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# Antibacterial activity of six essential oils on Gram-negative and Gram-positive bacteria

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## ABSTRACT

Essential oils are naturally occurring volatile compounds that are characterized by their strong odor and are isolated from different parts of aromatic plants. In this study, the antibacterial activity of six (Mint, Sage, lavender, Evergreen, Citronella and Marigold) essential oils on two strains gram-negative bacteria (Escherichia *coli* and *Pseudomonas aeruginosa*) and two strains of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) was investigated. These essential oils were extracted using steam distillation and their antibacterial activity was determined using the disk diffusion method. The investigated essential oils were all successful in inhibiting the growth of the two strains of gram-positive bacteria. The susceptibility of gram-negative bacteria was variable, depending on the essential oil. Marigold essential oil was proven to be the most effective as it was successful in suppressing the growth all tested bacterial strains and has the possibility of being a successful antibiotic for the treatment of multiple infectious diseases.


Keywords: Essential oils, Antibacterial, Antimicrobial resistance.

## INTRODUCTION

The emergence of new resistance mechanisms of microbes to antibiotics causes serious threats to the global public health (WHO, 2018). The antibiotic resistance crisis is attributed to many factors such as overuse of antibiotics, inappropriate prescription and the availability of few new antibiotics (Ventola, 2015).

According to the center for disease control, (CDC) at least 2.8 million people are infected with antibiotic resistant bacteria or fungi in the US alone and 35,000 people have died from it (CDC,2020). Due to the serious threats that antimicrobial resistant bacteria represent, scientist have been researching new ways to kill bacteria, including the use of essential oils.

Essential oils are naturally occurring volatile compounds that are characterized by their strong odor. They are extracted from different parts of aromatic plants such as flowers, leaves, stems and seeds (Akhtar et al, 2014). The main constituents of essential oils are generally terpenes (hydrocarbons made up of 2 isoprene units($C_5H_8$)), aromatic compounds and terpenoids (oxygenated terpenes) (Tongnuanchan at el, 2014). The composition of essential oil varies depending on the part of the plant that it is extracted from. The composition can change after extraction, due to the rapid ability of the components to become oxidized. This depends on the conditions in which the essential oil is stored (Vigan, 2010).

Essential oils can be extracted from plant organs through different methods such as distillation ( steam distillation, hydrodistillation and hydrodiffussion) and solvent extraction (Supercritical carbon dioxide and supercritical water extraction). Steam distillation is the method that is most widely used. The proportion of essential oils extracted through it is 93%, this illustrates how effective this method is (Tongnuanchan et al, 2014).

This study is conducted to investigate the antibacterial activity of six essential oils: Mint oil (*Mentha*), Sage oil (Salvia), Lavender oil (*lavandula*), Evergreen (*Thuja occidentalis*), Citronella oil (*Pelargonium citrosum*) and Marigold oil (*Tagetes erecta*). Two strains of gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and two strains of gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus,* were used.

The constituents of essential oils are responsible for their biological activities. The antibacterial activity of these constituents seems to mainly target the bacterial cell membranes. Gram-positive bacteria cell membranes are composed mainly of peptidoglycan. While gram-negative bacteria cell membranes consist of a peptidoglycan layer which is enveloped by an outer membrane made up of various proteins and lipopolysaccharides. The mentioned above bacterial strains were chosen for their easy accessibility and their involvement in human infections. These bacteria have all been identified as causes of food poisoning diseases (Mostafa et al, 2017). The threat that they pose on the public health through food poisoning brings forth interest in finding new antibacterial agents against them.

The findings of this study could expand the knowledge on essential oils and their antibacterial properties. It will contribute to the shaping of researches on novel plant based antibacterial agents.

## MATERIALS AND METHODS

i. Essential oils

Leaves, stems, and flowers from six plants

(*Mentha*, *Salvia*, *lavandula*, *T. occidentalis*, *P. citrosum* and *T. erecta*) were obtained, cut into small species and collected in a collection flask. The essential oils of the plant materials were extracted using steam distillation apparatus as shown in Figure 1. The plant materials were exposed to heat which caused them to burst and break their cell structures, thereby leading to the release of aromatic compounds (Tongnuanchan et al, 2014).

