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Feasibility study on squill production in north Wales

Final Report

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Cronfa Amaethyddol Ewrop ar gyfer Datblygu Gwledig:
Ewrop yn Buddsoddi mewn Ardaloedd Gwledig
European Agricultural Fund for Rural Development:
Europe Investing in Rural Areas

Llywodraeth Cymru
Welsh Government

Contents

1. EXECUTIVE SUMMARY

- Squill is a plant native to the Mediterranean and squill extracts have been used for many years as herbal medicine

- Squill essence is included in many 'Over The Counter' cough preparations (e.g. Covonia and Tixylix)

- Supplies of squill are under pressure given the political turmoil in the traditional production areas

- Bangor University have identified that squill grown in north Wales has higher levels of the main bioactive than reported for squill grown in native countries

- This therefore presents a potential market opportunity for Welsh farmers to grow squill (on marginal utility land) to meet market gap caused by constrained supply

- This project is a very early stage review aimed at evaluating the technological and commercial potential of this market opportunity

- The project demonstrates that :
    - Squill can grow at a number of locations across north Wales
    - It does contain a number of high value compounds
    - There is existing technology that can be adapted to plant and process squill at commercial scale
    - There are potential high value/low volume products in both animal and human health, but further work would be required to develop these
    - There is interest in squill as a ruminant feed additive and this would be a quick route to a lower value/high volume market

- However the project also demonstrates that :
    - Availability of seed bulbs will be an issue for any commercial exploitation
    - Additional information is required on the biology of the squill plant to be able to design an optimal growing/production methodology

2. SQUILL TECHNICAL & COMMERCIAL FEASIBILITY STUDY

2.1. Objective

This project is based on the discovery that squill grown in Bangor had higher levels of high value compounds than reported in traditional growing areas.

The aim of the project is to determine if this discovery presents an exploitable commercial diversification opportunity for farmers in north Wales.

The intention is to achieve this by establishing trial plots growing squill on a small number of diverse farms in north Wales.

The first objective for the project is to identify the technical issues associated with this concept, including :

- establish the demands of the plant including agronomy
- harvesting
- extraction techniques and their costs

The second objective is to investigate the potential markets for the high value compounds produced by this plant when grown in north Wales and thus get an indication of the commercial potential, should the technical investigation be successful.

2.2. Project outline

This project addresses three main areas:

- **Growing**
  In this element we established a number trial plots of squill across north Wales

- **Production**
  In this element we investigated a number of ways of processing squill with a view to identifying a scalable processing methodology for isolating the bioactive target compounds

- **Market Evaluation**
  In this element we reviewed available data on potential markets for the high value compounds produced by squill

3. TECHNOLOGICAL FEASIBILITY

3.1. Technical challenge

This project is based on the discovery that squill grown in Bangor had higher levels of high value compounds than reported in traditional growing areas.

The aim of the project is to determine if this discovery presents an exploitable commercial diversification opportunity for farmers in north Wales.

