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Research Article

Bioactive principles, antibacterial and anticancer properties of *Artemisia arborescens* L.

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Abstract

Artemisia arborescens is a medicinal and aromatic plant used in traditionally by the people of Saudi Arabia. This research attempts to evaluate the bioactive constituents of the plant using organic solvents, as well as the antibacterial and anticancer properties of plant extracts. The Phytochemical analysis of methanol extract revealed eleven bioactive constituents, identified by comparing their retention periods and GC-MS profiles to account for 52.45 percent of the studied extract. In the meantime, the extract of pet ether had demonstrated the presence of sixteen significant constituents, six of which were distinct sesquiterpene derivatives. In lipophilic plant extract, three higher alkanes made up 12.49% of the total. These higher alkanes were tetratriacontane (6.55%), hentriacontane (4.17%), and octacosane (1.77%). Studies on antimicrobial activity have revealed that both methanolic and petroleum ether extracts had a broad spectrum of activity against specific human pathogens. Both extracts, however, failed to exhibit any anti-*Candida albicans* activity. Methanolic extract not shown inhibition in the cell growth of MCF-7 cell, but petroleum ether extract had shown significant anti-cancer activity against MCF-7 cell with an IC_{50} of 13.49 µg/mL. the results obtained show that *A. arborescens* have a lot of potential for further research into variety of biological functions, against cancer and microbes.

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Keywords: antimicrobial; *Artemisia arborescens*; cytotoxic activity*;* phytochemical screening

Introduction

Herbs are a gift from nature that allow humans to live a life free of disease and illness. It has always been seen as a vital element of daily life by the great majority of people around the world. Artemisia is the largest genus in the Asteraceae family. The plant contains a wealth of essential oils used in aromatherapy and for their abortifacient, anthelmintic, anticonvulsant, antimalarial, antimicrobial, antioxidant, antispasmodic properties, antitoxic, cytotoxic, febrifuge, insecticidal, and repellent characteristics. The treatment of circulatory, digestive, genitourinary, immune, nervous, delivery, respiratory, nutritional, musculoskeletal, dermatological, and sensory problems has been described for a variety of animals (Al-Momani *et al*., 2007; Abad *et al*., 2012; Al Bratty *et al*., 2020).

The plant has anti-oxidant principles and has anti-viral properties specifically against herpes viruses. The leaf paste of *A. arborescens* can cure acne, burns, keratosis, skin infections, sunburns, and wrinkles (Araniti *et al*., 2013). Inhaling the vapors produced by the plant is an effective approach for treating a variety of respiratory problems, such as asthma, bronchitis, catarrhal, chest congestion, cough, and sinus. Women who are pregnant, infants, and persons whose skin is injured or sensitive should avoid encountering it (Araniti *et al*., 2013). The essential oil from the aerial parts was rich in β-thujone, camphor, β-carophyllene, myrcene, chamazulene and eudesmol (Biondi *et al*., 1993; Ballero *et al*., 2001; He *et al*., 2003; Boussaada *et al*., 2008; Abderrahim *et al*., 2010; Boachon *et al*., 2015; Bouabdallah *et al*., 2016; Balouiri *et al*., 2016; Bechkri *et al*., 2017; Baghbani *et al*., 2017; Al Bratty *et al*., 2020;). The aerial parts afforded artemitin, arborescin, sesamin, (+)-lirioresinol β-dimethyl ether, chrysoeriol, apigenin, β-sitosterylglucoside, dihydroridentin, chrysoeriol 4-glucoside and eudesmanolidejordanolide (Cheesbrough, 1981; Bouzenna and Krichen, 2013) germacrane derivatives ketopelenolides C and D (Chinery *et al*., 1997) and a nor-caryophyllane derivative, artarborol (Chou *et al*., 2012). According to the studies by Abderrahim *et al*. (2010), Boachon *et al*. (2015), and Al Bratty *et al*. (2020) the essential oil of *A. arborescens* can be a valuable tool in the fight against food-borne diseases. This is because it inhibits the growth of Listeria monocytogenes strains. The tone and amplitude of the ileum's phasic contractions were decreased by an aqueous plant extract (Boachon *et al*., 2015). *Rhizoctonia solani* and *Rhysopertha dominica* were both susceptible to the insecticidal and antifungal effects of essential oils (Biondi *et al*., 1993).

Plant extracts and α-thujone prevented fruit infestation by codling moth neonates (Yacouba *et al*., 2019). Plant extracts inhibited lettuce's germination and root growth processes (*Lactuca sativa* L.) (Cory *et al*., 2015; Silva *et al*., 2015). Essential oils demonstrated antibacterial action against Escherichia coli, Staphylococcus aureus, and *Pseudomonas aeruginosa* (He, 2003; Boachon *et al*., 2015; Al Bratty *et al*., 2020). By restoring liver functions, the essential oil of *A. arborescens* was able to protect against hepatotoxicity that was generated by estrogen and progesterone treatment (Githinji *et al*., 2010). It has been the purpose of this study to investigate the nature of the phytochemicals and screen the bioactivities of extracts of the plant grown in the Jazan region of Saudi Arabia using methanol and pet ether solvents. This was done with the knowledge that the whole plant of *A. arborescens* has a high reputation and a variety of different bioactivities.

