The Role of Essential Oils in Fighting AMR

The Global Problem of Antimicrobial Resistance (AMR)

The discovery of penicillin by Sir Alexander Fleming in 1928 was one of the greatest medical victories of the 20th century that significantly reduced deaths from common bacterial infections. Excessive and inappropriate use of antimicrobials such as antibiotics, antivirals and antifungals in human and veterinary medicine, animal husbandry, agriculture and the food industry around the world has led to the emergence of a multitude of drug-resistant bacteria, fungi and yeasts. Consequently, the treatment of infections has again become a formidable challenge.

The situation is further exacerbated by the diminishing antimicrobial drug pipeline. Pharmaceutical companies are disincentivised by the time, technical challenges and costs associated with antimicrobial development. Furthermore, this is a medical category that is not as profitable as common "everyday" drugs that are needed to manage chronic health conditions such as heart disease, cancer and diabetes. Consequently, of the 22 new classes of new antibiotics discovered since the discovery of penicillin, only two classes have been approved since 1962 [1].

There are two ways of curbing AMR:

- prudent use of antimicrobials, as recommended by WHO [2], and
- the discovery of new classes of compounds with excellent antimicrobial efficacy and minimal ongoing contribution to AMR.

Dr. Suki Harding, Manuka Bioscience Ltd., December 2021

Essential Oils as Antimicrobials

Essential oils are naturally synthesized by plants in response to attacks by insects, herbivores, microbes and other creatures. They are complex mixtures of bioactive chemicals that are responsible for antimicrobial properties and other unique attributes of the plant. There are around 3000 known essential oils, of which 300 are commercially important in the pharmaceutical, agriculture, food, sanitary, cosmetic, and perfume industries [3].

The use of essential oils to kill germs goes back to 1867 when British surgeon Joseph Lister began using four essential oil constituents, menthol, cineole, thymol and methyl salicylate as an antiseptic during surgery [4]. This mixture, Listerine, is now used by millions of people as an antiseptic mouthwash.

Both thyme oil and eucalyptus oil were used as surgical antiseptics by some doctors in the 1880s and eucalyptus oil was used to treat respiratory infections.

Scientific and medical research over the past two decades show that, individually and in combination, essential oils such as basil, citrus, manuka, mint, rosemary, oregano, tea tree and thyme are a source of safe, highly effective and renewable antimicrobials for use in human and animal therapeutics and hygiene products such as hand and surface sanitisation. They have rich chemical diversity and few side effects compared to synthetic chemicals. Furthermore, plant essential oils do not give rise to AMR [5].

FIGURE 1:
Selected Essential Oil Sources

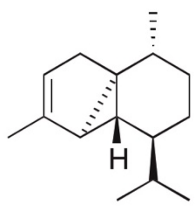
From Top Left: Manuka, citrus, thyme, rosemary and tea tree

Around 90% of essential oils consist of chemical compound family known as the terpenes that have antimicrobial properties. More than 55,000 terpene compounds have been discovered to date.

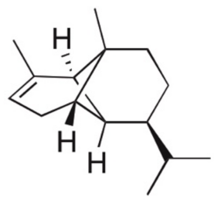
FIGURE 2 shows some examples of terpenes found in manuka and kanuka oils from New Zealand [6]. Manuka oil from the East Cape region of New Zealand also has a unique group of chemical compounds known as β-triketones. These compounds demonstrate outstanding antimicrobial efficacy against a wide range of bacteria fungi and viruses that include *Staphylococcus aureus*, *MRSA, Candida albicans, Trichophyton rubrum* and *Herpes simplex virus 1* and *2* [7, 8].

