

Research Concerning Antimicrobial Activities of Some Essential Oils Extracted from Plants

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Abstract

The principal components of some essential oils extracted from plants have been found to have microbial activity. Depending on the concentration, the members of this class are known to be bactericide or bacteriostatic. Their action mechanism is unclear, but some studies suggest that the compounds penetrate the cell, where they interfere with cellular metabolism. The purpose of this study was to evaluate the antimicrobial activity of 5 essential oils extracted from plants on *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus* and *Bacillus cereus* and to determine how different amount of the used oils can influence the results of inhibition tests. These results showed that mainly all the natural extracts presented an antimicrobial effect. Thereby, some extracts were more efficient than another and the order is: *Eucalyptus globulus* (eucalyptus), *Mentha piperita* (mint), *Lavandula angustifolia* (lavender), *Matricaria chamomilla* (chamomile), *Calendula officinalis* (calendula).

Keywords: bacteriostatic, essential oil, plant extract.

1. Introduction

For centuries, essential oils were used in a traditional way against infections and diseases globally. Today the utilization of these oils represents a growing market and therefore there is a considerably range of applications. Essential oils are usually utilized in food, beverage, perfume and cosmetics industry as well, they cover a large specter in biology. This fact led in an increase interest among researchers. In the last years, many studies were performed on microbial activity of essential oils and the results showed that all tested oils presented such an activity and some of them were more efficient than other. The principal components of some essential oils extracted from plants that have been found to have microbial activity are: thymol, carvacrol, linalool and eugenol.

Depending on the concentration, the members of this class are known to be bactericide or bacteriostatic. Their action mechanism is unclear, but some studies suggest that the compounds penetrate the cell, where they interfere with cellular metabolism. Other studies showed that phenols such as carvacrol and eugenol disturb cellular membrane and react with active sites of enzymes. In the last decade, there was an increased interest on essential oils and their antimicrobial activity due to the spread of antibiotic resistance. Since the discover of penicillin by Alexander Fleming in 1929, many new antibiotic classes have become available in bacterial infections treatments, but due to excessive consumption and often unnecessary in humans and animals, in every antibiotic available today has been reported bacterial resistance. The main components of essential oils are: hydrocarbons derived alcohols, aldehydes, ketones, esters, phenols and their derivatives, such

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as specific substances from the area where they are collected.

Besides these components, can be found also in smaller amounts some organic compounds such as olefin and acetylene, saturated aliphatic hydrocarbons, aromatic hydrocarbons, alcohols, ketones, phenols, acids, esters, lactones, carotenoids, flavones, lignans, nitrogen or sulfur derivatives. Essential oils are sensible at the action of oxidizing agents, which are present in unsaturated hydrocarbons composition. The peroxides and polymers which are formed in this process denature the quality of essential oils. In mature plants, essential oils are rich in hydrocarbons and some compounds which present a simple molecular composition and reproductive organs of plants are rich in oils which present in their composition oxygen. Volatile acids can have an important role in cellular metabolism because they are antioxidants and hydrogen donors. Also, some components, such as alcohol and ketones represent an intracellular moderator of oxidative processes and they protect against weather changes. The large diversity of essential oils requires some studies on their antimicrobial and antioxidant activity due their specific chemical composition.

Within European Union the highest use of essential oils is in food industry (as flavors), in perfume production (perfume and aftershave lotions) and in pharmaceutically industry [1-3]. In aromatherapy the use of essential oils represents approximately 2% from the total market [3]. The individual components of essential oils are also used as flavors in alimentation products, either extracts from plant material or from synthetic manufactured material [4].

The antimicrobial properties of essential oils are exploited in other industries, such as the production of some materials for dental root canal lining [5], the production of antiseptics [1, 6], and as supplements for sows and weaners [7, 8]. All the preservatives that contain essential oils are already available commercially. "DMC natural base" is a natural preservative produced by DOMCA S.A., Alhendí'n, Granada, Spain and contain essential oils from rosemary (50%), sage, citrus and glycerol (50%) [9]. "Protecta One" and "Protecta Two" are mixed extracts from medicinal plants produced by Bavaria Corp Apopka, FL, USA and are classified as food additives. Their content is not published by producers but probably these products contain one or more essential oils

and are dispersed in sodium citrate and sodium chloride solutions [10].

