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Research Paper

Chemical variability along the value chains of turmeric (*Curcuma longa*): A comparison of nuclear magnetic resonance spectroscopy and high performance thin layer chromatography ☆

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ABSTRACT

Ethnopharmacological relevance: Herbal medicine value chains have generally been overlooked compared with food commodities. Not surprisingly, revenue generation tends to be weighted towards the end of the chain and consequently the farmers and producers are the lowest paid beneficiaries. Value chains have an impact both on the livelihood of producers and on the composition and quality of products commonly sold locally and globally and consequently on the consumers. In order to understand the impact of value chains on the composition of products, we studied the production conditions for turmeric (*Curcuma longa*) and the metabolomic composition of products derived from it. We aimed at integrating these two components in order to gain a better understanding of the effect of different value chains on the livelihoods of some producers.

Materials and methods: This interdisciplinary project uses a mixed methods approach. Case studies were undertaken on two separate sites in India. Data was initially gathered on herbal medicine value chains by means of semi-structured interviews and non-participant observations. Samples were collected from locations in India, Europe and the USA and analysed using ^{1}H NMR spectroscopy coupled with multivariate analysis software and with high performance thin layer chromatography (HPTLC).

Results: We investigate medicinal plant value chains and interpret the impact different value chains have on some aspects of the livelihoods of producers in India and, for the first time, analytically assess the chemical variability and quality implications that different value chains may have on the products available to end users in Europe. There are benefits to farmers that belonged to an integrated chain and the resulting products were subject to a higher standard of processing and storage.

By using analytical methods, including HPTLC and ^{1}H NMR spectroscopy, it has been possible to correlate some variations in product composition for selected producers and identify strengths and weaknesses of some types of value chains. The two analytical techniques provide different and complementary data and together they can be used to effectively differentiate between a wide variety of crude drug powders and herbal medicinal products.

Conclusions: This project demonstrates that there is a need to study the links between producers and consumers of commodities produced in so-called 'provider countries' and that metabolomics offer a novel way of assessing the chemical variability along a value chain. This also has implications for understanding the impact this has on the livelihood of those along the value chain.

Abbreviations: AE, aqueous extract; C, cosmetic; DMSO, dimethylsulphoxide; Drh, dried rhizome; EE, ethanolic extract; F, food; FRh, fresh rhizome; GAP, good agricultural practice; HMP, herbal medicinal product; HPMC, hydroxypropyl methylcellulose; HPTLC, high performance thin layer chromatography; LEDC, less economically developed country; LCIRAH, Leverhulme Centre for Integrative Research on Agriculture and Health; M, medicinal; MEDC, more economically developed countries; MHRA, Medicines and Healthcare Products Regulatory Agency; NMR, nuclear magnetic resonance; PCA, principal component analysis; PRC, Peoples Republic of China; PW, Powdered drug; Rf, retention factor; SCF, super critical fluid extract; TCM, traditional Chinese medicine; THMPD, traditional herbal medicinal products directive; VIVC, vertically integrated value chain; WHO, World Health Organisation; WRT, white light

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1. Introduction

Ethnopharmacological research has generally focused on herbal starting materials and their composition and effects, or on the final products available to the consumer. There has been a limited focus on the interconnectivities linking producers and consumers (Booker et al., 2012).

On the other hand, global value chain analysis has been commonly applied to food sectors, including tea, coffee and cocoa (Kaplinsky, 2004; Gibbon and Ponte, 2005; Fullbright, 2008) but little work has been undertaken in this area regarding *medicinal plants*. Agricultural production is generally in developing countries, while the consumption is both in developing and developed countries. Clearly such a usage impacts on public health, potentially not only in a beneficial but also detrimental way and it has a direct relevance in the context of ethnopharmacology.

It is an important area for research since the production of high value products is an emerging but poorly understood economic activity within many developing countries, and particularly within India and China, both with a long history of trading in such commodities. According to the World Health Organisation (WHO), world trade in herbal medicine was estimated at $83 Billion in 2008 (Robinson and Zhang, 2012).

Much of the trade in medicinal plants has been based within national market systems. While global trade in high value products like spices and medicines has a long history, e.g. along the spice route (Freedman, 2003), over the last 30 years international trade has flourished and a main thoroughfare of this trade exists between Asia and Europe, the US and Australia.

While ethnopharmacology has historically focused on the 'traditional' uses in far away regions, it is now more and more accepted that the complex interrelationship between producers and consumers needs to be studied from a trans-disciplinary viewpoint (Heinrich et al., 2011).

From a global perspective Europe has been leading the way in supplying high quality herbal products, first with diverse national initiatives and also with the development of quality standards, for example, in the European Pharmacopoeia and, more recently, with the introduction of the EU-wide Traditional Herbal Medicinal Products Directive (THMPD, 2004/24/EC) (European Parliament, C, 2004) which requires well defined minimal standards of quality and safety to be assured before a product can be released onto the market. Therefore, compared with food crops, medicinal plants may be more attractive economically (Alam and Belt, 2009; Sharmin, 2004). However, the European quality requirements for medicinal plants, plant extracts and herbal medicinal products set a much higher entry bar than for foods requiring more sophisticated inputs along the value chain.

The traceability of raw materials used in herbal medicines has always been problematic. In Asia, it is common practice for plants to be collected from the wild or cultivated on small farms and then either stored, for sometimes long periods, sold in the local market, or auctioned at one of the designated auction sites (Kala et al., 2006; van de Kop et al., 2006; Alam and Belt, 2009). Middlemen are usually involved in the supply of plant material to herbal manufacturers and any information regarding the origin and primary processing is mainly lost. An alternative to this approach has been the implementation of vertically integrated value chains (VIVCs) (Fig. 1), where contracts are made between (in our case study) the farmer in Asia and the retailer in Europe.

A VIVC can be defined as when 'a lead organisation' is responsible for two or more intertwined steps of the manufacturing or value chain process (Strategy-Train, 2009). This has advantages for the farmer in that a premium is paid for the crop, and the order size and type of crop grown can be well defined in advance, allowing for an effective planting and cultivation strategy to be developed. For the buyer, the advantages include that the crop has better traceability, a price has been agreed in advance and so market fluctuations are not an issue, and knowledge that good cultivation practice has been employed. India was chosen as a research site as it is a fast developing country with a long tradition of medicinal plant use. It has an established herbal medicine manufacturing base and a thriving export market (Polshettiwar, 2006).

