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INTEGRATED GENETIC, METABOLOMIC AND BIOACTIVITY ANALYSES FOR THE DISCRIMINATION OF *Piper sarmentosum* IN PENINSULAR MALAYSIA

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ABSTRACT

Piper sarmentosum Roxb. (Piperaceae) or locally known as *Kadok* is a well-known traditional medicinal plant widely used in Southeast Asia particularly for managing diabetes. Despite extensive pharmacological studies, most have focused on crude extracts, providing limited information on genetic diversity and the correlation between metabolite composition and a-amylase inhibitory activity. This study investigates *Piper sarmentosum* collected from across Peninsular Malaysia using a multi-platform approach that integrates genetic analysis, metabolomics (LCMS and NMR), and a-amylase inhibition activity assessment. The aim is to evaluate the genetic diversity and its influence on metabolite composition and a-amylase inhibitory activity, with the goal of identifying bioactive marker compounds and developing a predictive model for quality control. Genetic analysis using simple sequence repeats (SSR) markers on 173 samples from over 80 locations revealed low clonal diversity, grouping the samples into Cluster A comprising five clones (Clone 1 to Clone 5) and a genetically variable Cluster B. Samples of the five identified clones of Cluster A were selected for chemical profiling using NMR and LCMS. LC-MS base peak chromatograms (BPCs) showed distinct profiles between the clones, whereas NMR fingerprints appeared similar. LCMS-based dereplication using SIRRIS identified 41 metabolites, mainly piperamides and flavonoids, mainly piperamides and flavonoids, and was validated with five isolated compounds namely andamanicin, a-asarone, N-[3-(4-methoxyphenyl)propanoyl] pyrrole, N-(3-phenylpropanoyl)pyrrole and sarmentosine. The compiled NMR spectral data of the dereplicated metabolites were established and used as reference profiles to identify corresponding signals within the crude extracts. A total of 30 metabolites comprising 18 secondary and 12 primary metabolites were successfully assigned in the ^{1}H NMR spectra of the *P. sarmentosum* clone extracts. Multivariate analysis (PLS-DA and HCA) using NMR data revealed two main groups: Group 1 (Clones 1, 4, 5) and Group 2 (Clones 2, 3). Meanwhile, LCMS-based PLS-DA showed clearer separation into three clusters: Group 1 (Clone 4), Group 2 (Clones 1 and 5), and Group 3 (Clones 2 and 3). Of the 41 dereplicated metabolites, 23 discriminatory metabolites (VIP> 1.00, $p<$ 0.05) distinguished the clones, including N-isobutyl-2£',4$_{J}$E'-hexadecadienamide, pellitorine, futoamide, dodeca-2£',4£'-dienoic acid isobutylamide, N-2'-methylbutyl-2£',4$_{J}$E'-decadienamide, and 9-(l,3-benzodioxol-5-yl)-l-pyrrolidin-l-ylnon-8-en-l-one. Bioactivity assessment revealed that a-amylase inhibitory activity at 100 ppm ranged from 54.89% to 80.35% across the 119 samples. One-way ANOVA *(p=* 0.0417) confirmed significant variation between clones, with Clones 1, 4, and 5 showing higher inhibitory activity. Interestingly, this trend was consistent with the NMR-based metabolomic data. Thus, a PLS predictive model for a-amylase inhibitory activity was constructed using NMR data. Key metabolites correlated with activity included piperolactam A, N-(3-phenylpropanoyl)pyrrole, N-2'-methylbutyl-2£',4$_{J}$E'-decadien amide, brachiamide B, maleic acid, N-(3-(4-methoxyphenyl)propanoyl)pyrrole, pellitorine, and andamanicin. Most of these compounds were mostly abundant in Clones 1, 4, and 5. The predictive model showed high accuracy, with a root mean squared error of prediction (RMSEP) of 5.89. This research has established a comprehensive framework for studying *P. sarmentosum* that integrates genetic analysis, advanced metabolomics, and bioactivity modelling, offering a potential quality control strategy to identify superior clones with optimal medicinal properties and thus minimizing the need for routine bioactivity testing.

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CHAPTER 1
INTRODUCTION

1.1 *Piper sarmentosum* Roxb.: A Promising Herbal Medicine

For all of humanity across the world, traditional medicines have served as a remedy for various ailments and health issues for thousands of years. In recent times, there has been a renewed interest in an evidence-based assessment of the value of traditional medicines, promoted by the World Health Organization (WHO, 2013), and driven by a growing fascination with natural remedies and a preference for holistic healthcare approaches. A notable advantage of traditional medicines is the accessibility (availability and affordability) to populations in remote and rural areas who lack access to contemporary medical facilities and medications (Maroyi, 2013; Truter, 2007). Embedded within these communities, traditional healers possess an intimate understanding of available plant materials as well as local customs and traditions, enabling them to deliver culturally appropriate care tailored to individual needs. Often perceived as safe and efficacious, there have been relatively few cases of reported adverse effects resulting from the usage of plant-based medicinal products (Barnes, 2016). These adverse effects could potentially arise from various sources, including contamination of the products with toxic metals, the addition of pharmacologically active synthetic compounds, the mi si dentifi cation or substitution of plant components, inadequate or inappropriate processing, the preparation of products, and medicinal plant-drug interactions (Azam et al., 2018; Ba§aran et al., 2022; Basher et al., 2018). Recognizing these concerns, there is now a growing institutional (e.g., World Health Organization, European Commission, etc.) and public demand for elevation in the overall quality, safety, demonstrated efficacy, and consistency of plant-based medicines for the patient (WHO, 2013). This effort is cantered on the implementation of rigorous standardization practices and comprehensive traceable quality control measures applied to both the sustainability of the raw materials and the final products, and which relate to safety and efficacy (Daley & Cordell, 2022).

The traditional production techniques used in the medicinal plant industry often result in high yield losses, and the original quality of the plant materials can be