

## ANTIMICROBIAL ACTIVITY OF TWO ESSENTIAL OILS

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**Abstract:** The aim of the study was to evaluate the antimicrobial activity of essential oils *in vitro* for possible application to reduce the content of microorganisms in the air of animal houses. The essential oils of *Cymbopogon citratus* L. and *Malaleuca alternifolia* L. were screened against bacteria *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and yeast *Candida albicans*. The minimal inhibitory concentration of the active essential oils was tested using broth dilution assay. The essential oils concentrations ranged from 0.1–50.0%. The combined effects of essential oils were tested for *Malaleuca alternifolia* L. and *Cymbopogon citratus* L. concentrations ranged from 0.005–50.0%. The oils showed a wide spectrum of antibacterial activity. Concentrations of 0.1–0.5% of *Cymbopogon citratus* L. and *Malaleuca alternifolia* L. reduced total microorganisms count of *Proteus mirabilis* and *Candida albicans*. High antibacterial activity was also revealed for *Cymbopogon citratus* L. with bactericidal concentrations of 0.8% for *Escherichia coli*, 5.0% for *Enterococcus faecium*, 5.0% for *Pseudomonas aeruginosa* and 8.0% for *Staphylococcus aureus*. Bactericidal concentrations of *Malaleuca alternifolia* L. were 5.0% for *Pseudomonas aeruginosa* and *Enterococcus faecium*, and 8.0% for *Staphylococcus aureus*. The essential oils of *Cymbopogon citratus* and *Malaleuca alternifolia* may be a promising alternative of air disinfection in animal houses.

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## INTRODUCTION

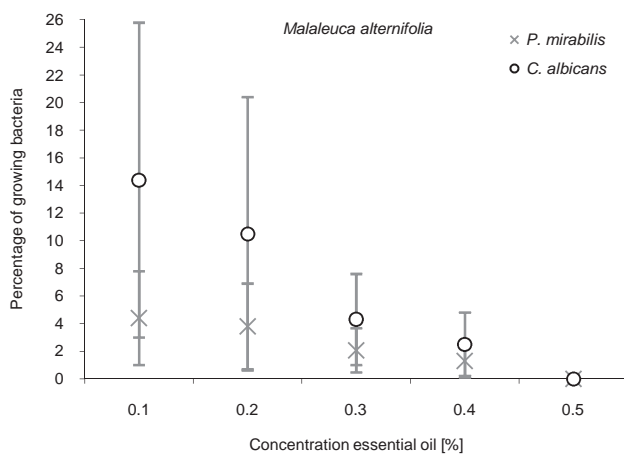
The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s [9].

Plant essential oils are generally isolated from non-woody plant material by distillation methods, usually steam or hydrodistillation, and appear as variable mixtures of principally terpenoids, specifically monoterpenes [C<sub>10</sub>] and sesquiterpenes [C<sub>15</sub>] although diterpenes [C<sub>20</sub>] may also be present, and as a variety of low molecular weight aliphatic hydrocarbons (linear, ramified, saturated and unsaturated), acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally as nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpro-

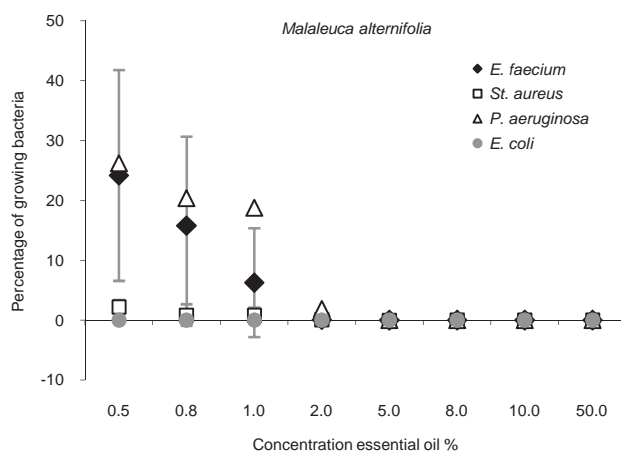
panoids. Terpenes are amongst the chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants. Most terpenes are derived from the condensation of branched 5-carbon isoprene units, and are categorized according to the number of these units present in the carbon skeleton [9].

