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# The antibacterial effects of *Melaleuca alternifolia*, *Pelargonium graveolens* and *Cymbopogon martinii* essential oils and major compounds on liquid and vapor phase

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**ABSTRACT**

Essential oils (EOs) are natural products from plant secondary metabolism. The antibacterial activity of EOs from *Melaleuca alternifolia*, *Pelargonium graveolens* and *Cymbopogon martinii* and terpinen-4-ol, citronellol and geraniol were investigated both in their liquid and vapor phases against Gram-positive and Gram-negative strains. From microdilution tests, geraniol showed a minimal inhibitory concentration (MIC) value of 0.05% v/v against almost all strains. According to the inverted plate assays, *Klebsiella pneumoniae* was highly sensitive (inhibitory zone of 31 mm) to terpinen-4-ol and 100% of reduction under vapor microenvironment assays were recorded. The effectiveness of compounds as antibacterial agents was demonstrated, highlighting the damage caused to strains by *C. martinii* EO and geraniol vapors through transmission electron microscopy, and it was observed that geraniol was probably responsible for the antibacterial effect of *C. martinii* EO.

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## Introduction

Essential oils (EOs) are secondary metabolites from plants and highly enriched in compounds with an isoprene structure, terpenes. Such compounds may contain additional elements, usually oxygen, and are denominated terpenoids, which are synthesized from acetate units and originate from fatty acid metabolism (1).

These products are typically liquid, volatile, clear and rarely colored, characterized by a strong odor and display antibacterial, antiviral, antifungal, insecticidal (2), antioxidant (3), anti-inflammatory (4), antimicrobial (5), antidepressant and anxiolytic activities (6). Traditional treatments or therapy (e.g., aromatherapy) using EOs volatilization has also been reported (7).

EOs are used in the medical, food and cosmetics industries due to their antimicrobial action and they have been studied both in liquid form (5, 8) and as vapors (9), although studies have strongly focused on methods to clarify their properties based on their liquid phase (10).

The use of EOs in the vapor phase could offer several advantages for antimicrobial activities, such as efficacy without requiring direct contact, and ease of application (11), in a addition, EO vapors could be used in the disinfecting air process (12).

However, in fumigation processes with medical purposes, the efficacy and safety of EOs in their gaseous state should be checked, although their antimicrobial and cytotoxicity activities have been measured in their liquid phase. Thus, standard procedures for evaluating their antimicrobial activities in liquid phase have already been established, while few studies with EOs at vapor phase have been performed (13).

Infectious diseases constitute a worldwide problem, and bacterial controls are usually by disinfection with liquid disinfectants. However, liquid disinfectants have an antibacterial effect in topical use (e.g., liquid phenol disinfectants) and aseptic environments, such as surgical rooms, clinical and food microbiology laboratories and the pharmaceutical industry require special attention (14).

Community and nosocomial pathogens, including *Staphylococcus aureus*, *Salmonella*, *Enterococcus* sp.,

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*Escherichia coli* and *Pseudomonas aeruginosa*, are the main multidrug-resistant bacteria (15) and natural products (e.g., herbal derivatives) with antimicrobial properties have been investigated with the aim of measuring their biological properties (16).

*Cymbopogon martinii* (Poaceae) is used in the perfume industry as well as employed traditionally in diabetes treatment and it has been documented in Ayurvedic medicine as having anti-inflammatory and diuretic properties in urinary tract infections (17).

Tea tree EO, extracted from the Australian native plant *Melaleuca alternifolia* (Myrtaceae), is widely employed incorporated as the active ingredient in topical formulations against cutaneous infections as well as marketed as a drug for several diseases (18).

The *Pelargonium graveolens* (Geraniaceae) leaves are popularly used as flavoring, insect repellent, in perfume and during aromatherapy procedures for the treatment of gastrointestinal diseases and throat infections (19).