Due to the great diversity of the essential oils that are available on the market requires many studies on their antimicrobial and antioxidant properties. The purpose of this study was to evaluate the antimicrobial activity of 5 essential oils extracted from plants on *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus* and *Bacillus cereus* and to determinate how different amount of the used oils can influence the results of inhibition tests.

2. Material and methods

Utilized essential oils: lavender (*Lavandula angustifolia*), eucalyptus (*Eucalyptus globulus*), mint (*Mentha piperita*), chamomile (*Matricaria chamomilla*), calendula (*Calendula officinalis*).

Utilized bacterial cultures: *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, which initially were seeded and indentified in growth medium and then passed on nutrient broth.

Working technique: dilution technique is used usually in order to determine the sensibility of germs at the action of some essential oils. The working technique is the following: in sterile test tubes is added 4.5 ml of nutritive broth; dilutions are made so therefore in each tube with broth is added 4 different quantities of studied essential oil: 200 μ l, 300 μ l, 400 μ l and respectively 500 μ l. In each dilution and in another control tube is seeded a constant quantity, 75 μ l, from the 24 h culture. The tubes were incubated at 37°C for 24h. The results were investigated with the help of an Eppendorf photometer by reading the absorbance at 600 nm. The strain sensibility or the bacteriostatic titre was indicated by the absorbance at 24 h.

3. Results and discussions

The purpose of this study was to evaluate the antimicrobial activity of the essential oils and to determinate how the inhibition was made by the different amounts of used oils.

The inhibitory activity of essential oils was determinate on liquid growth medium with the help of a protocol conducted for determination of the inhibitory activity upon microorganisms. This

protocol implied the dilutions technique which were made in growth media followed by seeded with an equal microbial culture quantity. In parallel were conducted control samples in which the tested oils were not added, and where the microbial cultures must develop according to specific cultural characteristics of the species.

The results of the conducted determinations for knowing the antimicrobial activity on the bacterial strains that were made using the method described before are detailed in Table 1. It was observed that all the results are relatively homogenous.

The results showed that volatile oil from *Eucalyptus globulus* has an inhibitory effect on microbial cultures. At *Staphylococcus* genus, the absorbance for control is 2.401 while at the samples with eucalyptus oil the absorbance is much lower, for example at 0.2 dilutions the absorbance was 0.666, at 0.3 dilution the absorbance is 0.678, at 0.4 dilutions is 0.644 and at 0.5 dilutions the absorbance is 0.606. The eucalyptus volatile oil has an inhibiting effect on the cultures from the *Bacillus* genus, where the absorbance of control samples was 2.089 and at the samples where eucalyptus oil was added the values were lower. The values differ from one dilution to another: at 0.2 the absorbance was 0.571, at 0.3 the absorbance was 0.535, at 0.4 dilutions the absorbance was 0.524 and at 0.5 dilutions the absorbance was 0.514.

The control samples which belong to *Escherichia* genus, the determinate absorbance was 1.965 and at the samples where the eucalyptus oil was added the absorbance presented lower values depending on the dilutions: at 0.2 dilution the absorbance was 1.094, at 0.3 dilution the absorbance was 0.969, at 0.4 the absorbance had a value of 0.843 and at 0.5 dilution the determined absorbance was 0.714.

The results on *Pseudomonas* genus showed that eucalyptus oil produced an inhibition. The absorbance at control samples was 1.901 and at 0.2 dilutions the analyzed absorbance was 1.877, at 0.3 dilutions the absorbance was 1.689 and at 0.4 dilutions it was 1.595. The lowest values were observed at 0.5 dilution where the absorbance was 1.478. The eucalyptus oil had an inhibiting effect also on the cultures from *Salmonella* genus where the absorbance at control samples was 2.088 in comparison to the samples where this oil was added. At 0.2 dilutions the absorbance was 1.484, at 0.3 dilutions it had a value of 1.454, at 0.4 dilutions it presented a value of 1.413 and at 0.5 dilutions the absorbance was 1.398.

