
# USING DNA METABARCODING TO INVESTIGATE THE MEDICINAL PROPERTIES OF HONEY

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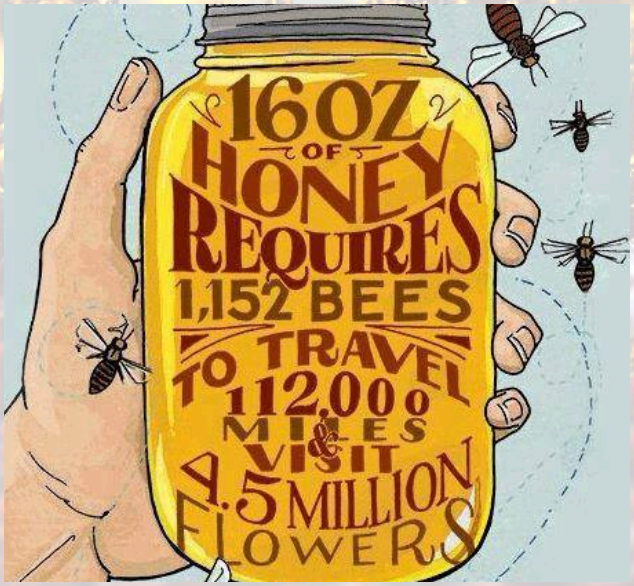
# UNDERSTANDING THE FLORAL COMPOSITION OF HONEY

Investigating honey bee foraging

Verification of the stated botanical source and geographic origin

Food safety and quality control

Determine links between medicinal properties of honey and plant derived compounds and their potential for new drug discovery routes



# ANTIBACTERIAL PROPERTIES OF HONEY

Bee defensin-1

Hydrogen peroxide

Methyl glyoxal (MGO)

Low pH

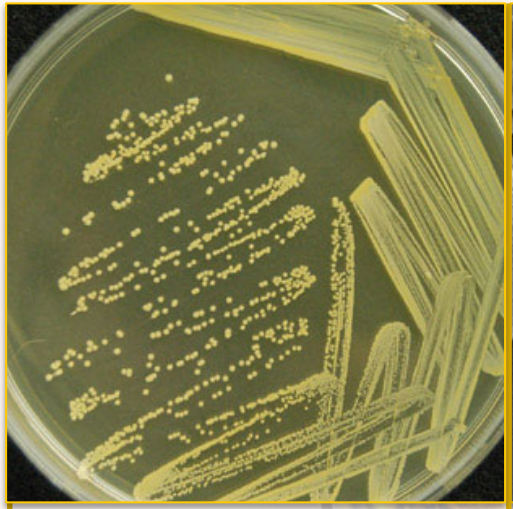
High sugar content

Low water content

**Plant-derived compounds**

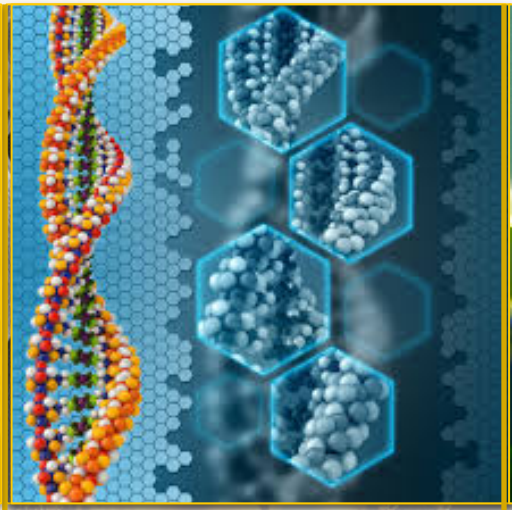
To identify plant-derived antimicrobial compounds from Welsh honey against methicillin-resistant *Staphylococcus aureus* (MRSA)

