Antibacterial activity and mode of action of the *Artemisia capillaris* essential oil and its constituents against respiratory tract infection-causing pathogens

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Abstract. Inhalation therapy using essential oils has been used to treat acute and chronic sinusitis and bronchitis. The aim of the present study was to determine the chemical composition of the essential oil of *Artemisia capillaris,* and evaluate the antibacterial effects of the essential oil and its main components, against common clinically relevant respiratory bacterial pathogens. Gas chromatography and gas chromatography-mass spectrometry revealed the presence of 25 chemical constituents, the main constituents being: α-pinene, β-pinene, limonene, 1,8-cineole, piperitone, β-caryophyllene and capillin. The antibacterial activities of the essential oil, and its major constituents, were evaluated against *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), MRSA (clinical strain), methicillin-gentamicin resistant *Staphylococcus aureus* (MGRSA), *Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae* and *Escherichia coli*. The essential oil and its constituents exhibited a broad spectrum and variable degree of antibacterial activity against the various strains. The essential oil was observed to be much more potent, as compared with any of its major chemical constituents, exhibiting low minimum inhibitory and bactericidal concentration values against all of the bacterial strains. The essential oil was most active against *S. pyogenes*, MRSA (clinical strain), *S. pneumoniae*, *K. pneumoniae*, *H. influenzae* and *E. coli*. Piperitone and capillin were the most potent growth inhibitors, among the major chemical constituents. Furthermore, the essential oil of *A. capillaris* induced significant and dose-dependent morphological changes in the *S. aureus* bacterial strain, killing >90% of the bacteria when administered at a higher dose; as determined by scanning electron microscopy. In addition, the essential oil induced a significant leakage of potassium and phosphate ions from the *S. aureus* bacterial cultures. These results indicate that the antibacterial action of *A. capillaris* essential oil may be mediated through the leakage of these two important ions. In conclusion, *A. capillaris* essential oil exhibits potent antibacterial activity by inducing morphological changes and leakage of ions in *S. aureus* bacterial cultures.

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Introduction

Upper respiratory tract infection (URTI) are disorders caused by acute infections, usually affecting the nose, sinuses, pharynx, or larynx. The various URTIs include the common cold, sinusitis, laryngitis and pharyngitis, amongst others (1). URTIs are usually caused by viruses, such as rhinoviruses, coronaviruses, parainfluenza virus and adenoviruses (2). However, numerous URTIs are also caused by bacteria, including *Streptococcus pyogenes, Streptococcus pneumoneae, Heamophilus influenzae, Corynebacterium diptheriae, Bordetella pertussis* and *Bacillus anthracis*. Bronchitis, which is an inflammation of the mucous membranes of the bronchi, can also be caused by certain types of bacteria. Approximately 10% of bronchitis cases are caused by bacteria, such as *Mycoplasma pneumoniae, Chlamydophila pneumoniae, B. pertussis* and *S. pneumoniae* (3,4).

Essential oils are composed of an odoriferous mixture of monoterpenes, sesquiterpenes and aromatic compounds, and form an important part of naturopathic therapy, where they are well-known for their antimicrobial properties. Essential oils were among the first topical and gastrointestinal antimicrobial agents used by mankind. Due to the recent, extensive use of conventional antibiotics and synthetic antimicrobial drugs, there has been an increase in the widespread development of drug resistant microorganisms, including methicillin-resistant *Staphylococccus aureus* (MRSA), and multidrug resistant strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These drug-resistant microbes pose a challenge for scientists to identify alternative ways to treat microbial infections. Essential oils are antimicrobial agents with multiple target sites. The essential oils and their components target

the bacterial cell wall and cytoplasmic membrane, resulting in permeabilization, which is followed by the loss of ions, reduction of the membrane potential, collapse of the proton pump and depletion of the ATP pool (5-7). Due to their multi-functionality, essential oils have a potentially large application in medicine and aromatherapy. Essential oils have been shown to exhibit potent antimicrobial actions against a wide range of both Gram-positive and Gram-negative bacteria (8).

Essential oils have also been used traditionally for treating respiratory tract infections, and are currently used as alternative medicines for the treatment of colds (9-11). Inhalation therapy using essential oils, has previously been used to treat acute sinusitis, and acute and chronic bronchitis. It has been reported that inhalation therapy using volatile essential oil vapors, is capable of enhancing the respiratory tract fluid output (12), maintaining the ventilation and drainage of the sinuses, and reducing asthma and inflammation of the trachea (13-15).

