Public summaries from completed BIV Projects – updated 26th June 2017

Unique Project ID
HVCFP-BIV-JUL14-001
Project title
Assessment and Optimization of Microwave Assisted Extraction (MAE) of Galanthamine and other Alkaloids from Daffodils for the UK Pharmaceutical Industry
Partners
Xianmin Chang, Royal Agricultural University & Kevin Stephens, Agroceutical Products Ltd
Summary of Public Project outcomes
The UK is the world's largest producer of daffodils and narcissi cut flowers, worth c. £23 million annually to the economy. More importantly, these plants also contain over 300 alkaloids, one of which is galanthamine which has been approved since 2001 for the treatment of early stage and moderate Alzheimer's disease, an age related dementia with an estimated 800,000 cases in the UK, rising to over one million in 2021. Galanthamine is often prescribed rather late in the progress of the disease due to its expense and shortage of supplies, adding to the burden on family carers and the NHS. Chemical synthesis is possible, but difficult and expensive. Therefore, isolation from daffodil biomass gives a new industrial biotechnology use for a non-food crop. Daffodils also contain other alkaloids which have potential pharmacological activities, such as, narciclasine and haemanthamine, with demonstrated anti-cancer, anti-leukemia or anti-inflammatory properties. Although there are over 30,000 varieties of daffodils worldwide only a few have identified which contain a significant level of galanthamine. The search for new varieties that can be used for galanthamine production is important both commercially and to Alzheimer's sufferers across the world. However, it is vital if we can significantly enhance the alkaloids extraction efficiency. The aim of this project was to assess and optimize Microwave Assisted Extraction (MAE) of alkaloids from daffodils to enhance the efficiency of alkaloids extraction, and ultimately to secure the sustainable and scalable production of galanthamine and other alkaloids for the UK Pharmaceutical Industry. The result of this project suggested that one minute low power level (136W) Microwave Assisted Extraction (MAE) significantly enhanced the efficiency of galanthmine and narciclasine extraction from daffodil fresh materials, and the MAE will also save the time of extraction. This result will encourage the commercial company to develop MAE instrumentation specifically for daffodil alkaloid extraction and the production line using fresh materials.

Unique Project ID
HVCFP-BIV-JUL14-002
Project title
Discovery of Novel Plant-Derived Agrochemicals
Partners
Ray Marriott, Bangor University & Apostolos Papadopoulos, Crop Intellect Ltd
Summary of Public Project outcomes
Crop Intellect ltd and Bangor University have worked collaboratively using an Innovation Voucher provided by the BBSRC sponsored group "high value chemicals from plants". The aim of the project was to identify novel plant-derived agrochemicals. Selected plant materials were used to be fractionated and tested for their efficacy for plant enhancement and protection effects. Bangor University produced several functional groups of chemicals and there were tested for their effects on model plants. The project resulted in identifying a fraction where the activity is isolated. Analytical work was also performed on the fraction to identify the presence of molecules that are responsible for the observed benefits. Further work will be performed to investigate synergies and aim at identifying the molecules responsible for the activity.

Unique Project ID
HVCFP-BIV-JUL14-003
Project title

Road Map for the use of *Sargassum muticum* in high value Bioactive Compound Discovery
Partners
Birthe Nielsen, University of Greenwich & David Bailey, IOTA Pharamaceuticals Ltd
Summary of Public Project outcomes
In spite of several eradication attempts, Japanese wireweed, S. muticum, has now become a permanent member of the European coastline's aquatic flora. At present, this macroalgae has no real economic value, and is therefore not harvested for commercial exploitation. Due to high heavy metal uptake, ash and water content, it is unlikely that this seaweed would be useful as a direct source of food or fuel. However, S. muticum grown under aquaculture conditions could produce a food supplement with health benefits due to naturally high levels of certain antioxidants (tocopherol, fucoxanthin, and other carotenoids and phenolic compounds). The precise metabolic pathways used by S. muticum for the production of these compounds are not clear and further research is required to determine both the spectrum of this alga's bioactive compounds and the preventative and therapeutic opportunities that they represent, although specific anti-inflammatory, anti-obesity, anti-tumour and antioxidant activities have been reported from S. muticum. In particular, fucoxanthin appears to have a range of therapeutic effects. The extraction of fucoxanthin and related metabolites from S. muticum as part of a biorefinery approach to exploit this invasive seaweed may merit further research. The most promising, commercially viable use of S. muticum thus appears to be to turn this 'menace' into medicines.

