Institution: De Montfort University

Unit of Assessment: 3

Title of case study: Novel DNA Authentication Methods for Stronger Regulation of the Global Herbal Medicines Industry to Improve Product Quality and Consumer Safety

Period when the underpinning research was undertaken: 2007–2018

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Prof. Adrian Slater	Professor of Biomolecular Technology;	1 January 1986–31 October 2016;
Dr Caroline Howard	Senior Research Fellow;	1 May 2013–25 January 2014
Dr Tiziana Sgamma	Senior Lecturer	5 May 2014–present

Period when the claimed impact occurred: January 2014–31 December 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

As the global herbal medicines market continues to grow at a rapid rate, the scarcity of quality herbal materials presents a security risk to supply chains and can lead to fatal outcomes. A substantial body of research at DMU has pioneered the development of novel DNA barcoding methods for the authentication of herbal medicines. These methods have led to more effective quality control protocols being implemented by the world's leading manufacturer of traditional herbal medicines in order to meet stricter EU regulatory requirements, giving the company 'a decisive advantage'. The techniques have since been made available for wider use across the industry and have shaped new, stronger regulatory and legislative standards to reduce dangerous contamination, protect consumers and increase product quality. Through direct collaborations with British and American regulatory agencies, these standards have significantly tightened the industry's quality control procedures, increasingly mandating the adoption of the sensitive DNA-based method as a validation requirement for compendial standards to ensure safe, high-quality medicines for human consumption.

2. Underpinning research

Up to 80% of the world's population use herbal medicines, according to the WHO 2011 report *Traditional Medicines: Global Situation, Issues and Challenges*. Usage is especially high in developing countries, but it has also been estimated that 70% of all medical doctors in France and Germany regularly prescribe herbal medicines. The WHO's *Global Report on Traditional and Complementary Medicine (T&CM) 2019* (pp.5) states that 'more and more countries are recognising the role of T&CM in their national health systems', with 124 countries having implemented regulations on herbal medicines. The WHO 2011 (pp.8) report describes the rapid growth of the global herbal medicines market, now thought to be worth in excess of USD100,000,000,000 and notes that 'most of the reported side effects associated with the use of traditional medicines are extrinsic to the product itself, arising instead from errors in plant identification, poor manufacturing practices and lack of product standardisation, contamination of products, substitution or incorrect preparations or dosage'. A 2019 study in *Frontiers in Pharmacology* found that 27% of 6,000 herbal products sold in 37 countries did not contain the ingredients specified on the label, quantifying the extent of the problem.

Since 2007, research within DMU's Biomolecular Technology Group (BTG) has applied the science of DNA barcoding to the development of novel, practical methods to monitor the quality of medicinal plant materials in supply chains, before adapting them to the needs of industry and regulators. The premise of using DNA barcoding to authenticate herbal medicines lay in the fact that DNA-based methods, unlike traditional botanical or chemical methods, would not be affected by the age of the plant material, growing conditions or the addition of dyes used to

deceive chemical tests. Using sequence data from the International Barcode of Life initiative, BTG researchers, led by Slater, designed PCR (polymerase chain reaction) primers specific to *Hypericum perforatum*, commonly known as St John's Wort, an over-the-counter (OTC) remedy often used to treat mild and moderate depression. They did this by identifying and targeting short 'microcode' sequences characteristic of the target species. These concepts were introduced in a 2009 paper that described a model of how barcode information can be used in the design of sequence-specific identification probes. This was the first publication to demonstrate the application of this concept to authenticate herbal medicines [R1].

The BTG then targeted the issue of contamination in herbal medicines, for which conventional DNA barcoding was ineffective. They developed an assay, PlantID, that could identify four closely related species within a mixture of seven species. This assay provided a means of detecting expected ingredients as well as adulterant materials in one reaction [R2]. To assess the sensitivity of the methods developed, the BTG successfully extracted very small DNA fragments from processed St John's Wort medicines and successfully identified the target species [R3].