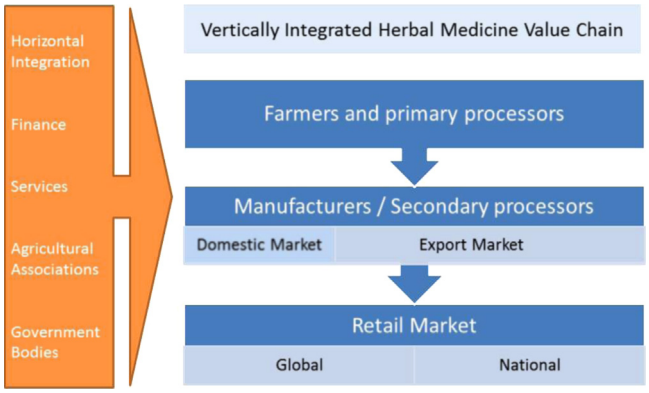

Fig. 1. The generic herbal medicine value chain.

In order to study these inter-relationships, metabolomics offers some novel and bioscientifically robust opportunities. It provides what has been called a 'holistic view' of the metabolites of a set of organisms (Kim et al., 2011) and has been used in a wide range of research fields like plant biotechnology, ethnopharmacology, ecological toxicology and plant physiology (e.g. Verpoorte et al., 2005; Kim et al., 2011; Liu et al., 2011; Michl et al., 2011), and is ideally suited for comparing large number of samples as one would encounter them, for example, along a value chain. At the same time it is a method which as such is not validated and needs to be compared to a standard method like high performance thin layer chromatography (HPTLC), widely used in the authentication and quality control of herbal substances (Reich and Schibli, 2007).

Following an online survey, turmeric (*Curcuma longa* L. Zingiberaceae) was chosen as a suitable case study. Turmeric's use as both food and a medicine presents an opportunity to show how a value chain may be different for a food and a drug and how investing in quality can lead to genuine value addition. There are concerns over the quality of material available commercially. It is acknowledged that the chemical composition is variable and misidentification of species and adulteration are frequently reported problems (Govindarajan, 1980; Dixit et al., 2009). There are several species that may be confused with *Curcuma longa*.

Curcuma aromatica Salisb. (wild turmeric) has been known to be used as an additive in order to improve the colour of cultivated *Curcuma longa*, which in turn raises its auction value, but clearly is an adulteration. In an integrated chain there is less opportunity (or necessity) for adulteration to take place as a price for the crop has already been agreed. An analytical assessment should be able to determine which products have been adulterated. *Curcuma aromatica* does not contain bis-demethoxycurcumin found in *Curcuma longa*.

Curcuma zanthorrhiza Roxb. (Javanese turmeric) is native to Indonesia and is commonly found in HMPs in Europe. It is listed in the British Pharmacopoeia. This species also lacks bis-demethoxycurcumin and is chemically similar *to Curcuma aromatica. Curcuma kwangsiensis* S.G. Lee and C.F. Liang is a Chinese species and favoured for use in traditional Chinese medicine (TCM) products.

This has led to the key research questions of this paper:

- How can the variation of the product composition be determined analytically? And how does this inform the debate about value chains of herbal medicines?

- Which methods are most useful? How does the information derived from NMR spectroscopy – metabolomics differ from that of HPTLC and how can these analytical tools be used best to study the interconnectivities between producers and consumers?

2. Methodology

2.1. Fieldwork in India

Two study sites were included, providing a contrast between products that derive from a 'buyers market' approach, typically to be found in India and compare these with products that have been produced through a VIVC. We sampled raw materials and products from each site and then compared them with other products that are available to consumers in India, Europe and USA.

The first case study examines a farm site near to Erode (Tamil Nadu), producing material sold through middlemen at an auction house. This case study represents the traditional route of supply where the plant material is bought by any buyer for a multitude of purposes, including being purchased by a local HMP manufacturing unit, where once traded, the traceability of the material is generally lost.

The second case study focuses on a farm site located near Shimoga, Karnataka, a farm in a chain, vertically integrated to a primary manufacturing site in Bangalore, and a secondary manufacturing site in the UK.

These farms provide a good comparison between an integrated and non-integrated approach, organic and non-organic, and high and low output, which allows for a wide angled view of the current situation and broad evaluation of the available data.

Several factors were considered to be of prime importance when choosing the sites:

- To be able to examine sites that had a large, established trade in Turmeric: Tamil Nadu and Karnataka are major growing areas for this crop and Erode is regarded as the main trading city in southern India.
- It was important to visit the sites during times of productivity; the busy period for turmeric production runs from November to April and this is the period when the majority of harvesting, drying, processing and trading takes place.

Fieldwork was conducted during a period of two months in January and February 2012 and again in October and November 2012. Our core sample was composed of farmers, farm workers, processors and market traders.

We used a case study approach employing semi-structured interviews and non-participant observations. Semi-structured interviews are useful in obtaining general and specific information from a sample of the population and gaining a range of insights on a specific issue. This methodology had advantages over a fully structured approach in that it was less intrusive, encouraging two way communication. It also provided data on the reasons behind their answers (FAO, 1990). As previously outlined by Handley (2012), observational methods offer an objective insight into what people do rather than what they say they do. Thoughtful and judicious use of observational methods provides one of the most effective ways for understanding activities 'in situ'.

2.2. Laboratory analysis

Throughout the fieldwork stage of the project, commercial samples were gathered from sites across India, USA and Europe. Samples taken included ethanol extracts, aqueous extracts, powdered medicinal products, food powders, powders used chiefly as cosmetics, whole dried rhizomes and tubers and fresh rhizomes. These samples were taken back to the UCL School of Pharmacy for analysis. Apart from the vertically integrated chain samples and the fresh rhizomes, there is no way of telling the true age of any of the HMP or market collected samples due to the lack of documented traceability regarding storage time.