Materials and Methods

Plant material

Aerial portions of *A. arborescens* L. were gathered from wild populations grown in the Jazan region of Saudi Arabia in November 2020. They were identified by Dr. Yahiya Masruhi, who works in the Botany Department of the Faculty of Science at Jazan University in the Kingdom of Saudi Arabia. A voucher specimen with the number JU/COP/19-2 has been placed in the herbarium of the department.

Extraction

The dried aerial parts were coarsely pulverized and extracted in a Soxhlet apparatus according to the described procedure with some slight modifications. The polar compounds were extracted with 1.5 liters of methanol, while the non-polar compounds were extracted with petroleum ether (Ali *et al.,* 2016). Using a rotary evaporator, the two extracts were collected before being filtered and then evaporated. After that, the polar and non-polar compounds were each weighed, and their yield percentage, expressed as a percentage of their dry weight, was calculated. The yield of the dark green extract from methanol was 57.5 grams (0.095%), and the light green extract from pet ether was 23.2 grams (0.046%). After that, the plant extracts were placed in the dark and refrigerated at a temperature of 4 °C.

Table 1. Chemical composition of polar and non-polar components of the aerial parts of *Artemisia arborescens* contributes in antimicrobial and anticancer activity

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Area% (MeOH)	Area% (Pet ether)	Nature of compound	Reported activity
1.	4.55	2-Methoxy-1-(2-nitroethenyl)-3-(phenylmethoxy)-benzene	$C_{16}H_{15}NO_4$	285	3.52	--	Aromatic compound	Antibacterial activity (Militello *et al.*, 2011)
2.	6.84	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	222	2.3	--	Siloxane derivative	Anti-cancer (Klaunig *et al.*, 2016)
3.	11.87	Octamethyl cyclotetrasiloxane	$C_8H_24O_4Si_4$	296	2.55	--	Siloxane derivative	Estrogenic activity (Praveen Kumar *et al.*, 2010)
4.	20.21	Decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	370	1.42	--	Siloxane derivative	Adenocarcinoma tumorigenesis (Kumaradevan *et al.,* 2015)
5.	28.61	Dodecamethylcyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	444	5.24	--	Siloxane derivative	emollient, de-foaming agent, antimicrobial (Kavanagh *et al.,* 1972)
6.	35.12	Tetradecamethyl-cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	518	7.67	--	Siloxane derivative	Antimicrobial, preservative (Khosravani *et al.,* 2020)
7.	39.86	5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2à(R*),5à]]-(Dovanone)	C15H24O2	236	--	4.88	Oxasesquiterpenic epoxide	Antibacterial (Presti *et al.*, 2007)
8.	40.11	Hexadecamethyl-cyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	592	3.12	--	Siloxane derivative	Antimicrobial (Khosravani *et al.,* 2020)
9.	42.96	Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester, [1à,2à(Z)]-(methyl jasmonate)	$C_{13}H_{20}O_3$	224		3.74	Methyl cyclopentane acetate	Antioxidant (Yang *et al.,* 2020)
10.	43.32	2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]-	$C_{15}H_{26}O$	222	--	3.04	Bicyclic sesquiterpenic alcohol	Cytotoxic activity (Quattrocchi, 2016)

		(β-Eudesmol,β-selineol)						
11.	44.80	2-(5-ethenyl-5-methyloxolan-2-yl)propan-1-ol (Lilac alcohol A)	$C_{10}H_{18}O_2$	170	--	1.47	Tetrahydrofuran heterocyclic compd	Insect repellent, flavoring agent (Sultana *et al.,* 2007)
12.	46.13	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-octasiloxane	$C_{16}H_{50}O_7Si_8$	578	3.15	--	Siloxane derivative	Anti-microbial (Kumaradevan *et al*., 2015)
13.	47.38	8-Cedren-13-ol	$C_{15}H_{24}O$	220	--	1.63	Tricyclic Sesquiterpenic alcohol	Antioxidant, anti-inflammatory (Saddi *et al.,* 2007)
14.	48.19	Methyl hinokiate	$C_{16}H_{24}O_2$	248	--	2.44	Bicyclic sesquiterpenoic acid ester	Ant proliferative activity (Sacco *et al.,* 1983)
15.	48.49	2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-, (E,E,E)-(fernesyl acetate)	$C_{17}H_{26}O_3$	278	--	1.14	Unsaturated aliphatic aldehyde	Antifungal, antibacterial (Boussaada *et al.,* 2008)
16.	52.37	n-Hexadecanoic acid (plamitic acid)	$C_{16}H_{32}O_2$	256	--	1.43	Fatty acid	anti-oxidant, nematicide, hypocholesterolenic, pesticidal, hemolytic, 5-Alpha reductase inhibitor, antiadrogentic (Parveen Kumar *et al*., 2010; Kumaradevan *et al.,* 2015)
17.	53.28	1-(1,5-Dimethyl-4-hexenyl)-4-methyl- benzene (alpha curcumene)	$C_{15}H_{22}$	202	--	1.83	Aromaticsesquiterpene	Antimicrobial, anti-inflammatory (Shareef *et al*., 2016)
18.	55.52	1-Nonadecene	$C_{19}H_{38}$	266	--	1.35	Higher alkene	Antibiotic (Ali *et al.,* 2016)
19.	56.4	spiro[4.5]decan-7-one, 1,8-Dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	236	--	2.79	Bicyclic sesquiterpenic epoxide	Anti-inflammatory activity (Shareef *et al.,* 2016)
20.	57.58	Naphtho[1,2-b]furan-2,6(3H,4H)-dione, 3a,5,5a,9,9a,9b-hexahydro-9-hydroxy-3,5a,9-trimethyl-	$C_{15}H_{20}O_4$	264	20.44	--	Bicyclic ketosesquiterpenic lactone	Antileishmanial, immunomodulatory (Githinji *et al.,* 2010)
21.	59.02	Germacra-1(10),4,11(13)-trien-12-oic acid, 6à-hydroxy-, ç-lactone, (E,E)-(Costunolide)	$C_{15}H_{20}O_2$	232	1.65	--	MegacyclicSesquiterpenic lactone	Antioxidant, anti-inflammatory, osteoblastic activity, neuroprotective, anticancer (Kim and Choi, 2019)
22.	63.07	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	--	2.31	Aliphatic alcoholic acetate	Anti-cancer, antioxidant, anti-inflammatory (Vijisaral and Arumugam, 2014)
23.	66.81	1,2-Benzenedicarboxylic acid, diisooctyl ester (Phthalic acid, diisooctyl ester)	$C_{24}H_{38}O_4$	390	1.36	0.83	Phthalic acid diester	Antibacterial, Anti-inflammatory, antipromastigotes activity and antiamastigotes activity, anti-pathogenic, antifungal, platelet phospholipase A2 inhibitor, testiculat toxicant and promote protein phosphorylation, antioxidant (Li *et al*., 2012)
24.	70.14	Octacosane	$C_{28}H_{58}$	394	--	1.77	Higher alkane	Antioxidant, anti-inflammatory activity (Tanod *et al*., 2019)
25.	75.24	Tetratriacontane	$C_{34}H_{70}$	478	--	6.55	Higher alkane	Hypoglycemic, antioxidant