FIGURE 2
Some Antimicrobial Compounds in East Cape Manuka Oil and Kanuka Oil from New Zealand

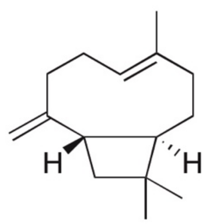
(a) sesquiterpenes



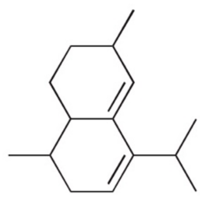
α-Cubebene
$C_{15}H_{24}$
Manuka



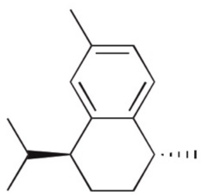
α-Copaene
$C_{15}H_{24}$
Manuka



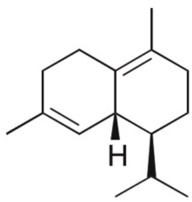
β-Caryophyllene
$C_{15}H_{24}$
Manuka



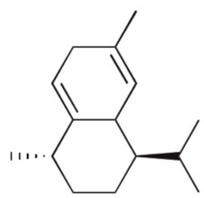
Cadina-3,5-diene
$C_{15}H_{24}$
Manuka



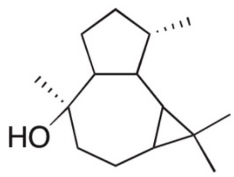
trans-Calamenene
$C_{15}H_{22}$
Manuka, Kanuka



δ-Cadinene
$C_{15}H_{24}$
Manuka

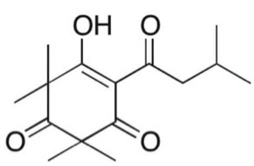


trans-Cadina-1,4-diene
$C_{15}H_{24}$
Manuka

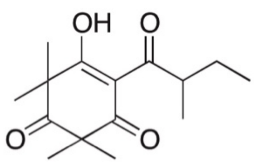


Viridiflorol
$C_{15}H_{26}O$
Kanuka

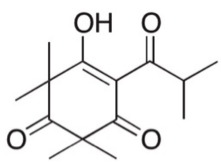
(b) Beta-triketones



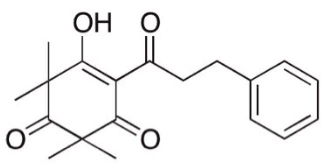
Leptospermone
$C_{15}H_{22}O_4$



Isoleptospermone
$C_{15}H_{22}O_4$



Flavesone
$C_{14}H_{20}O_4$



Grandiflorone
$C_{19}H_{22}O_4$

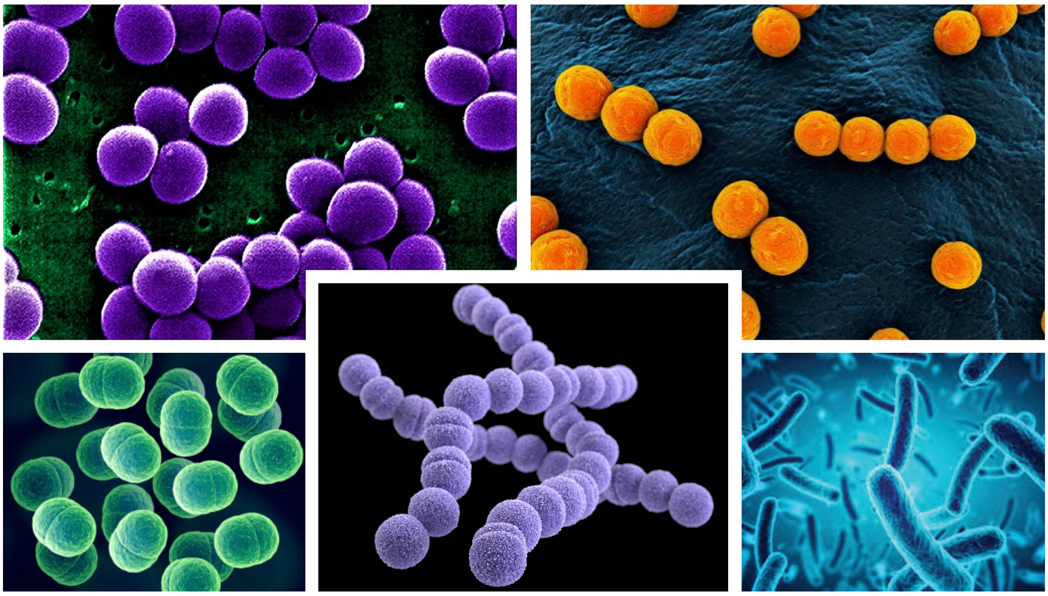
Source: [6]

About Bacteria

Bacteria can be divided into two major structural groups, called "gram-positive" and "gram-negative". FIGURE 3 gives some examples of gram positive and gram negative bacteria.