Due to the specific composition of lavender oil it was observed that this oil had an inhibition effect on all the studied cultures. At *Staphylococcus* genus, the control samples had an absorbance of 2.401 and at the samples where the oil has been added the absorbance was lower: 0.812 at 0.2 dilutions, a value of 0.721 at 0.3 dilutions, 0.643 at 0.4 dilution, and 0.621 cm^{-1} at 0.5 dilutions.

At microbial cultures from *Escherichia* genus the absorbance at control samples was 1.965 and at the samples with lavender volatile oil the absorbance had lower value depending on the dilutions: at 0.2 dilution the absorbance was 1.378, at 0.3 dilution the value was 1.321, at 0.4 dilution it presented a value of 1.297 and at 0.5 dilution the value was 1.277.

The oil extracted from *Lavandula angustifolia* had an inhibition effect on the cultures from *Pseudomonas* genus where the absorbance at control samples was higher in comparison to the samples where lavender oil has been added. According to the results, the absorbance at control samples had a value of 1.901 and at the other dilutions the measured absorbance was: at 0.2 dilution had a value of 1.578, at 0.3 dilution 1.432, at 0.4 dilution it was 1.225 and at 0.5 dilution it was 1.122.

The lavender oil had an inhibition effect on the microbial cultures from *Salmonella* genus, where the absorbance at the control samples was 2.088 and at the samples where the oil has been added it was observed lowest values which differ from one dilution to another: at 0.2 dilution the absorbance was 1.413, at 0.3 dilution it was 1.313, 1.222 at 0.4 dilution and 1.113 at 0.5 dilution.

The use of mint oil (*Mentha piperita*) had an inhibition effect on microbial culture from *Staphylococcus* genus where the absorbance at control samples was 2.401 while at the samples with mint oil the absorbance had lower values: at 0.2 dilution it was 0.797, at 0.3 dilution it was 0.796, at 0.4 dilution it was 0.795 and at 0.5 dilution the absorbance was 0.800.

Also, the mint oil had an inhibition effect on the cultures from *Bacillus* genus where the absorbance of control samples had a value of 2.089 and at the samples with mint oil the absorbance decreased: at 0.2 dilution it had a value of 0.797, at 0.3 dilution it was 0.796, at 0.4 dilution it was 0.795 and at 0.5 dilution it was 0.800.

At microbial cultures from *Escherichia* genus the absorbance at control samples was 1.965 while at the other test samples the measured absorbance

was lower. At the dilution of 0.2 the absorbance was 1.145, at 0.3 dilutions it was 0.711 at 0.4 dilutions it was 0.702 and the lowest absorbance of 0.682 was observed at 0.5 dilutions.

The *Mentha piperita* oil presented an inhibition effect on the cultures from *Salmonella* genus were

the absorbance at the control sample had a value of 2.088 in comparison with the other samples were the measured absorbance was lower: a value of 1.712 at 0.2 dilution, 1.585 at 0.3 dilution, 1.298 at 0.4 dilution and a value of 1.034 at 0.5 dilutions.

Table 1. Effects of essential oils on some pathogenic and commensally microorganisms

Natural Extract	<i>Staph. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>Ps. aeruginosa</i>	<i>Salmonella</i>
control	2.401	2.089	1.965	1.901	2.088
eucalyptus 0.2	0.666	0.571	1.094	1.484	1.877
eucalyptus 0.3	0.678	0.535	0.969	1.454	1.689
eucalyptus 0.4	0.644	0.524	0.843	1.413	1.595
eucalyptus 0.5	0.606	0.514	0.714	1.398	1.478
lavender 0.2	0.812	1.601	1.378	1.413	1.578
lavender 0.3	0.721	1.600	1.321	1.313	1.432
lavender 0.4	0.643	1.597	1.297	1.222	1.225
lavender 0.5	0.621	1.585	1.277	1.113	1.112
mint 0.2	0.797	0.743	1.145	1.712	1.883
mint 0.3	0.796	0.711	1.130	1.585	1.777
mint 0.4	0.795	0.702	1.062	1.298	1.698
mint 0.5	0.800	0.682	1.020	1.034	1.688
calendula 0.2	1.601	1.884	1.763	1.750	1.873
calendula 0.3	1.447	1.870	1.691	1.654	1.860
calendula 0.4	1.140	1.824	1.561	1.589	1.845
calendula 0.5	1.002	1.753	1.482	1.511	1.833
chamomile 0.2	1.324	1.793	1.834	1.765	1.765
chamomile 0.3	1.187	1.758	1.494	1.682	1.645
chamomile 0.4	1.134	1.775	1.234	1.598	1.622
chamomile 0.5	1.132	1.722	1.106	1.545	1.613

