Extracting and separating pigments from spinach

This activity is designed by ScienceGrrl Glasgow to celebrate the work of Dr. Tu Youyou a Nobel prize winning chemist who discovered the antimalarial drug artemisinin in sweet wormwood. Dr. Youyou is one of ScienceGrrl Glasgow's #UnsungHeroines – female scientists who we feel deserve to have their achievements celebrated for their contributions to our scientific knowledge.

Dr. Youyou undertook her work on artemisinin in China during the cultural revolution, when intellectuals were often persecuted for their work. Dr. Youyou was not recognised for her work for a long time due to the communist nature of the project and the fact that her discoveries were helping the Chinese army during the war efforts in Vietnam, so were top secret!

Dr. Youyou used a cold extraction process using hexane to extract artemisinin. We can follow a similar process using acetone to extract pigments from spinach.

Interested in methods to purify compounds from ancient Chinese medicines, Dr. Youyou collected over 2000 recipes looking for ways to isolate their active ingredients. One recipe she found was for a drink made from sweet wormwood in a book written in 340 AD by Ge Hong. The drink was intended to treat "bone steaming fevers" and the recipe was written on silk and bamboo. The parchment had partially degraded, but Dr. Youyou was able to follow the extraction protocol and eventually managed to improve it and purify the active compound – artemisinin.

Sweet wormwood doesn't grow in the UK in this activity we will extract molecules from spinach instead. Most people know that chlorophyll is responsible for the green pigmentation in plants, but many people don't realise that there are actually several coloured pigments that make up the green colouration. These colours can be easily extracted using materials found in most households.

#UnsungHeroines

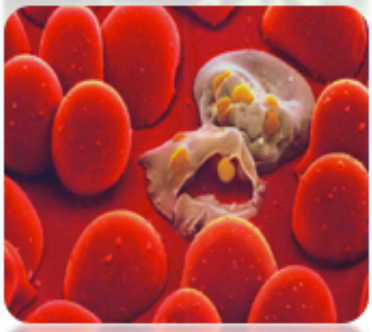
Dr. Tu Youyou

Chemist

1930-

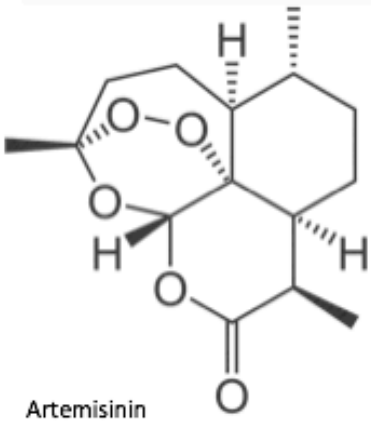
Tu Youyou won the **Nobel Prize in Physiology /Medicine** in 2015 for her work on **artemisinin, an anti-malaria drug** that she extracted from sweet wormwood using a recipe from the year 340. Artemisinin based drugs are now at the front line in the treatment of malaria and their discovery has contributed to **saving millions of lives**. Youyou's work is particularly impressive as it was carried out in China during Mao's Cultural Revolution when scientists were persecuted.

Sweet wormwood or *Artemisia annua*.



Malaria parasites bursting from red blood cells

Ge Hong (left) recorded his recipe to produce a plant extract curing "bone-steaming" fevers (middle) in a 1700 year old book (right) printed on silk and bamboo. Youyou used this recipe to extract and validate the drug *qinghao* (artemisinin in the West).



Artemisinin

The experiment

You will need

- Spinach
- Alcohol (around 40% abv)
- Pestle and mortar or similar
- Filter paper (e.g. from a coffee machine)
- Dropper to transfer liquid (cocktail sticks also work)
- Acetone-based nail polish remover
- Pencil
- Felt tip pens
- Jam jar/trough with stick over the top to attach filter paper

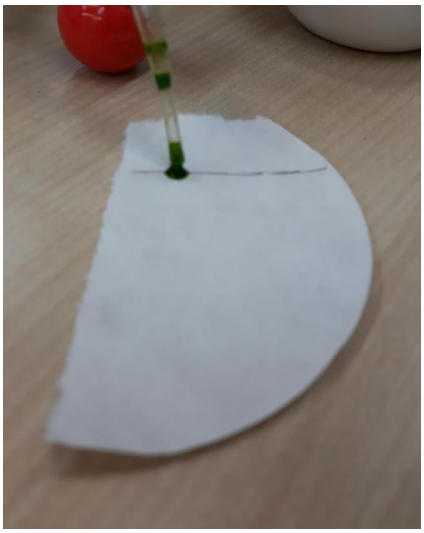
1. Grind spinach in a pestle and mortar (or blender or chop into fine pieces)

2. Add alcohol – e.g. use gin at 37.5% alcohol – and grind again

Grinding spinach and gin in a pestle and mortar

3. Using a pencil, mark a line on piece of filter paper 1 cm from the bottom

4. Spot the spinach juice onto the filter paper on the line using a dropper or cocktail stick – a smaller, more concentrated spot is better