# INVESTIGATING THE COMPOSITION OF HONEY



**Antibacterial analysis**

Screen honey samples against MRSA by successive neutralisation



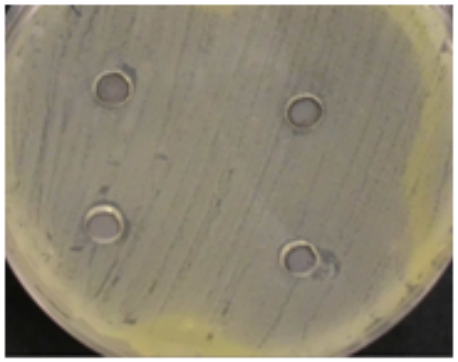
**DNA barcoding**

Use r*bcL* DNA marker identify the botanical species present in honey

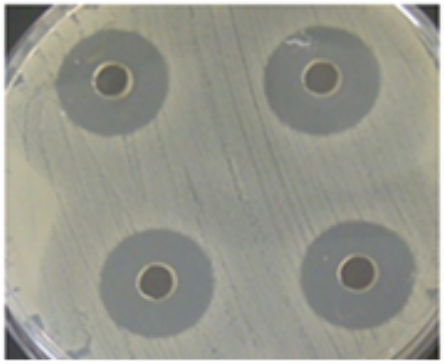

**Chemical analysis**

Extract and identify minor chemical components

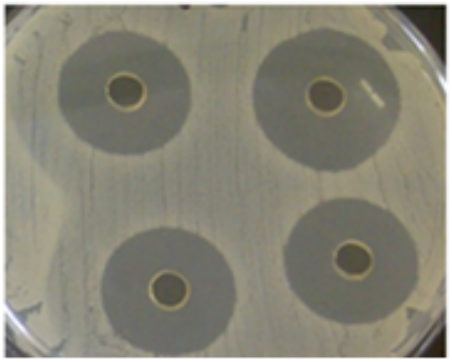
# ANTIBACTERIAL ANALYSIS



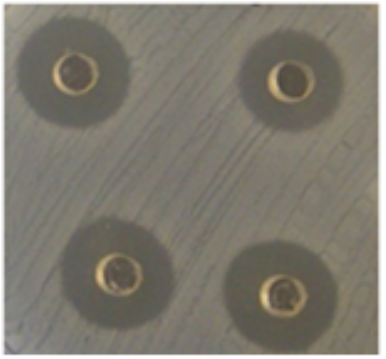
Sugar control



Manuka +10



Tywyn - raw



Tywyn - all known antibacterial factors removed

- 300 honey samples tested from hives located across the UK
- 88% of unprocessed honeys showed some level of inhibitory activity
- Activity was still seen following the removal of other known antibacterial factors from two samples from Tywyn and Bournemouth showed high levels of non-peroxide antimicrobial activity
- Suggests the activity is due to plant derived phytochemicals

# DNA ANALYSIS

*rbcL* DNA marker was used to act as unique identifier for plant species

DNA sequences were compared to Genbank which includes the Barcode Wales reference sequences for identification

**DNA extraction** → **Amplification** → **Tag annealing** → **NGS Sequencing** → **Analysis**

Qiagen kits, modified for honey samples

Core DNA barcode *rbcL*

Unique identification DNA tags adapted

Roche 454 and Illumina

Plant identification (BLAST)

de Vere N, Rich TCG, Ford CR, Trinder SA, Long C, Moore CW, et al. DNA barcoding the native flowering plants and conifers of Wales. PLoS ONE. 2012;7(6):e37945