The use of mint oil (*Mentha piperita*) had an inhibition effect on microbial culture from *Staphylococcus* genus were the absorbance at control samples was 2.401 while at the samples with mint oil the absorbance had lower values: at 0.2 dilution it was 0.797, at 0.3 dilution it was 0.796, at 0.4 dilution it was 0.795 and at 0.5 dilution the absorbance was 0.800.

Also, the mint oil had an inhibition effect on the cultures from *Bacillus* genus were the absorbance of control samples had a value of 2.089 and at the samples with mint oil the absorbance decreased: at 0.2 dilution it had a value of 0.797, at 0.3 dilution it was 0.796, at 0.4 dilution it was 0.795 and at 0.5 dilution it was 0.800.

At microbial cultures from *Escherichia* genus the absorbance at control samples was 1.965 while at the other test samples the measured absorbance was lower. At the dilution of 0.2 the absorbance was 1.145, at 0.3 dilutions it was 0.711 at 0.4 dilutions it was 0.702 and the lowest absorbance of 0.682 was observed at 0.5 dilutions.

The *Mentha piperita* oil presented an inhibition effect on the cultures from *Salmonella* genus were the absorbance at the control sample had a value of 2.088 in comparison with the other samples were the measured absorbance was lower: a value of 1.712 at 0.2 dilution, 1.585 at 0.3 dilution, 1.298 at 0.4 dilution and a value of 1.034 at 0.5 dilutions.

Calendula officinalis oil had an inhibition effect when was added on *Bacillus* microbial cultures. The control samples presented an absorbance of 2.089 in comparison to the other samples where the calendula oil was added. The measured absorbance where: 1.884 at 0.2 dilutions, 1.870 at 0.3 dilutions, 1.828 at 0.4 dilutions and 1.753 at 0.5 dilutions.

At microbial cultures from *Escherichia* genus the control samples had an absorbance of 1.965 and the sample where the calendula oil has been added, the absorbance decreased as following: 1.763 at 0.2 dilution, 1.691 at 0.3 dilution, 1.561 at 0.4 dilution and 1.482 at 0.5 dilution.

The measured absorbance at *Pseudomonas* genus showed that calendula volatile oil had an inhibiting effect on the samples where this oil has been added. The control presented an absorbance of 1.901 which was higher in comparison with tested samples. At 0.2 dilutions the value of the absorbance was 1.873, at 0.3 it was 1.860, at 0.4 dilutions it was 1.845 and at 0.5 it was 1.833.

For *Salmonella* microbial cultures, the calendula oil presented an inhibiting effect with values which depend on the used dilution. Control samples showed an absorbance of 2.088 and at the test samples the values decreased: 1.750 at 0.2 dilutions, 1.654 at 0.3 dilutions, 1.589 at 0.4 dilution and 1.511 at 0.5 dilutions.

The chamomile (*Matricaria chamomilla*) volatile oil due to its specific composition presented an inhibiting effect on all the tested cultures. At *Staphylococcus* genus the absorbance at control samples was 2.401 and the other samples where chamomile oil has been added showed lowest absorbencies levels. At 0.2 dilutions the measured absorbance was 1.324, at 0.3 dilutions it was 1.187, at 0.4 dilutions it was 1.134 and at 0.5 dilutions the value was 1.132.