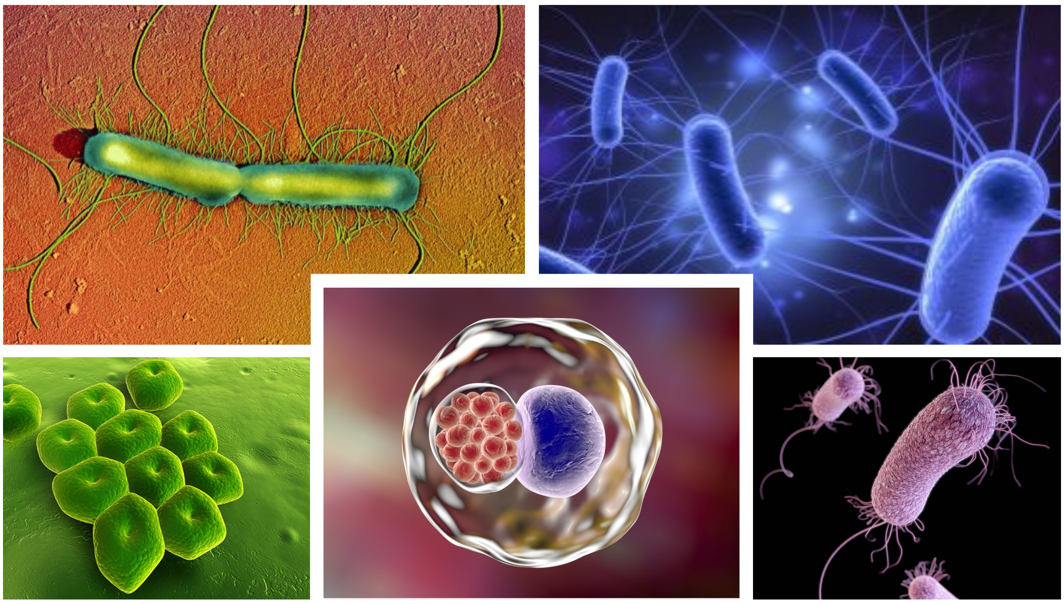
FIGURE 3
Examples of Gram Positive and Gram Negative Bacteria

(a) Gram Positive Bacteria



From Top Left: *Staphylococcus aureus, Enterococcus faecium, Cutibacterium acnes, Streptococcus pyogenes, Staphylococcus epidermidis* (**Source:** various)

(b) Gram Negative Bacteria



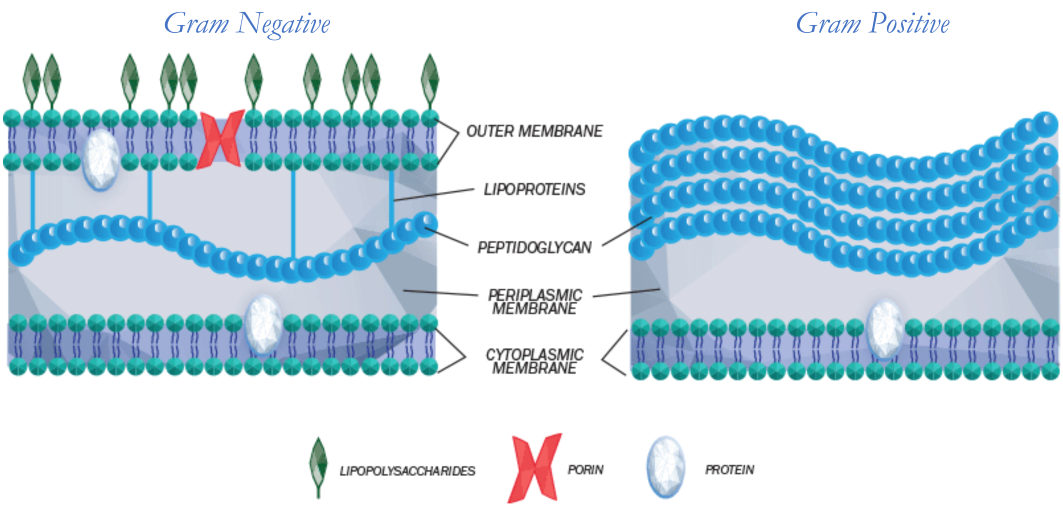
From Top Left: *Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Chlamydia trachomatis, Acinetobacter baumanni* (**Source:** various)