Antimicrobial properties of EOs from the leaves of *P. graveolens* have been associated with their high content of oxygenated monoterpenes and were more active against Gram-positive than Gram-negative bacteria, revealing an *in vitro* antibacterial activity, confirmed by low minimal inhibitory concentrations (20).

Thus, Gram-positive and Gram-negative American Type Culture Collection (ATCC) standard, human clinical and hospital environment strains were assayed with the aim of assessing their susceptibilities to tea tree (*M. alternifolia*), geranium (*P. graveolens*) and palmarosa (*C. martinii*) EOs and their major compounds terpinen-4-ol, citronellol and geraniol, respectively, using the liquid and vapor phases of the compounds. Transmission electron microscopy was also carried out to determine the effects of *C. martinii* EO and geraniol on bacterial structure.

## Experimental

### Essential oils and major compounds

Essential oils were purchased from 'By Samia Aromaterapia', which markets essential oils in São Paulo, Brazil, and their identification are: pure essential oil-By Samia Aromaterapia 10 mL, with batch numbers, tea tree (*M. alternifolia*) (Maiden & Betche) Cheel LOT 341105BS, geranium (*P. graveolens* L.) LOT 1221010BS and palmarosa (*C. martinii* L) LOT 2311NB5, and their chemical composition, achieved by gas chromatography coupled to mass spectrometry (GCMS) were provided by the company 'By Samia Aromaterapia', is presented in Table 1.

The compounds terpinen-4-ol, citronellol and geraniol were purchased from Sigma Aldrich® (purity > 98%) and chosen according to GC-MS analysis considering the highest concentration occurring in the essential oils tea tree, geranium and palmarosa, respectively.

### Bacterial strains

The standard ATCC strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 10100, *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* ATCC 43895 and *Pseudomonas aeruginosa* ATCC 2785, as well as the strains, isolated from human clinical specimens, *S. aureus, E. coli, Salmonella* Typhimurium, *P. aeruginosa* and *Klebsiella pneumoniae* and *S. aureus* obtained from the hospital environments, were assayed by susceptibility assays. This research project was approved by the Ethics Committee of Botucatu Medical School (CEP 3840-2011).

All strains were stored at −80°C in Brain Heart Infusion (BHI) broth plus glycerol in a culture collection from the Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu. Prior to use, strains were seeded in blood agar to check both viability and purity. All clinical isolates are maintained at the place of research above, therefore, available at the request of researchers.

### Bacterial sensitivity tests

#### Microdilution assays

Bacterial strains were previously cultured (37°C/24 hours) in BHI broth and sensitivity assays were performed by resazurin microtiter assay (REMA) to determine the minimal inhibitory concentration (MIC) values. Ninety-six well microplates with BHI plus Tween 80 0.5% were used and concentrations of 0.05; 0.1; 0.2; 0.5; 1.0; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0 and 10% v/v of each compound were prepared.

Each well received a volume of bacteria from the standardized suspensions to attain approximately $10^5$ CFU/mL (colony-forming units).

The microplates were incubated (37°C/24 hours) and the results were recorded after adding the indicator dye redox resazurin 0.01%. Bacterial growth is indicated by a color change from violet to pink (or shade) and the lowest concentration without a color change was taken as the MIC value (21).

#### Vapor diffusion assay by the inverted plate method

Sterile filter paper discs 9 mm in diameter containing 15µL of each substance with and without ethyl acetate (1:1) were fixed inside the covers of Petri dishes with Mueller-Hinton Agar (MHA) and inoculated with ATCC standard bacteria and *K. pneumoniae* from the hospital environment. These strains were standardized at 0.5 MacFarland scale, then placed in an inverted position, so that only the vapor of

**Table 1.** Density and chemical profile by gas chromatography-mass spectrometry (GC-MS), supplied by By Samia Aromaterapia/São Paulo, Brazil.