As these highly sensitive detection methods were being developed, the BTG applied them to studies that directly targeted the detection of toxic species in widely used herbal products. This had become an issue of particular concern with reported cases of toxicity related to the contamination of some Asian herbal medicines with other highly toxic substituted plant species. For instance, several women had been hospitalised with nephrotoxicity after taking a slimming product thought to comprise of *Stephania tetrandra* (Chinese name: Han Fang Ji) but subsequently found to contain *Aristolochia fangchi* (Chinese name: Guang Fang Ji) that is damaging to the kidneys. The novel sensitive methodology developed by the BTG has enabled the detection of very low levels of just 2% contamination (corresponding to 50 copies of DNA) proving the ability to detect small fragments of DNA with specificity [R4]. In addition to being able to do this, the research has also demonstrated that other herbal plants used in many homes are in fact misidentified. For example, a study [R5] of the Indian medicinal plant Tulsi (Holy Basil; commonly used in Ayurvedic medicine) found that this species may have been substituted during the Indian diasporic migration to East Africa and onwards to the UK, to the extent that an African species of Tulsi is now found in many British Asian homes.

To better understand and address barriers to entry for DNA-based authentication methods, the BTG partnered with Schwabe, Europe's largest phytopharmaceutical company [G1], to develop an industrially viable DNA barcoding method, based on quantitative PCR, to rapidly identify and separate plant raw material in medicinal plants for quality assurance [R6].

3. References to the research

All outputs were published in leading international journals in the field, publishing rigorously peer-reviewed research. All outputs are reference points for further research beyond the original institution as evidenced by increasing citation numbers.

[R1] Howard, C., Bremner, P.D., Fowler, M.R., Isodo, B., Scott, N.W. and Slater, A. (2009) 'Molecular identification of *Hypericum perforatum* by PCR amplification of the ITS and 5.8S rDNA region', *Planta Medica*, 75(8): 864–869; http://dx.doi.org/10.1055/s-0029-1185397

[R2] Howard, C., Socratous, E., Williams, S., Graham, E., Fowler, M.R., Scott, N.W., Bremner, P. and Slater, A. (2012) 'PlantID – DNA-based identification of multiple medicinal plants in complex mixtures', *Chinese Medicine*, 7: art 18; https://doi.org/10.1186/1749-8546-7-18

[R3] Kazi, T., Hussain, N., Bremner, P., Slater, A. and Howard, C. (2013) 'The application of a DNA-based identification technique to over-the-counter herbal medicines', *Fitoterapia*, 87: 27–30; https://doi.org/10.1016/j.fitote.2013.03.001

[R4] Sgamma, T., Masiero, E., Mali, P., Mahat, M. and Slater, A. (2018) 'Sequence-specific detection of Aristolochia DNA – a simple test for contamination of herbal products', *Frontiers in Plant Science*, 9: art 1828; https://doi.org/10.3389/fpls.2018.01828

[R5] Jürges, G., Sahi, V., Rios Rodriguez, D., Reich, E., Bhamra, S., Howard, C., Slater, A. and Nick, P. (2018) 'Product authenticity versus globalisation – the Tulsi case', *PLoS ONE*, 13(11): e0207763; https://doi.org/10.1371/journal.pone.0207763

[R6] Sgamma, T., Lockie-Williams, C., Kreuzer, M., Williams, S., Scheyhing, U., Koch, E., Slater, A. and Howard C. (2017) 'DNA barcoding for industrial quality assurance', *Planta Medica,* 83(14/15): 1117–1123; https://doi.org/10.1055/s-0043-113448

Grant:

[G1] VITANGO, EU FP7 Marie Curie Action: Industry–Academia Partnerships and Pathways scheme (Coordinator: DMU), 2012–2016, EUR449,391 https://cordis.europa.eu/project/id/286328

4. Details of the impact

The BTG have translated bench-top research into the development of novel DNA barcoding methods for the authentication of herbal medicines into safer, more efficient industrial quality control protocols and new global standards. Having recognised that DNA testing of medicinal plants could reduce dangerous contamination, benefit consumers and increase product quality and therefore confidence in the industry, they worked with the industry leader to establish commercially viable quality assurance methods. Concurrently they worked with global regulators to implement new standards and legislative changes that required the wider herbal medicines industry to change its approach and strengthen quality control procedures.