The present study aimed to determine the chemical composition of the essential oil of *Artemisia capillaris* as well as to evaluate the antibacterial effects of *Artemisia capillaris* and its primary components against common clinically relevant respiratory bacterial pathogens. Gas chromatography mass spectrometry was used to study the chemical composition of the oil. In addition, Agar well diffusion assays and micro-well dilution methods were used to study the antibacterial susceptibility of the microbes towards the essential oil. Furthermore, Scanning Electron Microscopy (SEM) was used to study the morphological changes which occurred following oil exposure.

Materials and methods

Materials. The major essential oil components: α-pinene, β-pinene, limonene, 1,8- cineole, piperitone, β-caryophyllene and capillin were all purchased from Sigma-Aldrich (St Louis, MO, USA).

Plant material. The aerial portions of the *Artemisia capillaris* plant were collected from a local region in Jianguo, China, between June and July 2013. The plant was identified by an experienced taxonomist.

Essential oil isolation. The extraction of the essential oil from the aerial parts of *A. capillaris,* was carried out by hydrodistillation for 3 h using Clevenger-type apparatus (Lianyungang Hightborn Technology Co., Ltd, Jiangsu, China), as recommended in the European Pharmacopoeia (16). Three samples of the dried aerial parts (200 g) were subjected to hydrodistillation at separate times. The essential oil was collected, dehydrated with Na_2SO_4 (Sigma-Aldrich), and stored at 4˚C until further use.

Essential oil analysis. The essential oil was analyzed by a combination of gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analytical techniques.

GC-FID analysis. GC-FID was carried out using a Perkin Elmer AutoSystem XL Gas Chromatograph 8500 series (Perkin Elmer, Waltham, MA, USA), with a flame ionization detector and head space analyzer, using a fused silica capillary column HP-5 (30 m x 0.25 mm x 0.25 μm) coated with dimethyl polysiloxane. The oven temperature was programmed from 50-260˚C at 2˚C/min, with an injector temperature of 250˚C and a detector temperature of 260˚C. The injection volume was 0.8 μl, and nitrogen was used as the carrier gas (1.2 ml/min).

GC-MS analysis. GC-MS analysis was conducted using a Varian Gas Chromatograph series 3800 (Varian Medical Systems, Palo Alto, CA, USA) fitted with a VF-5 MS fused silica capillary column (60 m x 0.25 mm, x 0.25 μm), using split/splitless injection, and coupled with a 4000 series mass detector. The GC-MS was conducted under the following conditions: injection volume 0.8 μl with a split ratio of 1:80, helium was used as the carrier gas (1.5 ml/min constant flow mode), an injector temperature of 250˚C, and the oven temperature was programmed from 50-260˚C at 2˚C/min. Mass spectra was produced with an electron impact (EI+) mode 70 ev, and ion source temperature 260˚C. The mass spectra were recorded at a range between 50-500 a.m.u.

Identification of components. Identification of the essential oil constituents was based on the Retention Index, which was determined with respect to a homologous series of n-alkanes (C5-C28; Polyscience, Niles, IL, USA), which underwent the same experimental conditions; co-injection with standards (Sigma Aldrich and standard isolates); an MS Library search (NIST 05 and Wiley); and by comparing the MS data of the present study with the previous MS literature data (17).

Antibacterial testing

Bacterial strains and culture media. *S pyogenes* [American Type Culture Collection (ATCC) 12344], MRSA (ATCC 43300), MRSA (clinical strain), Methicillin-gentamicin resistant *S. aureus* (MGRSA; ATCC 33592), *S. pneumoniae* (ATCC 2730), *K. pneumoniae* (ATCC 27853), *H. influenzae* (ATCC 33391), *E. coli* (clinical strain) were used in the present study. All of these bacterial strains were obtained from the State Key Laboratory of Microbial Resources, the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). The bacterial strains were grown on nutrient agar plates, at 37˚C, and maintained on nutrient agar slants. Cell suspensions of the micro-organisms in 0.5% NaCl, were adjusted at 0.5 Mcfarland to obtain ~10^6 cfu/ml.