								(Sivakumar and Gayathri, 2015)
26.	79.92	Hentriacontane	$C_{31}H_{64}$	436	--	4.17	Higher alkane	Anti-tumor (Takahashi *et al.,* 1995)

GC-MS analysis of the extract

The dried extracts were evaluated using a GC-MS (Thermo Scientific, USA) equipped with an AS 3000 autosampler; trace ultra-GC and ISQ detector. The components were separated using TR 5MS (Thermo Scientific, USA) with dimensions of 30 m × 0.25 mm (internal diameter) × 0.25 m (film thickness). Helium, at a flow rate of 1.2 mL/min (constant flow mode), was used as a carrier gas. A volume of 2 µL of sample extracts was injected in split less mode. The injection port was set at 320 °C and temperature of the oven was initially set at 70 °C for 5 minutes. The oven temperature was subsequently ramped to 205 °C at a rate of 5 °C /min for 5 minutes, 280 °C at a rate of 5 °C /min for 5 min, 290 °C at rate of 5 °C /min for 5 min and finally to 300 °C at rate of 5 °C /min for 5 min. Maximum oven temperature was set at 320 °C. Mass spectrometer was operated in an electron ionization (EI) mode within the mass range of 60-900 amu with 0.6 scan times (min). The MS transfer line temperature and ion source temperature were kept at 320 °C and 350 °C, respectively with electron multiplier voltage of 1 Kv.

Identification of constituents

GC-MS identified most constituents by comparing their retention indices with authentic standards available in the library, which were in close agreement with the reference samples. The mass spectra's fragmentation patterns achieved further identification compared with those stored in the spectrometer database using the NIST08 and Wiley 9n/Adams mass spectral library of GC/MS data system. They confirmed with the aid of retention indices from published sources. The relative percentage of separated compounds was calculated from FID chromatograms. The relative concentration of each compound was quantified according to the peak area integrated by the analysis program. Table1 shows the GC MS of methanol and Petroleum ether extracts of aerial parts of *A. arborescens.*

Assessment of antibacterial potentiality

Sample analyte

5% w/v of the sample analyte was prepared by dissolving methanolic and petroleum ether extracts of *A. arborescens* individually in Milli-Q water and heating at 60 °C for 10 min. The pH of sample analyte was determined as 7.1. The sample analyte was further sonicated to disperse uniformly for 5 mi using a laboratory probe sonicator (CPX ultrasonic processor, Cole Parmer Instruments Co, USA) at 40 % amplification.

Bacterial strains

The bacterial strains used in the study were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 254992), *Proteus mirabilis* ATCC 257440, *Klebsiella pneumonia* ATCC 254656 and *Staphylococcus aureus* ATCC 29213. Briefly, a 24 h culture was established and standardized using a nutrient broth dilution gradient from 10^{-1} to 10^{-7}. The vitality of a bacterial culture was determined by measuring the number of colony forming units per milliliter (CFU/mL).

Determination of Minimum inhibitory concentration (MIC)

Detection of the minimum inhibitory concentration (MIC) was adopted for the crude extracts of *A. arborescens* against the bacteria using broth dilution method. Test bacterial cultures (100 µL of bacterial culture containing 10^{-5} CFU/mL) were inoculated into tubes containing different concentrations of both polar and non-polar extracts of 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 mg/L and incubated overnight at 37 °C. The values were determined by detecting the inhibition of visible growth in the culture tubes. The minimum

concentration of inhibiting the bacterial growth was determined based on the visibility of bacterial growth in the broth (Mee *et al.,* 2008; Ivanescu *et al.,* 2015).