We analysed the samples using ^{1}H nuclear magnetic resonance (NMR) spectroscopy and multivariate analysis software. The samples were grouped according to the product information accompanying each sample, e.g. the label information. The groupings were validated through the use of authenticated reference material and through the collection of a proportion of samples directly from the cultivation site. For comparison, the samples were then analysed 'blind' using HPTLC techniques. This analysis was not to provide a direct comparison of sample metabolites, but to compare the approaches. Therefore, different extraction solvents were used, but to discover what information could be determined regarding each sample using each analytical technique.

2.2.1. ^{1}H NMR spectroscopy methods

2.2.1.1. Sample preparations for ^{1}H NMR spectroscopy analysis. The method for the extraction of plant samples was developed from a method described by Kim and Verpoorte (2010). Initial experiments were carried out using chloroform-D, and DMSO-D6 and methanol-D4 but it was found that DMSO-D6 was a better extracting solvent because the ^{1}H NMR spectra showed more peaks in the 0–10 ppm range than with the other solvents tried. Approximately 20 mg of all the samples were weighed into a 1.5 ml reaction tube and 1.0 ml of deuterated DMSO-D6 containing 0.05%TMS was added. The mixture was mixed on a rotary mixer for 60 s, sonicated in an ultrasound bath for 15 min, and allowed to stand for 1 h. The solutions were centrifuged for 10 min at 14,000 rpm. Eight hundred microlitres of the supernatant was added to a 5 mm diameter NMR spectroscopy tube and the samples were submitted for NMR analysis.

2.2.1.2. ^{1}H NMR spectroscopy – metabolomic analysis. ^{1}H NMR spectra of the turmeric samples were recorded at temperature 298 K on a Bruker Avance 500 MHz spectrophotometer (Bruker Analytik, Rheinstetten, Germany) equipped with a 5 mm cyroprobe head and operating at proton frequency of 500.13 MHz. The spectra were acquired with 256 scans, requiring approximately 10 min acquisition time.

The following parameters were used during the acquisition: ~0.16 Hz/point, pulse width (PW)=30° and relaxation delay=1 s. FIDs were Fourier transformed with LB=0.30 Hz. The spectra were referenced to the internal TMS peak. TOPSPIN version 1.3 software was used for spectra acquisition and processing of the ^{1}H NMR spectra which were manually corrected for phase and baseline distortions. The spectra were exported to Topspin Plot editor (3.5 Bruker) and thoroughly examined visually for appropriate reference peaks for the identification of curcuminoids in the sample. The ^{1}H NMR spectra of all the samples were imported to the AMIX software 3.5 and simplified by using the bucketing function. The spectra in the range 0–12 ppm were divided into 301 buckets of 0.04 ppm. The residual solvent signals for DMSO (2.65–2.45 ppm) and water (3.60–3.10 ppm) were excluded. This data was imported to Microsoft EXCEL where the samples were re-labelled S1–S52. The data was then imported to SIMCA, version 13.0 software, for principal component analysis (PCA).

2.2.2. HPTLC methods

2.2.2.1. Sample preparations for HPTLC analysis. The method for the extraction of plant samples was developed from a standardised

method used at CAMAG laboratories based on a method of the British Pharmacopoeia. Approximately 200 mg of all the samples was weighed into a 10 ml reaction tube and 4.0 ml of solvent was added (methanol for curcuminoids, toluene for determination of essential oils and water for sugars). The mixture was mixed on a rotary mixer for 60 s and shaken for 20 min. The solutions were centrifuged for 10 min at 14,000 rpm. The supernatant solution was transferred to a vial and the samples were submitted for HPTLC analysis.

2.2.2.2. HPTLC analysis. HPTLC was carried out using Merck HPTLC glass plates 20 × 10 cm, Si 60 F254.

HPTLC Plates: Samples were applied using the CAMAG Automatic TLC Sampler 4. Three developing systems were used, for curcuminoid determination, (Toluene, acetic acid 4:1), for sugars (Acetonitrile: acetone: water 40:40:20) and for essential oils (Dichloromethane). The lab temperature was 23 °C and tank humidity within the developing tank was controlled at 33%. The developed plates were derivatised by dipping with anisaldehyde reagent (curcuminoid and essential oil systems) and aniline-diphenylamine-phosphoric acid for sugars, using a CAMAG chromatogram immersion device III and heated to 100 °C on a plate heater. The plates were inspected using a CAMAG visualiser under white light and at UV 366 nm, photographed and uploaded into the computer software (WinCats).

3. Results and discussion

3.1. Fieldwork in India

Two farm sites were compared in southern India. The first site in Erode, Tamil Nadu grows sugar cane as its main crop and turmeric as a secondary crop. Turmeric suffers from leaf rot and thin rhizomes and so different proprietary herbicides, fungicides and pesticides are used together with fertilisers including potash, urea and manure. After harvesting, the turmeric is boiled using a mobile steam generator which is hired from a third party. The boiling step is used to decrease microbial load and help to remove water from the roots and rhizomes. Following the boiling step, the material is dried in the sun for 20 days before polishing. The polishing gives the product a more intense yellow colour, making it more attractive to buyers. Both the root and the rhizome are used and both can be re-planted. The root tends to be used more for use in paints and dyes and for cosmetic purposes.

In 2011 the auction price of turmeric was 18,000 rupees (US$ 280) per quintal (100 kg) in 2012 it had fallen to 4000 rupees (US$ 60) per quintal. At this price it is difficult for farmers to recoup their costs and so they may choose to store the crop until prices rise. At the government regulated market, they sell about 2000 bags of turmeric per day at about 4000 rupees per bag, depending on quality. Samples are displayed for buyers prior to the auction for organoleptic inspection and subsequently the buyers will bid for each lot. Buyers are generally aware (through the available documentation), from which farms the lots are sourced, but little information regarding the cultivation and processing methods is provided. The auction market receives 2% of the sale price as commission. Farmers who do not want to sell their product immediately, send their harvest to one of the approximately 350 storage units, situated in Erode. Turmeric can be stored in a facility such as this for up to five years. When the crop is sold, the storage unit owner also receives 2% as commission. The turmeric is stored in jute sacks and evidence of weevil infestation and fungal moulds were evident.

The second case study is a 30 acre farm set in 450 acres of forest that is managed by a conservancy trust. The farm has been certified organic since 2004. The farm cultivates *Areca catechu* L. (Arecaceae) as a main crop and medicinal herbs are grown as secondary crops. The farm also produces food crops: chili, allspice, cinnamon, nutmeg, pepper, bananas, coconut and ginger, which are sold at local markets.