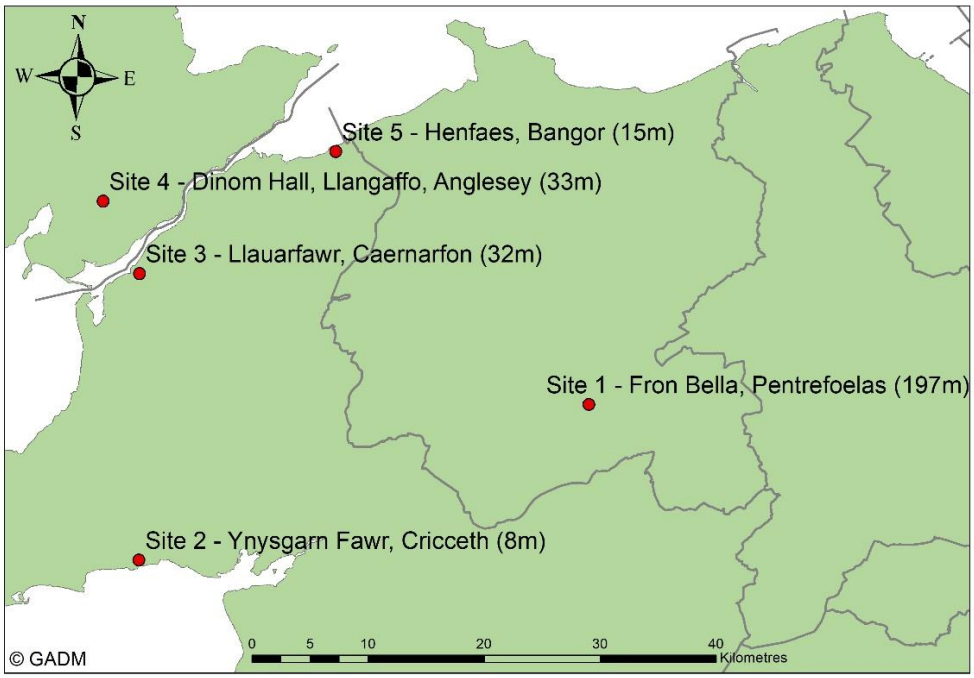
Therefore the technical issues that need to be explored are:

- Will it grow in various locations across north Wales?
- Will it have suitable levels of the high value compounds?
- Are we able to process the bulb biomass at a scale that would support commercial production?
- Are we able to isolate the high value compounds?

3.2. Will squill grow in north Wales ?

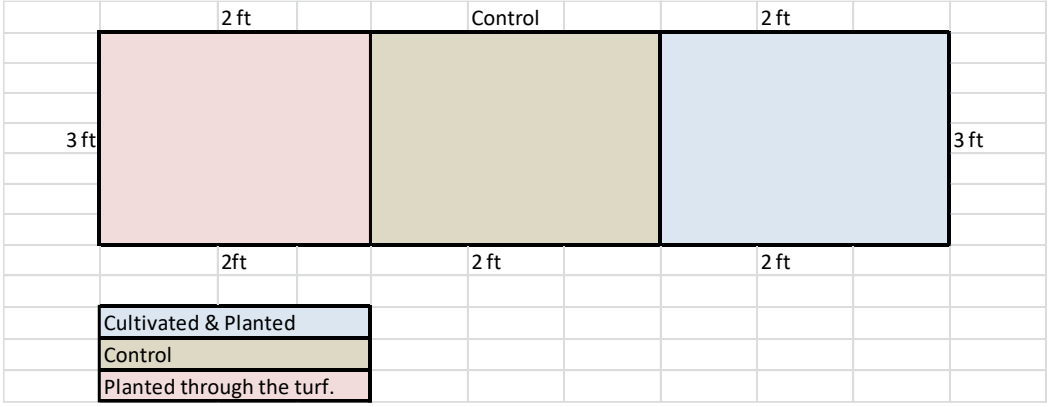
The original work carried out by Bangor University demonstrated that squill can be grown at Bangor, but it was important to demonstrate that plants would grow at a number of locations and that there was NOT something specific to the growing site in Bangor that produced anomalous results.

A number of trial sites were established across north Wales with varying differences such as rainfall, altitude and distance from the coast.



The Squill 'seed bulbs' are quite variable in size, but typically are reminiscent of a small swede:

These were planted by hand into small trial plots as per the trial design below:



These trial sites were monitored throughout the year.

Interestingly there were challenges from wild birds attacking the bulbs and one of the trial plots was irreparably damaged by cattle. In addition, some of the harvested bulbs showed evidence of slug attack.

The biomass planted and harvested from each site was:

Site No	Planted Kgs	Harvested Kgs	
Site 1	14	34	242.86%
Site 2	15	32	213.33%
Site 3	14	35	250.00%
Site 4	15	36	240.00%
Site 5	14	34	242.86%
Site 6	15	0	0.00%

Interestingly, a significant proportion of the squill bulbs reportedly did not produce foliage, which could have been caused by the environmental factors but also could be part of the plants' growth cycle. While this could have a significant impact on the agronomic and commercial potential for this plant, it also indicates that there is a significant gap in our knowledge of the plant that needs further research.

