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Non-destructive chemical analysis of a *Garcinia mangostana* L. (Mangosteen) herbarium voucher specimen

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ABSTRACT

Herbarium voucher specimens are used primarily for taxonomic confirmation. However, they also afford a record of the metabolic profile of a plant, potentially at the time it was collected, or at the very least, at the time of analysis. Even with the enhanced sensitivity of modern analytical techniques, analysis of the metabolites of a herbarium voucher requires removal and consumption of at least part of an entire specimen. We present herein a non-destructive method to analyze the metabolites of herbarium voucher specimens with the droplet-liquid microjunction-surface sampling probe (droplet probe) coupled to ultra-performance liquid chromatography and high-resolution mass spectrometry. As proof of concept, a herbarium voucher specimen of *Garcinia mangostana* (mangosteen) was utilized due to the well-characterized xanthones biosynthesized by this plant, which are of interest as potential anticancer agents. Also, the juice of the fruits of this plant is used widely in the United States and in other countries as a botanical dietary supplement. Metabolite profiles of the sampled surfaces were compared to a subset of xanthone standards. Using this innovative method on the herbarium voucher specimen, we were able to readily identify cytotoxic prenylated xanthones while maintaining the integrity of the entire specimen.

1. Introduction

Herbarium voucher specimens have been integral to science as far back as 1556, when the Italian botanist, Luca Ghini, first produced them from dried plants, employing sheets of heavy paper bound together and stored vertically, as formal and permanent documentation (Stearn, 1971). Subsequently, Carl Linnaeus in 1735 laid the foundation for taxonomic identification of plants *via* herbarium vouchers (Müller-Wille, 2006). In addition to being a permanent record of a plant, these specimens present a unique opportunity to study plants and other preserved species over time. They are a snapshot that researchers can examine, providing opportunities to look at the phenotype, genotype, and chemotype during a specific window of time (Culley, 2013; Willis et al., 2017), albeit with the recognition that some degradation or other changes may occur to the voucher during storage. Herbarium specimens provide a glimpse into the past that researchers of the present (and perhaps future) are capable of studying with modern and next-generation techniques, documenting and investigating changes over time (Drabkova, 2014; Jiang et al., 2005). However, to do this, some techniques require the destruction of a portion of the sample for DNA and/or metabolite analysis (Willis et al., 2017). While valuable, the sampling process is permanent, visible, and invasive, forever altering the integrity and scientific value of the voucher.

Over the past three years, our team has been utilizing the droplet-liquid microjunction-surface sampling probe (droplet probe) as a tool to examine the chemistry of fungal cultures *in situ*. Essentially, this sampling system dispenses a droplet of approximately 4 μl on the surface of a sample, enabling a microextraction, which can then be analyzed by UPLC-HRMS (Paguigan et al., 2016; Sica et al., 2015, 2016a; Sica et al., 2017). In addition to a suite of applications on fungal cultures, we have also shown its potential in studying various plant parts *in situ*, including fruits, seeds, stems, and even rarely studied flower petals (Sica et al., 2016b). In the current study, we turned our attention to the use of droplet probe for the examination of herbarium vouchers. In principle, they should behave in a manner similar to plant tissues, which have been sampled with this technique previously (Sica et al., 2016b).

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However, due to the delicate, and at times precious, nature of these specimens, we wanted to ensure that the specimen's appearance would not be altered by the analysis, as the physical characteristics of these specimens are critical for taxonomic studies.

Garcinia mangostana L. (Clusiaceae), commonly known as mangosteen, was chosen because this plant is known for its numerous biological applications, including as an antimicrobial, anti-inflammatory, and antioxidant, among many other purposes (Benatrehina et al., 2018; Chen et al., 2008; Chin and Kinghorn, 2008; Chin et al., 2008; Williams et al., 1995). Mangosteen has been used traditionally to treat disorders ranging from skin infection to dysentery to abdominal pains (Ibrahim et al., 2016). Xanthones are the major metabolites that have been isolated and investigated from this plant. Modern studies have shown antibacterial, antifungal, antihistamine, anti-HIV, antineoplastic, aromatase inhibitory, cancer chemopreventive, and cytotoxic activities from this class of compounds (Balunas et al., 2008; Chairungsrilerd et al., 1996; Chen et al., 1996; Chitchumroonchokchai et al., 2013; Chomnawang et al., 2007, 2009; Gopalakrishnan et al., 1997; Han et al., 2009; Ibrahim et al., 2016; Jung et al., 2006; Matsumoto et al., 2003; Phongpaichit et al., 2006; Suksamrarn et al., 2003; Tousian Shandiz et al., 2017).

In this study, we investigated six xanthones of mangosteen: α-mangostin (**1**), β-mangostin (**2**), γ-mangostin (**3**), gartanin (**4**), 9-hydroxycalabaxanthone (**5**), and cratoxyxanthone (**6**) (Fig. 1) (Ji et al., 2007; Walker, 2007). Many of these compounds are found in one or more of the pericarp, stem, and whole fruit of *G. mangostana* (Obolskiy et al., 2009). Using a herbarium voucher specimen of *G. mangostana*, our goal was to extract chemical knowledge *via* the droplet probe technique without marring the physical appearance of the voucher.