| Essential oils | Popular name | Density (mg/mL) | Compounds (%) |
|---|---|---|---|
| *Melaleuca alternifolia* | Tea Tree | 858 | terpinen-4-ol (45.48), γ-terpinene (18.77), α-terpinene (8.67), α-terpineol (4.18), para-cimene (3.66),1.8 cineole (3.45), α-terpinolene (3.23), α-pinene (2.44), limonene (0.90), α-tujene (0.90), mircene (0.82), β-pinene (0.71), α-phelandrene (0.35) |
| *Cymbopogon martinii* | Palmarosa | 874 | geraniol (57.49), geranyl acetate (13.56), linalool (1.71), β-caryophyllene (1.07), ocimene (0.27) |
| *Pelargonium graveolens* | Geranium | 848 | citronellol (28.57), geraniol (20.99), menthone (5.76), α-muurolene (1.83), neryl acetate (1.50), isomenthone (1.32), rose oxide (1.26), α-bourbonene (1.15), geranyl acetate (0.92), citronellyl acetate (0.72) |

each compound was in contact with the agar surface. After 37°C/24 hours, the inhibitory zones were recorded in millimeters. Assays were performed in triplicate and ethyl acetate was used as negative control (11). Ethyl acetate was used as solvent control because it is less toxic than other organic solvents containing halogens and benzenes, and volatilizes efficiently at room temperature (22).

### *Bacterial reduction count in vapor microenvironment*

Open MHA RODAC Petri dishes (120 $cm^3$ of air) were inoculated with 5 µL from standardized suspensions of strains of ATCC and *K. pneumoniae* from hospital environment at inoculums containing $10^6$ CFU/mL. Essential oils and their major compounds in a concentration of 1000 µg/$cm^3$ of air, with or without dilution in ethyl acetate (1:1), were placed on strips of filter paper 10 × 1.7 cm in dimension, placed inside the Petri dishes and incubated at 37°C/24 hours along with RODAC Petri dishes.

The Petri dishes were sealed with parafilm to preserve the vapor microenvironment generated by the compounds. This methodology was adapted from Inouye (22). After this period, colony-forming units (CFU) were recorded from RODAC plates. The percentage of bacterial colonies was compared to the percentage of control without oil and compounds, considered as 100%. The assays were performed in duplicate, with control strains and ethyl acetate used as negative control.

### *Transmission electron microscopy (TEM)*

*C. martinii* EO and geraniol were chosen for viewing the damage caused by vapors from these antimicrobials and because of the results of geraniol in the microdilution assays. Also, as this is the major compound of C. *martinii* EO, both EO and geraniol were tested against Gram-positive and Gram-negative strains and conduced together with bacterial reduction count in a vapor microenvironment test.

*S. aureus*, *P. aeruginosa*, *S.* Enteritidis and *E. coli* ATCC standard strains were incubated overnight in BHI at 37°C. RODAC plates containing MHA were then inoculated with 5 µL standardized bacterial suspension at $10^6$ CFU/mL before placed in open Petri dishes (120 $cm^3$ of air). Then 15 µL of each compound was used to achieve 1000 µg/$cm^3$ air. *C. martinii* EO and geraniol were placed on 10 × 1.7 cm strips of filter paper inside the Petri plates and incubated. All treatments and controls were incubated at 37°C and centrifuged after 2 hours of incubation. Cells were washed twice with 0.1 M phosphate buffered saline -PBS (pH 7.4) and fixed with 2.5% (v/v) of glutaraldehyde in 0.1 M PBS overnight at 4°C. Then, cells were post-fixed with 1% (w/w) $OsO_4$ in 0.1 M PBS for 2 hours at room temperature and washed three times with the same buffer before dehydration through a graded series of ethanol solutions (30%, 50%, 70%, 90%, and 100%). Stained bacteria were photographed using a transmission electron microscope.

