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Original Article

Effect of storage time on metabolite profile and alpha-glucosidase inhibitory activity of *Cosmos caudatus* leaves – GCMS based metabolomics approach

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ABSTRACT

Cosmos caudatus, which is a commonly consumed vegetable in Malaysia, is locally known as "Ulam Raja". It is a local Malaysian herb traditionally used as a food and medicinal herb to treat several maladies. Its bioactive or nutritional constituents consist of a wide range of metabolites, including glucosinolates, phenolics, amino acids, organic acids, and sugars. However, many of these metabolites are not stable and easily degraded or modified during storage. In order to investigate the metabolomics changes occurring during post-harvest storage, *C. caudatus* samples were subjected to seven different storage times (0 hours, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, and 12 hours) at room temperature. As the model experiment, the metabolites identified by gas chromatography-mass spectrometry (GC-MS) were correlated with α-glucosidase inhibitory activity analyzed with multivariate data analysis (MVDA) to find out the variation among samples and metabolites contributing to the activity. Orthogonal partial least squares (OPLS) analysis was applied to investigate the metabolomics changes. A profound chemical alteration, both in primary and secondary metabolites, was observed. The α-tocopherol, catechin, cyclohexen-1-carboxylic acid, benzoic acid, myo-inositol, stigmasterol, and lycopene compounds were found to be the discriminating metabolites at early storage; however, sugars such as sucrose, α-D-galactopyranose, and turanose were detected, which was attributed to the discriminating metabolites for late storage. The result shows that the MVDA method is a promising technique to identify biomarker compounds relative to storage at different times.

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1. Introduction

The importance of plants to human life can be seen in their diverse utilization, such as medicine and food. Recently, researchers have been trying to profile their active compounds to scrutinize their beneficial effects. Plants form a major part of the diet of many consumers worldwide, because of the diverse health benefits that can be derived from them [1]. These health benefits have been confirmed by different studies which indicate a sharp decline in the occurrences of certain diseases along with increased consumption of plant-based foods [2,3].

Diabetes mellitus is a disease which is characterized by the increased level of blood sugar (hyperglycemia) caused by the inability of the body to produce or use insulin, a hormone responsible for the regulation of blood glucose. Diabetes mellitus, indeed, takes the largest proportion in terms of prevalence among the endocrine disorders globally. An estimated 382 million people were reported to be living with this disease in 2013 and this figure is projected to increase to about 592 million by 2035 [4]. This disease can be divided into two kinds, namely type 1 (insulin-dependent) and type 2 (noninsulin-dependent). Although some antidiabetic drugs are effectively used as medicine [5,6], they have some serious side effects, which obligate finding alternative ones. For example, miglitol and voglibose [7], which act as inhibitors of α-glucosidase activity [8], are available commercially. The prolonged usage of these medications is frequently associated with undesirable side effects, including liver toxicity and other gastrointestinal symptoms [7,9]. Therefore, the search for effective and safe α-glucosidase inhibitors from natural sources in order to develop a physiologically functional food, or to design drugs for use against diabetes, is of great interest. Several medicinal plants are used in different parts of the world for the treatment of diabetes. Numerous studies have shown the potency of the crude extracts of different plants as well as specific bioactive compounds in lowering blood glucose levels [10]. The ability of polyphenolic compounds in plants to inhibit the activities of digestive enzymes due to their potential to form complexes with protein has been reported [11]. *Cosmos caudatus* is originally from Mesoamerica, and is a well-known herb and vegetable in Malaysia and other tropical countries [12]. In the Malay society, it is called "Ulam Raja", and is consumed raw or cooked, which adds a pleasant aroma and taste to food. It belongs to the Asteraceae family and has several varieties [12,13]. All of its parts are traditionally used for several purposes, including food additives, medicines, and perfumes [12,14]. Profiling data for the investigation of the role of metabolites is necessary to generate reliable metabolites. Thus, further research is required to evaluate the metabolites content and their efficiencies. Many approaches have been successfully used in analyzing and evaluating the metabolites variation of organism, such as metabolomics.

The metabolomics approach can be defined as qualitatively and quantitatively comprehension assessment of all existing metabolites and their variation in biological systems of organisms [15–17]. Metabolomics has also been used for monitoring plant metabolic changes [18]. In many physiological circumstances, a wide range of metabolites were found to be changed, including phenolic compounds, amino acids, fatty acids, organic acids, carbohydrates, and sterol based compounds [19]. Changes in environmental patterns and processing methods can alter metabolic contents [20]. Medicinal herbs are usually subjected to long time storage in the chain of production of various products [21–23]. Studies to determine the effect of storage on medicinal plants provide important information, not only for traditional healers and consumers, but also in designing sustainable harvesting methods for these plants.

With improved knowledge of the "shelf-life" of plants used in traditional medicine, better sustainable harvesting measures can be implemented [24]. This differentiation can be measured via the metabolomics approach based on several techniques and the implementation of sophisticated statistical analysis. Gas chromatography-mass spectrometry is one of the most utilized techniques for analyzing and identifying the constituents of biological samples [25,26]. However, there is no published data currently available on the effect of storage time on antidiabetic activity of this herb. Therefore, the aim of the present study is to determine the effect of storage time on α-glucosidase inhibitory activity of *C. caudatus* samples by using the GC-MS based metabolomics approach. This information acquired will be useful as the basis for recommendation regarding the suitability of the storage time in order to preserve the metabolic features and beneficial values of the herbal material.

2. Materials and methods

2.1. Plant material

Seeds of *C. caudatus* were obtained from the nursery at the Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia and identified by voucher specimens (SK 1934/11), before being deposited to herbarium of the Laboratory of Natural Products, Universiti Putra Malaysia. The planting was implemented in an open plot, which was divided into seven sections, each one containing seven plants. The necessary needs and care for good germination were well provided. For sampling, seven replicated leaf samples of the 8-week-old plants were randomly collected in the early morning from each section. Any damaged leaves were discarding by using laboratory scissors [27]. For consistency in the resulting data due to possible technical differences in the sampling and sample preparation, these processes were done in groups and in a parallel manner.

2.2. Preparation of plants

The collected leaves were washed and dried with tissue paper to remove any debris prior to immediate grinding with a mortar and pestle under liquid nitrogen. *C. caudatus* samples were divided into seven parts; one part was immediately used for the preparation of the extract and the other parts were kept at room temperature for different times (2 hours, 4 hours, 6 hours, 8 hours, 10 hours, and 12 hours) before extraction. The samples were then subjected to freezing in a deep freezer (−80°C) and freeze drying for 3 days. The dried matrix was ground and sieved through a sieve of 0.70 mm particle size and then the obtained fine powder was kept in a chiller at 4°C before further