The same effect was observed at microbial cultures of *Bacillus* genus, where the absorbance at control samples was 2.089 and the values decreased at the samples where chamomile oil was added. It was observed an absorbance of 1.793 at 0.2 dilutions, 1.758 at 0.3 dilutions, 1.775 at 0.4 dilution and 1.722 at 0.5 dilutions.

At microbial cultures of *Escherichia coli* the absorbance of control samples had a value of 1.965 and the values decreased at the samples with chamomile oil. At the samples where 0.2 dilution was performed the absorbance had a value of 1.834, at 0.3 dilution the absorbance was 1.494, at 0.4 the value was 1.234 and at 0.5 dilution the measured absorbance was 1.722.

The chamomile volatile oil presented the same inhibitory effect on the cultures of *Pseudomonas* genus where the control samples had the measured absorbance of 1.901, while the samples where this oil has been added the values presented a decrease: at 0.2 dilution the absorbance was 1.765, at 0.3 dilution it was 1.645, at 0.4 dilution it was 1.622 and at 0.5 dilution it was 1.613.

At *Salmonella* genus the same inhibitory effect was observed. The values of the absorbance at tested tubes was lower in comparison to control where it has a value of 2.088. So, at 0.2 dilutions the measured absorbance was 1.765, at 0.3

dilutions it was 1.682, at 0.4 dilutions it was 1.598 and at 0.5 dilutions the absorbance was 1.545.

4. Conclusions

The great diversity of the essential oils that are on the market implies some studies to evaluate their antimicrobial and antioxidant activity.

The purpose of this research was to evaluate the antimicrobial activity of some essential oils and to determinate the inhibitory action which was influenced by the different quantity that was used. After the experiments that were carried out by using different amount of oil extract (eucalyptus, lavender, mint, calendula and chamomile oils) on some microorganism's strains we observed that they present an inhibitory effect on microbial growth.

The results of this study showed that all the essential oils presented a good antimicrobial activity, but some of them had a stronger effect than another that presented a lower activity. It was observed that the selected oils were able to partial inhibit the developing of some pathogen strains such as: *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Staphylococcus aureus* and *Bacillus cereus*.

Eucalyptus oil (*Eucalyptus globulus*) showed that in comparison to control samples, the oil presence had an inhibitory effect main on *Staphylococcus aureus* and *Bacillus cereus* where the survival rate was approximately of 25% regardless the quantity of the oil used. The results at *Pseudomonas* and at *Salmonella* showed that the survival rate was of 75% and 90% respectively.

Lavender oil (*Lavandula angustifolia*) had an inhibitory effect main on *Staphylococcus aureus* where the inhibitory rate was up to 30% regardless the quantity of the oil used, and a lower activity was observed at *Bacillus cereus* where the survival rate was 75%.

Mint oil (*Mentha piperita*) had an inhibitory effect on *Staphylococcus aureus* and *Bacillus cereus* where the survival rate was up to 25% while at *Pseudomonas aeruginosa* and *Salmonella* where the survival rate was up to 90%.

The calendula oil (*Calendula officinalis*) presented an inhibitory activity in comparison with control sample, main on *Staphylococcus aureus* where the survival rate was approximately of 50% while at *Pseudomonas aeruginosa*, *Escherichia coli*,

Bacillus cereus and *Salmonella* where the survival rate was approximately 90%.

Chamomile oil had an inhibitory effect mainly on *Staphylococcus aureus* where the survival rate was up to 50% while the survival rate of *Pseudomonas aeruginosa*, *Salmonella*, *Bacillus cereus* was up to 90%. At *Escherichia coli* the inhibitory rate depended by the quantity of the oil. These results showed that mainly all the natural extracts presented an antimicrobial effect. Thereby, some extracts were more efficient than another and the order is: *Eucalyptus globulus* (eucalyptus), *Mentha piperita* (mint), *Lavandula angustifolia* (lavender), *Matricaria chamomilla* (chamomile), *Calendula officinalis* (calendula).

All these essential oils showed an antimicrobial effect and all have a good potential in medicine industry with the purpose to replace as much as possible the use of antibiotics which are capable to induce a resistance to microbial strains.

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