3.3. Will it have suitable levels of the high value compounds?

Two approaches to laboratory processing were explored:

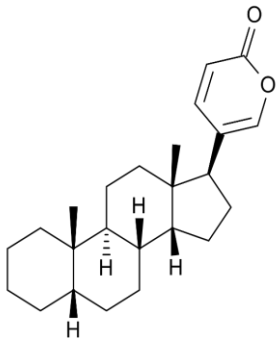
- Dry squill material and macerate
- Use fresh squill

The cross section of a squill bulb is akin to an onion and is made up of layers. These layers were separated and then sliced up individually. This method ensured that the sections of squill were small enough to dry adequately. Once cut, the samples were

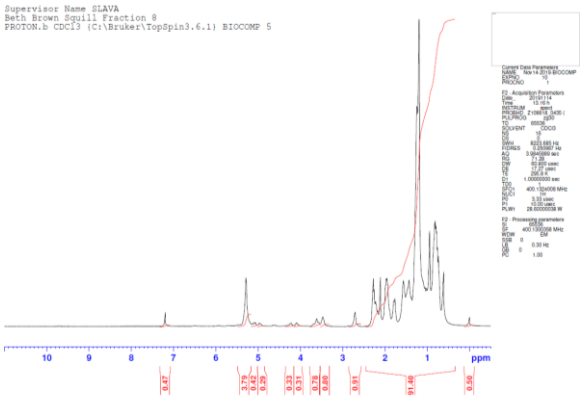
placed in the kiln and dried for 48-72 hours. Once prepared and dried, the samples were then placed into an industrial blender and milled to a powder.

Sample material was then submerged in solvent for several days, before the organic matter was filtered off and the solvent concentrated and analysed.

The samples were analysed by gas chromatography mass spectrometry (GC/MS) and nuclear magnetic resonance spectrometry (NMR):



Bufadienolide structure



As expected, all the trial samples were seen to contain Bufadienolides, fructans and phytosterols much in line with the literature with limited site to site variation.

The differences in biomass yields were seen to be more significant in terms of overall yield in particular the dry matter (DM) percentage of site 2.

Site	Altitude (m)	Squill Bulbs Extract (% DM)	Real yield (%)	Lipid (%)	Active Compounds (%)	Fructans (%)
1	**197**	**5.63**	**22.48**	**15**	**24**	**61**
2	**8**	**6.98**	**27.92**	**20**	**16**	**64**
3	**32**	**4.87**	**19.48**	**16**	**17**	**67**
4	**33**	**4.83**	**19.32**	**18**	**17**	**65**
5	**15**	**4.76**	**19.04**	**19**	**14**	**67**

The greater proportion of biomass vs water (in the total biomass weight) in the samples from site 2 then translated through to greater recoverable yield.

Site	Dried Squill	Real yield	Total extract	Lipid	Active Compounds	Fructans

	(kg)	(per DM %)	(kg)	(kg)	(kg)	(kg)
1	6.8	22.48	1.52	0.228	0.365	0.927
2	6.4	27.92	1.78	0.356	0.285	1.139
3	7	19.48	1.36	0.217	0.231	0.912
4	7.2	19.32	1.48	0.266	0.252	0.962
5	6.8	19.04	1.294	0.246	0.181	0.867

Without replicated trials at each site this can only be indicative rather than definitive and further work would be required to identify repeatable and reproduceable results identifying particular aspects of growing conditions that have a particular impact.

However, it does seem likely that the initial hypothesis that low altitude, free draining, sandy soils (akin to squill's natural growing environment) will facilitate growth, more interestingly there is no obvious indication from this early work to show that environmental factors have a significant impact on levels of high value compounds.