Determination of antibacterial susceptibility

Briefly, Muller Hinton agar plates were prepared for performing the antibacterial study (Janackovic *et al*., 2015). Bacterial subcultures were prepared from the stock culture, and after 24 h incubation, the culture was subjected to antibacterial studies. Agar well diffusion technique was performed for sample analytes, and disc diffusion was utilized for standard ciprofloxacin disc (5 mcg/disc). The inoculation was done by dipping a sterile cotton swab into the standardized (CFU/ml) culture individually with various organisms and streaking on the MH agar plate by rotating the Petri dish to distribute the culture evenly. The plates were allowed to dry for about 10 min before administration of sample analytes. The agar well diffusion technique was performed by punching holes on the inoculated MH agar plates using a standard sterile stainless-steel borer. The plates were incubated at 37 °C for 24 h, and the development of inhibitory zones assessed the antibacterial spectrum after 24 h incubation.

Assessment of antifungal potentiality

Sabouraud dextrose (SD) agar plates were prepared for evaluating the antifungal study. The organism was subcultured in Sabouraud dextrose broth and incubated at 25 °C for 72 h. Following incubation, the broth culture was diluted serially from 10^{-1} to 10^{-5}. A 10^{-3} dilution was chosen as the optimal concentration based on the growth of *Candida albicans* ATCC 254625 as determined by the crowded plate technique. The spread plate technique was used for growing the *Candida albicans* ATCC 254625. The sample analyte was analysed using the agar well diffusion method. A conventional sterile stainless-steel borer was used to create holes in SD agar plates that had already been inoculated to carry out the agar well diffusion procedure. The samples were inserted into the wells of SD agar plates, which had a diameter of 10 mm. Disc diffusion technique was used for the standard Amphotericin-B AP 20 mcg/disc (Hi media, India). The activity of EG against *Candida albicans* was compared with amphotericin.

In vitro cytotoxicity study

Cell line and culture

MCF - 7 human breast cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, and penicillin (100 U/mL), streptomycin (100 µg/mL), and incubated at 37 °C with 5% CO_2.

Cell growth assay

The cytotoxicity profiles of the formulations were determined by MTT viability assay as described previously. Briefly, 1×10^6 cells were seeded into 96 well plate and incubated for 24 hours. The attached cells in 96 well plats were treated for 72 hours with various concentration of extracts (highest was 100 µg/mL). Each plate was included with untreated cell controls and blank cell-free control. After incubation, MTT (5 mg/mL) was added to each well and the plates were incubated for a further 4 h after which the media was removed. DMSO (100 µL) was added into each well to solubilize the formazan crystals. The absorbance was read at a wavelength of 490 nm using a microtiter plate reader (BioTek Instruments, Winooski, VT, USA). The percentage of cellular viability was calculated with the appropriate controls considered. The experiment was done in triplicate. The cytotoxicity of our formulations on cancer cells was expressed as IC50 values (sample concentration reducing the cell count of treated cells by 50% with respect to untreated cells).

Results and Discussion

Phytochemical screening

The GC MS analysis of *A. arborescens* extract revealed the presence of various bioactive constituents such as organic siloxane derivatives, sesquiterpene compounds, cyclopentane derivative, tetrahydrofuran, aliphatic compounds, fatty acid, phthalic acid di ester, higher alkene and alkanes. Figure 5 and Figure 6 represents some important bioactive compounds contribute in antimicrobial and anticancer activities based on literature survey. Retention time (RT), compounds, molecular formula (MF), molecular weight (MW), area percentage of methanol and pet. Ether extracts with reported activities are given in Table 1. GC MS profiling for methanol and petroleum ether extracts are represented in Figures 1 and 2 respectively.

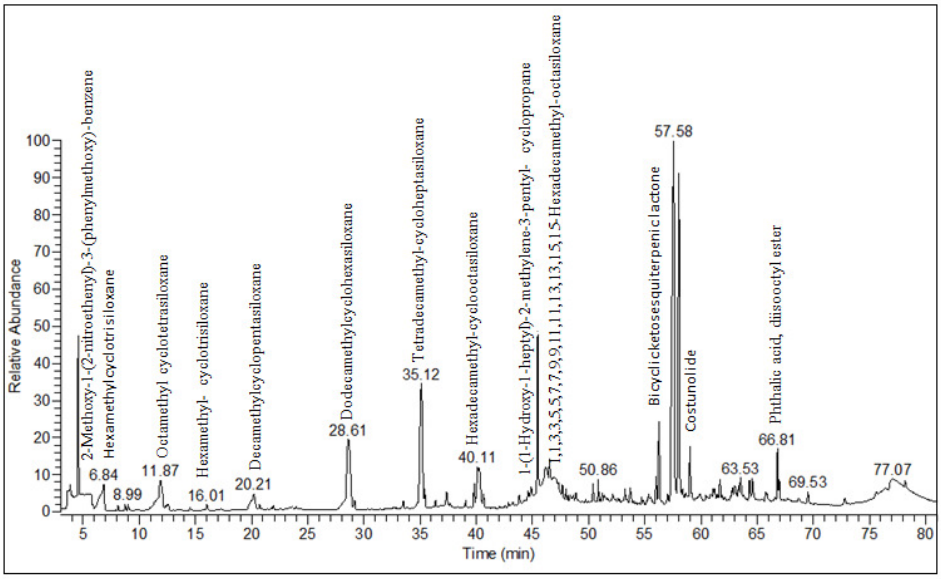

Figure 1. GC–MS chromatogram of the hot methanol extract of aerial parts of *Artemisia arborescens*