Gram-positive bacteria have a simple cell wall with a single type of molecule called "peptidoglycan". These bacteria do not have an outer membrane, and is therefore more permeable to small molecules such as antibiotics and essential oils. Common gram positive bacteria *S. aureus* can cause minor skin infections such as impetigo and boils, as well as serious medical conditions such as blood poisoning, surgical wound infections and flesh eating bacterial disease.

Gram-negative bacteria have a complex, double-layered cell wall with an outer membrane, which makes it less permeable to drugs and other substances. They also have higher levels of transport proteins that remove toxic substances such as antibiotics from the cells [9]. As such, gram negative pathogens cause serious illnesses such as serious wound infections, cholera, bubonic plague, bacterial meningitis, pneumonia and gonorrhoea.

FIGURE 4 shows the structural differences in gram positive and gram negative bacteria.

FIGURE 4:
Membrane Structure of Gram Negative and Gram Positive Bacteria



Terminology:

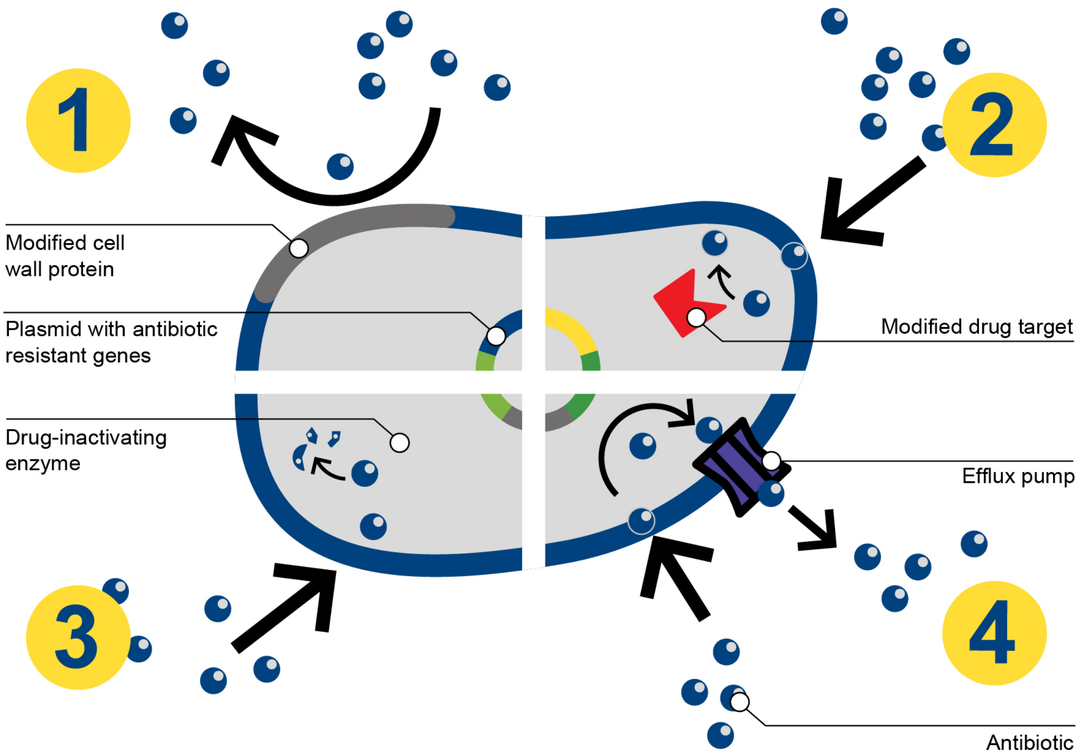
- The *outer membrane* protects gram-negative bacteria against toxins such as antibiotics.
- *Lipoproteins* play an important role in bacterial physiology and virulence, including nutrient uptake, cell wall metabolism, cell division, antibiotic resistance and adhesion to host tissues during infection.
- *Peptidoglycan* is a polymer comprising sugars and amino acids that makes up the cell wall of most bacteria.
- The *periplasm*, a compartment filled with periplasmic fluid that has a gel-like consistency, contains a number of chemical entities such as proteins and ions that perform functions such as nutrient binding, transport, folding and alteration of substances toxic to the cell.
- *Lipopolysaccharides* are a major component of the outer membrane of gram-negative bacteria, contributing greatly to the structural integrity of the bacteria and protecting the membrane from some chemical attacks.
- *Porins* are a family of proteins on the outer membranes of gram-negative bacteria that act as molecular filters for hydrophilic compounds.
- Bacteria use *proteins* as structural components, as enzymes or for transport. They are highly toxic to humans and animals. Antibiotics that prevent protein synthesis are used to cure bacterial infections.
- *Cytoplasmic membrane* separates the interior of the bacterial cell from the outside environment.

