	>2.5	>2.5	>2.5	>2.5
C. albicans ATCC 10231	2.5	2.5	1.25	1.25

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