Seven different derivatives of siloxanes detected in alcoholic extract are frequently utilized in a variety of industrial processing and consumer goods, including paper coatings, cosmetics, shampoos, and textiles. Its numerous advantageous traits and crucial chemical feedstocks for the synthesis of variety of silicone products (Kavanagh, 1972). Dodecamethylcyclohexasiloxane (5.24%), tetradecamethyl-cycloheptasiloxane (7.67%), hexadecamethyl-cyclooctasiloxane (3.12%) and 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-octasiloxane (3.15%) were having anti-microbial properties (Kavanagh, 1972; Kim *et al*., 2019; Khosravani *et al*., 2020), while hexamethylcyclotrisiloxane (2.3%) was anticancer in nature (Klaunig *et al.,* 2016), octamethyl cyclotetrasiloxane (2.55%) was pharmacologically estrogenic compound (Praveen Kumar *et al*., 2010). Decamethylcyclopentasiloxane (1.42%) is a cyclic siloxane used in the formulation and production of variety of consumer products (Kumaradevan *et al*., 2015).

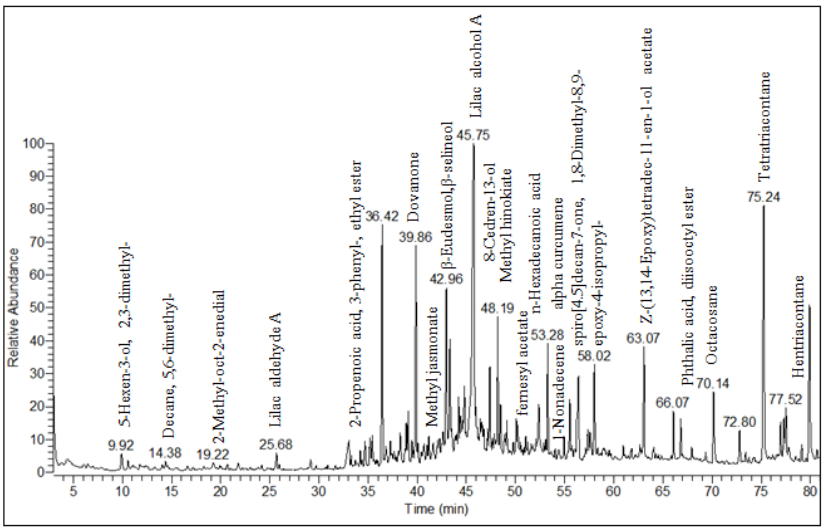

Figure 2. GC–MS chromatogram of the hot petroleum ether extract of aerial parts of *Artemisia arborescens*

Sesquiterpene lactones are bitter, colourless and lipophilic compounds found primarily in genus *Artemisa*. They exhibit a wide range of biological activities, like antitumor, anti-ulcer, anti-bacterial, anti-viral, anti-fungal, anti-inflammatory, analgesic, anti-parasitic and insect deterrent effects (Lai *et al*., 2007). Naphtho[1,2-b]furan-2,6(3H,4H)-dione, 3a,5,5a,9,9a,9b-hexahydro-9-hydroxy-3,5a,9-trimethyl- (20.44%) and Germacra-1(10),4,11(13)-trien-12-oic acid, 6à-hydroxy-, ç-lactone, (E,E)- (1.65%) also known as costunolide were two sesquiterpene lactones reported in methanol extract of aerial parts with antioxidant, anti-leishmanial and immunomodulatory (Li *et al*., 2012) activities. 2-Methoxy-1-(2-nitroethenyl)-3-(phenylmethoxy)-benzene (3.52%) an aromatic compound exhibited antibacterial properties (Militello *et al*., 2011).

Sixteen important bioactive compounds were identified in pet ether extract of the plant. Six of them were different derivatives of sesquiterpenes like 5-hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2à(R*),5à]]- (4.88%), 2-naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]- (3.04%), 8-cedren-13-ol (1.63%), methyl hinokiate (2.44%), 1-(1,5-dimethyl-4-hexenyl)-4-methyl- benzene (1.83%) and spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl- (2.79%). The characterized sesquiterpenes were reported with multiple medicinal importance such as antibacterial (Presti *et al*., 2007), anti-cancer (Quattrocchi, 2016), anti- proliferative (Sacco *et al*., 1983), anti-oxidant, anti-inflammatory effects (Saddi *et al*., 2007; Said *et al*., 2016).

Among three higher alkanes, hentriacontane is a solid long chain alkane with good anti-tumor properties in concentrations of 0.65 ng/ml (Takahashi *et al*., 1995) while antioxidant, anti-inflammatory (Tanod *et al*., 2019), and hypoglycemic properties were reported in tetratriacontane (6.55%) and octacosane (1.77%) (Sivakumar and Gayathri, 2015. 1-Nonadecene (1.35%) was an antibiotic identified in non-polar extract (Sinico *et al*., 2005). An unsaturated aliphatic aldehyde called 2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-, (E,E,E)- (1.14%) has antifungal and antibacterial properties (Boussaada *et al*., 2008). Z-(13,14-epoxy)tetradec-11-en-1-ol acetate (2.31%), an aliphatic alcoholic acetate has been proven for anti-cancer, antioxidant and anti-inflammatory qualities (Vijisaral, and Arumugam, 2014).