Unique Project ID
HVCFP-BIV-OCT14-004
Project title
Identifying high value bioactive complex carbohydrates/polysaccharides from a high exopolysaccharide (EPS) forming strain of microalgae
Partners
Paul Knox, University of Leeds & John Dodd, AlgaeCytes Ltd.
Summary of Public Project outcomes
A strain of cultured microalgae secreting large amounts of a soluble polysaccharide (an exo-polysaccharide or EPS) into the culture media has been analysed using large sets of molecular probes / antibodies. Two major features, characteristic of large arabinogalactan-proteins in land plants have been identified to link to two separate components of the microalgae EPS. These molecular markers for microalgae EPS will be useful to further define the structures and bioactivities of microalgae EPS.

Unique Project ID
HVCFP-BIV-OCT14-005
Project title
Pinitol isolation from Senna seeds
Partners
Ana Winters, Aberystwyth University & Roger Jones, Phytovation Ltd
Summary of Public Project outcomes
D-Pinitol is a known insulin mimicker and as a result there is a growing interest in its application as a food supplement because of its reported efficacy in lowering blood glucose levels. This compound has been identified in senna seeds, a waste product generated in the production of a laxative product from senna pods. This project investigated the potential of the liquid-liquid separation technique, high performance counter current chromatography (HPCCC) to isolate pinitol. This study showed that because of its highly polar nature, this method is not suited to isolation of pinitol. However results showed that phase separation with a range of solvent systems is potentially an effective method for separating pinitol from less polar metabolites. This indicates that HPCCC has potential for isolating other less polar metabolites in senna seeds which may have a commercial value. Use of ion exchange resins (including a cost effective resin which is used in the food industry) proved effective for

purifying pinitol. Treatment with a commercial cellulose preparation broke down gel forming polysaccharides, improving the flowability of the solution and thereby facilitating pinitol extraction. A concentration of 50% ethanol was demonstrated to be most effective for extracting pinitol from senna seeds.

In summary, this study has identified factors which improved extraction of pinitol from senna seed and can be applied in the development of a cost-effective extraction and isolation process. HPCCC may have a role in a process involving co-extraction of pinitol and other valuable metabolites with a lower polarity from senna seed.

Unique Project ID
HVCFP-BIV-OCT14-006
Project title
Pyrethrum liquid extraction (liquid-liquid fractionation of components of a naturally occurring insecticide)
Partners
Ana Winters, Aberystwyth University & Bryan Shand, Agropharm Ltd
Summary of Public Project outcomes
The project has provided evidence of the potential for HPCCC to rapidly and simultaneously separate a range of closely related natural plant insecticidal actives at preparative scale. There are a range of biological activities of the isolated pyrethrins that could be of use in human/animal medicines and this technology can provide a cost effective mechanism for isolating these natural pesticides for further testing and analysis.

Unique Project ID
HVCFP-BIV-JAN15-007
Project title
Characterisation of the bioactives from Mugwort roots active against the oomycete pathogen of carrot and parsnips, *Pithium* spp.
Partners
Cathie Martin, John Innes Centre & Howard Hinds, Root Crop Consultancy Ltd.
Summary of Public Project outcomes
This project was founded on an observation by a Yorkshire farmer, MH Poskitts and the Agronomy consultant Howard Hines, growing parsnips and carrots, that the incidence of cavity spot in both crops was reduced significantly in fields infected with the invasive weed, Mugwort (Artemesia vulgaris). Cavity spot is caused by the oomycete pathogen, Pythium. Several species of Pythium may be involved but the most common species infecting carrots and parsnips is Pythium violae. Cavity spot causes an estimated £12 million of damage every year in UK root crops, through the formation of black unsightly cavities which make the vegetables unsellable. Roots are susceptible to infection throughout the growing season and also in storage. Infection may be facilitated by nematode damage allowing easier penetration of the root tissues by the pathogen. The current treatment for cavity spot is Metalaxyl-M applied as a drench shortly after planting. The fungicide has suffered severe resistance problems particularly in use against Phytophthora infestans. This work which was funded by the HVCfP voucher developed techniques to test the fungicidal and nematicidal activity of extracts from the leaves and roots of Mugwort. We identified that extracts from the leaves of mugwort plants are only able to suppress the growth of a specific species of Phythium, P. attrantheridium which is a major cause of cavity spot disease. Root extracts do not suppress Phythium, but do kill free-living nematodes, that are a major cause of root deformation “fanging” in carrot and parsnip. Culture systems based on root tissue have been established as a step towards largescale production. Future work

will identify the compounds that deliver the fungicial and nematicidal activity and develop methods for the production of the compounds found in the leaf extracts.