(1) INDUSTRY UPTAKE OF DNA-BASED QUALITY ASSURANCE METHODS

Schwabe Pharmaceuticals, part of the Dr Wilmar Schwabe Group, is the world-leading manufacturer of traditional herbal medicines (annual turnover: EUR900,000,000). Schwabe recognised that DNA-based tests offered an opportunity to further consolidate their market-leading position and respond to EU requirements for DNA-based testing to obtain market approval for herbal medicine products [C1]. The BTG secured EU FP7 funding to partner with Schwabe [G1] and led the development of DNA-based tests to authenticate some of Schwabe's key commercial medicinal plants. By February 2016, BTG and Schwabe had sufficient evidence to conclude that the preferred method was the optimised, highly sensitive quantitative PCR developed at DMU. This technical approach required a high initial industrial infrastructure outlay, but Schwabe felt this was offset by the technology's sensitivity, speed and capability of assessing the purity of the sample, as well as the fact that BTG methods could be developed to the company's own specification [C1].

BTG researchers developed DNA-based tests to identify *Rhodiola rosea*, used in herbal medicine to reduce fatigue and stress, and differentiate it from other *Rhodiola* species. This addressed a significant challenge that Schwabe had been trying to resolve with a problematic supply chain issue. The company had identified an unacceptable variation in *Rhodiola rosea* found in the raw materials supplied to European buyers and this had resulted in significant variations in constituents present in its Rhodiola products [C2]. Schwabe therefore required a scientific method to standardise purchasing, particularly because *Rhodiola rosea* herb is the active ingredient of Vitano®, one of its best-selling products. In their study of *R. rosea*, the BTG team obtained DNA sequences for four DNA barcode regions and 10 potential contaminant species. They identified the most appropriate region from the DNA sequences on which to base the design of PCR primers, allowing them to distinguish between medicinal plants and the contaminant or non-target plants. Schwabe were able to adopt the quality control protocol developed by BTG for the authentication of *R. rosea* [C3].

In another project, BTG developed a DNA-based method to differentiate between two closely related Pelargonium species [C4]. Marketed by Schwabe under its Kaloba range, this medicinal product is used to relieve the symptoms of upper respiratory tract infections (pelargonium is listed under self-care treatments for acute coughs in NICE Guideline 120). The new method enabled Schwabe to protect their claim to the most potent species, protecting their competitive advantage and allowing them to meet EU pharmaceutical approval procedures [C1].

The Rhodiola assay was later published as a case study in the 2017 *Planta Medica* paper co-authored with the Schwabe team [R6], exemplifying a universal DNA barcoding authentication strategy for use across the industry. Schwabe notes: 'As a company with the most expertise in phytochemical and pharmacological studies, we weren't experts in developing or using DNA-based test systems to discriminate medicinal plant at DNA level. Therefore, for our company this collaboration project with the BTG at DMU was a very excellent and profitable experience' [C1:pp.2]. The BTG helped to embed this new technology into company processes by training two research scientists and three technical staff, who were seconded by Schwabe to DMU. The company concludes that this 'has given Schwabe as a company a decisive advantage in preparing or adjusting to the ever-increasing quality requirements and analytical methods that are increasingly required by the authorities like the German Federal Institute for Drugs and Medical Devices (BfArM) during approval procedures and that are slowly being introduced as a quality standard in e.g. the British and Chinese Pharmacopoeias' [C1:pp.2].

(2) REGULATORY UPTAKE OF DNA-BASED STANDARDS TO INCREASE PRODUCT QUALITY AND REDUCE DANGEROUS CONTAMINATION

Slater and Howard recognised that DNA testing for medicinal plants would only be taken up across the herbal medicines industry if regulations stipulated it. Herbal medicines in the UK are regulated by the Medicines and Healthcare Products Regulatory Agency (MHRA), and the standards applied to specific products are written into 'monographs' within the British Pharmacopoeia (BP). Following discussions between BTG and the MHRA, Howard was appointed, in January 2014, to a new post within the BP in order to develop and embed an in-house DNA testing capability. This led to the founding of the BP Herbals Laboratory at the National Institute for Biological Standards and Controls (NIBSC) as an MHRA centre; with several new posts created to service the facility [C5, minutes 8th June 2016:pp1]. The BP Laboratory has been shaped by BTG research. Howard was closely involved in crystallising the core concepts of DNA testing and transferred these to the new laboratory, while Slater was recruited to the BP Commission Panel of Experts DNA Identification Techniques Steering Committee, and subsequently appointed Chairman of the Expert DNA Panel [C5, minutes 8th June 2016: pp2].