n-Hexadecanoic acid (1.43%) a well-known fatty acid detected in the extract has been reported to have anti-oxidant, nematicide (Kim *et al*., 2019), hypocholesterolenic, pesticidal, hemolytic, 5-alpha reductase inhibitor, antiadrogentic properties (Sivakumar and Gayathri, 2015). (Parveen Kumar *et al*., 2010; Kumaradevan *et al*., 2015). 2-(5-ethenyl-5-methyloxolan-2-yl)propan-1-ol (1.47%) was powerful insect

repellent (Sultana *et al*., 2007) respectively. Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester, [1à,2à(Z)]- (3.74%) commonly known as methyl jasmonate has antioxidant activity (Yang *et al*., 2020)

1,2-benzenedicarboxylic acid, diisooctyl ester present in both the extracts of *A. arborescens* in different concentrations, was previously isolated from many plant species like unripe fruits of *Nauclea latifolia*, aerial parts of *Gremlina asiatica* Linn., *Murraya koenigii*, roots of *Plumbago zeylanica*, *Trichodesma indicum*, seeds of *Entada pursaetha* etc. According to USDA 2016, it is used in textile, furniture, dyestuffs, glass making, cars, clothing, boots, perfumes, and cosmetics. Phthalic acid diester is bioactive compound with antibacterial, antioxidant, anti-inflammatory, antipromastigotes, antiamastigotes, anti-pathogenic, antifungal, platelet phospholipase A2 inhibitor, testiculat toxicant and promote protein phosphorylation (Li *et al*., 2012).

Antimicrobial activity

The MIC and MBC values of the aerial parts of *A. arborescens* were calculated to be in the range of 125-250 and 250-500 µg/mL respectively against all bacterial strains tested as shown in Table 2. The investigation revealed that the antibacterial activity of petroleum ether extract was superior to that of methanolic extract (Figure 3). *Proteus mirabilis* (22 ± 2 mm) > *Klebsiella pneumonia* (21.3 ± 1.5) > *Staphylococcus aureus* (10.3 ± 1.5 mm) against methanolic extract; *Proteus mirabilis* (21.3 ± 2.5 mm) > *Escherichia coli* (21 ± 2.6 mm) > *Staphylococcus aureus* (19 ± 2 mm) > *Klebsiella pneumonia* (11.3 ± 3.5) against petroleum ether extract (Figures 3A and 3B). Interestingly both methanolic and petroleum ether extracts were showing higher activity against *Proteus mirabilis*. Methanolic extract was inactive against *E. coli* and *P. aeruginosa* whereas petroleum ether extract was inactive against *P. aeruginosa*. Furthermore, both methanolic and petroleum ether extracts were inactive against the fungal strain *Candida albicans*. In contrast to our findings, a recent investigation found that both methanolic and hot petroleum ether extracts were effective against *Candida albicans* (Plescia *et al*., 2022). According to Jaradat *et al.* (2022), essential oil from *A. arborescens* revealed antibacterial properties against MRSA and *P. vulgaris*. According to a previous study, the essential oil isolated from *A. arborescens* was efficient against Listeria monocytogenes but ineffective against enterobacteria and salmonellas (Militello *et al*., 2011).

Table 2. Antimicrobial study

Organism	Methanol extract			Petroleum ether extract		
	Zone of inhibition	MIC (µg/mL)	MBC (µg/mL)	Zone of inhibition	MIC (µg/mL)	MBC (µg/mL)
E. coli	-	-	-	21 ± 2.6	125	500
K. pneumonia	21.3 ± 1.5	250	500	11.3 ± 3.5	250	500
P. mirabilis	22 ± 2	125	500	21.3 ± 2.5	125	500
P. aeruginosa	-	-	-	-	-	-
S. aureus	10.3 ± 1.5	500	500	19 ± 2	250	500
C. albicans	-	-		-	-	-

(-) No activity. Each value is the mean of n=3 batches with standard deviation.

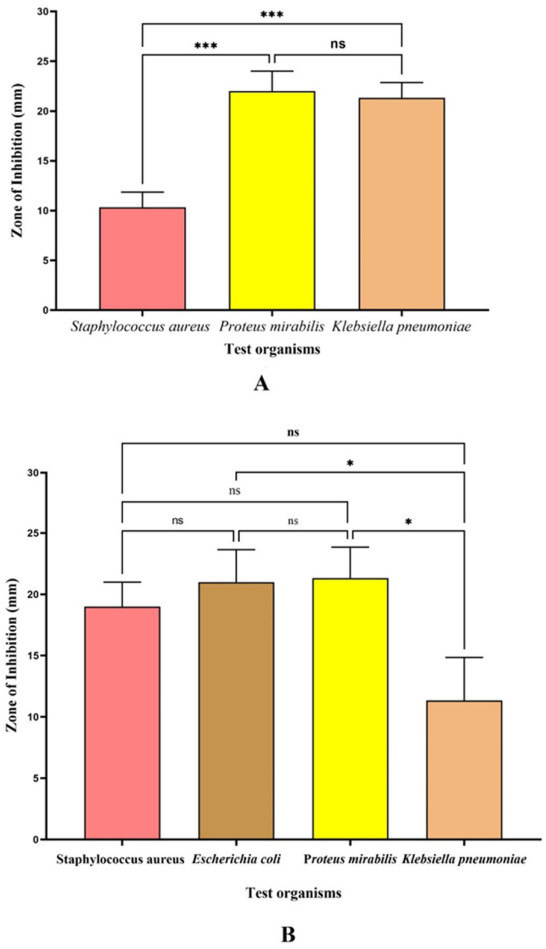

Figure 3. Anti-bacterial study **(A)** Effect of hot methanolic extract against selected human pathogenic bacteria **(B)** Effect of hot petroleum ether extract against selected human pathogenic bacteria

Cell growth inhibition

To determine the effect of *A. arborescens*, two different extracts of this plant were tested against the MCF-7 cells for 72 h. We have set 100 µg/ mL as the maximum concentration to be used in this study. As observed from the results, methanol extract has shown no inhibition in the cell growth, but the pet ether extract had shown significant activity with an IC_{50} of 13.49 µg/mL (Figure 4).