The before and after soil analysis (see Appendix) shows no significant changes to soil macro-nutrient balance as a result of growing the crop. As squill is a bulbaceous plant, it obviously carries with it a massive quantity of reserves necessary for growth and therefore, a multi-year trial would be necessary to examine the impact of environmental macro/micro nutrient factors on the squill itself.

3.4. Are we able to grow and process the bulb biomass at a sensible scale?

For the sake of expedience in this trial the squill "bulbs" were planted by hand. For more field scale production a degree of mechanisation would be required.

The following picture is of a daffodil bulb planter specifically designed for Welsh conditions:

While squill bulbs are significantly larger than daffodil bulbs, the design of this machine is such that it would be readily adapted to planting squill bulbs.

For ease of harvesting the bulbs at the end of the crop it would be preferable to plant the squill bulbs into a prepared seed bed rather than directly into pasture. Given that one of the trial plots was damaged by cattle, then a co-cropping (under pasture) approach is unlikely to work for this crop.

Importantly as the harvested material is the bulbs themselves, then it is effectively a destructive harvest – which puts a premium on the ability to source 'seed bulbs' for planting future crops.

The squill from the trials were harvested by hand, but given the size of the bulbs at harvest then a stone rake would seem to be a sensible approach to harvest mechanisation.

A mechanical macerator was used quite effectively to break up the squill bulbs into small pieces suitable for immersion in solvent for extraction of the high value components.

The organic matter needs to be filtered out of the solvent prior to onward processing. The following picture shows a mobile screw press unit that could be used to recover the remaining solvent from the filtered biomass and thus improve recovery yields.

4. COMMERCIALISATION

Squill is already used in the UK and Europe as an ingredient in various health related products.

4.1. What compounds does squill contain?

The main components of squill extract are Bufadienolides and flavonoids (10-12%) and polyfructan blend (75%-80%) with the remaining fraction made up of lipids.

4.2. What is the potential applications for these compounds?

The desk study highlighted that research has shown that Mediterranean squill contains a number of compounds that have either been shown to have bioactivity or are closely related to compounds from other sources that have been shown to have bio-activity, including, anti-bacterial (a candidate for tackling antibiotic microbial resistance); anti-cancer; diabetes and arthritis.

Anti-cancer: there is published research identifying compounds that have demonstrated to have specific anti breast cancer activity. The structure of these compounds is very similar to squill bufadienolides.

Pre-biotics/probiotics/silage additive: the anti-bacterial properties of squill compounds could have positive benefits in bacteria nutrition either as prebiotics/probiotics acting on the rumen microbiome, or as possible grass additive to improve lactobacilli fermentation within silage.

There is ethnobotany evidence that squill and related plants have been used to improve efficiency in animal nutritional and the fact that we saw cattle damage to one of our trial plots indicates that it is not unpalatable.

Animal health – pulmonary diseases: a bufadeniloide compound extracted from a related plant (Helleborus) which is very similar to squill bufadeniolides has an established use in several EU countries to control pulmonary health problems in cattle and pigs.

There is also a product incorporating a squill extract sold in Australia to address pulmonary issues with horses.

https://www.amacron.com.au/products/exocough-red-25l

The combination of anti-bacterial and pulmonary activity of squill could make it a particularly valuable treatment for pasturella, which is particularly problematic in the Welsh sheep flock.

4.3. Regulatory constraints

Squill extracts have been used for more than 10 years in parts of the EU as phytotherapy and/or homeopathy and there is an existing pharmacopeial standard for the German homeopathy product and a range of information already in the public domain.

Typical estimates of cost for registration with the European Medicines Agency (EMA) as a new human drug is £500k for registration of the compound and £500k for registration of the formulation.

The public domain prior art should facilitate product registration and thus reduce the time/cost required to register new products incorporating Squill and its possible that as little as £100k would be required for a characterisation, toxicity and eco-toxicity studies.

There will be a level of traceability and quality control required around the growing and processing of squill used for health purposes.