### *Statistical analysis*

Kruskal–Wallis one-way analysis of variance on ranks ($p < 0.05$) was used in the microdilution assay, and the Mann–Whitney Rank Sum Test ($p < 0.05$) was used in reverse plating tests.

## Results and discussion

The composition of EOs (Table 1) purchased from By Samia Aromaterapia are in agreement with those reported in literature, and the major compounds of *M. alternifolia*, *P. graveolens* and *C. martinii* essential oils are terpinen-4-ol, citronellol and geraniol, respectively (23–28).

The geraniol showed the highest inhibitory effects against the tested bacterial strains with MIC around 0.05% v/v (Table 2), except against *P. aeruginosa*, whose MIC was around 8–10% v/v. Results of lower sensitivity in *P. aeruginosa* strains, with MIC around 10% v/v to palmarosa, were found using *P. aeruginosa* from clinical specimens in other studies (29, 30).

Geraniol appeared to be a potent inhibitor of efflux mechanisms; geraniol had a synergistic effect with β-lactams and was effective in restoring antibiotic susceptibility in *Enterobacter aerogenes*, *E. coli*, *P. aeruginosa*, and *Acinetobacter baumannii* bacteria, increasing the susceptibility to the β-lactams, ampicillin and

**Table 2.** MIC (%v/v) from microdilution test with palmarosa EO, geraniol, geranium EO, citronellol, tea tree EO and terpinen-4-ol against Gram-positive and Gram-negative strains.

| Bacterial Strains | Palmarosa | Geraniol | Geranium | Citronellol | Tea Tree | Terpinen-4-ol |
|---|---|---|---|---|---|---|
| *S. aureus* 25923 | 0.5 | 0.05 | 0.5 | 0.2 | 0.5 | 0.5 |
| *S. aureus*(human clinical) | 0.5 | 0.05 | 0.5 | 0.5 | 0.5 | 0.2 |
| *S. aureus*(hospital environment) | 0.5 | 0.05 | 1.0 | 0.2 | 0.5 | 0.2 |
| *S. epidermidis* 12228 | 0.5 | 0.05 | 0.05 | 0.05 | 0.5 | 0.05 |
| *E. faecalis* 10100 | 0.05 | 0.05 | 0.05 | 0.05 | 0.1 | 0.1 |
| *S.* Enteritidis 13076 | 0.5 | 0.05 | 0.05 | 0.2 | 0.5 | 0.05 |
| *S.* Typhimurium(human clinical) | 0.5 | 0.05 | 0.2 | 4 | 0.5 | 0.1 |
| *E. coli* 43895 | 0.5 | 0.05 | 0.5 | 0.1 | 0.2 | 0.1 |
| *E. coli*(human clinical) | 0.5 | 0.05 | 1.0 | 2.0 | 0.5 | 0.05 |
| *P. aeruginosa* 27853 | 10.0 | 8.0 | 7.0 | 7.0 | 4.0 | 4.0 |
| *P. aeruginosa*(human clinical) | 9.0 | 10.0 | 8.0 | 8.0 | 4.0 | 0.5 |
| *K. pneumoniae*(hospital environment) | 0.5 | 0.05 | 0.5 | 0.1 | 0.5 | 0.1 |

Note: The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference when $p > 0.05$.

penicillin and to the fluoroquinolone norfloxacin (31). This study partly explains the highest antimicrobial activity of geraniol against almost all bacterial strains in our study.

Vaara et al. (32) reported that the outer membrane of the Gram-negative wall is impermeable to macromolecules and allows only limited diffusion of hydrophobic substances through its lipopolysaccharide-covered surface. Besides wall hydrophobicity, *P. aeruginosa* synthesizes an exopolysaccharide, the alginate, in response to environmental conditions to protect against adversity in its surroundings and also enhances adhesion to solid surfaces (33).

Thus, *P. aeruginosa* was less sensitive to natural products in this research than the other tested Gram-negative bacteria and this may also be due to the exopolysaccharide of this bacteria. In our study, *P. aeruginosa* was resistant to citronellol and geraniol, both of which are monoterpenes even as terpinen-4-ol.

