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Comparative characterization of nucleotides, nucleosides and nucleobases in *Abelmoschus manihot* roots, stems, leaves and flowers during different growth periods by UPLC-TQ-MS/MS

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ABSTRACT

Nucleotides, nucleosides and nucleobases have been proven as important bioactive compounds related to many physiological processes. *Abelmoschus manihot* (L.) Medicus from the family of Malvaceae is an annual herbal plant of folk medicine widely distributed in Oceania and Asia. However, up to now, no detailed information could be available for the types and contents of nucleotides, nucleosides and nucleobases contained in *A. manihot* roots, stems, leaves as well as the flowers. In the present study, an UPLC-TQ-MS/MS method was established for detection of the twelve nucleotides, nucleosides and nucleobases. The validated method was successfully applied to identify the 12 analytes in different parts of *A. manihot* harvested at ten growth periods. 2′-deoxyinosine was not detected in all of the *A. manihot* samples. The data demonstrated that the distribution and concentration of the 12 compounds in *A. manihot* four parts were arranged in a decreasing order as leaf > flower > stem > root. Based on the results, the leaves and flowers of *A. manihot* could be developed as health products possessed nutraceutical and bioactive properties in the future. This method might also be utilized for the quality control of the *A. manihot* leaves and other herbal medicines being rich in nucleotides, nucleosides and nulecobases.

1. Introduction

Nucleotides, nucleosides and nucleobases are the basic components of all cells, and have been proven as important bioactive compounds related to many physiological processes ranging from cardiovascular activity to neurotransmission to cellular proliferation [1]. For instance, nucleosides and their corresponding nucleotides play significant roles in cell physiology by functioning as both nutrients and modulators of cellular homeostasis [2]. Meanwhile, these compounds exhibit multiple pharmacological activities such as anti-arrhythmic [3], anti-cancer [4], anti-viral effects [5] and repairing ischemia/reperfusion induced vascular damage [6]. In addition, nucleotides, nucleosides and nucleobases have also been selected as the quality control marker of several traditional Chinese medicine and functional foods, such as *Cordyceps sinensis* [7], *Mactra veneriformis* [8] and royal jelly [9].

Abelmoschus manihot (Linn.) Medicus from the family of Malvaceae is an annual herbal plant of folk medicine and widely distributes in Papua New Guinea, Vanuatu, Fiji, New Caledonia, and China [10]. Modern studies have shown that the roots and aerial parts of *A. manihot* are extensively used in traditional medicine [11]. For example, *A. manihot* flowers, the main medicinal part, have been used in clinical practice for the treatment of chronic glomerulonephritis, oral ulcers and burn in China for centuries [12,13]. *A. manihot* leaves have been demonstrated that it had the analgesic activity as well as the providing bone-sparing effect [14,15]. The larvicidal activity of the roots has been reported by using the larvae of mosquitoes in the genera Anopheles and Culex [16]. Additionally, the root juice is applied to treat sprains in Nepal [11]. *A. manihot* stems own the bioactivities of anti-inflammatory and wound healing [17,18]. Collectively, these examples highlight the activities of *A. manihot* in many medical conditions.

Up to now, information on the types and contents of nucleotides, nucleosides and nucleobases contained in *A. manihot* roots, stems, leaves as well as the flowers remains unknown. The exploration of

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the profiles of these compounds in *A. manihot* would be very helpful for improving the potential values of *A. manihot* as food or other uses, and also be convenient for their quality control. Moreover, the most common application mode of *A. manihot* in the present is picking its flowers for medicine, which no doubt causes enormous waste of resources and environmental pollution as numbers of *A. manihot* roots, stems, leaves are discarded or burned.

The detection and quantitation of nucleotides, nucleosides and their bases have become increasingly important in the field of phytochemistry and biomedical research. So far, a large number of methods have been established for their determination, including ion-pairing liquid chromatography (IPIC), capillary electrophoresis (CE), flow injection-chemiluminescence (CL), and GC–MS/MS [19,20]. However, some of these mentioned methods have the disadvantages of time-consuming, high cost, low sensitivity, and complicated pretreatment. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) has become state-of-the-art technology for nucleotides, nucleosides and nucleobases analyses, and over the past few years this approach has superseded earlier methods by providing increased selectivity, higher sensitivity, reduced sample preparation, and shortened assay times [21]. In our research, we applied the ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-TQ-MS/MS) to separate and detect 12 nucleotides, nucleosides and nucleobases, which could show great advantages such as adequate retention of highly polar compounds; rapid and excellent separation over traditional HPLC; specific and sensitive ESI–MS/MS detection using MRM transitions to exclude false-positive results.

In this study, we performed a complete research on four parts of *A. manihot* at different growth periods with the aim of highlighting the similarities and/or differences in the composition of nucleotides, nucleosides and nucleobases in order to on one hand screen and identify organ specific marker compounds and on other hand explore the growth tendency of *A. manihot*. The results will be useful for the utility value of *A. manihot* disused parts.