The World Health Organization noted that the majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are a major source of natural organic compounds widely used as medicine. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [3, 16]. Some oils have been used in cancer treatment [31].

Some oils have been used in the food preservation [11], aromatherapy and fragrance industries [3]. Extracts from



**Figure 1.** Effect of essential oil from *Malaleuca alternifolia* L. oil on *P. mirabilis* and *C. albicans*.



**Figure 2.** Effect of essential oil from *Malaleuca alternifolia* L. oil on *E. faecium*, *St. aureus*, *P. aeruginosa* and *E. coli*.

aromatic plants, particularly essential oils, are a rich source of biologically active compounds showing antimicrobial properties [21]. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential [7].

Gram-negative bacteria such as *Pseudomonas*, *Escherichia coli*, *Salmonella* species and other important pathogens are ubiquitous, omnipresent in our immediate environment. Inhaled bacterial and viral pathogens, organic dusts, components of the cell wall of Gram-negative bacteria (endotoxins, lipopolysaccharides) and Gram-positive bacteria (lipoteichoic acid) or fungal spores ( $\beta$ -glucans) may have infectious, allergic and immunostimulatory properties [25, 29].

Epidemiological studies have shown that in occupational environment the high concentration of inhaled lipopolysaccharide is strongly and consistently associated with reversible airflow obstruction [22]. The consequences are non-specific respiratory symptoms such as cough, expectoration and dyspnoea, the severity of which depends on the dose and individual susceptibility. At higher endotoxin levels, flu-like symptoms of toxic pneumonitis can be observed [17, 28]. A long-term exposure to endotoxins promotes irreversible changes in the lung function that can significantly reduce work ability. Several epidemiological studies have shown that the content of endotoxins in dust is related to long-term adverse effects pulmonary in exposed workers [28]. Asthmatic subjects and persons with other chronic respiratory diseases are more susceptible to the toxic effects of endotoxins [17].

Particulates suspended in the air in and emitted from animal houses include dust and airborne microorganisms [2, 26]. Airborne microorganism are adsorbed on dust particles smaller than 5  $\mu$ m in diameter, inhaled by respiration, and deposited in the respiratory tract or lung, which can induce respiratory disorders, such as pneumonia, asthma, bronchitis, and rhinitis. The incidence of these respiratory symptoms and diseases are commonly widespread among

farmers working in confinement swine houses that are managed in an almost enclosed condition to keep the pertinent thermal environment constant [19, 27].

The aim of the study was to evaluate the antimicrobial activity of essential oils *in vitro* for possible application to reduce the content of microorganisms in the air of animal houses.

## MATERIALS AND METHODS

Essential oils of *Cymbopogon citratus* L. (lemongrass) and *Malaleuca alternifolia* L. (tea tree) were obtained from a commercial source (Oil manufacturer Sensient Essential Oils, GmbH, Germany), for producing essential oils used steam distillation method.

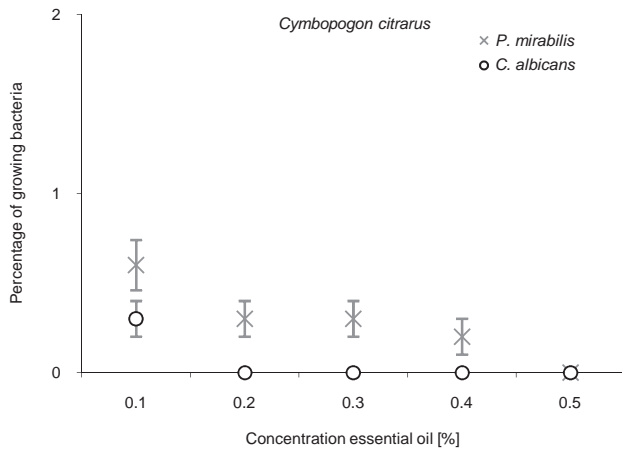
The plant species *Cymbopogon citratus* L. is from the *poaceae* family and has cytoprotective, antioxidant, anti-inflammatory, antibacterial properties. Essential oils from *Cymbopogon citratus* L. contain active ingredients, such as myrcene, citronella, citronellol and geraniol.

