Students of Boroughbridge High School

Essential oils as antimicrobial agents

The team from Boroughbridge High School met Professor Laura Piddock of Antibiotic Action.

Key words
antibiotics
essential oils
microbiology
bacteria

Since the discovery of antibiotics, many believe that the pathogenic microorganism threat to the population has been removed, with fatal infections and infectious diseases brought under control. This has discouraged research into drugs containing natural compounds such as essential oils. However, we are now 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.

Figure 1 shows that there is a worrying decline in the number of new drugs in research, as there is little prospect of the pharmaceutical companies making a profit. Campaigning groups, such as Antibiotic Action are trying to alert all to this crisis.

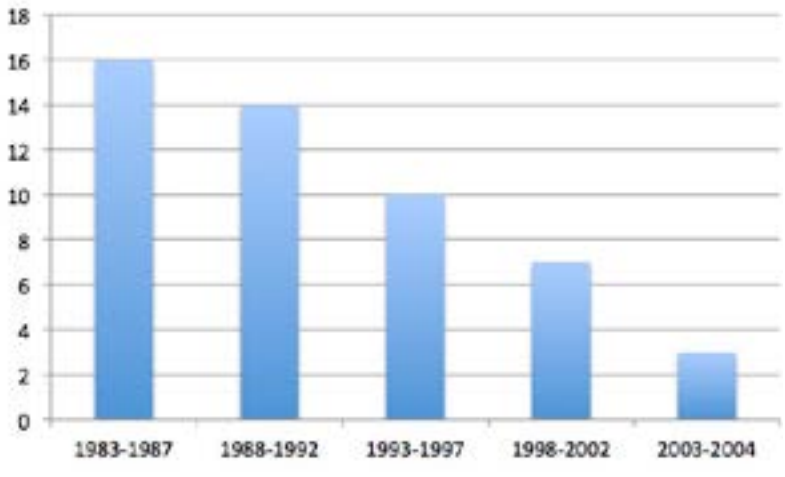


Figure 1 *The number of antibiotic approvals has been in decline since 1983.*

Our microbiology club

Our school is Boroughbridge High School in North Yorkshire. We were very fortunate that a teacher at our school, Colin Inglis, began a microbiology club. Prof. Kerr from Harrogate Hospital came to talk to the club about the looming antibiotic crisis. This stimulated our teacher to apply for a Schools Partnership Grant from The Royal Society, which funded the specialist equipment needed to carry out our experiments, including Pipette tips, microtitre trays and an autoclave.

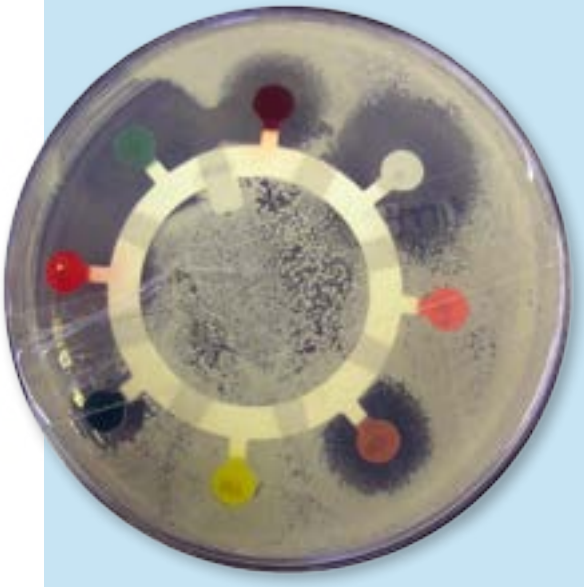
It was important to learn correct techniques for handling biological materials.

We tested five different essential oils (Lavender, Geranium, Cedar Wood, Rosemary and Thyme) against two different types of bacterium: *Escherichia coli* (common Gram Negative gut bacteria) and *Staphylococcus epidermis* (common Gram Positive skin bacteria, a close relative of MRSA).

A preliminary study was conducted using the disc-diffusion method (see Box A). This commonly-used method relies on the essential oil spreading through the agar by diffusion. Therefore, the size of the zone of inhibition is affected by the rate of diffusion which itself is affected by a number of variables, including molecule size. It was decided, therefore that this method was not suitable for future work.

BOX A The disc diffusion method

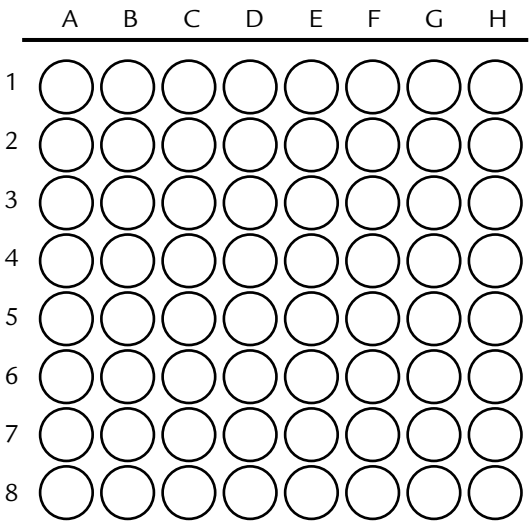
The disc diffusion method is widely used to test the effectiveness of antibiotics. A lawn of bacteria is prepared on a nutrient agar plate. Sterile discs impregnated with the test substance are placed on the lawn. After 24 to 48 hours of incubation the plates can be inspected. Clear areas around the discs show inhibition of bacterial growth.