The global bacterial biomass is greater than that of all plants and animals combined, and bacterial species have existed for about 3 billion years. As a result, they are extremely adapt at survival [4].

Mechanisms of Antibiotic Resistance

There are numerous and complex ways in which bacteria fight back when exposed to antibiotics. FIGURE 5 illustrates some of these AMR mechanisms.

FIGURE 5:
Resistance Mechanisms: How do Bacteria Resist Antibiotics?



Source: amr.biomerieux.com

RESISTANCE MECHANISMS

1. **Impermeable barrier:** the bacterial cell membrane develops an impermeable barrier which blocks the entry of antibiotics.
2. **Target modification to make the interaction less effective:** modification of bacterial components which are targeted by the antibiotic prevents the antibiotic from binding properly to the its target site in order to destroy the bacteria.
3. **Antibiotic modification:** the cell produces substances (usually a protein called an "enzyme") that inactivate the antibiotic before it can harm the bacteria.
4. **Efflux pump mechanism**: the antibiotic is actively pumped out of the bacteria so that it cannot harm the bacteria.

Terminology:

- *Plasmids* are a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm.
- *Cytoplasm* is a thick solution of water, salts and proteins that fills each cell.

CASE STUDY
Multi-drug Resistant *Pseudomonas aeruginosa*

The gram negative bacteria *P. aeruginosa* is a common cause of health care-related infections [10]. These include: surgical wound, skin and soft tissue infections, blood stream infections, urinary tract infections, catheter associated infections, corneal and conjunctival erythema, meningitis and pneumonia.

Until about 15 years ago, these infections were most common amongst critically ill or immunocompromised patients who were treated with powerful broad-spectrum antibiotics such as carbapenems. The incidence of multi-drug resistant *P. aeruginosa* infections are on now on the rise, particularly in relatively healthier patients [11].