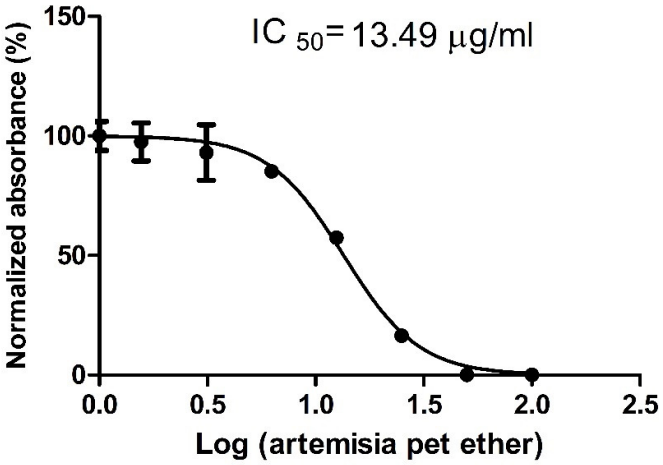

Figure 4. Effect of pet ether extract on the cell growth of MCF-7 cells
Cells were treated with extract for 72 h and then the growth inhibition were determined by MTT assay. Data are the mean ± SD of three replicates

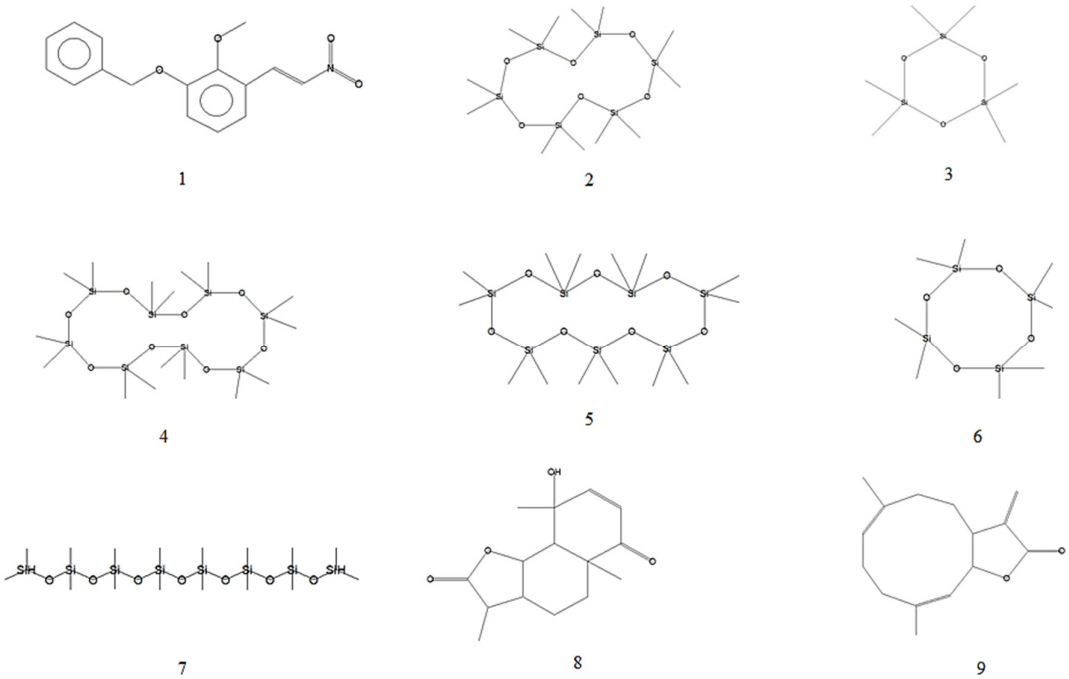

Figure 5. Bioactive compounds identified in methanol extract of aerial parts of *Artemisia arborescens*
(1) 2-Methoxy-1-(2-nitroethenyl)-3-(phenylmethoxy)-benzene; (2) Dodecamethyl cyclo- hexasiloxane; (3) Hexamethylcyclotrisiloxane; (4) Hexadecamethyl-cyclooctasiloxane; (5) Tetradecamethyl-cycloheptasiloxane; (6) Octamethyl cyclotetrasiloxane; (7) 1,1,3,3,5,5,7, 7,9,9,11,11,13,13,15,15-Hexadecamethyl- octasiloxane; (8) Naphtho[1,2-b]furan-2,6(3H,4H)-dione, 3a,5,5a,9,9a,9b-hexahydro-9-hydroxy-3,5a,9-trimethyl-; (9) Germacra-1(10),4,11(13)-trien-12-oic acid,6à-hydroxy-, ç-lactone, (E,E)-