Following harvesting, the crops are cleaned by hand, washed using a tiered system, and then drained and dried, initially in a drying room, before being laid out in the sun on plastic sheets for further drying. At this farm the turmeric rhizome is supplied to a primary processing company in Bangalore. The company in Bangalore has a contract with the farmer and does not generally buy material from market sources. The farmer receives a 10–40% premium for spices, foods and medicinal plants, above the market price because of the complexity of cultivation and the good agricultural practice (GAP) processes employed (specifically for ginger 30–40%, turmeric 10%, coffee 10%, pepper 20%, white pepper 30%). The premium is adjusted depending on the conventional market price and so the farmer receives the agreed price for their crop irrespective of the market price in a similar fashion to the

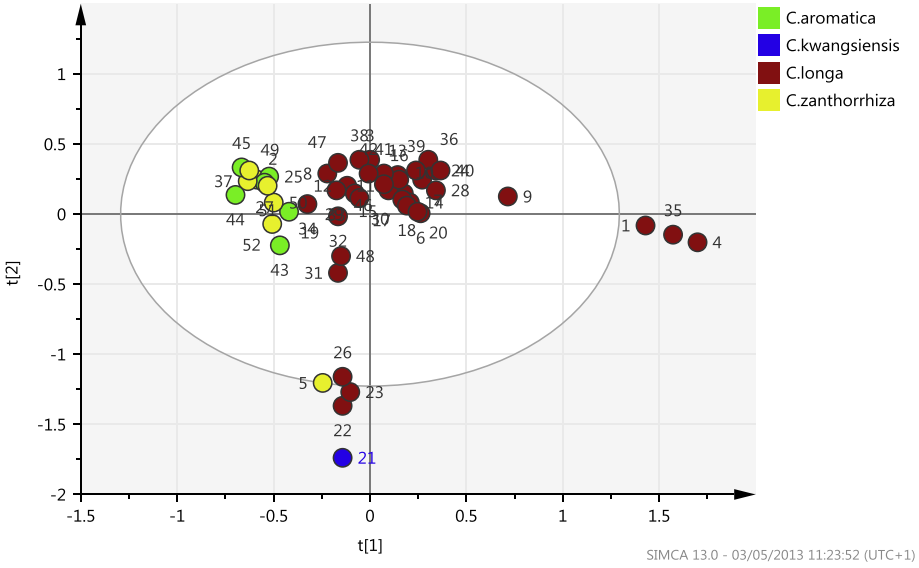

Fig. 2. Scores plot showing samples grouped according to species, PC1 vs PC2.

Fairtrade® scheme. After cutting and grinding the turmeric is shipped to the UK for secondary manufacture.

3.2. Laboratory analysis

3.2.1. ^{1}H NMR spectroscopy

The samples were analysed using ^{1}H NMR spectroscopy coupled with SIMCA multivariate analysis software and by HPTLC. Using both of these techniques it was possible to order the products into different groups. With the SIMCA software the groups were separated with reference to species (Fig. 2) and to dosage form/morphology (Fig. 3). Using this method it was possible to differentiate three separate species based on their principal component variability: *Curcuma longa* L., *Curcuma aromatica* Salisb., *Curcuma zanthorrhiza* Roxb., and a range of different dosage forms, comprising ethanolic extracts, a super critical fluid extract, aqueous extracts, powdered medicinal drugs, powders used as food, mixed powder and extract products, dried cut rhizomes, whole dried rhizomes and fresh turmeric rhizomes.

The contribution plot provides information on how PCA separates the samples based on the differences of the intensity of compounds at particular chemical shifts i.e. it describes where the main variability in chemical composition occurs and the software enables us to demonstrate these differences graphically. Comparing an ethanol extract (sample 1), against the average for all samples, sample 1 is higher in the chemical shift regions associated with curcuminoids (6–10 ppm) but lower in the carbohydrate region (typically 3–6 ppm) and essential oils/fatty acid region (typically 0.4–3 ppm) (Fig. 4).

PCA was able to differentiate four different species but the initial analysis failed to differentiate *Curcuma aromatica* and *Curcuma zanthorrhiza* satisfactorily (Fig. 2). However, when the powder samples were analysed without the extracts, the PCA differentiated them more effectively (Fig. 5). In Fig. 2, sample 5 appeared to be grouped with the *Curcuma longa* groupings and in Fig. 3, sample 5 was grouped with the aqueous extracts, suggesting that it was an aqueous extract *of Curcuma longa*. However, this was not concordant with the product information that was held on this product. It was observed in the contribution

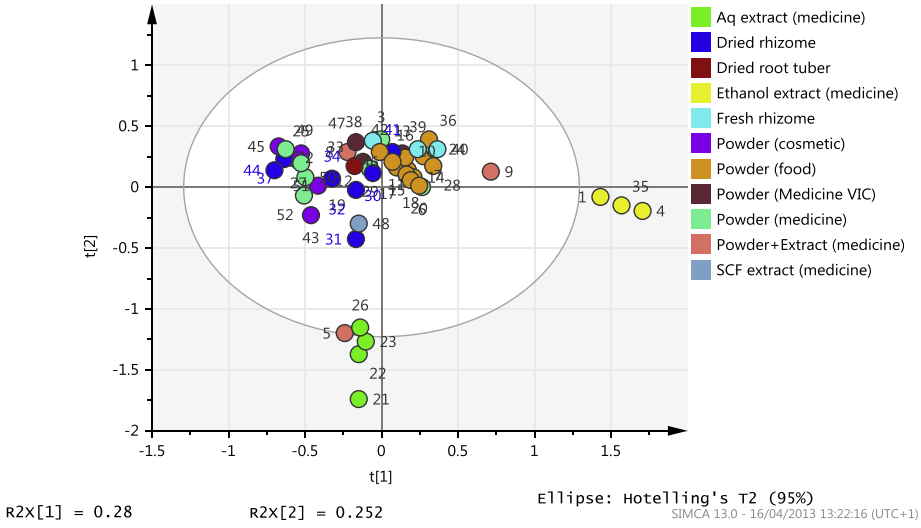

Fig. 3. Scores plot showing samples grouped according to form, PC1 vs PC2.