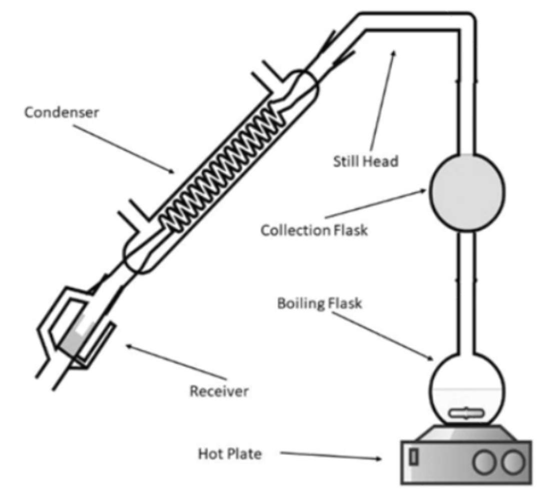


**Figure 1**: Steam Distillation apparatus (Nelson, 2018).

ii. Bacterial strains

Two strains of gram-negative bacteria (*E. coli* and *P. aeruginosa*) and two strains of gram-positive bacteria (*S. aureus* and *B. cereus*) were used in this study. The strains of bacteria were purchased from Carolina Biological Supply. Subsequently, they were cultured in Mueller-Hinton broth and then incubated at 37°C.

iii. Antibacterial activity

The antibacterial activity the essential oils were investigated using the disc diffusion method as described by Bachir et al, with some modification (Bachir et al 2012). Bacteria was spread evenly on Mueller-Hinton agar in sterile petri dishes. Sterile dicks were loaded with 7µl of essential oils at a 100% concentration and placed on top of the petri dishes with agar and bacteria. The petri dishes were then incubated for 24h at 37°C. The zone of inhibition was measured in mm.

iv. GC-MS analysis

The essential oils samples were sent to the university of Nebraska GC-MS facility for analysis. The gas chromatography coupled with mass spectrometry (GC-MS) analysis of the essential oils was performed with a thermo Scientific Trace 1310 GC oven coupled to a Thermo Scientific ISQ-LT mass spectrometer (single quadrupole) operated in electron ionization mode. The carrier gas was He at 2ml/min; the ion-source temperature was 200°C and transfer line temperature 250 °C. A split ratio of 1:25 at 250 °C was used.

DB-5 (5% diphenyl, 95% dimethyl polysiloxane) 30 m x 0.32 mm column of 0.5 um film thickness was employed. The oven temperature was programed as: 40 °C for 3 minutes, ramp at 5 °C to 160 °C and ramp at 15 °C to 240 °C. The samples were diluted with diethyl ether and 1µl of the sample was injected. Data acquisition was performed at a mass range of 34 to 650 amu. Each sample took about 33 minutes.

## RESULTS

i. Antibacterial Activity

The inhibition zones obtained from the antibacterial trials were recorded in Table 1 and looked as illustrated in Figure 2.

The result revealed that the investigated essential oils were all successful in inhibiting the growth of the two strains of gram-positive bacteria used in this study. The susceptibility of gram-negative bacteria was variable depending on the essential oil.

*T. erecta* oil was proven to be the most effective as it was successful in suppressing the growth all tested bacterial strains. The least effective oil was *Lanvandula* oil as it was only effective against gram-positive bacteria and had the smallest zones of inhibition (3.5 mm & 4mm). The most resistant bacteria were *P. aeruginosa*; They only susceptible to *T. occidentials* oil.

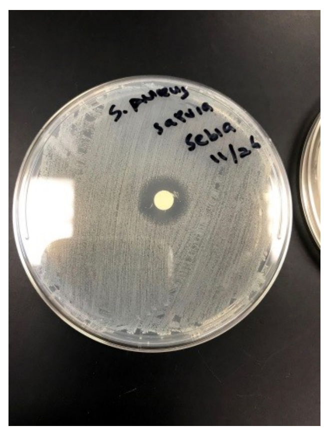

**Figure 2:** Zone of inhibition of bacterial strain caused by an essential oil

ii. GC-MS analysis

The GC-MS analysis of *Mentha*, *T. occidentalis*, *T. erecta*, *Salvia*, *Lavandula* and *P. citrosum* essential oils revealed that they contain 30, 41, 77, 62, 57, and 55 components respectively. By reason of the lengthiness of the tables with all the components for each oil sample, the five components with the highest concentration for each sample are tabulated below in Table 2-7.

**Table 1.** Inhibition zones (mm) of essentials oils on Gram (+eV) and Gram (-eV) bacteria

| EOs | *E. coli* | *P. aeruginosa* | *S. aureus* | *B. cereus* |
|---|---|---|---|---|
| ***P. citrosum*** | 4.0 | 0.0 | 7.0 | 6.0 |
| ***Mentha*** | 5.0 | 0.0 | 7.5 | 6.0 |
| ***Salvia*** | 0.0 | 0.0 | 8.5 | 5.5 |
| ***Lanvandula*** | 0.0 | 0.0 | 3.5 | 4.0 |
| ***T.occidentalis*** | 6.0 | 5.0 | 5.5 | 6.0 |
| ***T. erecta*** | 8.5 | 0.0 | 6.5 | 7.0 |
| ***Control*** | 0.0 | 0.0 | 0.0 | 0.0 |