The plant species *Malaleuca alternifolia* L. is from the *myrtaceae* family and has antibacterial, antifungal, anti-septics properties. *Malaleuca alternifolia* L. is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols.

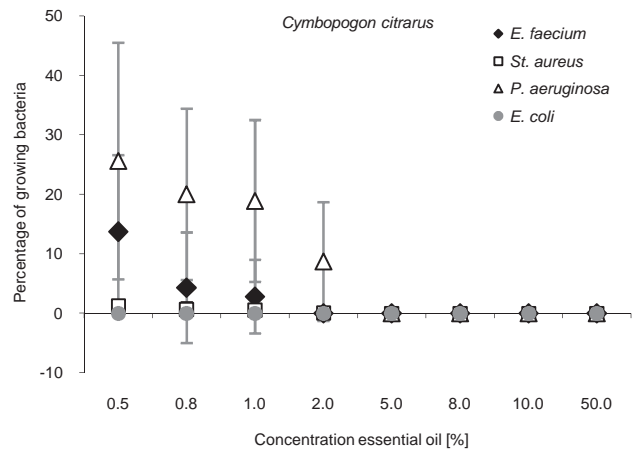
In the Animal Welfare Laboratory of the Veterinary Academy of LUHS the essential oils from *Cymbopogon citratus* L. and *Malaleuca alternifolia* L. were tested on specific bacterial cultures: *Staphylococcus aureus* DSM-No. 799, *Enterococcus faecium* DSM-No. 2918, *Pseudomonas aeruginosa* DSM-No. 939, *Escherichia coli* DSM-No.1077, *Proteus mirabilis* DSM-No. 788 and the yeast *Candida albicans* DSM-No. 1386.

The broth dilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards [24].

All tests were performed in 0.1% sterile peptone water (Oxoid, Basingstoke, UK). Microorganisms were suspended



**Figure 3.** Effect of essential oil from *Cymbopogon citratus* L. oil on *P. mirabilis* and *C. albicans*.



**Figure 4.** Effect of essential oil from *Cymbopogon citratus* L. oil on *E. faecium*, *St. aureus*, *P. aeruginosa* and *E. coli*.

in sterile peptone water 0.1% with turbidity visually corresponding to 0.5 Mc Farland (approximately  $10^8$  CFU ml<sup>-1</sup>). A dilution of the essential oils was prepared in the concentration range of 0.1–50.0%, in 0.1% sterile peptone water.

Standardized microorganisms suspensions were inoculated into microwells. 50 µl aliquots of microorganism suspension and 50 µl aliquots of essential oil (different concentrations of 0.1–50.0%) were added to individual wells and incubated at 37°C for 24 h. Then, 100 µl aliquots of microorganisms-peptone water-essential oil suspension were dissolved in 9.9 ml tubes with 0.02% Tween 80 (Sigma). 100 µl aliquots of the suspension were plated onto agar plates: *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, *Proteus mirabilis* were cultivated on Blood Agar Base N. 2 (Oxoid, UK) at 37°C for 24 h, *Pseudomonas aeruginosa* was cultivated on Blood Agar Base N. 2 at 30°C for 24 h, and *Candida albicans* was cultivated on Malt Extract-Agar (Oxoid, UK) at 25°C for 48 h.

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. All the experiments were carried out 5 times.

**Statistical analysis.** The data were analysed using the “SPSS for Windows” package, version 12.0 and Microsoft Office Excel 2003 for calculating the mean of values ( $\bar{x}$ ), standard deviation (SD) and coefficient of variation (CV). The p-value of 0.05 was set as a limit for statistically significant difference in the studies.