Agar well diffusion assay. The antibacterial susceptibility test was conducted using the agar well diffusion assay. The overnight bacterial cultures were added to 30 ml of liquid nutrient agar. The contents of the tubes were then transferred to petri plates. Following 20 min solidification of the agar petri plates, at 25˚C, the wells of the plates were filled with 20 μl neat *A. capillaris* essential oil and the major chemical constituents. The plates were then incubated for 24 h at 37˚C. Following the incubation, the antimicrobial efficacy of the essential oil and its individual constituents was determined by calculating the width of the inhibition zone. The inhibition zones were expressed in mm. All of the experiments were repeated in triplicate. Ampicillin and vancomycin, (10 μg/disc) were used as positive controls.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The MIC of the essential oil was determined using the micro-well

dilution method, as recommended by the National Committee for Clinical Laboratory Standards, as reported by previous methods (18). The essential oil and its chemical constituents were dissolved in dimethylsulfoxide (2 mg/ml) and diluted to prepare concentrations in the range of 0.10-392.8 μg/ml (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 24.6, 49.2, 98.4, 196 and 392.8 μg/ml). The inoculum suspensions, with a final concentration of $0.5x10^6$ cfu/ml, were added to 96-well microplates. A total of 150 μl Mueller Hinton (MH) broth was added to the wells of the 10th column, which was reserved for the bacterial growth control. The wells of the 11th column were reserved for the control of broth sterility. The wells of the final column were used as a negative control, containing 150 μl of nutrient broth and 5 μl of inoculum. Following a 24 h incubation period at 37˚C, the plates were screened visually for broth turbidity. The MIC was defined as the lowest concentration of the essential oil and the components at which the bacteria did not exhibit any visible growth, following the 24 h incubation at 37˚C. The MBC was defined as the lowest concentration of the essential oil and the components at which 99.9% of the bacterial population were killed, following the 24 h incubation at 37˚C.

SEM. The essential oil-treated bacterial cells were obtained for SEM. Briefly, an overnight culture of *S. aureus* (clinical strain), grown on MH agar at 37˚C, was added to a saline solution containing 0.1% Tween®-80 (Shanghai Sungo Technology and Trade Co., Ltd, Shanghai, China). Four different concentrations of the *A. capillaris* essential oil (20, 40, 60 and 80 μg/ml) were prepared and added to the suspension, which was then incubated at room temperature. Following a 24 h incubation, the bacterial cells were centrifuged at 8000 x g for 15 min. The bacterial cells were then washed with 0.1 mol/l Tris-acetate buffer (pH 7.1), fixed in Tris-acetate buffer containing 1.5% glutaraldehyde, and freeze-dried. Each of the bacterial cultures was observed using a SEM (Shenzhen Jinliyang Technology Co., Ltd., Guangdong, China), at magnification 10,000x. A bacterial cell suspension in saline, with no essential oil treatment, was used as a positive control.

Determination of potassium and phosphate ion efflux. Overnight bacterial cultures of *S. aureus* were harvested and washed three times in deionized water. The bacterial cells were treated with 50 μl *A. Capillaris* essential oil and the concentration of extracellular potassium and phosphate ions was estimated, using an ion selective electrode and a phosphorous inorganic kit 670-A (Sigma-Aldrich), respectively. The numerical values were compared with standard calibration curves of HPO_4 and KCl for determination of phosphate and potassium ion efflux, respectively. To observe the dead and viable cells both prior to resuspension in water, and during the incubation period at 37˚C in the absence of an antimicrobial agent, 70 μl of the bacterial cultures were transferred to 250 μl MH broth, and variations in optical density were recorded using a Bioscreen (Shanghai Vizai Trade and Development Co., Ltd, Shanghai, China).

Results

Chemical composition of the essential oil of A. capillaris. The yield of the essential oil obtained from the dry aerial parts of *A. capillaris* was ~1.6% w/v. The chemical components of the essential oil were identified using GC and GC-MS techniques (Table I and Fig. 1). A total of 25 compounds were identified in the *A. capillaris* essential oil, accounting for 90.1% of the total oil composition. The major components of the essential oil were: α-pinene (4.3%), β-pinene (12.1%), limonene (4.5%), 1,8-cineole (6.2%), piperitone (4.2%), β-caryophyllene (5.2%), capillin (24.2%), and germacrene D (3.9%) (Fig 2). The essential oil of *A. capillaris* was dominated by the presence of monoterpene hydrocarbons (29.3%), oxygenated monoterpenes (18%), sesquiterpene hydrocarbons (12.4%), amongst others (24.2%). Previous studies have also demonstrated that various *Artemisia* essential oils contain α-pinene, β-pinene, limonene, 1,8-cineole and capillin as major constituents (5,18,19).