**Table 2.** Major five components with the highest concentration in *Mentha* essential oil

| Component | Percentage |
|---|---|
| Carvone | 65.27% |
| (1R)methyl-5-(1-methylethenyl)cyclohexene | 11.10% |
| Eucalyptol | 8.46% |
| Trans-2-methyl-5-(1-methylethenyl)cyclohexanone | 4.96% |
| 4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane | 2.29% |

**Table 3.** Major five components with the highest concentration in *T. occidentalis* essential oil

| Component | Percentage |
|---|---|
| (+)-3-Carene | 35.63 % |
| (1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene | 27.49% |
| Cedrol | 12.23% |
| ß-Phellandrene | 4.65% |
| (1R)-2,2-dimethyl-3-methylenebicyclo [2.2.1]heptane | 2.58% |

**Table 4.** Major five components with the highest concentration in *T. erecta* essential oil

| Component | Percentage |
|---|---|
| (1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene | 21.89% |
| 3-Carene | 19.20% |
| Cedrol | 16.70% |
| a-Terpinyl acetate | 5.78% |
| m-Cymen-8-ol | 3.63% |

**Table 5.** Major five components with the highest concentration in *Salvia* essential oil

| Component | Percentage |
|---|---|
| (4aS,7S,7aR)-4,7-Dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-1(4aH)-one | 33.43% |
| (4-Methyl-pent-3-enyl)-cyclohexane | 23.34% |
| Caryophyllene oxide | 6.76% |
| (4aS,7S,7aR)-4,7-Dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-1(4aH)-one | 4.05% |
| (-)-ß-Bourbonene | 2.37% |

**Table 6.** Major five components with the highest concentration in *Lavandula* essential oil

| Component | Percentage |
|---|---|
| Eucalyptol | 18.14% |
| (1S)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one | 13.15% |
| 3-Carene | 11.80% |
| Caryophyllene | 9.76% |
| (1Z,4Z,7Z)-1,5,9,9-tetramethylcycloundeca-1,4,7-triene | 8.21% |

**Table 7.** Major five components with the highest concentration in *P. citrosum* essential oil

| Component | Percentage |
|---|---|
| Geraniol | 26.87% |
| Citronellol | 18.26% |
| (1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene | 8.20% |
| l-Menthone | 5.90% |
| 2-((2S,4aR)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl)propan-2-ol | 5.87% |

## DISCUSSION

As the results suggest, essential oils evaluated in this study exhibited antibacterial activity against gram-positive and gram-negative bacteria. The effectiveness of these essential oils differs depending on the bacterial strain and the source of the essential oil. Gram-positive bacteria were more sensitive than gram-negative bacteria. Several studies have come to the same findings (Mumivand et al., 2019, Helal et al, 2019).

The antibacterial activity of essential oils is usually attributed to the destructive effect that their hydrophobic components have on the membrane structure of bacteria (Nazarro et al, 2013). In fact, multiple previous studies have shown that many mechanisms of antibacterial activity of essential oils are affiliated to membrane alterations. This is including but not limited to: increase in membrane permeability leading to leakage of cell contents, disruption the cell membrane architecture, decrease in intracellular pH, reduction of membrane potentials, decreased ATP synthesis and coagulation of the cytoplasm (Boire et al, 2013; Nazzaro et al, 2013; Swamy et al , 2016; Akhtar et al, 2014).

This could explain the difference in the susceptibility of gram-negative and gram-positive bacteria. The peptidoglycan layer of gram-positive bacteria allows hydrophobic compounds such as the ones found in essential oils to easily penetrate the cell. While, the peptidoglycan layer of gram-negative bacteria ( which is surrounded by an outer membrane made up of various proteins and lipopolysaccharides) does not. Lipopolysaccharides consist of polysaccharide which are hydrophilic and their interactions with the hydrophobic essential oils do not allow easy access of the interior cell. The composition of the cell wall of gram-negative bacteria makes them more complex and more resistant to essential oils (Nazarro et al, 2013).

In the case of this study, the major components in *Mentha*, *T. occidentalis, T. erecta*, *Salvia*, *Lavandula* and *P. citrosum* essential oils were Carvone 65.27%, (+)-3-Carene 35.63%, (1S)-2,6,6Trimethylbicyclo[3.1 .1]hept-2-ene 21.89%, (4aS,7S,7aR)-4,7-Dimethyl5, 6,7,7a-tetrahydrocyclopenta[c]pyran-1(4aH)-one 33.43%, Eucalyptol 18.14% and Geraniol 26.87% respectively. Although major components can compose up to 85% of the essential oils (Chouhan et al, 2017),past studies have shown evidence that the antibacterial activities of essential oils are not only attributed to major components but the interactions between the minor and major components (Chouhan et al, 2017). The major components of the essential oils used in this study are either terpenes or terpenoids. Terpenoids have been found to exhibit better antimicrobial activity than terpenes (Guimarães et al, 2019). However, further studies would need to be conducted to determine which compounds of the investigated essential oils show better antimicrobial activity.

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