## RESULTS

The inhibitory effects of *Malaleuca alternifolia* L. oil were different. MIC for *P. mirabilis* and *C. albicans* was 0.5% of *Malaleuca alternifolia* L. oil (Fig. 1). At a concentration of 0.1% of essential oil the growth rates of the 2 bacteria were observed at:  $4.4 \pm 3.4\%$  – *P. mirabilis* ( $p > 0.05$ ) and  $14.4 \pm 11.4\%$  – *C. albicans* ( $p > 0.05$ ). *Malaleuca alternifolia* L. oil at a concentration of 5.0% was already sufficient to inhibit the growths of all other bacteria strains (Fig. 2). When applying 0.5% of *Malaleuca alternifolia* L. oil, the growth rates of the bacteria were:  $26.2 \pm 19.05\%$  – *P. aeruginosa* ( $p > 0.05$ ),  $24.0 \pm 17.6\%$  – *E. faecium* ( $p > 0.05$ ),  $2.2 \pm 1.2\%$  – *St. aureus* ( $p > 0.05$ ) and  $0.014 \pm 0.03\%$  – *E. coli* ( $p > 0.05$ ).

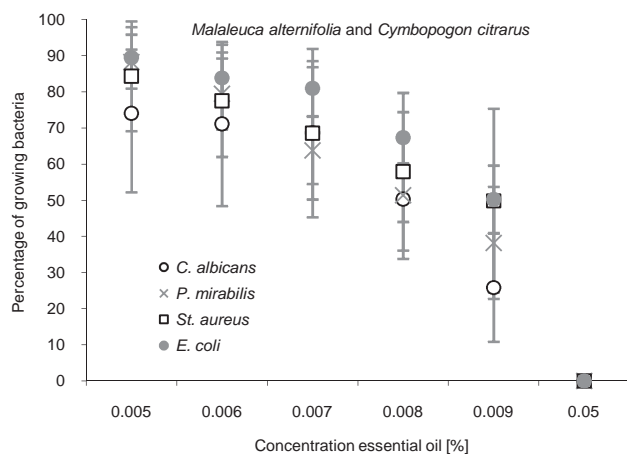
The results in Figure 3 indicate that *Cymbopogon citratus* L. oil is considerably potent against *P. mirabilis* and *C. albicans*. Growth rate at 0.1% oil concentration was already reduced to  $4.4 \pm 3.5\%$  – *P. mirabilis* ( $p > 0.05$ ) and  $14.4 \pm 11.4\%$  – *C. albicans* ( $p > 0.05$ ). A full growth inhibition with *Cymbopogon citratus* L. oil is reached for *C. albicans* and *P. mirabilis* at a concentration of 0.5%.

*Cymbopogon citratus* L. was able to exert antimicrobial activity on *E. coli*, *E. faecium*, *P. aeruginosa*, *St. aureus* up to 0.5% concentration: *E. coli* ( $0.009 \pm 0.008\%$ ,  $p > 0.05$ ), *E. faecium* ( $13.7 \pm 12.9\%$ ,  $p > 0.05$ ); *P. aeruginosa* ( $25.6 \pm 19.9\%$ ,  $p > 0.05$ ), *St. aureus* ( $1.2 \pm 0.8\%$ ,  $p > 0.05$ ).

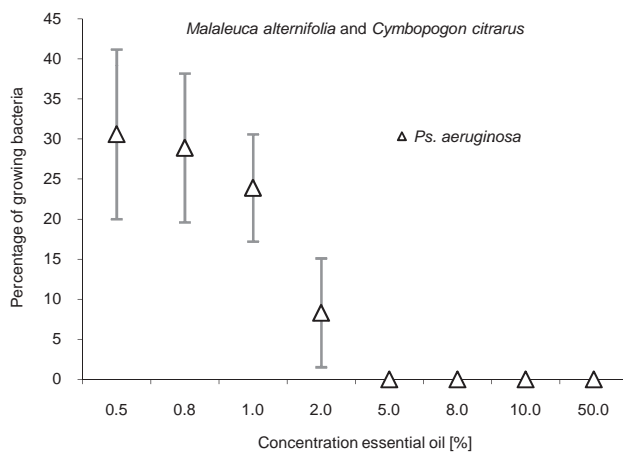
A concentration of 5.0% of essential oil *Cymbopogon citratus* L. reduced total bacterial counts of *E. faecium* and *P. aeruginosa* while a concentration of 0.8% of *Cymbopogon citratus* L. reduced total bacterial counts of *E. coli* and *St. aureus* to below 8.0% (Fig. 4).