Antibacterial activity. The antibacterial activities of the *A. capillaris* essential oil, and its major constituents (α-pinene, β-pinene, limonene, 1,8-cineole, piperitone, β-caryophyllene and capillin) were evaluated against various clinically significant, and respiratory infection causing, bacterial strains. These included: *S. pyogenes*, MRSA, MRSA (clinical strain), MGRSA, *S. pneumoniae*, *K. pneumoniae*, *H. influenzae* and *E. coli*. The potency of the essential oil, and its chemical constituents, was assessed by measuring inhibition zones, and MIC and MBC values. The essential oil of *A. capillaris* and its major constituents exhibited a broad spectrum and variable degree of antibacterial activity against the various tested bacterial strains. The essential oil exhibited potent growth inhibition against *S. pyogenes* (MIC = 52 and MBC <52), MRSA clinical strain (MIC= 56, MBC= 98), *S. pneumoniae* (MIC = 32, MBC= 56), *K. pneumoniae* (MIC = 32, MBC = 56), *H. influenzae* (MIC = 26, MBC = 72), and *E. coli* (MIC = 24, MBC = 64) μg/ml (Table II). The two bacterial strains MRSA (ATCC 43300) and MGRSA (ATCC 33592) were less susceptible to the effects of the essential oil, and exhibited higher values of MIC and MBC. This reduction in susceptibility may arise from the drug-resistant nature of these bacterial strains.

To identify which of the chemical compounds present in the essential oil of *A. capillaris,* were active against the respiratory bacteria, a further *in vitro* experiment was conducted to evaluate the antibacterial effects of the major constituents: α-pinene, β-pinene, limonene, 1,8-cineole, piperitone, β-caryophyllene and capillin (Table III). Almost all of the tested essential oil constituents exhibited moderate to potent antibacterial effects against the various bacterial strains. However, the essential oil remained much more potent, as compared with the individual chemical constituents. These results indicate there is a possible interplay between the various chemical constituents of the essential oil. Among the chemical compounds, piperitone and capillin exhibited more potent growth inhibition of the bacterial strains. *K. pneumoniae*, *H. influenzae* and *E. coli* were the most susceptible bacterial strains towards the effects of piperitone and capillin. Piperitone treatment resulted in MIC/MBC values of 86/>86, 72/>72 and 72/>72 μg/ml against *K. pneumoniae, H. influenzae* and *E. coli* respectively; whereas capillin treatment resulted in MIC/MBC values of 72/>72, 64/>64 and 64/>64 respectively against the above bacterial strains. Among all of the chemical constituents, capillin had the most potent effects against all of the bacterial strains. Capillin was capable of inhibiting the growth of drug resistant bacterial strains, including MRSA

Table I. Chemical components identified in the essential oil of *Artemisia capillaris*.

Compound	RI	Relative peak area (%)
Tricyclene	919	2.1
α-Thujene	924	1.2
α-Pinene	931	4.3
Camphene	943	1.6
β-Pinene	981	12.1
Limonene	1021	4.5
1,8-Cineole	1038	0.9
(z)-Ocimene	1038	0.9
Artemesia Ketone	1045	3.5
γ-Terpinene	1057	2.6
4-Terpineol	1179	0.7
Citronellol	1213	1.6
Piperitone	1220	4.2
Pulegone	1224	1.8
Eugenol	1356	1.7
β-Cubebene	1382	0.7
β-Caryophyllene	1420	5.2
β-Farnesene	1438	0.9
Capillin	1457	24.2
Germacrene D	1479	3.9
δ-Cadinene	1520	1.7
Nerolidol	1564	2.0
Spathulenol	1578	2.6
Globulol	1587	0.3
α-Cadinol	1652	1.3
Monoterpene Hydrocarbons		29.3
Oxygenated Monoterpenes		18
Sesquiterpene Hydrocarbons		12.4
Oxygenated Sesquiterpenes		6.2
Others		24.2
Total (%)		90.1

RI, Retention index, calculated using a RTX-5 column.