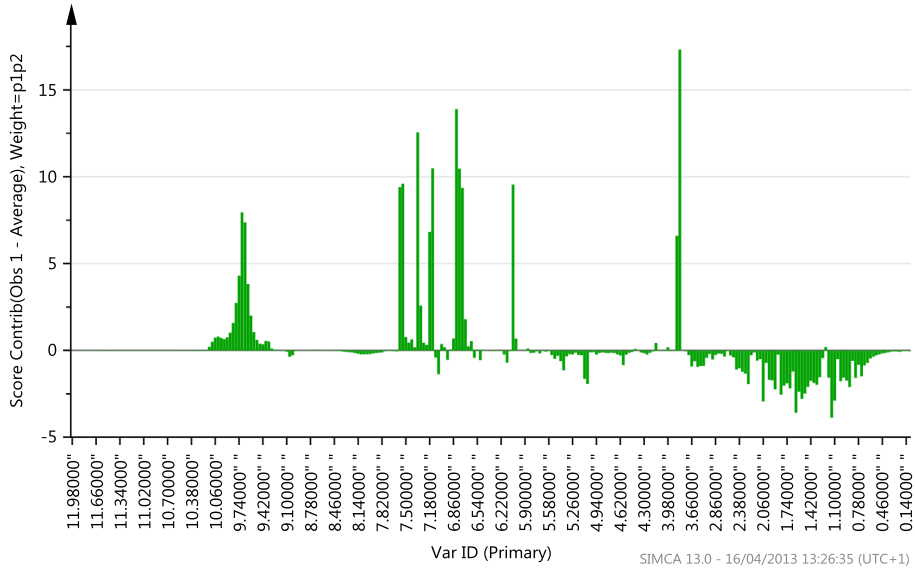

Fig. 4. Contribution plot showing the metabolite differences between sample 1, an ethanol extract and the group average.

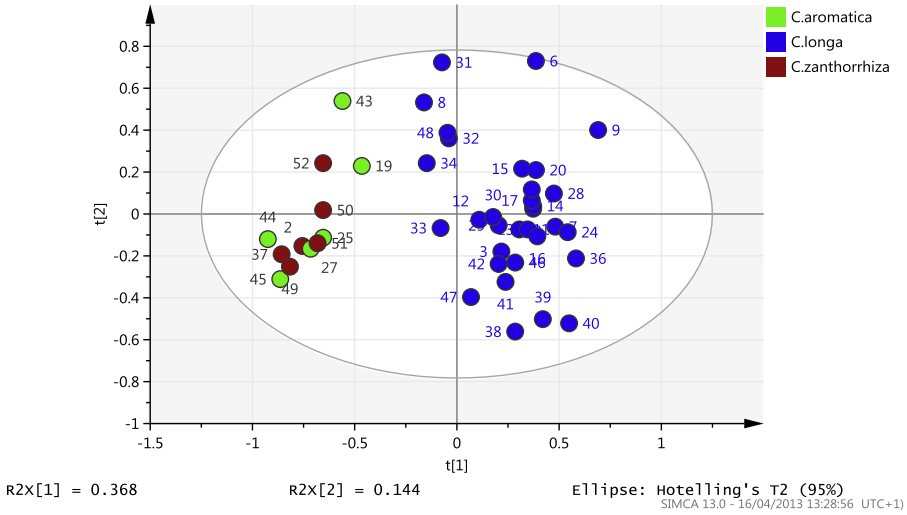

Fig. 5. Scores plot comparing all powders, roots and rhizomes, grouped by species, PC1 vs PC2.

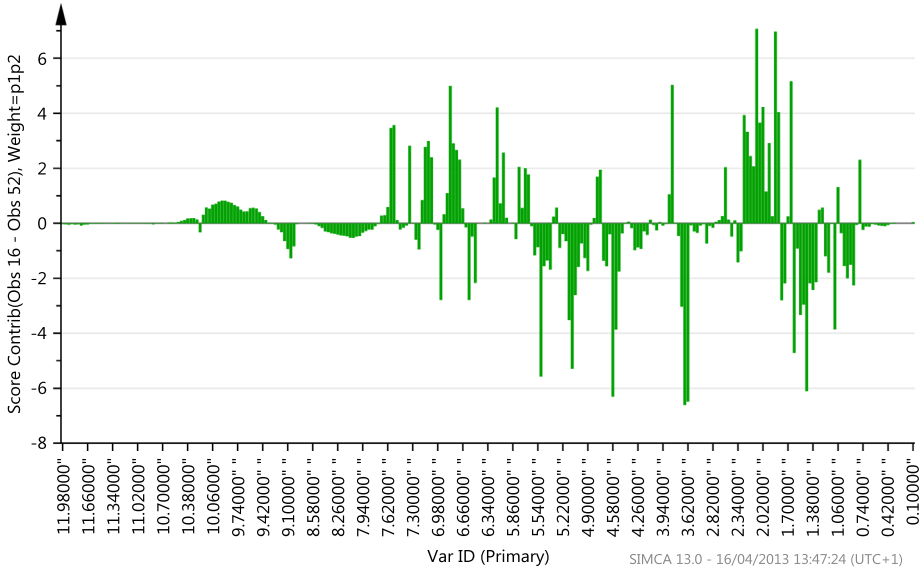

Fig. 6. Contribution plot showing where the main differences in metabolite composition between *Curcuma longa* and *Curcuma zanthorrhiza*.

plot (Fig. 4) that sample 5 was high in the carbohydrate region, consistent with high sugar content. Subsequent HPTLC analysis confirmed that this was not an aqueous extract and was not *Curcuma longa* as the PCA suggested (see Fig. 9).

The metabolite variability between species was chiefly in the curcuminoid region (in *Curcuma aromatica* and *Curcuma zanthorrhiza*, bis-demethoxy curcumin was absent, a phenomenon confirmed by HPTLC) and variation was also observed in the lower range of chemical shift where one would expect to see essential oils (Fig. 6). One reported difference between *Curcuma longa* and *Curcuma aromatica*/*Curcuma zanthorrhiza* is the absence of tumerone in the later species, the essential oil which gives *Curcuma longa* its distinctive aromatic smell. This was confirmed by running a tumerone reference standard. Sample 19 was interesting as although sold as *Curcuma longa* it appeared to be contaminated with *Curcuma aromatica* or *Curcuma zanthorrhiza* (Figs. 5 and 9).