The level of pharmaceutical regulation (GMP = Good Manufacturing Practice) around squill products will depend on whether powdered whole squill is used as the final product or whether a bio-active component is extracted from the squill for incorporation into a final formulated product. The greyed areas in the following table indicate when GMP regulations start to apply to human medicines.

Type of Manufacturing	Application of this guide to steps (shown in grey) used in this type of manufacturing				
Chemical Manufacturing	Production of the API starting material	Introduction of the API starting material into process	Production of intermediates	Isolation and purification	Physical processing and packaging
API derivedfrom animal sources	Collection of plant	Cutting and initial extraction	Introduction of the API starting material into the process	Isolation and purification	Physical processing and packaging
API extracted from plant sources	Collection of plants and/or cultivation and harvesting	Cutting/comminuting	Introduction of the API starting material into the process	Isolation and purification	Physical processing and packaging
Herbal extracts used as API	Collection of plants	Cutting and initial extraction		Further extraction	Physical processing and packaging
API consisting of comminuted or powdered herbs	Collection of plants and/or cultivation and harvesting	Cutting/comminuting			Physical processing and packaging
Biotechnology: fermentation/cell culture	Establishment of master cell bank and working cell bank	Maintenance of working cell bank	Cell culture and/or fermentation	Isolation and purification	Physical processing and packaging
"Classical" fermentation to produce an API	Establishment of cell bank	Maintenance of working cell bank	Introduction of the cells into fermentation	Isolation and purification	Physical processing and packaging

4.1. Potential customer interest

As part of this project we have established contact with an Irish animal feed formulation company who are interested in incorporating dried/powdered squill into their ruminant feed formulations. This would provide a quick route to market, but at a lower value than more medical applications.

Exploratory discussions are ongoing about volumes and prices that they may be interested in.

4.2. Competition

Squill products already on the market come from collecting wild plants either in Libya or India. These are areas with considerably lower costs of production than in Wales, however, as this is a destructive harvest approach the scalability and sustainability of squill production in this way is questionable.

5. APPENDIX 1 : POSSIBLE DISEASE INDICATIONS OF SQUILL COMPOUNDS

Diseases	Causes (Bacteria or Parasite)	Effects and Signs	Activity of bufadienolides or related molecules	Existing products
Abortion-Chlamydial	Chlamydial (*Chlamydophila abortus*)	accounting for about half of all infectious abortions. ---------- Pregnant goats or sheep infected with the bacteria will have late term abortions, stillbirths, or early delivery of weak lambs	Cunha Filho GA, Schwartz CA, Resck IS, Murta MM, Lemos SS, Castro MS, Kyaw C, Pires OR Jr, Leite JR, Bloch C Jr, Schwartz EF. Antimicrobial activity of the bufadienolides marinobufagin and telocinobufagin isolated as major components from skin secretion of the toad Bufo rubescens. Toxicon. 2005 May;45(6):777-82. Yeo, Eun-Ju; Kim, Kee-Tae; Han, Ye Sun; Nah, Seung-Yeol; Paik, Hyun-Dong Antimicrobial, anti-inflammatory, and anti-oxidative activities of Scilla scilloides (Lindl.) Druce root extract Food Science and Biotechnology (2006), 15(4), 639-642.	
Abortion – Toxoplasmosis	*Toxoplasma gondii*	The second most common cause of abortion in animal Infection later in pregnancy may result in abortion, still births and weakly lambs, often accompanied by a mummified foetus	Related with leishmaniasis Tempone AG, Pimenta DC, Lebrun I, Sartorelli P, Taniwaki NN, de Andrade HF Jr, Antoniazzi MM, Jared C. Toxicon. 2008 Jul;52(1):13-21. Antileishmanial and antitrypanosomal activity of bufadienolides isolated from the toad Rhinella jimi parotoid macrogland secretion.	
Clostridial Disease	Clostridia bacteria (anaerobic, spore forming)	The bacteria produce spores that can survive in the environment for a very long time, and it is fatal diseases that strike cattle and sheep suddenly, often causing death before any clinical signs are seen. It might appear as lamb dysentery, tetanus, pulpy kidney, black disease, blackleg, struck and braxy	Fructans reduce clostridium	Vaccines
Coccidiosis	*Eimeria ovinoidalis* & *Eimeria crandallis*. Are the main two types in UK	A parasitic disease of the intestinal tract of animals caused by coccidian protozoa The common signs of coccidiosis are a rapid loss of weight and diarrhoea containing mucus and flecks of blood, causing staining of the perineum and tail.		
Johnes Disease (Paratuberculosis)	Mycobacterium avium subspecies paratuberculosis	a chronic enteritis of adult cattle and sheep diarrhoea, poor milk yield and weight loss in cattle three to five years-old with onset often following calving		