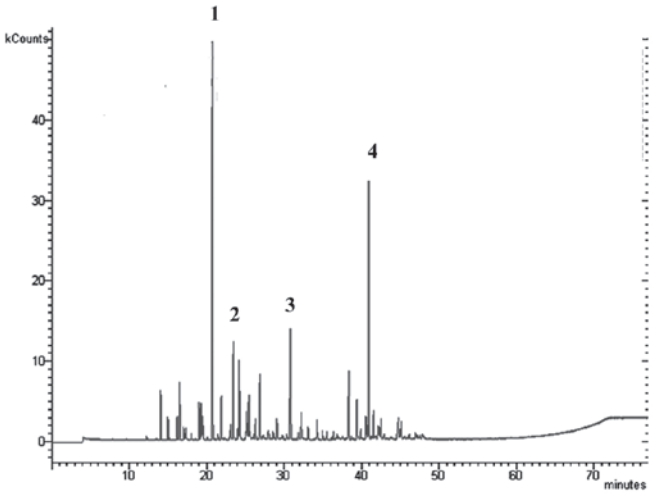

Figure 1. Gas chromatography-mass spectrometry total ion chromatogram of the leaf essential oil of *Artemisia capillaris*. Numbers represent major components: 1, β-pinene; 2, 1, 8-cineole; 3, β-carophyllene; 4, capillin.

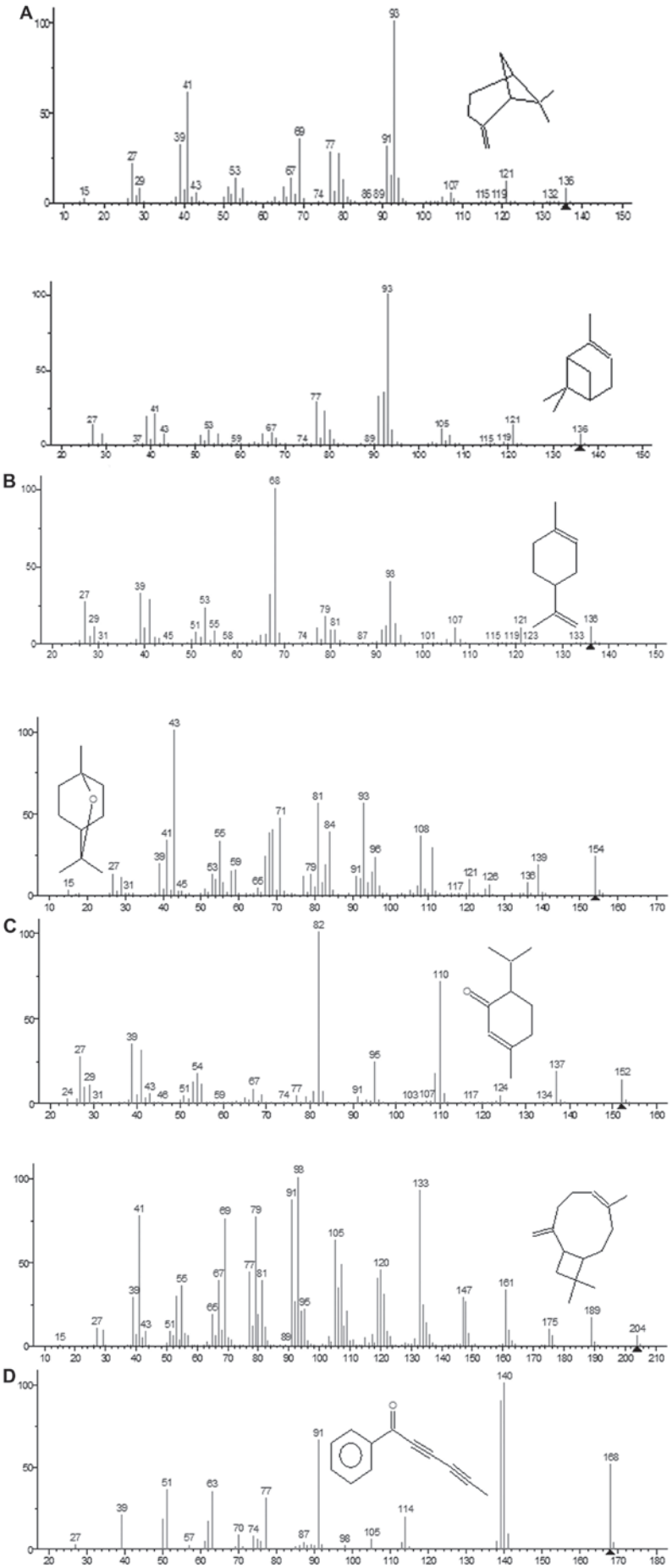

Figure 2. Mass spectra and molecular structures of the major identified compounds present in the essential oil of *Artemisia capillaris*, (A) β-Pinene and α-Pinene. (B) Limonene and 1,8-Cineole. (C) Piperitone and β-Caryopyllene. (D) Capillin.

Table II. Antibacterial activity of the *Artemisia capillaris* essential oil, minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) values.

	Essential oil (μg/ml)		Ampicillin (μg/ml)		Vancomycin (μg/ml)	
Bacterial strain	MIC	MBC	MIC	MBC	MIC	MBC
Streptococcus pyogenes (ATCC 12344)	52	>52	0.7	>1.5	0.5	1
MRSA (ATCC 43300)	72	112	0.9	2	0.5	1.5
MRSA (Clinical strain)	56	98	0.9	2	2	4
MGRSA (ATCC 33592)	98	>98	0.5	1.2	1	2
Streptococcus pneumoniae (ATCC 2730)	32	56	2	4	1	2
Klebsiella pneumoniae (ATCC 27853)	32	56	2	4	2	4
Haemophilus influenzae (ATCC 33391)	26	72	1	2	2	
Escherichia coli (Clinical strain)	24	64	1	2	1	2

MRSA, methicillin-resistant *Staphylococcus aureus*; MGRSA, methicillin-gentamicin resistant *Staphylococcus aureus*; ATCC, American Type Culture Collection.