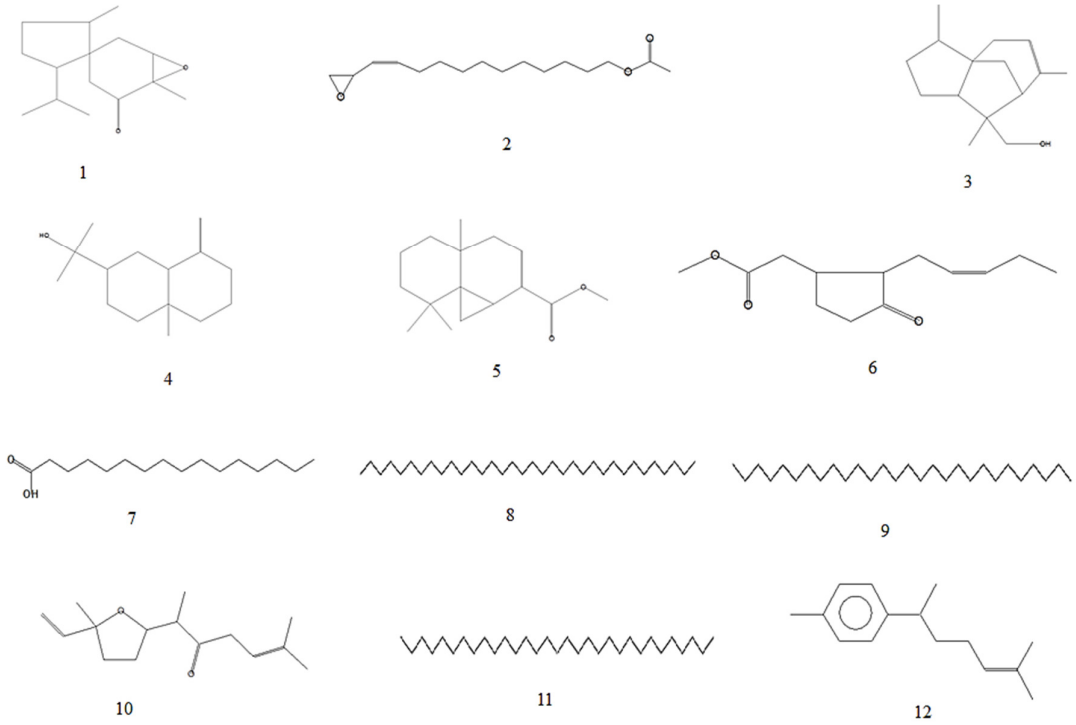

Figure 6. Bioactive compounds identified in petroleum ether extract of aerial parts of *Artemisia arborescens* (1) Spiro[4.5]decan-7-one, 1,8-Dimethyl-8,9-epoxy-4-isopropyl-; (2) Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate; (3) 8-Cedren-13-ol; (4) β-Eudesmol,β-selineol; (5) Methyl hinokiate; (6) Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester, [1à,2à(Z)]- ; (7) n-Hexadecanoic acid; (8) Tetratriacontane; (9) Octacosane; (10) 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2à(R*),5à]]- (11) Hentriacontane; (12) 1-(1,5-Dimethyl-4-hexenyl)-4-methyl- benzene.

Even though there are no literatures available regarding the anti-cancer effect of *Artemisia arborescens*, the species *Artemisia* is well known for its ability to inhibit cancers (Velez *et al*., 2007; Vijisaral, and Arumugam, 2014). Additionally, the phytochemical analysis performed in the current study had exhibited a various range of chemicals, in which few of them has been shown cytotoxic activity earlier. Either they exert direct cell cytotoxicity, or via another mechanism. One among them is antioxidant compounds. They either exert cell death in cancer cells, and in some cases, it can synergistically assist the cytotoxic behaviour of other chemicals. Earlier it is documented that antioxidants such as vitamin E and pyrrolidine dithiocarbonate can enhance the cytotoxic activity of DNA-damaging chemotherapeutic agents 5-fluorouacil and doxorubicin in colorectal cancers (Wang *et al*., 2008). In the current study, we have found the presence of antioxidant compound Methyl cyclopentane acetate (Yang *et al*., 2020), tricyclic sesquiterpene alcohol (Saddi *et al*., 2007), fatty acids (Kim *et al*., 2019) and some Higher alkane (Zarga *et al*., 1995; Younes *et al*., 2012). The presence of these compounds strongly supports our assumption of the role of antioxidants in the cytotoxicity activity. Moreover, the cytotoxic compounds present in the pet ether extracts such as Bicyclic sesquiterpene alcohol (Quattrocchi, 2016) and Bicyclic sesquiterpenoid acid ester (Sacco *et al*., 1983) may also be taken part an important role in the observed cytotoxic activity.

Conclusions

The study's findings revealed that *A. arborescens* is a promising plant that has an abundance of phytochemicals, the majority of which have medicinal effects. This comprehensive research enables it to be used as a natural alternative to various synthetic drugs. The chemicals identified in this plant have antibacterial and anticancer capabilities, according to our research.

Authors' Contributions

Conceptualization: SS; Methodology and Investigation: HAM, GK; Project administration and funding: HAH, HEH; Project administration and result analysis: SM, AN; Methodology and result analyses: MAB; Software and statistical analysis: SNMNU; Supervision AZ.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Notes: