Investigating the chemistry of the Uncha plant

Sharing Aboriginal and Torres Strait Islander peoples’ knowledge and perspectives

Prepared by: Dr Susan Semple, Dr Bradley Simpson and Dr Chi Ndi (University of South Australia: Clinical and Health Sciences) and Mr David Claudie (Chuulangun Aboriginal Corporation)

Notes for teachers

The following notes provide further information about three of the techniques used in testing Uncha for medicinal properties, to accompany the embedded videos in the PowerPoint presentation ‘Investigating the chemistry of the Uncha plant – Sharing Aboriginal and Torres Strait Islander peoples’ knowledge and perspectives’.

Rotary evaporation

This procedure is used to remove organic solvents from the extract, or from fractions of the extract before they are tested for anti-inflammatory activity or used in further separation processes.

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| Steps |
| * The plant extract is transferred into a round bottom flask. The plant material was extracted in a mixture of 50% methanol and 50% dichloromethane then filtered to remove the leaf material.
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| * The flask is connected to the rotary evaporator.
* The sample is rotated which increases the surface area for evaporation.
* The flask is put into a water bath which has a temperature of 40°C to warm the solvent.
* The rotary evaporator is attached to a vacuum pump - so that the sample is under vacuum.
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| * The rotary evaporator has a condenser. Cool water inside the coil condenses the solvent that has evaporated.
* The solvent collects in another flask.
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| * Once all the solvent has been removed, the dried plant extract is ready to use.
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Solvent partitioning

This procedure is used as an initial method to clean up the extract and separate the different components of the extract based on their polarity. The three fractions produced from this process were then tested for anti-inflammatory activity.

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| Steps |
| * In this procedure the extract will be partitioned between immiscible solvents.
* The first step involves mixing the extract in 70% methanol which is a polar solvent.
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| * Hexane is then added which dissolves more of the non-polar compounds in the extract.
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| * The extract and solvents are transferred to a separating funnel.
* The funnel is then carefully shaken to allow the extract components to partition into the different solvents.
* When allowed to stand, the solvents will form two separate layers.
* The hexane is less dense than the lower methanol/water solution and thus sits on top.
* Once the layers have settled the lower layer is collected by running it out through the tap at the bottom.
* The top layer is then collected as the hexane fraction.

(Usually this process is then repeated) |
| * The methanol/water fraction will now be further partitioned with dichloromethane.
* Dichloromethane will dissolve the extract components with intermediate polarity.
* The same process is used. This time the dichloromethane is the denser solvent, so it will sit at the bottom with the methanol / water layer at the top.
* After the partitioning process is complete, there are three extract fractions.
* These can now be dried, tested and then separated further using chromatographic methods.
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Thin Layer Chromatography (TLC)

Thin layer chromatography can be used to guide the separation process. This is an example using the three fractions from the solvent partitioning process and comparing these with the crude (whole) Uncha extract and two of the known compounds found in the Uncha extract.

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| Steps |
| * A TLC plate has a thin coating of silica gel on the surface (this is normal phase TLC). The TLC plate is initially marked to show the different samples that will be spotted on the plate.
* This includes the hexane, dichloromethane and 70% methanol fractions, as well as the crude plant extract for comparison.
* In this example, two of the known compounds from the plant will also be spotted on the right side of this plate. This allows us to see whether they are contained in the fractions.
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| * The samples are spotted onto the TLC plate.
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| * The plate is placed in the TLC tank which already contains the solvent mobile phase. Some chromatography paper is also in the tank to saturate the tank with the mobile phase vapour. The mobile phase in the tank is 90% dichloromethane and 10% methanol with 0.1% formic acid.
* The mobile phase starts to move up through the TLC plate carrying the compounds. Compounds will move at different rates depending on the strength of their interactions with the silica gel on the plate (e.g. the more polar compounds move less because they have stronger interactions with the silica gel).
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| * The plate is allowed to develop until the solvent reaches the marked line (solvent front).
* The plate is taken out of the tank.
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| * The plate is now sprayed with a reagent to help visualise the spots.
* In this example an anisaldehyde spray reagent is used.
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| * The plate will now be heated to show the spots more clearly.
* It is placed in an oven.
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| * In the heat of the oven the spots will be revealed.
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| * The plate is removed from the oven.
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| * The plate can now be examined.
* It shows the hexane fraction contains spots of the more non-polar compounds that have moved up the plate the furthest.
* The dichloromethane fraction has in the intermediate polarity compounds.
* The 70% methanol fraction has the most polar compounds. Some of these have remained on the origin.
* We can also see some of the compounds from the extracts which have a dark grey colour after spraying with anisaldehyde– these are the anti-inflammatory clerodane compounds that will be discussed later in the presentation.
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