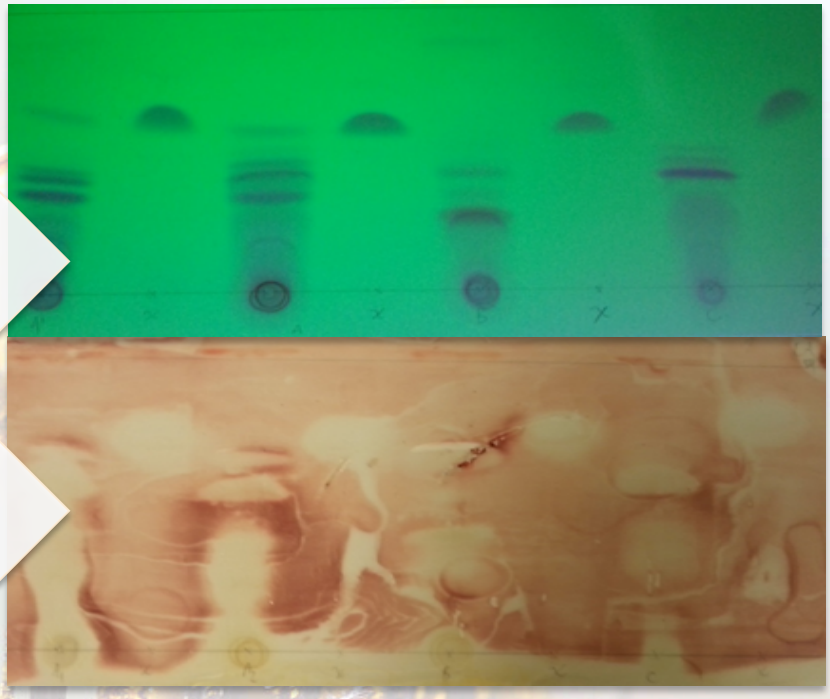
# SEQUENCING RESULTS

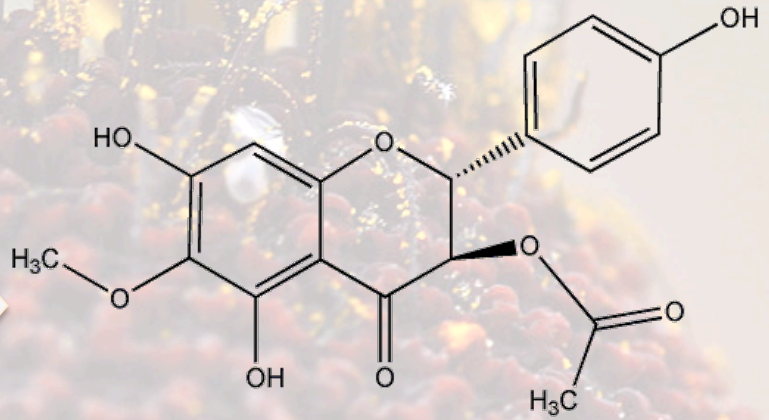
# FLORAL COMPOSITION OF TYWYN HONEY

- Galium odoratum
- Trifolium
- Hyacinthoides non-scripta
- Quercus
- Rubus
- Taraxacum
- Trifolium repens
- Brassicaceae
- Rosaceae
- Muscari

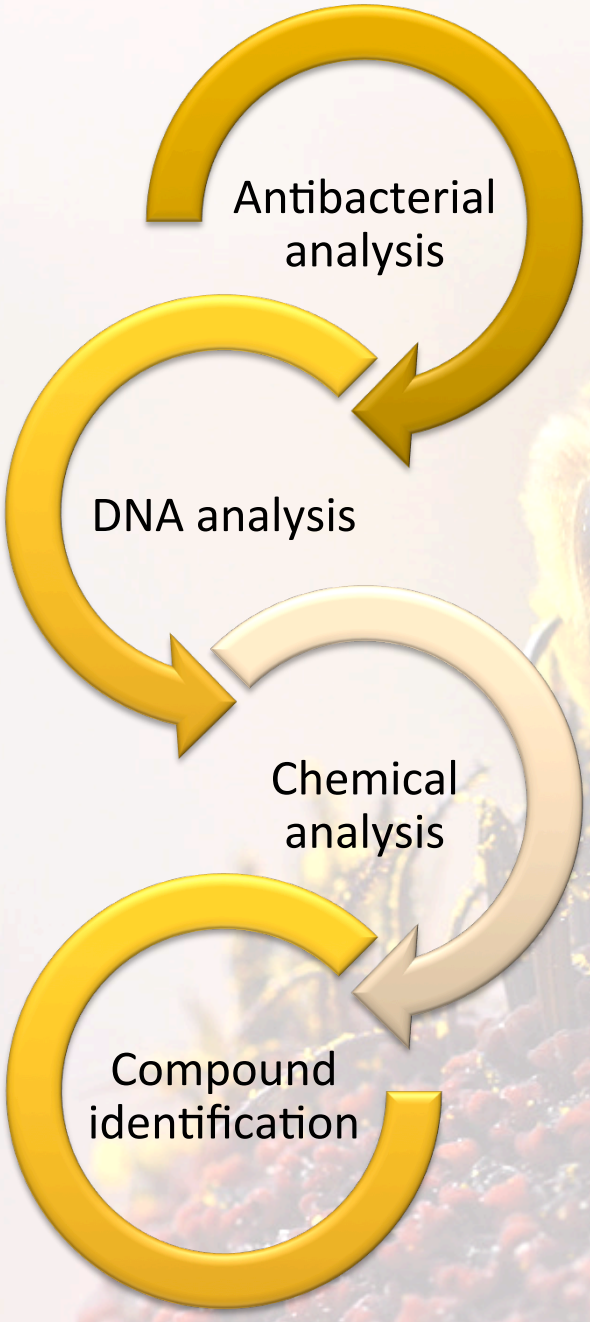
# CHEMICAL ANALYSIS

| | |
|---|---|
| Organic extractions | Hexane<br>Ethyl acetate<br>Methanol |
| Separation | Sephadex<br>TLC<br>Amberlite |
| Antibacterial activity | Bioautographic overlay assay |
| Isolation | Remove individual compounds |
| Identification | Mass Spectrometry<br>NMR<br>HPLC |






Pinobanksin-3-O-propionate




# CONCLUSIONS

- Compounds from honey show good levels of antibacterial activity against hospital associated pathogens
- DNA metabarcoding was successfully used to characterise the plants which contribute to the making of the active honey samples
- Compounds identified using these approaches included pinobanksin derivatives and unknown compounds suggesting that the plants may be the original source of bioactive compounds
- Compounds may provide new leads that could serve as selective agents against antibiotic resistant bacteria

Travel Award

6th International Barcode of Life Conference

Barcodes to Biomes

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**THANK YOU FOR LISTENING!**