Curcuma aromatica samples were not well separated from *Curcuma zanthorrhiza* samples. The curcuminoid composition between *Curcuma zanthorrhiza* and *Curcuma aromatica* is not a major variable and so in order to find differences between these two species it was necessary to examine different principal components. A pattern was observed between the species and samples 49–52 were particularly well grouped (Fig. 7). These four samples came from the same geographical location. Although sample 2 was labelled as complying with the BP and the company claimed it was *Curcuma zanthorrhiza*, our data suggests that it was more similar to the metabolite profile of *Curcuma aromatica*.

We compared sample 47 to an ethanolic turmeric extract (sample 1, Fig. 8) where we observed that sample 47 was far lower in the curcuminoid region but higher in the essential oil region. This may be a result of the high temperature processing employed in the extraction process leading to the loss of volatile compounds. Consequently, sample 47 may be richer in these compounds as it has been collected fairly soon after harvesting and has not been subjected to high temperature processing. Direct comparison with the tumerone reference standard confirmed that sample 47 was relatively high in this compound.

3.2.2. HPTLC analysis

The samples were analysed blind by HPTLC in order to determine what additional or different information could be found.

In Fig. 9, group 1 includes *Curcuma aromatica* and *Curcuma zanthorrhiza* and group 2 consisted of *Curcuma longa*. We observed

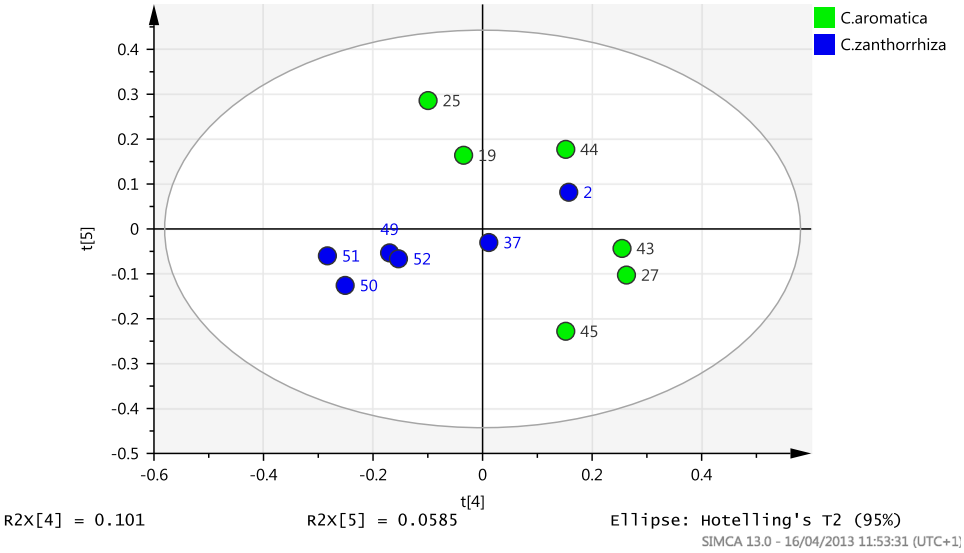

Fig. 7. Scores plot showing differentiation of *Curcuma aromatica and Curcuma zanthorrhiza*, PC4 vs PC5.

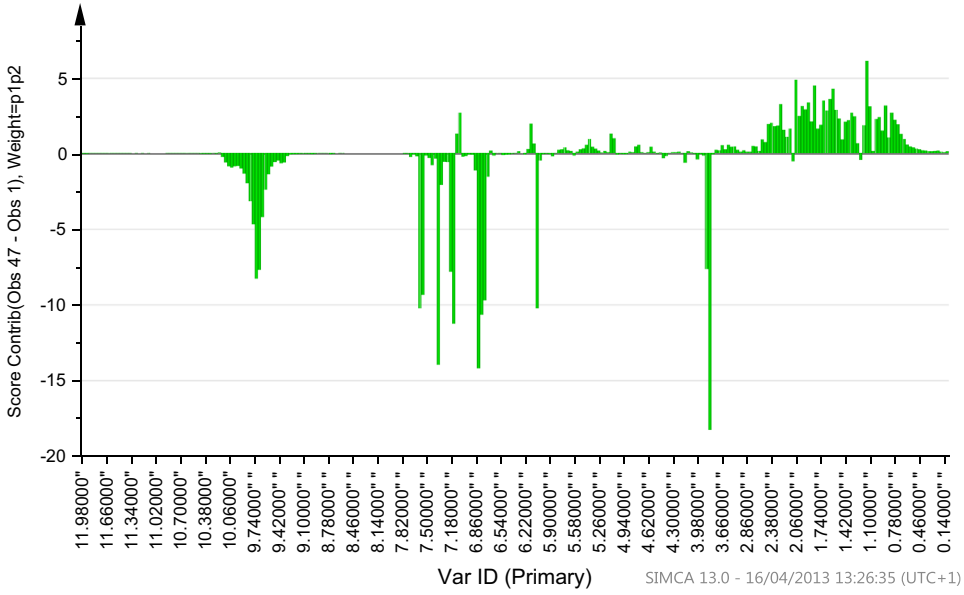

Fig. 8. Contribution plot showing metabolite differences between sample 47 and an ethanol extract, sample 1.