In 2015 BP published the world's first pharmacopoeia barcode [C6]. This was for Holy Basil, and the decision was underpinned by DMU's research on this genus [R4]. This gave the global industry access to a validated, DNA-based method to unambiguously identify plant material for the first time. To provide a 'positive control' for this method, a novel reference material was brought to market, with the BTG performing the fundamental Second Laboratory validation of the material and protocol [C7]. Over the impact period, the barcode sequences of eight key medicinal plants were published in the BP, each individually endorsed by the Expert Panel for DNA. The use of this technology increased the stringency of all benchmarks in the BP for herbal drugs by verifying the material used in their production. Identifying adulterated and mixed samples within the reference samples gave insight into how these materials appear in all analyses, allowing standards to be set that would rule them out.

The Chairman of the British Herbal Medicines Association provides an overview of the significance of these changes to industry standards:

> The issues of adulteration and contamination are complex and may be caused intentionally or through misidentification. Recent research carried out by the BHMA suggests that this may affect up to 40% of material available on the UK marketplace. This issue is harmful to the industry as it can reduce the potency of the medicines administered, could cause ill health and reduce consumer confidence. For these reasons there is an urgent need for novel and accessible quality control measures, such as those published by the Biomolecular Technology Group of De Montfort University. The impact of these publications, and guest speaker appearances by several [BTG] members at BHMA seminars, has been to enable our members to understand the potential of these methods to answer their supply chain questions [C8].

The BP has a wide international reach as it is used as a reference standard in more than 100 countries [C9] and forms an inherent part of established medicines legislation in Commonwealth

countries. For example, Australia and Canada have adopted the BP as their national standard, and therefore its DNA testing requirements. Through informing the BP, the research presented in this case study has influenced how the quality of herbal products should be assessed and enhanced in many countries around the world.

DMU research has had a direct impact on approaches to DNA barcoding in the United States. The US Pharmacopoeia (USP) is in the process of developing its own DNA tests. They invited Howard to be a keynote speaker at their 2018 workshop on DNA authentication and their strategy for developing DNA tests is based on the 2009 DMU paper [R1, C10]. USP state that DMU's 2017; pp.1 paper [R6] in particular has had 'a major influence' on their work, noting that 'these comprehensive guidelines are very important for researchers and industry' [C10: pp.1]. They write: 'Publications from the group informed and influenced the direction of our work regarding the suitable methods and the validation requirements for compendial standards'. This included, in 2018, the use of the methods described in R1 to investigate species–species identification of the American ginseng, Asian ginseng and Tienchi ginseng [C10]. The USP says this could 'contribute to the introduction, for the first time, of the genomic methods for botanical identification to complement the current compendial identification methods' [C10: pp.1].

DMU research [R6] has also informed USP policy towards developing new stricter quality standards that will be mandatory for herbal medicine manufacturers. It writes:

> We are also referring to the group's guidance regarding quality control decision points to update the USP General Chapter <563> Identification of the Articles of Botanical Origin to incorporate guidance regarding out-of-specification investigations. If specifications of a botanical monograph cite the analytical methods and acceptance criteria in a General Chapter, the requirements become mandatory for manufacturers who claim compliance with the monograph [C10: pp.1].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[C1] Corroborating statement from Schwabe Pharmaceuticals.

[C2] Booker, A., Jalil, B., Frommenwiler, D., Reich, E., Zhai, L., Kulic, Z. and Heinrich, M. (2016) 'The authenticity and quality of Rhodiola Rosa products', *Phytomedicine*, 23(7): 754–762; https://www.ncbi.nlm.nih.gov/pubmed/26626192; a peer-reviewed journal paper co-authored by Schwabe's pre-clinical research group.

[C3] Schwabe's quality control protocol for Rhodiola rosea authentication.

[C4] Standard Operating Protocol for DNA-based testing developed by DMU and Schwabe.

[C5] BP DNA minutes.

[C6] The British Pharmacopoeia's DNA barcode for Holy Basil leaf – the first DNA barcode to be incorporated into the BP.

[C7] Second Laboratory validation of the first British Pharmacopoeia DNA test.

[C8] Corroborating statement from the Chairman of the British Herbal Medicines Association.

[C9] British Pharmacopoeia webpage that confirms the BP's 'wide international reach': https://www.pharmacopoeia.com/the-british-pharmacopoeia

[C10] Corroborating statement from the United States Pharmacopoeia.