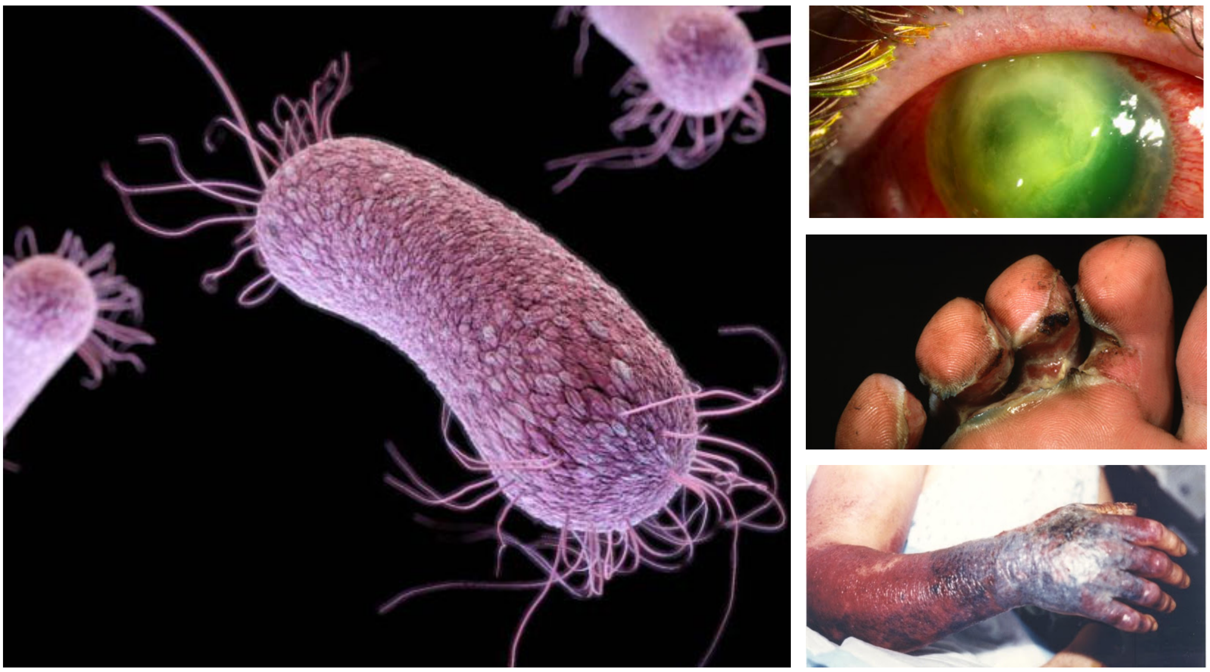
The factors influencing the growing antibiotic resistance in *P. aeruginosa* include:

- the low permeability of its cell wall;
- multiple efflux pump mechanisms;
- the production of antibiotic-inactivating enzymes.

P. aeruginosa has the genetic capacity to harbour all these resistance mechanisms and switch between them to render nearly all classes of antibiotics ineffective.

FIGURE 6 depicts *P. aeruginosa* and some of the serious infections it causes.

FIGURE 6:
***Pseudomonas aeruginosa* Infections**



Source: various

Antimicrobial Action of Essential Oils

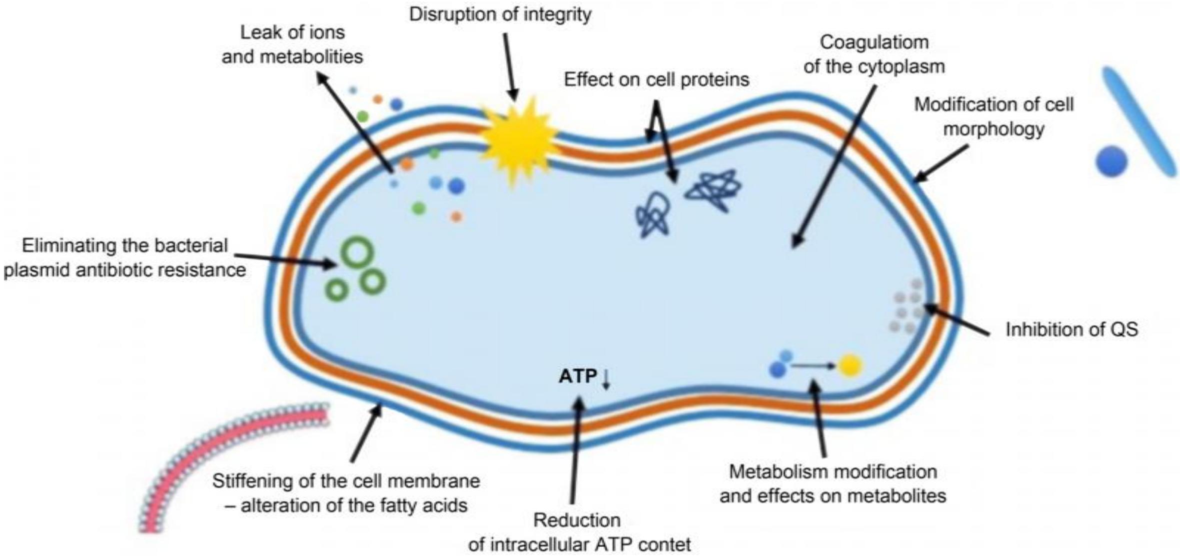
Different essential oils possess different modes of antimicrobial action, depending on their chemical composition, concentration of key antimicrobial constituents, and the affinity of these chemical compounds to different target sites within bacterial cells.