2. Experimental

2.1. Chemicals and materials

Acetonitrile and formic acid (HPLC-grade) were purchased from Merck (Darmstadt, Germany), and deionized water (H_2O) was purified by an EPED superpurification system (Eped Technology Development Co., Ltd., Nanjing, China). Ammonium acetate and ammonium formate were of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Chemical standards of thymidine (1), thymine (2), 2′-deoxyuridine (3), adenine (6), 2′-deoxyinosine (7), inosine (8), cytidine (9), 2′-deoxyadenosine-5′-monophosphate (11) and cytidine-5′-monophosphate (12) were from Sigma Chemical Co. (St. Louis, MO, USA). Reference compounds of uracil (4), 2′-deoxyadenosine (5) and guanine (10) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of each compound was above 98%, determined by HPLC analysis. The chemical structures of these reference compounds are shown in Fig. 1.

2.2. Sample collection and preparation

The samples of *A. manihot* were harvested in Herbal Plantation of Nanjing University of Chinese Medicine from July 20 to October 20, 2014 in 10 periods of growth (G1–G10). After collection, each sample was divided into four parts (roots, stems, leaves and flowers)

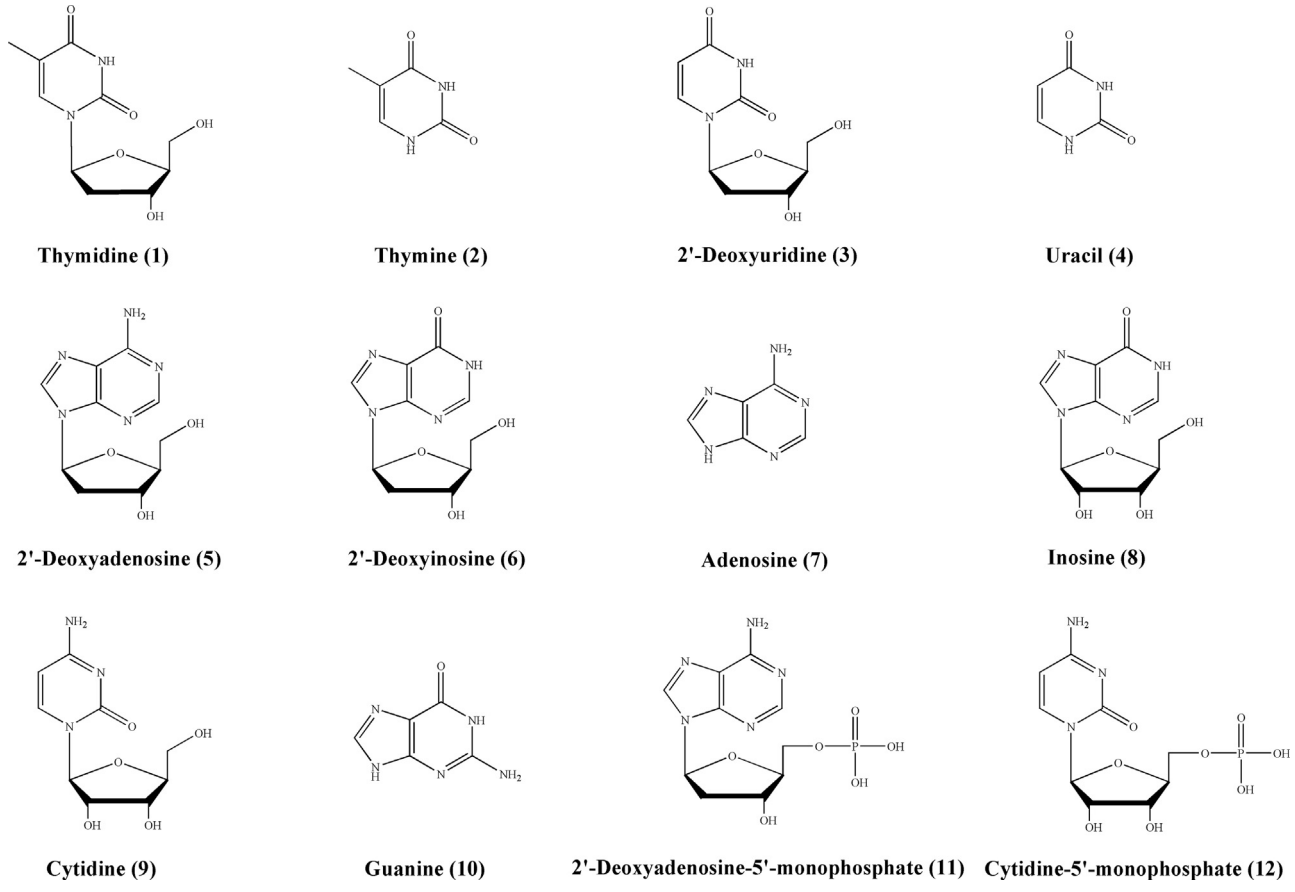

Fig. 1. Chemical structures of the 12 identified compounds.