The result of a typical disc diffusion experiment. The clear areas around the discs show that bacterial growth is suppressed. The size of each clear area may reflect the amount of suppression but it also depends on molecular size and other factors.

Once we had determined that the selected essential oil had an effect on bacterial growth using disc-diffusion, we went on to use the broth dilution method to determine the minimum concentration at which the oils affected growth.

In this method micro-titre trays were used, allowing the Minimum Inhibitory Concentration of oils on bacteria in vitro (in glass) to be determined. **Figure 2** shows a tray in which each column (A to L) has a different concentration of the selected oil and each row is a replicate.



Essential oil concentration in row

1 = 4%
2 = 2%
3 = 1%
4 = 0.5%
5 = 0.25%
6 = 0.125%
7 = 0.0625%
8 = empty

Figure 2 *A micro-titre tray showing dilutions of oils used in the experiments*

A chemical indicator TTC (Triphenyl Tetrazolium Chloride) is present in each compartment of the micro-titre tray. This is a redox dye, obtained as a white powder soluble in water in which it forms a colourless solution. In respiration in living cells, dehydrogenase enzymes cause the reduction of the TTC (by addition of electrons), which turns it red. Therefore, any red colour in a micro-titre tray cell shows the presence of living bacteria.

None of the essential oils had an effect on the growth of *Staphylococcus epidermidis*. However, the *E. coli* showed sensitivity to the four essential oils, especially Lavender and Thyme, both of which inhibited growth during the first 24 hours at the lowest concentration of 0.0625%.

Figure 3 *Typical results*

Figure 3 shows a typical set of results showing the minimum inhibitory concentration of *E. coli* when exposed to Cedar Oil (no effect, all cells red), Rosemary, Geranium, Lavender and Thyme (top row).

The bottom row shows an experiment with *Staphylococcus epidermis* in which virtually all cells show red, thus no effect from any of the oils. Any differences in the intensity of red are due to dilution effects; it is the presence of red which shows that respiration is happening.

selected The Royal Society summer science exhibition 2013

On show

The Royal Society was so impressed with the progress we had made they invited us to take part in their annual Summer Science Exhibition in London representing the Partnership Grant Schools. The exhibition involved 12 hour days from Monday July 1st to Sunday July 7th, and included two black tie and cocktail dress soirees.

The Exhibition allowed us to present our findings to a broad audience of visitors, including other school groups, specialists in science, fellows of the Royal Society, members of the general public, exhibitors from University departments, MPs and invited VIPS, including Lord Robert Winston, the impressionist Jon Culshaw and the BBC's Evan Davis. We received a great reception. Everybody that we met was interested in the work we were doing and we had some very positive feedback.

We thoroughly enjoyed the whole experience. Although being surrounded by exhibitors from universities and specialist agencies was at first overwhelming, we did grow more confident in engaging with them and sharing our ideas by the end of the week. Whilst we were primarily there to present our investigation, we got to involve ourselves in the other exhibits and learn all about their work too. Being amongst professionals and others that are all so enthusiastic about their careers and research was both inspiring and encouraging to continue with education in science and possibly pursue our own careers in science.

Rebecca Longbottom (year 12 pupil) explains our project to Martyn Poliakoff, the Royal Society Foreign Secretary and Vice-president.

Laura Tee plays the iPad app 'Plague inc' with one of our younger visitors. Epidemiology is accessible to all!

A member of the public is very pleased with an explanation given.

Tom Kennedy and Kayleigh Coates speaking to Evan Davies of the Radio 4 Today programme

Next steps

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 to analyse the many different components in the oils used.

We have been invited to publish summaries about our project in a number of journals which will help us develop our writing and communication skills. So far we have given an interview to the BBC world service and our teacher was interviewed for a podcast for the Guardian newspaper.

We hope to visit Oxford University, which is a great opportunity for us to see where penicillin, the first antibiotic, was developed.

A number of other schools would like to develop links with our project so they can become involved. So go on, do some real research and see what exciting opportunities it opens up for you!

We are grateful to **Colin Inglis** *of Boroughbridge High School for many of the images used in this article.*

The microbiology club
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Tom Kennedy
Joe Headford

together with
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Colin Inglis
Assistant Head
John List
Teaching Assistant
Tracy Acaster
Teaching Assistant
Melanie Blackburn

Look here!

See our video on the Royal Society Summer Science Exhibition website: *sse.royalsociety.org/2013/exhibits/antimicrobial-agents/*
BBC World Service podcast: *www.bbc.co.uk/programmes/p01b9cb0*
Guardian science podcast: 8 July 2013