Papadopoulos et al. (34) reported the tolerance of *P. aeruginosa* to tea tree EO and terpinen-4-ol due to the MexAB-OprM efflux pump system. Thus, this system may contribute to the tolerance to some components of tea tree EO, including 1,8-cineole and terpinen-4-ol. 'The resistance of *P. aeruginosa* also occurred with other monoterpene compounds by this efflux pump system.

EO vapors could reduce surface and airborne levels of bacteria, including methicillin-resistant *S. aureus* (MRSA) (12); they show the potential to be used in the treatment of MRSA infections (35) and antimicrobial activity for foodborne microorganisms (36).

In the inverted plate test (Table 3) inhibitory zones may be less than 9 mm, although disk filter papers are 9 mm, because it is not in direct contact with the culture medium and the inoculated bacterium, and inhibitory zones are formed directly by the vapor of these compounds.

Tea tree EO produced inhibitory zones, except with *P. aeruginosa* and *E. faecalis,* but the largest inhibitory zone was produced by its major compound, terpinen-4-ol, against *K. pneumoniae* (31 mm).

*S. aureus* and *S. epidermidis* were susceptible to the antibacterial effect of all compounds in the inverted plate test, ranging from 6.0 to 21.0 mm. *S. aureus* strains show inhibitory zones using orange EO in a disc-diffusion vapor assay from 17.8 to 78.8 mm (37).

According to the reduction percentage of bacterial numbers in the vapor microenvironment (Table 4), most of the strains showed the greatest sensitivity to tea tree EO, including 96.3% against *S.* Enteritidis and 100% against *K. pneumoniae*. *K. pneumoniae* strain was the most sensitive bacterial strain of all and is a human nosocomial pathogen

**Table 3.** Mean inhibitory zones (mm) from plate reverse test of each compound alone (C) and formed with 30 µL of ethyl acetate added to each compound (1:1) (EA) for Gram-positive and Gram-negative strains.

| | Palmarosa | | Geraniol | | Geranium | | Citronellol | | Tea Tree | | Terpinen-4-ol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial Strains | C | EA | C | EA | C | EA | C | EA | C | EA | C | EA |
| *S. aureus* 25923 | 7.0 | 9.0 | 12.5 | 12.0 | 10.0 | 11.5 | 12.0 | 15.5 | 12.0 | 16.0 | 17.5 | 21.0 |
| *S. epidermidis* 12228 | 6.0 | 12.0 | 9.0 | 15.0 | 12.0 | 9.0 | 12.0 | 18.0 | 18.0 | 6.0 | 11.0 | 20.0 |
| *E. faecalis* 10100 | 0.0 | 0.0 | 0.0 | 6.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.0 |
| *S.* Enteritidis 13076 | 0.0 | 0.0 | 0.0 | 0.0 | 6.0 | 0.0 | 6.0 | 0.0 | 17.5 | 14.0 | 20.0 | 23.0 |
| *E. coli* 43895 | 0.0 | 0.0 | 0.0 | 0.0 | 14.5 | 0.0 | 0.0 | 0.0 | 15.5 | 19.0 | 18.0 | 19.5 |
| *P. aeruginosa* 27853 | 0.0 | 0.0 | 0.0 | 6.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *K. pneumoniae* (hospital environment) | 0.0 | 0.0 | 8.5 | 6.0 | 0.0 | 0.0 | 0.0 | 17.0 | 22.5 | 25.0 | 26.5 | 31.0 |

Note: The difference in the median values between the groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference when $p > 0.05$.