Unique Project ID
HVCFP-BIV-APR15-008
Project title
Purification of high value molecules using vibrational membrane filtration
Partners
Ray Marriott, Bangor University & Carolyn Carver, Blue Sky Botanics Ltd
Summary of Public Project outcomes
Extraction of high value labile molecules from plants using water or aqueous solvents is encouraged from a green chemistry perspective but presents unique difficulties for individual molecule separation. In this Project we investigated recent innovations in membrane filtration in which a high frequency vibration is applied across the membrane as a potential solution to this problem, to allow the separation of labile molecules without the application of heat or the use of high cost chromatographic methods. This project has demonstrated that vibrational membrane filtration can be used to fractionate and concentrate complex aqueous solutions of botanical extracts. However the method cannot solely rely on the quoted molecular weight cut off for the membranes selected but need to be determined by practical trials with model solutions of known composition. Once the parameters have been established this is a robust and scalable technology.

Unique Project ID
HVCFP-BIV-APR15-011
Project title
Screening microalgae species for waste water phosphate removal/recovery and high value chemicals production
Partners
Christopher Ennis, Teeside University & Pattanathu Rahman, TeeGene Biotech Ltd
Summary of Public Project outcomes
Microalgae are a promising source of biofuel and high value chemicals and nutraceuticals. However, there are major technological challenges limiting the extent to which microalgae can be used in this way. This project explored suitable algal feedstock for input to a novel integrated biorefinery capable of overcoming these challenges and of cleaning waste water, recovering essential nutrients, and providing high value algal-derived chemicals and materials. This Project has established the viability in principle of an integrated biorefinery based upon a hydrothermal enabling technology. This concept is focussed on deployment of the integrated biorefinery in the context of waste water treatment and the project has established that there is technical viability for the recovery of phosphorus from waste water and the production of lipids that are of value to the biofuels and nutraceutical industries. Further value addition potential lies in the fuel value of solid coproducts from the central hydrothermal technology.

Unique Project ID
HVCFP-BIV-APR15-010
Project title
Establishing genome resources for improving the production of high value chemicals from simple plant systems
Partners
Anil Day, University of Manchester & John Dodd, Algaecytes Ltd
Summary of Public Project outcomes

Microalgae are a sustainable source of high value natural products requiring simple inputs of light, water and low levels of nutrients. Production is precisely controlled by growing these simple plants in advanced photobioreactors. The manufacturing process greatly reduces the energy costs and pollution associated with conventional chemical synthesis. AlgaeCytes' Omega 3 algal EPA and Omega 6 ARA oils are high value natural products used in the personal cosmetic and health care markets for clinical applications in cardiovascular and cognitive health. Increasing demand particularly for Omega 3 (EPA – Eicosapentaenoic acid) by an ageing population is unlikely to met by traditional methods involving extraction from the diminishing stocks of oily fish. Fish get the source of Omega 3/6 fatty acids by eating algae in the oceans but the sustainability of the harvesting of high Omega containg fish is now in doubt. Understanding the molecular processes that are required for production of EPA by algae will allow better control of the process and provides opportunities to improve the product quality and yield to serve the part of market need in the future and is a vegetarian source.

The project isolated algal cultures that were suitable for molecular analysis and metabolic enhancement. This required the formulation of complex media that sustained growth. Analysis of the growth of microalgae in five different media identified specific compounds that stimulated an increase in algal cell density. These growth promoting compounds will allow faster production of biomass when required. The activity of genes is reflected in their products. A library of these gene products was made using a technique called RNA-seq. The library contains the information required to identify and monitor the activities of genes required for EPA production in the microalgae studied. The project outputs, which include a gene library and clones of microalgae, provide the resources needed to guide improvement of high value products in these simple plant cells.

