Traditional laboratory projects using tried and tested methodology create room for experimentation

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Background and rationale

The project outlined is an example of one of many laboratory projects from our Biology Dissertation, a 40-credit final year project-based module, undertaken as part of degree requirements for BSc Biology or Environmental Science. Intended learning outcomes for the module are:

- Critically review relevant literature (essential preparation for the project, both as initial background in devising the project proposal and in informing the Introduction and Discussion of the thesis).
- Devise a goal and plan an appropriate study to achieve it (a research proposal with aims, objectives, project management plan, and completed risk assessment is submitted – and approved! – before work starts).
- Implement a study using relevant methodology and appropriate techniques (carrying out the proposal – with supervisor guidance, etc where necessary).
- Critically evaluate your own work (to encourage the student to criticise their project's shortcomings, etc. and suggest remedial action/further work, part of the Discussion).
- Produce an appropriately structured dissertation (a conventional bound thesis of 10,000 words).
- Communicate your work to a non-specialist audience (a short oral presentation of the investigation to their peers to develop communication skills other than written; takes place about 6 weeks before submission so feedback can inform and improve the write-up.

How to do it

In summary, candidate liquid plant extracts are applied to filter paper discs which are placed atop a microbial lawn growing on agar. The plant extract diffuses from the disc and either kills microbes or does not. The killing zone (zone of inhibition — ZOI) appears translucent relative to the rest of the lawn. The diameter of the ZOI is measured and compared between different extracts, control treatments, etc.

The project uses standard microbiological techniques and should be suitable for most bioscience departments. Where appropriate, students may be involved in all stages of the preparation of media, production of lawns, sterilisation of equipment, etc. Otherwise, a decision needs to be made regarding how much 'hands-on' involvement the student has in the — usually — 'behind-the-scenes' preparatory work. We encourage students to source details of techniques, etc. themselves — using past copies of undergraduate theses and published literature as inspiration. This is discussed with the supervisor before any practical work begins and in conjunction with project management planning and risk assessments.

Because of all the variables — plant/seaweed species, plant part, developmental stage of plant, harvest season, etc. that could be considered in any project, it is difficult to be too prescriptive regarding how to do it. The following is offered as a guide to the considerations that need to be applied in each study.

There are three main stages to the investigation:

i) Microbial cultures and preparation of agar plates

Choice of microbes is important and needs to be relevant to the investigation. For health and safety reasons, we generally use relatively harmless microbes, or non-pathogenic isolates of otherwise harmful ones. To cover a range of microbes, students generally use a Gram-positive bacterium, e.g. *Staphylococcus albus* (as a member of the same genus as the MRSA bug), a Gram-negative bacterium — e.g. *Escherichia coli* (as a proxy for a major stomach-upset causing organism), and a fungus — e.g. *Saccharomyces cerevisiae*. Microbes are usually purchased from Philip Harris (Ashby de la Zouch, Leicestershire LE65 1NG).

Each microbe has its own preference for growth media and culture conditions (the supplier can advise on this). For those mentioned above we generally use:

Organism	Growth medium	Incubation temp. (°C)
Staphylococcus albus	Nutrient agar	37
Saccharomyces cerevisiae	Sabouraud's agar	30
Escherichia coli B	MacConkey agar	37

Further details and formulations of the growth media can be found in standard microbiology texts, manufacturer's catalogues, or reputable internet sites.

Lawns are made by spreading c. 0.15 ml of microbial culture over the appropriate agar in a Petri dish and incubating overnight.

ii) Preparation of plant material and discs

In the past students have used fresh material (leaves, roots, stems, flowers, etc.), dried material, commercial preparations either solid (e.g. dried herbs) or liquid (e.g. essential oils). The goal is to get an extract that can be mixed/dissolved in an appropriate solvent so that it can be soaked up onto a filter paper disc, and made up in a reproducible formulation. This may take some experimenting (and can be both a fun and frustrating! part of the investigation); solvents routinely used include water, ethanol, industrial methylated spirit (IMS). Factors to vary include the time of shaking of extract and solvent, and the degree to which plant material is crushed or cut up before extraction.

Issues that can arise include pigmentation of extract solutions which can obscure identification/measurement of any ZOI, and difficulty of dissolving extracts (e.g. Aloe mucilage). If plant material does not dissolve, it should be possible to obtain an extract by shaking the plant material with a suitable solvent; again, some trial-and-error is almost inevitable here. In some cases it may be possible to use discs of plant material directly applied to the agar. An alternative to use of rather crude whole plant samples is to consider steam-distillation of plant material to release essential oils, which can then be tested. This may be done in an attempt to narrow down the range of potential anti-microbial agents.

iii) Incubation of plates and measurements of ZOI

Sterile discs (we routinely use 13 mm Whatman filter papers) are dipped in the plant extract, drained of excess liquid and dried in a sealed sterile Petri dish. Up to four discs can be accommodated in a Petri dish (but if the extract is highly active ZOIs can merge between one disc and another) and are applied to the surface of the agar containing the microbial lawn. The dishes are then sealed with tape (and not opened again — useful safety practice) and incubated for 24, 48 hours or longer at appropriate temperatures (depending on the investigation). Dishes are inspected for microbial death, which can be identified by a clear/translucent zone around the filter paper discs. The diameter of this zone of inhibition (ZOI) is measured and recorded and is the basis for comparison of effectiveness of anti-microbial activity of plant extracts/solvents.