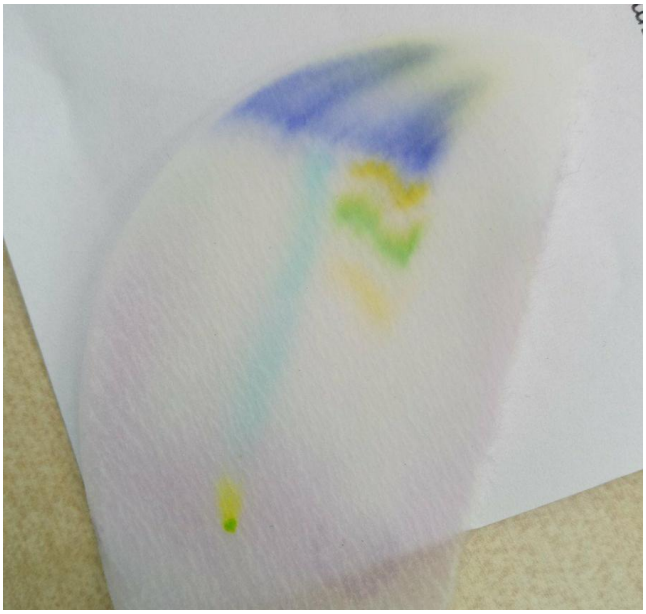
Spotting the spinach/gin juice onto the filter paper

5. As controls add spots of felt tip pens on the line next to the spinach (green would be a suitable control)

6. Dip the end of the filter paper into acetone-based nail polish remover in a jam jar or trough. You can attach it to a stick over the top of the jar. Don't let your coloured spots touch the liquid. Allow the liquid to travel up the paper, bringing the colour with it.

Chromatography. The pigments travel up the paper with the solvent front – the liquid acetone.

7. The green from the spinach should separate into three distinct bands – green, yellow and orange. The controls may also separate into different colours, but, unless they are plant-based pigments, are unlikely to travel the same distance as the colours in the spinach.



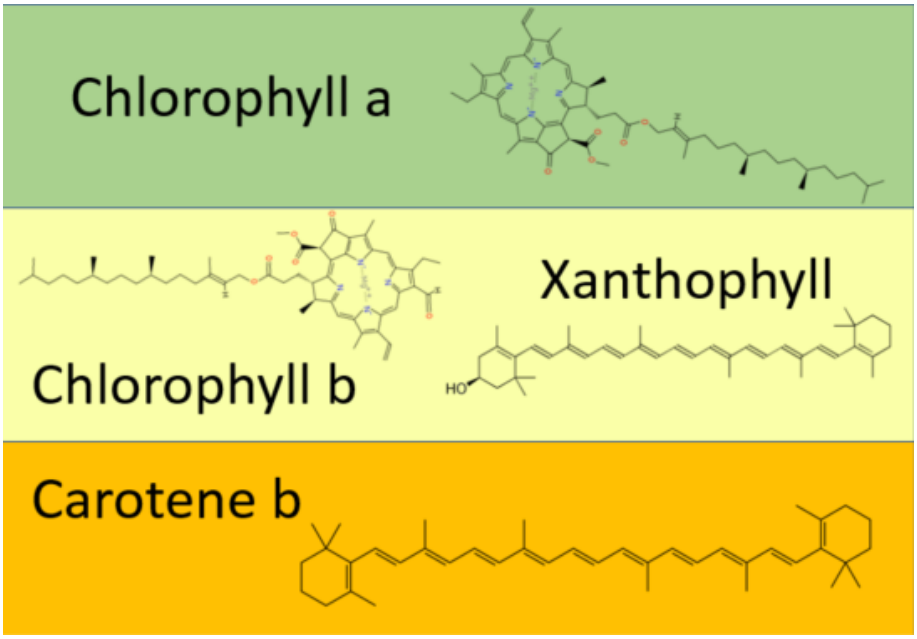
Separation of coloured pigments from spinach (right) and felt tip pen (left). The blue at the top is ink from the name at the top – better to write names in pencil!

Results

Most people will be able to separate the spinach colour into the three bands and some people will get four – green, yellow and two orange bands travelling different distances in the acetone.

The controls usually separate into different colours if they contain water soluble or acetone soluble inks. Pens that are alcohol resistant or permanent usually don't separate.

Since the ink colours don't travel the same distance as the colours in the spinach, we can conclude that they are not the same pigments.



Pigments in spinach. Chlorophyll a is green, xanthophyll and chlorophyll b are yellow and carotene b is orange.

Extensions to the activity

- A complementary activity, Smelly molecules, has been developed by ScienceGrrl Glasgow. See our website (www.sciencegrrlglasgow.wordpress.com) or email sciencegrrlglasgow@gmail.com for further details.
- Use molecular building kits to build artemisinin and/or the pigments

Questions related to the activity

- What is the difference between Carotene b and xanthophyll?
- Why do these molecules appear different colours?
- What other useful molecules are derived from plants?
- How many molecules might there be in plants that we have yet to discover?

References

- Read our blog about Tu Youyou on the Biochemist https://thebiochemistblog.com/2017/10/31/how-a-combination-of-ancient-and-modern-medicine-led-to-a-discovery-that-saved-millions/
- Tu Youyou Biography. https://www.nobelprize.org/prizes/medicine/2015/tu/auto-biography/
- Haynes RK, Vonwiller SC. Extraction of artemisinin and artemisinic acid: preparation of artemether and new analogues. Trans R Soc Trop Med Hyg. 1994 ;88 Suppl 1:S23-6.