The combined effect between *Malaleuca alternifolia* L. ( $\gamma$ -terpinene, terpinen-4-ol) and *Cymbopogon citratus* L. (neral, geranial) showed strong activity against *St. aureus*, *P. mirabilis*, *C. albicans*, and *E. coli*. The combination of the 2 essential oils of *Malaleuca alternifolia* L. (concentration 0.0025%) with *Cymbopogon citratus* L. (concentration 0.0025%) – the total concentration of combination was 0.005% – remarkably decreased the growth of *St. aureus* ( $84.3 \pm 15.2\%$ ,  $p < 0.05$ ), *P. mirabilis* ( $88.2 \pm 3.5\%$ ,  $p < 0.05$ ), *C. albicans* ( $74.02 \pm 21.8\%$ ,  $p < 0.05$ ) and *E. coli* ( $89.4 \pm 8.5\%$ ,  $p < 0.05$ ). The MIC found for these four bacteria was 0.05% (Fig. 5).

The inhibitory effects of *Malaleuca alternifolia* L. combined with *Cymbopogon citratus* L. were weaker on



**Figure 5.** Effect of the combination of *Malaleuca alternifolia* L. and *Cymbopogon citratus* L. oils on *C. albicans*, *P. mirabilis*, *St. aureus*, *E. coli*.



**Figure 6.** Effect of the combination of *Malaleuca alternifolia* L. and *Cymbopogon citratus* L. oils on *Ps. aeruginosa*.

*P. aeruginosa* and *E. faecium*. MIC on *P. aeruginosa* was 5.0% (Fig. 6) and MIC on *E. faecium* was 8.0% (Fig. 7).

## DISCUSSION

Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the essential oils inhibited bacterial growth, but their effectiveness varied.

*Malaleuca alternifolia* L. presented similar antimicrobial activity as *Cymbopogon citratus* L. Thus, generally lower bactericidal concentrations were required for the *Cymbopogon citratus* L. and *Malaleuca alternifolia* L. against bacteria *Proteus mirabilis* and yeast *Candida albicans*, amounting to 0.5% ( $p > 0.05$ ).

*Malaleuca alternifolia* L. at a concentration of 5.0% was already sufficient to inhibit the growths of all 4 bacteria strains (*St. aureus*, *E. faecium*, *Ps. aeruginosa*, *E. coli*).

A concentration of 5.0% of essential oil *Cymbopogon citratus* L. reduced total bacterial counts of *E. faecium* and *P. aeruginosa*. A concentration of 0.8% of *Cymbopogon citratus* L. reduced total bacterial counts of *E. coli* and *St. aureus* to below 8.0%.

*Malaleuca alternifolia* L., for the most part, is bactericidal in nature, although it may be bacteriostatic at lower concentrations. While most bacteria are susceptible to *Malaleuca alternifolia* L. at concentrations of 1.0% or less, MICs in excess of 2.0% have been reported for organisms such as common skin commensal staphylococci and micrococci, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* [4].

Prior to the availability of data, assumptions about its mechanism of action were made on the basis of its hydrocarbon structure and attendant lipophilicity.

Hydrophobic substances such as hydrocarbons, can modify interactions and thereby determine the operational and structural stability of the microbial cell and its macromolecular systems and disrupt their vital functions [31].

*Malaleuca alternifolia* L. and its components were also presumed to behave in this manner. This premise is further supported by data showing that *Malaleuca alternifolia* L. permeabilizes model liposomal systems [5]. In previous work with hydrocarbons, not found in *Malaleuca alternifolia* L. [15, 33], and with terpenes found at low concentrations in *Malaleuca alternifolia* L. [1, 32] lysis and the loss of membrane integrity and function manifested by the leakage of ions and the inhibition of respiration, were demonstrated.