The antimicrobial activity of essential oils is attributed to several different mechanisms that include the following [12].

1. Essential oil bioactives such as carvacrol, citral, *p*-cymene, thymol and eugenol increase membrane permeability and lead to the swelling of cellular membranes, resulting in the disintegration of cell walls, leakage of cell contents, and eventual cell death.
2. The proteins present in the cytoplasmic membrane (as shown in FIGURE 4) are also the target of essential oils. For example, cinnamon oil inhibits these proteins in gram negative *Klebsiella aerogenes,* that cause skin and soft tissue, urinary tract and respiratory infections.
3. Essential oils can inhibit bacteria by attacking components in cytoplasmic membrane such as the electron transport system. For example, mint essential oil has been shown to kill staph in this manner.
4. Phenolic compounds in essential oils, such as eugenol in clove oil, are known to cause the disruption of various chemical processes within the cell, resulting in the coagulation of cell contents.

FIGURE 6 illustrates these and other modes of antibacterial action of essential oils [10]. In this context, it is important to remember that plants have essential oils to guard against pest attacks and microbial infections. As such, like bacteria, these oils have been evolving for millions of years for this very purpose.

FIGURE 6:
Mode of Antibacterial Action of Essential oils



The Role of Essential Oils in Preventing AMR

Research undertaken around the world show that essential oils and their components are highly effective against a wide range of multi-drug resistant methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Mycobacterium tuberculosis* and *Acinetobacter baumannii.* For example, a German study shows that East Cape manuka oil and key bioactive constituent β-triketone compounds are highly effective against MRSA [7]. Other essential oils that are effective against MRSA include eucalyptus, thyme, French lavender, tea tree, peppermint and geranium [4, 12,13]. This research highlights the important role of essential oils in the fight against AMR.

The role of essential oils in enhancing the effectiveness of antibiotics to treat diseases caused by gram positive, gram negative and multi-drug resistant bacteria is also being studied [12]. For example, it has been reported that cinnamon oil and the antibiotic amikacin have a synergistic antimicrobial effect on *Acinetobacter* bacteria. Similarly, combining different essential oils that have synergistic chemical constituents can increase antimicrobial efficacy. A possible mechanism of action may involve the combined effect of (natural or synthetic) antimicrobial compounds with different sites and modes of action, both inside and outside the cell.

Pathogenic bacteria do not become resistant to essential oil antimicrobials for a number of reasons:

- In contrast with antibiotics that have just one or two active ingredients, essential oils are a complex mix of many active compounds (sometimes a hundred or more) with different mechanisms of action. It is therefore difficult if not impossible for bacteria to develop multiple resistance mechanisms to negate the efficacy of all these bioactive constituents.
- Essential oils can sometimes reverse antimicrobial resistance in bacteria by inhibiting bacterial communication systems known as "quorum sensing". This prevents bacteria from becoming virulent. ("Virulence" here includes rapid multiplication, biofilm formation, and the secretion of bacterial toxins). When bacteria are 'quiescent' they often do not pose a threat to health, even though they have not been eliminated.
- Resistance in bacteria to antibiotics generally happens through genetic mutations that are passed rapidly to other bacteria. However "…*it is difficult to envisage a single genetic mutation or series of mutations that would render microbial membranes impervious to essential oils yet allow normal cell functioning. Any potential mutations to membrane properties that confer resistance to essential oils are likely to also negatively impact general microbial growth and survival.*" [14].
- Drug efflux is a is a key antimicrobial resistance mechanism in gram negative bacteria. Efflux pumps enable these bacteria to spit out antibiotics before it has time to cause fatal cell damage. Both grapefruit oil and eugenol (the major constituent of clove oil) have been shown to turn off efflux pumps in some bacteria, thus removing their resistance to antibiotics.
- Further mechanisms include that of p-cymene and myrcene, which have little actual antibacterial effect, but vastly aid the absorption of other substances into the bacteria, such as antibiotics or other antimicrobial essential oil constituents [15].