Unique Project ID
HVCFP-BIV-JUL15-012
Project title
Natural UK-absorbing compounds from Living stones – evaluation of bioactivity and commercialisation for the cosmetic market
Partners
Alison Smith, University of Cambridge & Franck Michoux, Evonik Nutrition & Care GmbH
Summary of Public Project outcomes
"Living stones" (Lithops spp.) are unique southern African plants that live partly underground in extremely hot and dry conditions. This underground life makes it difficult to get enough sunlight to photosynthesise while still conserving as much water as possible. Lithops has many adaptations to help it do just this, including a top surface with "windows" that allows light to penetrate to photosynthetic tissues deep within the subterranean leaf. The mottled window of the plant's modified leaf look solid but are actually translucent if you cut off the tops and hold them up to the light. Cleverly, these translucent windows have sunscreen properties to block out harmful UV light that these desert plants would be exposed to. The bio-cosmetic industry are interested in discovering new natural ingredients for products such as sun-screens. The UV-blocking chemicals in these Lithops have the potential to act as a natural sun screen ingredient. This exciting new collaboration between the University of Cambridge and Evonik Nutrition & Care GmbH assessed whether these chemicals have the correct properties, novelty and the ability to be mass produced to meet the demands of the cosmetic industry. We were able to extract, separate and detect a large number of compounds from the semi-translucent (mainly pink and red pigmentation) upper leaf window of 19 Lithops species that have UV absorbing properties that may be of interest to the biocosmetic industry. Generally, sunscreens need to absorb UV-A (320-400nm, accounts for 95% of solar radiation and penetrates deep into the skin), UV-B (290-320nm, accounts for 5% of solar radiation and can cause direct DNA damage) and UV-C (290-100nm, mainly filtered out by the ozone layer and unlikely to cause skin damage). The detected compounds all strongly absorb in these UV

regions. Evonik Nutrition & Care GmbH were able to successfully establish several cultures of Lithops tissue.

Unique Project ID
HVCFP-BIV-APR15-009
Project title
Extraction of a novel chelate from native UK plant species
Partners
Vesna Nadjanovic, Lancaster University & David Marks, Levity CropScience Ltd
Summary of Public Project outcomes
Iron fertilisation is a major input in global agriculture, particularly in regions where soils are calcareous. In these regions iron becomes locked up in soil, and the use of chemical chelating compounds such as EDDHA is widespread. EDDHA is associated with environmental pollution (freeing up of heavy metals), and has agronomic issues. Novel alternative to synthetic chelates extracted from native UK plant species was investigated in this project, aiming to develop a new product from renewable resources for sale to farmers via distributors. Levity Cropscience and Lancaster University have worked together using an Innovation Voucher provided by the BBSRC sponsored group “High Value Chemicals from Plants”. Addition of extracts to cheap but otherwise inefficient iron sources increased dramatically the fertilizer efficiency on cucumber plants. These results will pave the way to avoid the widespread reliance on synthetic EDDHA-Fe chelates which is expensive and associated with negative environmental impacts.

Unique Project ID
HVCFP-BIV-OCT15-013
Project title
Bio-derived compounds from levoglucosenone – 3 steps from sawdust to solvents
Partners
James Clark, University of York & Tony Duncan, Circa Sustainable Chemicals Ltd
Summary of Public Project outcomes
Cellulose is the most abundant renewable feedstock on the planet. The most easily accessed high volume source of cellulose is from the lumber industry, with sawdust an underutilised by-product. Sawdust is the ideal feedstock currently employed in Circa’s pilot plant for the simple and inexpensive one step production of levoglucosenone, a reduced sugar with interesting functionality. The current petroleum route to this molecule is complex and expensive and it has received little attention other than in niche applications. The new bio-based version has the potential to open up a new field of chemistry. In this project, levoglucosenone has been reacted with other readily available bio-derived platform molecules to produce a novel family of bio-based compounds with distinct functionalities. Their simple synthesis makes them potentially very interesting for a range of important applications. Further reactions have proved their suitability as monomers for the formation of bio-plastics, as polar constituents in bio-surfactants and as formulation modifiers. These new applications will be further perused by both Circa and The University of York, with the aim to find commercialisation opportunities.