**Table 4.** Percentage reduction of bacterial strains treated with 1.000 µg/cm³ of air for each compound alone (C) and with ethyl acetate (1:1) (EA).

| | Palmarosa | | Geraniol | | Geranium | | Citronellol | | Tea tree | | Terpinen-4-ol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial Strains | C | EA | C | EA | C | EA | C | EA | C | EA | C | EA |
| *S. aureus* 25923 | 60.5 | 45.8 | 56.0 | 45.8 | 94.0 | 36.6 | 76.4 | 55.0 | 85.2 | 96.6 | 93.0 | 86.6 |
| *S. epidermidis* 12228 | 84.6 | 20.8 | 89.0 | 78.3 | 79.3 | 41.7 | 82.8 | 59.3 | 81.2 | 68.1 | 90.0 | 69.2 |
| *E. faecalis* 10100 | 60.0 | 45.0 | 59.6 | 51.6 | 78.2 | 33.3 | 61.3 | 33.3 | 57.2 | 86.6 | 56.7 | 33.3 |
| *S.* Enteritidis 13076 | 0.0 | 13.0 | 0.0 | 30.0 | 12.3 | 0.0 | 11.0 | 0.0 | 96.3 | 100.0 | 0.0 | 70.0 |
| *E. coli* 43895 | 0.0 | 48.2 | 13.6 | 74.6 | 41.7 | 6.6 | 16.6 | 65.3 | 81.1 | 86.9 | 30.5 | 33.3 |
| *P. aeruginosa* 27853 | 71.0 | 0.0 | 45.0 | 31.8 | 71.0 | 0.0 | 53.0 | 5.0 | 75.2 | 26.6 | 47.0 | 22.2 |
| *K. pneumoniae* (hospital environment) | 99.0 | 14.2 | 98.0 | 53.3 | 100.0 | 51.0 | 99.0 | 52.3 | 100.0 | 85.7 | 100.0 | 33.3 |

and an important agent in community-acquired infections (e.g., pneumonia and urinary tract infections) (38).

The percentage inhibition found by López et al. (39) against *E. faecalis* and *L. monocytogenes* with clove and cinnamon EOs did not exceed 35%. Our results showed that tea tree EO was able to inhibit *E. faecalis* growth, with an 86.6% bacterial count reduction.

A consistent mechanism of action has been described concerning tea tree EO, including loss of intracellular material, inability to maintain homeostasis, and inhibition of respiration after treatment with tea tree EO and/or components, involving loss of membrane integrity (18).

The ATCC *S. epidermidis* and *S. aureus* strains were susceptible, even though resistance to antiseptic solutions has increased globally (15). An *in vitro* antimicrobial resistance assay showed the resistance of *S. epidermidis* ATCC 12228 to methicillin and tetracycline, whereas this strain was susceptible to fusidic acid, vancomycin, oxacillin, erythromycin, rifamycin, chloramphenicol and fluorquinolone (40).

In our research, it was observed that with different methodologies and for all antimicrobial compounds, the *S. epidermidis* ATCC 12228 strain was highly inhibited by the vapor state as well as in the liquid phase of these natural products.

Goñi et al. (41) reported no statistical differences in the EO inhibition zones and the isolated compounds combination', which agrees with our results which also found no statistical difference between inhibition by EOs and their major compounds using gaseous phase assays.

Visual information is useful in providing insights into the microstructures of cells, characterizing the type and magnitude of changes on cell composition during treatments. Transmission electron microscopy may help in the understanding of how and why a treatment is effective against a particular organism, illustrate changes and explain possible mechanisms of action, including disruption of plasma membranes by localized hyper-acidification and disruption of membrane transport and/or electron transport systems (42).

Transmission electron microscopy (Figure 1) data revealed that there was uniformity in cell walls. Control Gram-negative bacteria (B,C,D) were rod-shaped, with double layers of the outer membrane closely apposed to the cytoplasmic membrane, and dispersed nuclear material.

In treatments with EO vapours (A1, A2, B2, D1), there was some evidence that the cytoplasmic membranes were bulging and/or ruptured, and cells appeared to be discharging intracellular materials. The cell wall became separated from the wall after treatment (C1, D2) and some vacuolization appeared in B1 and D2.