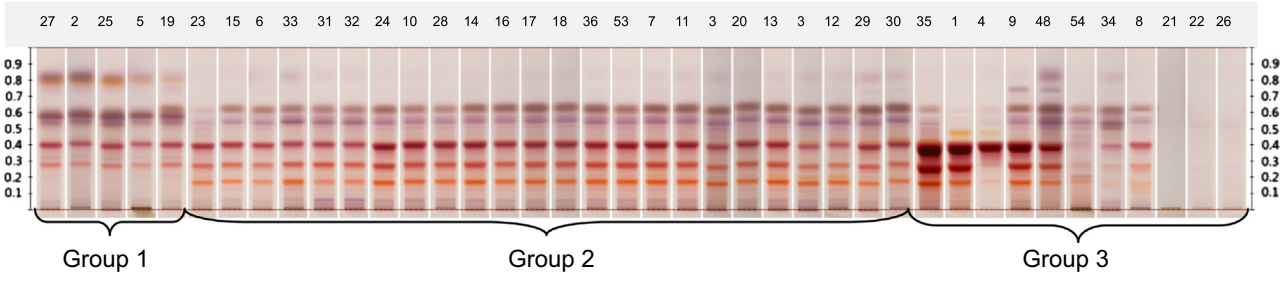

Fig. 9. Derivatised plate under white light. Mobile phase toluene, acetic acid 4:1 (curcuminoid determination).

that bis-demothycurcumin (Rf 0.18) was only present in *Curcuma longa*. Group 3 are the ethanolic/SCF extracts, aqueous extracts and some unknown products. We also observed that group 1 exhibited strong zones at Rf 0.58 and at Rf 0.82. These zones were observed faintly in some of the *Curcuma longa* samples indicating possible contamination from other species.

Sample 48 (group 3), a SCF extract, appeared to contain more compounds than the other extracts. The samples in the last three tracks were aqueous extracts and it was observed that no curcuminoids or essential oils were detected. Sample 23 was unusual in that it was an aqueous extract but contained curcuminoids. Sample 19 was sold as turmeric but was likely to be

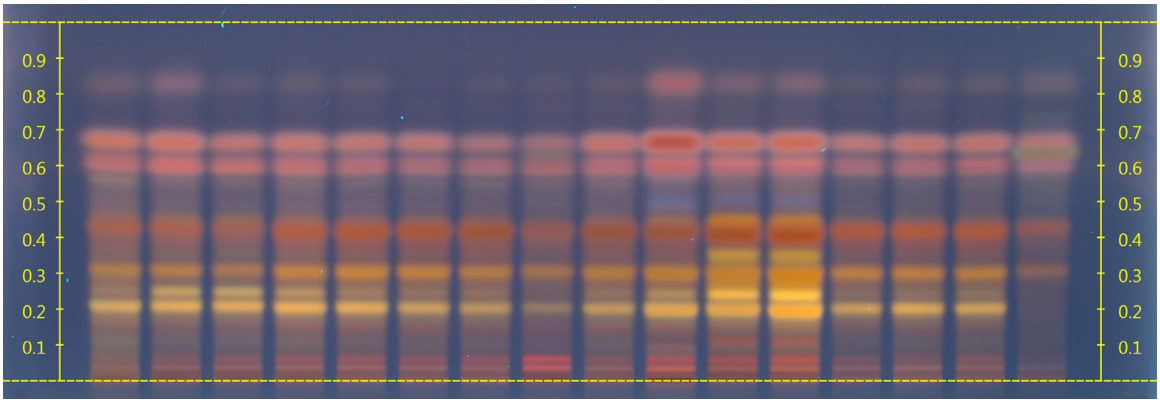

Fig. 10. Derivatised plate under UV 366 nm. Mobile phase toluene, acetic acid 4:1 (curcuminoid determination).

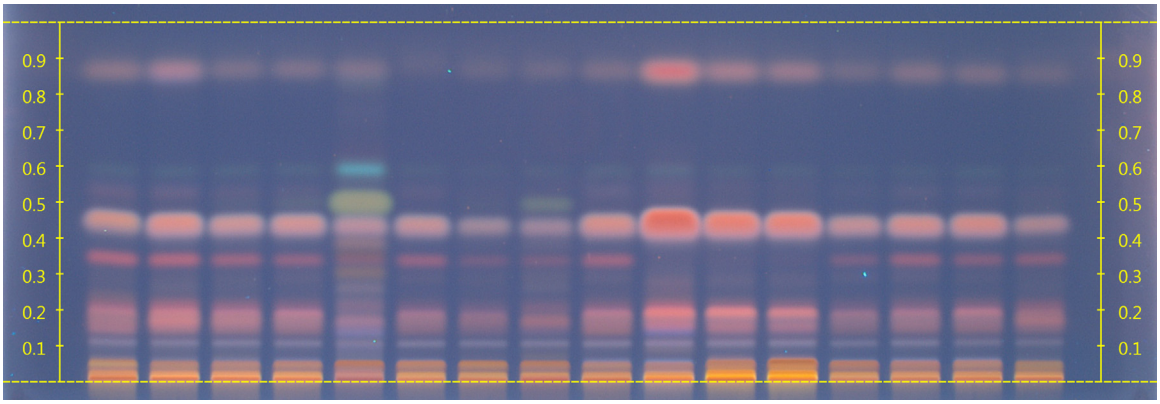

Fig. 11. Derivatised plate under UV 366 nm. Mobile phase dichloromethane (essential oil determination).

Curcuma aromatica or *Curcuma zanthorrhiza*. Sample 34 a TCM product appeared different to other *Curcuma longa* chromatograms and may possibly be *Curcuma kwangsiensis* S.G. Lee and C.F. Liang.

HPTLC was also used to determine if there were any differences between samples that were sold as fresh rhizomes, samples obtained through the integrated chain and those that had been boiled, dried, polished and stored (the traditional method of processing). Our first hypothesis was that the samples obtained through the integrated chain would have a metabolite profile that was closer to that of a fresh rhizome as the boiling, sun-drying, polishing and storing steps were not used. Our second hypothesis was that samples obtained through the traditional route may be adulterated with *Curcuma* species other than *Curcuma longa*.

In Fig. 10, we observed that more compounds were present in the freshest samples 38, 39 and 40. These were fresh rhizomes that had not been boiled or dried. The integrated chain samples 47, 12 and 13 displayed a strong zone at Rf 0.25 which was also observed for samples 38, 39 and 40. There was also a stronger zone for the integrated chain samples and the fresh rhizomes at Rf 0.83. Sample 19 displayed a different pattern, having an extra zone at Rf 0.65 and the bis-demethoxy curcumin zone at Rf 0.2 was missing (consistent with *Curcuma aromatica* and *Curcuma zanthorrhiza*) indicating that this sample was not *Curcuma longa*.

In the HPTLC analysis of the essential oil system sample 19 stood out as being different from the rest of the group and was likely to be *Curcuma aromatica* (Fig. 11). Sample 32 was a polished rhizome from Erode and exhibited a band at Rf 0.5 indicating possible adulteration with *Curcuma aromatica*. Sample 47 (track 2) showed a strong zone at Rf 0.86 that was also observed with the fresh rhizome samples.

