Essential Oils, the Answer to Antibiotic Resistance?

Abstract
Essential oils have been known to inhibit bacterial growth so more and more companies are using them in their products as an alternative to ‘harsh chemicals’. We wanted to understand if, with increased usage, bacteria could become resistant to essential oils in a similar way to bacteria becoming resistant to antibiotics. This was a useful way to understand the threat of antibiotic resistance. Would bacteria evolve resistance, or adapt? These two possibilities may seem identical however if a bacterial cell were to evolve resistance it will forever have that resistance but if a bacterial cell adapts to the exposure of the essential oils, if you were to remove the bacterium from that environment the resistance would be reduced until it was non-existent. So what we are testing is if they adapt or evolve. We are doing this by putting a strain of E. coli in an environment where it can still grow but at a reduced rate because of the essential oils in its environment which may lead to the E. coli evolving or adapting.

Funding Statement
Royal Society Partnership Grant

The Project
Introduction:
At Boroughbridge High School, our Year 10 gardeners have agreed to grow a medicinal herb garden with the plants chosen by students working on the Royal Society Partnership Grant project. These plants are also found in the Booke of Sovereign Medicines circa 1570¹, an electronic copy of which was given to the school by the Prior of Ampleforth Abbey. The plants will be used to teach pupils not only the many uses of the plants, but also the processes used to extract the oils. This will make the garden a valuable resource for the whole school and visiting primary school pupils. Since the discovery of antibiotics, many believe that the pathogenic micro-organism threat to the population has been removed, with fatal infections and infectious diseases brought under control. Since synthetic drugs have shown such great success, there has been less research into drugs containing natural compounds. But now we are facing the rising problem of antibiotic resistance and must look at alternatives. New drugs are failing to keep up with the emergence of antibiotic resistant strains of pathogens that cause many fatalities every year.

Summary of methods and results:
Mr Inglis a teacher at Boroughbridge High School, started a weekly lunchtime microbiology club after a conversation with Professor Kevin Kerr, Head of Pathology at Harrogate Hospital. Prof. Kerr came into the school to discuss the looming antibiotic crisis with us and he suggested that studying essential oils might help us to understand antimicrobial properties. A number of plants produce aromatic oils which are described as the ‘essence’ of the plant; this is what gives lavender and rosemary their smell. Essential oils are popular in fringe medicine and alternative remedies but it is difficult to obtain reliable references concerning their pharmacological merits. The specialist equipment needed to carry out our experiments included Gilson multi-channel pipette and tips, microtitre trays and an autoclave.

Each member of our group suggested an oil to investigate, so we tested 5 different essential oils:
- Lavender
- Geranium
- Cedar Wood
- Rosemary
- Thyme
against 2 different types of bacterium: Escherichia coli (common gram-negative gut bacteria) and Staphylococcus epidermis (common gram-positive skin bacteria, also a close relative of MRSA).

We used disc-diffusion methods for a preliminary study to see if the oils affected bacterial growth. Cedar wood oil had no effect on the bacteria. We didn’t know if this was because it had no antimicrobial effects or because the oil molecules were so large they didn’t diffuse very well through the agar. The size of the clear areas around the discs, the zone of inhibition, is affected by a number of variables including the size of the oil molecules. Essential oils comprise a complex mix of molecules all different shapes and sizes, right down to very small volatile compounds. As we don’t know which actual molecules affect bacterial growth, we decided to use a doubling dilution of nutrient broth since this will allow us to identify the concentration at which the oil molecules inhibit growth. The concentrations used were 4%, 2%, 1%, 0.5%, 0.25%, 0.125%, 0.0625%. A chemical indicator TTC (Tetrazolium Chloride) was added. This changed from a clear colourless liquid to red by growing bacteria. The broth-dilution method using microtitre trays was used as this allowed us to quickly and easily identify the Minimum Inhibitory Concentration. Unfortunately, none of the essential oils had an effect on the growth of Staphylococcus epidermidis. However, the E.coli showed sensitivity to 4 of the 5 essential oils, in particular lavender, thyme and geranium, which inhibited growth during the first 24 hours, at the lowest concentration of 1/32%; Rosemary at 1/16% dilution. These results allow us to conclude that some essential oils do inhibit bacterial growth, even at quite low concentrations; that they contain antimicrobial agents.

Contacts with experts:
During our research we visited Chris Bax of ‘Taste the Wild’ a local bush-craft expert who described some traditional plant based remedies. We also held a Skype video conference with a pharmaceutical company representative, who explained the different stages a new medicine must successfully pass through before it is cleared to be given to humans. Throughout our investigations, we have learned how antibiotics are becoming increasingly ineffective in fighting infections because bacteria are developing resistance to current antibiotics. Despite this, there is a worrying decline in the number of new drugs in research, since there is increasingly little prospect of the pharmaceutical companies making a profit. Our work attracted the attention of Professor Laura Piddock of the University of Birmingham and the UK-led Antibiotic Action group. She campaigns tirelessly to urge Governments to take action over the problems caused by antibiotic resistance; our teacher, Mr Inglis was awarded ‘Antibiotic Champion’ status by the Antibiotic Action group and they offered to support our exhibit by providing a variety of resources. There was great excitement when we were also interviewed by the BBC’s World Service and featured in a Guardian Science Podcast. Lots of opportunities were forthcoming, including an invitation to the University of Oxford to visit the laboratory where penicillin was developed.

Editor’s note: Readers may be interested in the related Longitude Prize, which gets a mention in the interview with Lord Rees in this issue. He chairs the Longitude Prize, which is offered for a significant development in the research into antibiotic resistance.

Further work:
We have a list of plant products (spices and oils) which are reputed to have antimicrobial properties; we are going to establish the Minimum Inhibitory Concentration and then the Minimum Bactericidal Concentration for each one. We are also hoping to form links with York University chemistry department to enable us analyse the many different components in the oils used.
Acknowledgements
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References

We are a very passionate group of year 10 students at Boroughbridge High School, and since we started a weekly lunchtime microbiology club after a conversation with Professor Kevin Kerr, Head of Microbiology at Harrogate Hospital, we’ve really fallen in love with science and even more with this project.

Authors: Alexander Stamos, Adam Dickson, Kayleigh Coates, Rebecca Longbottom, Holly Barnes, Emma Scott-Spivey, Joe Headford, Georgina Gill, Charles Grasby, Thomas Kennedy, Laura Bickerdike, Caprice Aspey, Laura Tee, Bethany Grout, Georgia Armitage, Bethany Grant