2. Results and discussion

The main objective of this project was to identify prenylated xanthones from the herbarium voucher specimen of *Garcinia mangostana*, as compared to isolated reference standards, without damaging the voucher. The sampling of the herbarium voucher specimen was performed using the droplet probe technique, in which a droplet of approximately 4 µl of 50:50 DMSO:H_2O was dispersed onto the surface of the sample. Metabolites from the herbarium voucher specimen were dissolved and analyzed using the droplet probe system (Fig. 2).

The herbarium voucher was sampled at four sites of both the leaf and the stem (Fig. 3). Essentially, the droplet probe performs a microextraction from the surface of the voucher, and the concentrated droplet is then analyzed *via* UPLC-HRMS. This permitted a comparison of chromatographic and spectrometric data to that of the reference standards (Figs. 1 and S1), while keeping the whole sample intact and undamaged in appearance for taxonomic posterity.

A herbarium specimen is important for future taxonomic studies. To demonstrate this innocuous property of this technique, photographic close ups of the voucher, both before and after droplet probe analysis are included (Fig. 4). Chemical sampling of the *G. mangostana* voucher *via* droplet probe was non-destructive, and at least at the optical level, deterioration of the specimen was not observed. This was important since a non-destructive chemical analysis of a voucher has not yet been reported, although recently there has been a non-destructive analysis of DNA from herbarium specimens (Shepherd, 2017).

Previous methods from our laboratory have utilized water and methanol as the solvent combination for droplet microextractions (Paguigan et al., 2016; Sica et al., 2015, 2016a,b), but the xanthones were found to be semi-soluble in methanol and not at all soluble in water. The droplet probe technique relies on droplets that contain water, which maintains surface tension when making contact with the sample. Thus, DMSO was included in the microextraction solvent because of its improved solubility with the xanthones.

In preliminary studies, a total of five microextractions per droplet were used to sample the herbarium voucher specimen; however, the xanthones were not well detected. In order to concentrate the microextraction further, the number of microextractions was increased by five until α-mangostin (**1**), β-mangostin (**2**), and γ-mangostin (**3**) could be detected consistently on the herbarium voucher specimen. In total, 15 microextractions per droplet were performed, which was three times more than required previously with fungal cultures (Sica et al., 2015).

The ability to retain a droplet on the tip of the syringe and concentrate the microextractions *via* repeated sampling is a benefit of the droplet probe system. However, in doing so, some amount of the solvent may be retained on the sample, and this could result in inconsistencies and variability in sampling. Therefore, we sought an internal standard that could be used to normalize the data, and to solve this

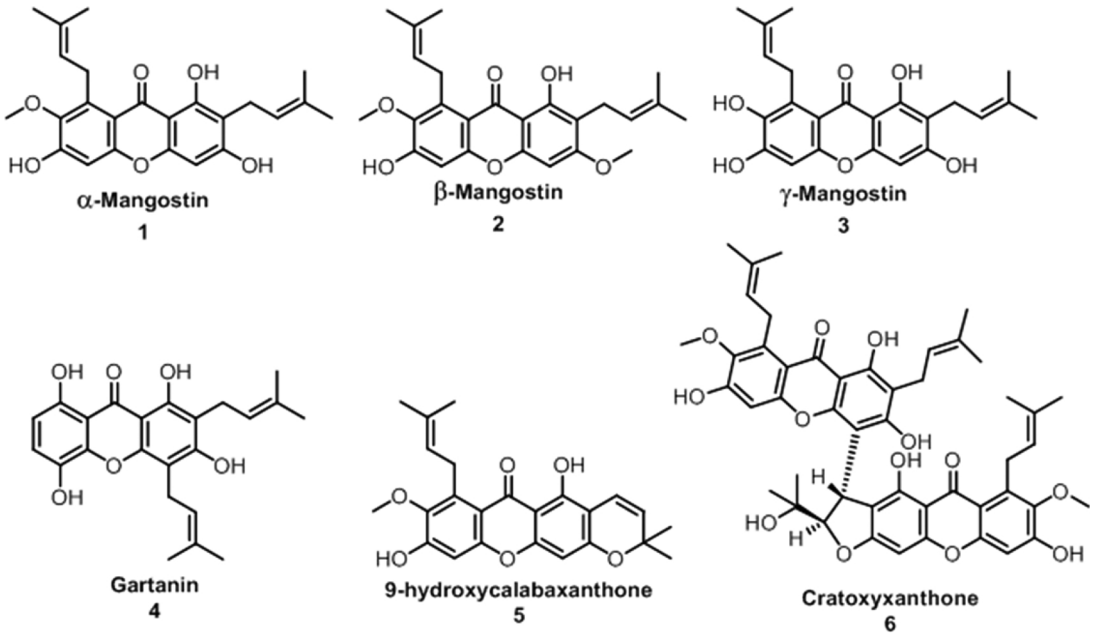

Fig. 1. Six prenylated xanthones previously isolated from *Garcinia mangostana* fruit or stem bark (Han et al., 2009; Jung et al., 2006). These were used as reference standards when analyzing the droplet probe data from the herbarium voucher.