4. General discussion

This research both has implications in the context of developing analytical (metabolomic) methods and for the use of such techniques in understanding the interconnectivities of producers and consumers. This has been done in the context of comparing two analytical techniques, ^{1}H NMR spectroscopy and HPTLC, and assessing their relative strengths and weaknesses in connection with the analytical investigation of herbal value chain products (see Table 1).

Both techniques were shown to be useful, but clearly, the metabolomic analysis provided a sharper picture. As is generally the case quality assurance needs to be developed for each botanical drug and, therefore, turmeric is a specific example. As we show below, it is the combination of these two methods which has enabled us to understand both the general variability and the specific differences between products.

For example, a low price for cultivated turmeric can force farmers to store rather than sell (Booker et al., unpublished) and this storage can be detrimental to the product as shown by the comparison with the fresh rhizome samples. In the HPTCL and in the ^{1}H NMR spectroscopy it was observed that adulteration with other species was a problem. Sample 19, sold as *Curcuma longa* was determined to be *Curcuma aromatica* and sample 32, was *Curcuma longa* clearly adulterated with *Curcuma aromatica*. Conversely, we have hypothesised that by establishing a well-managed and well monitored vertically integrated value chain, end-companies can exert a degree of control over processes and procedures that can lead to a better-quality product. Samples obtained from the integrated chain are more similar in composition to the fresh rhizomes tested and free of contamination by other species

Table 1
Comparison of key strengths and weaknesses of 1H NMR spectroscopy and HPTLC.

1H NMR	HPTLC
Equipment expensive	Equipment relatively cheap
High throughput technique with sample preservation	High throughput technique with sample preservation
One system needed to view entire range of extractable metabolites	Three systems needed to view entire range of extractable metabolites
Multivariate analysis is able to group the samples according to the metabolite composition	Grouping of samples is achieved manually by visual inspection
Multivariate analysis can be confounded by multi-ingredient samples leading to errors in groupings	Separate systems and visual inspection reduces grouping errors
Manipulation of data allows for individual samples to be compared in detail against other samples or to the whole group	Comparisons are generally achieved visually

As with other botanical drugs, traditional methods of cultivating and selling *Curcuma longa* can lead to adulteration with incorrect *Curcuma* species. In addition, we have shown that some of the sampled products, specifically the aqueous extracts, contain almost no compounds that would generally be regarded as therapeutically relevant or beneficial. In our study we have observed and measured how some products have different chemical characteristics to others, how some are different species, different extracts and different potencies.

Only two of the medicinal quality *Curcuma longa* products differ from the mass of ground turmeric available as a food ingredient (based on both ^{1}H NMR spectroscopy and HPTCL data). One of these is a Chinese medicinal product and one is from the integrated chain. The ^{1}H NMR spectroscopy and HPTLC data suggest that this is linked to variability in the chemical composition of the essential oil (e.g. concentration of tumerone, zingiberene). It is these more volatile compounds that are likely to be lost during long term storage or due to the heat caused in the traditional grinding process and this data supports the traditional Indian view that the product should be used within three months (Booker, 2012; unpublished fieldwork interviews).

Curcuma longa is an important medicinal and spice plant in terms of its economic significance, and usage within Europe and it has potential as a licensed herbal medicinal product. The quality differences and problems that are inherent within the turmeric market help us to understand better the influence of quality standards on the value chain and how a product may be prone to adulteration, especially when lacking suitable controls and without a vertically integrated system in place.

For the farmers and primary processors, developing and implementing working practices that conform to international requirements is a priority. If high quality raw materials can be produced for the export market, this may lead to a shift in equity towards the beginning stages of the value chain. Moreover, by forming partnerships and alliances with well-established European companies, small farmers may expect greater benefits in terms of governance, training and supply of essential inputs.

EU and WHO guidelines on GAP and Collection Practice (GACP) stipulate that the production of a HMP of acceptable quality begins with the harvest of the starting material. In the global pharmaceutical industry the quality of raw materials has been an important factor for many years but in the paradigm of traditional medicines, the focus has been chiefly on the end stages of supply, with companies investing heavily into packaging, marketing and advertising and other strategies that add value to the finished product. The source and quality of the plant material has in many instances been largely ignored, a problem highlighted in detail in this study.

5. Conclusion and future work

In this study we used two analytical tools, ^{1}H NMR spectroscopy and HPTLC, in order to understand the composition and quality of herbal medicinal products along value chains. Both methods provide different and complementary data and together they can be used as tools to differentiate between a wide variety of crude drug powders and herbal medicinal products. ^{1}H NMR spectroscopy coupled with multivariate analysis provides a quick method of separating different samples but is sometimes fooled by multi-component products or by the addition of excipients. Coupling with HPTLC helps to avoid this pitfall and together the two techniques provide a clear picture of sample composition, offering new ways to understand local livelihoods and the fast developing market links of ethnopharmacological commodities.

These analytical data are interpreted in the context of existing value chains for turmeric. Turmeric medicinal product samples obtained from within a vertically integrated value chain show a closer resemblance to fresh samples of turmeric rhizome with respect to their metabolite content than samples obtained from the general market. Samples of turmeric obtained from a non-integrated chain have shown to be contaminated with different *Curcuma* species other than *Curcuma longa*. Most of the products sold as aqueous extracts contained little trace of compounds that were present in the powder and that would generally be regarded as quality indicators, some contained polysaccharides but the HPTLC analysis confirmed that others only contained simple sugars. Future work should focus on isolating and identifying those compounds that were present only in the fresh samples of turmeric and in the vertically integrated medicinal product. There still are some conflicting results regarding the differences in chemical composition of *Curcuma aromatica* and *Curcuma zanthorrhiza* and how effectively ^{1}H NMR spectroscopy or HPTLC can separate these species and we are currently conducting further research in this area.

This study also provides a proof-of-concept for using combined analytical techniques in the context of value chains of herbal medicines and food supplements. These analytical techniques have shown to be effective platforms for the rapid and accurate determination of plant and plant product composition and future studies should focus on their further development and refinement using them not only as a quality assurance tool at the end of a value chain, but as a method to improve the production and supply of such globalised commodities.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.12.042.

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