Unique Project ID
HVCFP-BIV-JAN16-014
Project title
Novel tomato compounds with anti-ageing effects in a human skin bioassay using MALDI-IMS
Partners
Jelena Gavrilovic, University of East Anglia & Jonathan Clarke, Persephone Bio
Summary of Public Project outcomes

The cosmetics and skincare industry exploits plant natural products (Naturals) within a wide range of skin tonics, lotions and topical skin applications. Persephone Bio has developed a tomato based production system where, through metabolic engineering, bioactive compounds can be made in high concentrations. Compounds produced in this way require validation of their biological activity (mode of action). These bioassays have previously been undertaken using animal models, human cell cultures or using qualitative tests in human trials. In all methods, the true biological activity has been difficult to discern. The University of East Anglia has recently established a human skin bioassay platform which has the potential to overcome these limitations. In preliminary tests we have recently found that aqueous extracts of tomatoes change the activity of 86 genes (1.5-fold or above) in the human skin biopsy bio-assay. We compared extracts of tomatoes generated by Persephone Bio, with those of a control tomato using the human skin bio-assay platform and observed effects on markers for skin aging in response to sunlight. We developed the MALDI-IMS technique to study protein changes in skin layers and cell types. In the future this will provide data which will allow Persephone Bio to identify not only compounds that have an anti-ageing activity, but also which human skin cells are affected. This knowledge will direct the future development of new treatments/cosmetics from tomato and provide evidence about which natural products are most appropriate for the ongoing application of metabolic engineering in both proprietary and heritage tomato lines in the future.

Unique Project ID
HVCFP-BIV-APR16-015
Project title
Generation of novel aroma active compounds from waste plant material for use in the flavouring and fragrance industry
Partners
Ian Fisk, University of Nottingham & Arthur Kay, bio-bean Ltd
Summary of Public Project outcomes
Waste plant materials contain significant levels of chemical precursors that could be used to generate aroma active compounds. This project successfully formed a new collaboration between research and industry to quantify the precursors in a specific waste plant material and its potential to generate aroma active compounds for use in the Flavour and Fragrance Industries. The proposed approach is both novel and viable, as the flavourings and fragrances that will be formed

Unique Project ID
HVCFP-BIV-JUL16-017
Project title
Discovery of a systemic bio-fungicide derived from the Asteraceae family
Partners
Peter Rose, University of Lincoln & Apostolos Papadopoulos, Crop Intellect Ltd
Summary of Public Project outcomes
We have recently identified a wild plant of the Asteraceae family, indigenous to the UK that was found to be a useful companion species when grown alongside cereals. Chemicals released by this companion plant appear to suppress the growth of several common fungal species on cereal crops grown nearby and reduce fungal disease severity. However, the active compounds present are potentially unknown. Some preliminary experiments have already been conducted which showed that extracts made from the companion plant are very effective at reducing fungal growth. In this collaborative study we aimed to identify the active compound(s) present in extracts, measure the activity on glasshouse plants and test for any unwanted toxic effects. A desk study took place to consider alternative ways to produce the active compound as commercial-scale production from the identified companion plant is unlikely to be feasible. Options may include transfer of the compound to a bacterial production system or identification of an alternative, commercially viable plant source. The

aim is to secure all potential IP with further input on the research aiming at commercialising the actives.
The support from the BBSRC group HVCfP has been vital for the performance of this project at the highest of its potential and has strengthened significantly the collaboration with the University of Lincoln. Specifically the project is of high commercial priority for Crop Intellect Ltd and it is aimed at the development of IP. Crop Intellect Ltd is focused on researching for novel actives in plants for use in agriculture. The aim of this research was to get closer to identifying novel molecules from a plant extract of known activity to strengthen the potential for protection through IP. Crop Intellect Ltd has a track record of licensing IP to multinationals where they find the market place.

Unique Project ID
HVCFP-BIV-APR16-016
Project title
Improving the anti-malarial and anti-cancer properties of a natural product
Partners
Colin W. Wright, University of Bradford & Matthew Hodges, Oxford Biotrans Ltd
Summary of Public Project outcomes
Many natural plant materials possess properties valuable to human health. The species we wished to investigate is endemic to Guatemala, where it is a traditional remedy for malaria. This Project relied on the traditional knowledge of indigenous communities to direct modern research techniques; the Nagoya Protocol and associated EU regulations require that appropriate access and benefit sharing measures are formally put in place before any R&D with both the Guatemalan plant and associated traditional knowledge could begin. An extensive period of engagement with the Guatemalan authorities and other relevant local contacts began, but it was not possible to gain access to plant material for research use in the required timescales. Given the delays experienced, the decision was taken to terminate the Project due to inability to access material within the short time period of a BIV award. These experiences, although disappointing, are still extremely valuable at a time when researchers are just beginning to attempt to negotiate access to genetic resources under the terms of the Nagoya Protocol and will be of benefit to the wider research community.