The features presented by bacteria treated with EO vapors were very similar to those subjected to direct contact with other products (11, 42).

Geraniol is the major compound of *C. martinii* EO and its concentration was 57.49% in the EO sample. According to the results of microdilution assays and through the damage found by transmission electron microscopy, one may suggest that geraniol was probably responsible for the antibacterial activity of *C. martinii* EO.

According to Soković (43), EOs have a potential clinical use because of their very high specific activity; i.e. they may be used at low and non-toxic concentrations for the prevention and treatment of intestinal diseases in animals and humans caused by *E. coli*, *Salmonella* and other pathogenic bacterial species.

There are few published researches on the toxicity of EO vapors *per se*; however, in the future this needs to be explored before they can be utilized as commercial antimicrobial agents.

Thus, the data revealed the effectiveness of these EOs and their respective major components as antibacterial agents, either by direct or vapor contact, and highlight the damage caused by these vapours to bacteria with clinical importance.

Finally, the high resistance of *P. aeruginosa* may be due to its exopolysaccharide layer; however, this protection by alginate should be the subject of future studies. The transmission electron microscopy illustrated the damage caused by EO vapors and reinforced the idea that geraniol is responsible for the antibacterial effect of *C. martinii* EO.

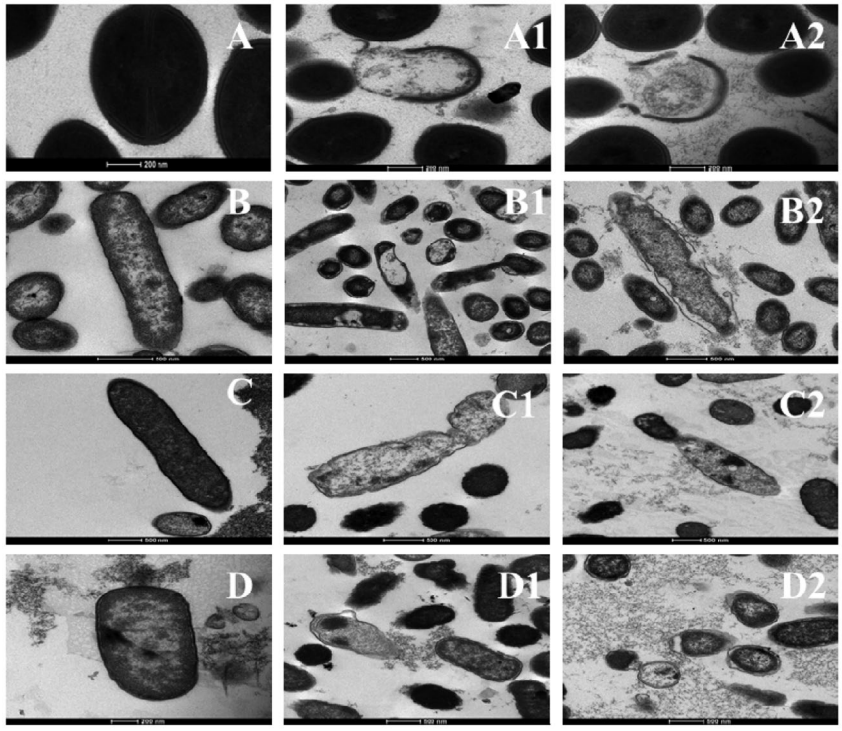


**Figure 1.** Transmission electron microscopy from ATCC standard bacteria, A- *S. aureus*, B- *P. aeruginosa*, C- *S.* Enteritidis, D- *E. coli* controls; A1, B1, C1, D1- treatments with 1000 µg/cm$^3$ of *C. martini* EO; A2, B2, C2, D2-treatments with 1000 µg/cm$^3$of geraniol.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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