Pasteurellosis	*Pasteurella* genus bacteria	It is one of the most common causes of mortality in all ages of sheep. _____ Septicaemia- Pneumonia and further complications	Antibacterial activity of Helleborus bocconei Ten. subsp. siculus root extracts. Puglisi S, Speciale A, Acquaviva R, Ferlito G, Ragusa S, De Pasquale R, Iauk L. J Ethnopharmacol. 2009 Aug 17;125(1):175-7. doi: 10.1016/j.jep.2009.06.011.	Antibiotic penicillin
Endoparasites (internal worms	Includes all internal parasites	Endoparasites are parasites that live in the internal organs of an animal	M.N. Shiva Kameshwari Chemical constituents of wild onion Urginea indica Kunth Liliaceae Int. J. of Pharm. & Life Sci. (IJPLS), Vol. 4, Issue 2: February: 2013, 2414-2420 2414.	
Liver Fluke	Parasitic trematodes (phylum Platyhelminthes)	Chronic parasitic disease of the bile ducts. Infection with this parasite occurs through eating fluke-infested, fresh-water raw or undercooked fish.		
Ectoparasites	fleas and other skin parasites	Permanent ectoparasites include scab mites, chewing lice and less common ones such as ear mites, mange mites, sucking lice and keds. Semi-permanent ectoparasites include blowfly strike, ticks and less common nasal bot flies and head flies ----------	Abdolhosseini et al. Evaluation of Treatment with Squill Vinegar in 10 Patients with Head Pediculosis Asian J Clin Case Rep Trad Alt Med. Jan-Mar 2017; 1(1): 57-64	ivermectin and clorsulon

6. APPENDIX 2 : BEFORE AND AFTER SOIL ANALYSIS OF TRIAL PLOTS

Squill Project

Soil Analysis Pre Planting and Post Harvest.

20/12/19

The analysis reports the soils to be loams of either silts or sandy loams.

Web searches state that light sandy/medium loams of free draining gritty nature are the most suitable for squill and that the plant is not sensitive to pH variability. As they are bulbs they have only a small requirement for nutrients P and K and this could easily be supplied by low applications of FYM in early spring. Reports state that they prefer dry, sunny aspects and free draining conditions. The data below will be interesting to see if the more difficult conditions impact on the chemical concentration of the plant. The plant needs bees to pollinate to flower.

Field Name	Inputs	Crop	Soil Analysis Results			
			pH	Phosphate	Potash	Magnesium
Site 1		Planting	5.3	2	2-	3
Site 1		Harvest	5.6	2	2-	3
Site 2		Planting	5.0	6	2-	2
Site 2		Harvest	5.1	6	2-	3
Site 3		Planting	5.7	6	2-	3
Site 3		Harvest	5.7	6	2-	3
Site 4		Planting	5.5	3	3	3
Site 4		Harvest	5.1	3	2-	2
Site 5		Planting	5.2	3	2-	2
Site 5		Harvest	5.6	3	1	2

The traffic light system in the table identifies that under normal farming and environmental practices the pH is significantly low, that phosphate levels on two sites are alarmingly high and that potassium and magnesium levels are near to perfect.

It would be interesting to compare site 2 and 3 against each other as pH is quite different but P levels both high. Then the other sites against them with similar pH but lower P levels.