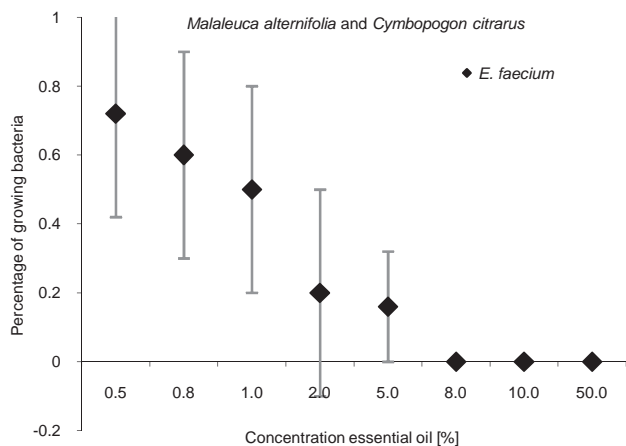
*Malaleuca alternifolia* L. compromises the structural and functional integrity of bacterial membranes. The loss of viability, inhibition of glucose-dependent respiration, and induction of lysis seen after *Malaleuca alternifolia* L. treatment, all occur to a greater degree with organisms in the exponential rather than the stationary phase of growth [6, 13]. The increased vulnerability of actively growing cells was also apparent in the greater degree of morphological changes seen in these cells by electron microscopy [6].

Further research, demonstrating that the membrane fluidity of *C. albicans* cells treated with 0.25% *Malaleuca alternifolia* L., significantly increased, confirms that the oil substantially alters the membrane properties of *C. albicans* [14]. Respiration in *C. albicans* was inhibited by approximately 95.0% after treatment with 1.0% *Malaleuca alternifolia* L. in a dose-dependent manner, and by approximately 40% after treatment with 0.25% *Malaleuca alternifolia* L. in a dose-dependent manner [5]. Glucose-induced medium acidification was also inhibited by *C. albicans* [14].

The synergistic interaction between individual components was reported to be too low to be of any practical importance [18].

The inherent activity of essential oil can be expected to relate to the chemical configuration of the components, the proportions in which they are present and to interactions between them [8, 9, 20]. An additive effect is observed when the combined effect is equal to the sum of the individual effects. Synergism is observed when the effect





**Figure 7.** Effect of the combination of *Malaleuca alternifolia* L. and *Cymbopogon citrurus* L. oils on *E. faecium*.

of the combined substances is greater than the sum of the individual effects [8]. Some studies have concluded that whole essential oils (EOs) have a greater antibacterial activity than the major components mixed, which suggests that the minor components are critical to the activity and may have a synergistic effect or potentiating influence.

The combined EOs – *Malaleuca alternifolia* L. and *Cymbopogon citrurus* L. – showed significant activity against species of *Staphylococcus aureus*, *Proteus mirabilis*, *Candida albicans* and *Escherichia coli*, tested by the broth dilution assay. The MIC ranged from 0.02–5.0%, indicating a notably different susceptibility among the species. *Enterococcus faecium* and *Pseudomonas aeruginosa* had much lower sensibility and MIC ranging between 5.0–50.0%. When the 2 essential oils are used together, they are more easily transported into the cell and, therefore, a synergistic effect is achieved. A mixture of essential oils shows an important inhibitory activity against bacterial species. Synergistic effects could be exploited so as to maximize the antibacterial activity of EOs and to minimize the concentrations required to achieve a particular antibacterial effect [12, 23].

## CONCLUSIONS

From this study it can be concluded that the essential oils *Malaleuca alternifolia* L. and *Cymbopogon citrurus* L. exhibited antimicrobial activity to varying degrees against all the tested strains when used individually. The maximum antimicrobial activity was observed against *St. aureus*, *P. mirabilis*, *C. albicans*, *E. coli* when a combination of *Malaleuca alternifolia* L. and *Cymbopogon citrurus* L. essential oils was used. This study confirms that the combination of these 2 essential oils show a greater antibacterial effect than the individual essential oils. Synergism between components of essential oils requires more study before these combined substances can be used reliably in antimicrobial applications.

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