The Future

Further scientific and clinical research will help develop promising essential oils into new classes of powerful antimicrobials for global consumption.

Some say that *"the complexity of essential oils is their advantage and disadvantage simultaneously. Multicomponent composition gives benefit in overcoming bacterial resistance; however, at the same time, this creates problems with standardization of essential oils as medical preparations and slows their scientific and practical implementation."* [16].

Others see this variability in essential oil composition as an advantage. For example, Dr. Raphael D'Angelo, who developed an antibacterial tablet, "AromaTab", based on palmarosa, white kunzea, oregano, lemon, tea tree, ravintsara, and basil essential oils, says that he intentionally varies the relative proportions of the essential oils in the product in order to minimize the risk of antimicrobial resistance [17].

REFERENCES

[1] Upadhyay, A. et al., *Controlling Antibiotic Resistance Using Plant-derived antimicrobials,* Chapter 10, Kon, K .et al., (2016) Antibiotic Resistance, Mechanisms and New Antimicrobial Approaches, Elsevier Inc.

[2] World Health Organization (2012), *The evolving threat of antimicrobial resistance: options for action.*

[3] Chavez-Gonzalez, M.L. et al., *Essential Oils: a Natural Alternative to Combat Antibiotics Resistance*, Chapter 11, Kon, K .et al., (2016) Antibiotic Resistance, Mechanisms and New Antimicrobial Approaches, Elsevier Inc.

[4] Tisserand, R., (2015) *Resistance is Futile* https://tisserandinstitute.org/resistance-is-futile/

[5] Ruddaraju, L.K. et al., (2020), *A review on anti-bacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nano technological combinations*, Asian Journal of Pharmaceutical Sciences, January 2020, 15(1), Pages 42-59

[6] Manuka Essential Oil, http://www.mbtk.org.nz/what-msstk/manuka-essential-oil/

[7] Christoph, F. et al., (2000), *A comparative study of the in vitro antimicrobial activity of tea tree oils with special reference to the activity of β-triketones*, Plantamed (66), 556-560

[8] Reichling, J. et al., (2009) *Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties - an overview*, Forschende Komplementärmedizin, 16(2):79-90.

[9] Chaudhuri, A & M., *Why are gram-negative bacteria resistant to antibiotics?*, The Biochemists, https://medium.com/the-biochemists/why-are-gram-negative-bacteria-resistant-to-antibiotics-c732fe9afd06

[10] Leja, K., (2019) *Antibacterial effect of natural oils – an opportunity to solve the problem of antibiotic resistance on the example of pseudomonas spp.*, Advancements of Microbiology, May 2019, 58(2)

[11] Lesho, E.P. et al. (2019), *The Slow-Motion Catastrophe of Antimicrobial Resistance and Practical Interventions for All Prescribers,* Mayo Clinic Proceedings, June 2019, 94 (6), 1040-47

[12] Aumeeruddy-Elalfi, Z. et al., *Antimicrobial and antibiotic potentiating activity of essential oils from tropical medicinal herbs and spices* Chapter 13, Kon, K .et al., (2016) Antibiotic Resistance, Mechanisms and New Antimicrobial Approaches, Elsevier Inc.

[13] Orchard, A. et al., (2017), *Commercial Essential Oils as Potential Antimicrobials to Treat Skin Diseases*, Hindawi Evidence-Based Complementary and Alternative Medicine Volume 2017, Article ID 4517971, 92 pages

[14] Hammer K. A. et. al (2011) Antibacterial and antifungal activities of essential oils. In: Thormar, H. (ed) *Lipids and Essential Oils as Antimicrobial Agents*. Wiley, Chichester.

[15] Langeveld, W. T. et al. (2014), Synergy between essential oil components and antibiotics: a review. *Critical Reviews in Microbiology*, 40, 76–94.

[16] Kon, K. V. et all., (2012). Plant essential oils and their constituents in coping with multidrug-resistant bacteria. *Expert Review of Anti-Infective Therapy*, 10(7), 775–790.

[17] D'Angelo, R., AromaTab™ – A Novel Essential Oil Tablet, https://tisserandinstitute.org/aromatab-a-novel-essential-oil-tablet/