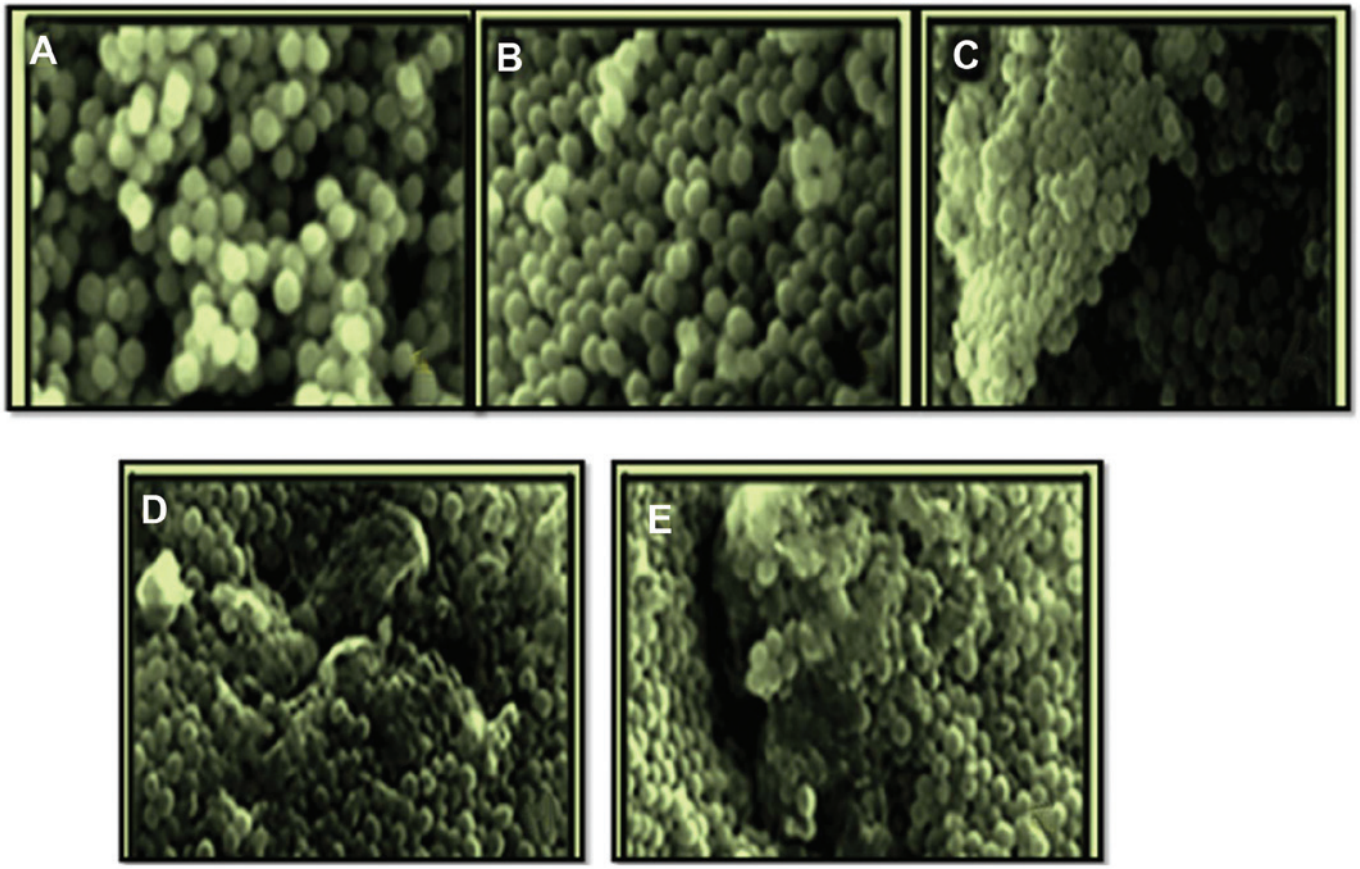

Figure 3. Scanning electron micrographs of *Staphylococcus aureus* treated with various concentrations of *Artemisia capillaris* essential oil. (A) *S. aureus* treated for 12 h in saline. (B-E) *S. aureus* treated for 12 h, with 20, 40, 60 and 80 μg/ml of the *A. capillaris* essential oil, respectively. Magnification, x10,000.

Table III. Antibacterial activity of the major chemical constituents of *Artemisia capillaris*.

	α-Pinene		β-Pinene		Limonene		1,8-Cineole		Piperitone		β-Caryophyllene		Capillin	
Bacterial strain	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. pyogenes	132	>132	144	>144	156	>156	112	>112	102	>102	126	>126	98	>98
MRSA	210	>210	212	>212	332	>332	244	>244	112	>112	330	>330	156	> 156
MRSA(Clinical)	172	>172	170	>170	150	>150	152	>150	122	>122	144	>144	112	>112
MGRSA	256	>256	256	>256	330	>330	256	>256	156	>156	332	>332	156	>156
S. pneumoniae	172	>172	170	>170	198	>198	132	>132	112	>112	122	>122	64	>64
K. pneumoniae	178	>178	170	>170	156	>156	102	>102	86	>86	64	>64	72	>72
H. influenzae	126	>126	132	>132	128	>128	98	>98	72	>72	64	>64	64	>64
E. coli (Clinical strain)	98	>98	102	>102	112	>112	102	>102	72	>72	92	>92	64	>64

MIC, minimum inhibitory concentration; MBC, minimum bacteriocidal concentration; *S. pyogenes, Streptococcus pyogenes;* MRSA, methicillin-resistant *Staphylococcus aureus;* MGRSA, methicillin-gentamicin *Staphylococcus aureus; S. pneuomoniae, Streptococcus pneumoniae; H. influenzae, Haemophilus influenzae; E.coli, Escherichia coli.*

Figure 4. Extracellular concentration of potassium and phosphate ions in aliquots of *Staphylococcus aureus* untreated (squares) and treated (circles) with 100 µl *Artemisia capillaris* essential oil. The data-points represent the results from two independent experiments (coefficient of variation <5%). Min, minutes; ppm, parts per million; ex, extracellular.

and MGRSA, resulting in MIC/MBC values of 112/>112 and 156/>156, respectively, against these bacteria.