Appropriate controls are essential: principally this is a disc dipped in the solvent used to extract the plant material. Another 'control' we employ is to use an alcoholic extract of garlic (3 g crushed fresh garlic and 10 ml IMS, shaken for 10 minutes) against the microbes to be tested. Garlic inhibits/kills all of those microbes mentioned above and is a good test of the student's aseptic technique as well as demonstrating a 'positive' result. Sufficient replicates are also needed to permit sensible statistical analysis of the results. This latter points needs to be borne in mind when designing the investigation — the demands on time for preparation of the large number of plates often needed, can be considerable! Subsequent analysis of results can then be undertaken using standard statistical tests.

- Refinements (these are but a few — encourage students to be imaginative!).
- Minimum inhibitory concentration (MIC) — how dilute can the plant extract be before it does not inhibit microbial activity? A series of doubling dilutions can provide useful information on this aspect of the investigation. Of necessity this is a relatively crude measure because the concentration of the active principle(s) is often unknown, but it can help to rank extracts' effectiveness pending further analysis of chemicals involved.
- Steam-distillation to release essential oils (see above), which can then be tested.
- Whether there is seasonal activity of any anti-microbial activity found.
- Whether anti-microbial activity is dependent upon stage of growth of plant.

Assessment of the Dissertion

Each project is written up as a standard scientific dissertation and submitted in duplicate. One copy is marked by the supervisor; the other independently by another member of staff. A table of marking criteria for the various sections of the thesis is completed; scores are given for each section and the scores discussed between the two markers. In the exceptional case where consensus is not reached on an overall mark, a third marker looks at the thesis. The agreed mark is reduced to a maximum of 85%; the remaining 15% of the final project mark is at the discretion of the supervisor having regard to the student's commitment and dedication to the project, adherence to agreed laboratory times, supervisory meetings, etc.

Advice on using this approach

- Ensure the students are thoroughly briefed on health and safety issues — get them to research and write the first draft of the health and safety/ risk assessment for discussion with their supervisor — before undertaking the work.
- Impress upon the students the need to be organised and manage their time appropriately — there are potentially a lot of plates to work with!
- As with every project, things can go wrong and the unexpected can happen — part of the learning outcome of the module is how students cope with these matters.
- Depending on the plant material investigated/ microbes used, increasing levels of care/control/ containment may be needed in carrying out work/ disposing of used materials.

Troubleshooting

The commonly-encountered main problems are:

1) Not finding any anti-microbial activity. Make this a 'positive' result (not all plants are anti-microbial) by ensuring they do a 'dummy run' with garlic and microbes (that does work).
2) Poor aseptic technique. This should be overcome with practice.
3) Issues in finding the appropriate diluent for plant material. This issue requires experimentation to overcome it (which should be part of the fun of doing research!).

Problems specific to particular test organisms include getting the microbes to grow suitably (ideally, as a lawn) so that anti-microbial kills can be identified and quantified.

Does it work?

Although an important aim of any dissertation is for students to get experience of a proper research project, too many traditional laboratory investigations can get bogged down in trying to get the methodology to work, without generating results. This study uses proven methodology and should mean the student spends more time on planning the investigation, and obtaining and analysing their results. As a result, this sort of investigation is ideal for undergraduate projects. Not only is there the enjoyment and satisfaction of undertaking a project that works (usually!), it is an investigation that is highly relevant to topical concerns about the unexploited biomedical potential of plant resources. There is great scope for student imagination in selecting the microbes to use, the treatment combinations to apply, the plant/seaweed species to investigate, and then which parts and which solvent to use, determining MICs of extract, whether to investigate seasonal microbiocidal activity, etc.

The method is straightforward, and is tried-and-tested both within our department and the published literature - see Harding and Maidment (1996), Maidment et al., (2006); Maidment et al., (2001); Maidment et al., (1999); and Maidment et al., (1998). It also lends itself to students suggesting their own ideas of material to test (chrysanthemums against 'jock-itch' is one memorable investigation suggested by a student!), so they have 'ownership' of the project. Additionally, this investigation often has the bonus that — if done well — it may lead to publishable results (several of the above papers arose from such student projects).

Further developments

No significant changes are planned — it works very well as it is! However, with over 250,000 species of flowering plants there is plenty of scope to broaden plant material tested and microbes investigated. Having identified 'interesting' plant material, a logical step is to attempt separation and identification of active ingredients (those technical considerations are beyond the scope of this case study).

References

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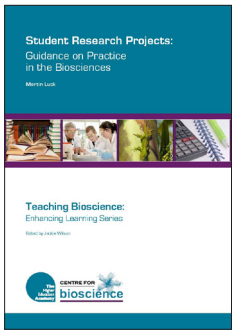
Additional materials



This case study was included in the Teaching Bioscience: Enhancing Learning guide entitled *Student Research Projects: Guidance on Practice in the Biosciences*, written by Martin Luck and published by the Centre for Bioscience. The associated website (www.bioscience.heacademy.ac.uk/resources/TeachingGuides/) contains a downloadable version of this case study

The gel diffusion assay technique has been used for many investigations of anti-microbial properties within undergraduate projects — e.g. New Zealand shrubs, Aloes, spice plants, Mediterranean herbs, commercial garlic preparations, essential oils, UK littoral seaweeds, and honeys. Some of the projects have been written up and published, while others are in preparation. Interested parties requiring further information are invited to contact the author for detailed instructions.

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