SEM results. The treatment of S. *aureus* with *A. capillaris* essential oil, induced marked morphological changes in the bacteria, as determined by SEM. The *S. aureus* bacterial cells were bloated, 12 h following *A. capillaris* essential oil treatment (20, 40, 60 and 80 µg/ml). The bacterial cells were crushed and collected 48 h following the treatment with the essential oil of *A. capillaris*. The essential oil induced cell morphological changes in the *S. aureus* bacterial cells, in a dose-dependent manner, whereas the untreated control cells did not show any changes in cell morphology (Fig. 3). Treatment of *S. aureus* with 80 µg/ml of the essential oil, for 12 h, killed >90% of the bacterial cells. These results indicate that the *A. capillaris* essential oil produces bactericidal effects against *S. aureus* microbes.

Leakage of phosphate and potassium ions. A 50 µg/ml concentration of *A. capillaris* essential oil was administered to a culture of ~1x10^6 cfu/ml *S. aureus*. The treated bacterial cultures exhibited significant potassium and phosphate ion leakage following essential oil administration, as compared with the untreated controls (Fig. 4). These results indicate that the antimicrobial actions of the essential oil are mediated

through the leakage of potassium and phosphate ions. It has previously been reported that essential oils may induce the loss of ions which are integral for the sustenance of bacterial strains, and this is known to be one of the predominant mechanisms by which essential oils exhibit their bactericidal effects (20).

Discussion

Essential oils have been used in traditional medicine for the treatment of bacterial, viral and fungal infections worldwide for centuries (19). Currently, essential oils are used mainly in alternative and holistic medicine, for similar purposes, and may be administered orally, topically or by aromatherapy. An increasing number of studies have recently focussed on elucidating the specific mechanisms of action of essential oils and their components. Emerging evidence has shown that many essential oils have both non-specific and specific mechanisms of action, which vary based on the relative abundance and chemical composition of the components. Elucidation of the mechanism of action of these compounds may enable identification of new antibiotic targets, and exploitation of novel biochemical pathways which are not currently targeted by existing antibiotics. Furthermore, a combination of existing drugs with essential oils and/or their components, may provide an alternative approach to combat emerging drug resistance. Antibiotic resistance is currently outpacing the research and development required to identify new drugs; therefore, scientists are facing a return to the 'pre-antibiotic era'. Essential oils are currently used in the food and beverage industries, and in perfumes and cosmetics. In addition to this, essential oils have been shown to exhibit a broad spectrum of biological activity, which has led to increased interest among scientists. There has been extensive recent research conducted to discover and determine the antimicrobial activity of essential oils (21). The mechanism of action remains unclear, but some studies have suggested that essential oil compounds may penetrate the cell, where they interfere with cellular metabolism (22). Other studies have shown that phenols, such as carvacrol and eugenol, disrupt the cellular membrane and react with the active sites of enzymes. Essential oils and their components may interact with the cell membrane and accumulate in the lipid bilayer of bacteria, occupying a space between the chains of fatty acids (23, 24).

It has previously been demonstrated that various *Artemisia* essential oils, as well as the major components found in *A. capillaris* essential oil, may possess antimicrobial activities. For example, *A. indica* (6), *A. absinthium* (22), *A. biennis* (22), *A. cana* (22), *A. dracunculus* (22), *A. frigida* (22), *A. longifolia* (22), *A. ludoviciana* (22) *A. chamaemelifolia* (23), *A. turcomanica* (23), *A. annua* (24) and *A. fragrans* (25), which are used in herbal medicines, have been reported to possess antimicrobial properties. Furthermore, the most abundant compounds present in the *A. capillaris* essential oil: Germacrene D, α-pinene, β-pinene, 1,8-cineole, limonene, have been reported to exhibit antimicrobial activity. (6,22,24). The present study is, to the best of our knowledge, the first to report on the antimicrobial activities of the essential oil of *A. capillaris*. These results further support the importance of the *Artemisia* species containing biologically active metabolites for drug development.

In conclusion, the present study has demonstrated that the essential oil of *A. capillaris* produces potent antibacterial effects against various respiratory tract infection-causing microbes. The bactericidal effects of the essential oil are mediated by the induction of significant morphological changes in bacterial cells, as well as promoting the leakage of potassium and phosphate ions from the bacterial cells. The results of the present study are significant, since numerous bacterial strains used have attained drug resistance, and many conventional drugs are not effective against them.

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