LC-MS profiling of *Moringa oleifera* identifies structurally unreported metabolites of potential pharmacological interest

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ABSTRACT

Introduction: Natural products have played an essential role as therapeutic agents for many years and are the foundation of modern drug discovery. Due to its diverse chemical constituents and its reported pharmacological properties, *Moringa oleifera* is a medicinal plant of considerable interest. Phytochemicals have been identified from its leaves but existing studies mainly described familiar compounds, with the plant's chemical composition insufficiently explored. As a result, metabolites that are found in low abundance or have unusual structural characteristics may not be detected. Comprehensive chemical profiling is therefore required to expand our knowledge of the phytochemical diversity of *M. oleifera* by using biochemical profiling to identify compounds that may have been overlooked in previous investigations. To evaluate the chemical composition of aqueous *M. oleifera* leaf extract and to determine known and potentially unreported metabolites, high-resolution LC-MS was employed. **Materials and Methods:** Shade-dried *M. oleifera* leaves were powdered and extracted in distilled water under ambient conditions, then filtered and lyophilized. The dried extract was reconstituted in HPLC-grade water and analyzed by high-resolution LC-MS/TOF-MS in positive ion mode. Injection volumes of 100 µL and 1000 µL were tested to optimize sensitivity and reproducibility. Chromatographic separation was performed on a reverse-phase C18 column using a linear acetonitrile water gradient with 0.1% formic acid. Molecular features were detected through automated peak-picking, refined by manual verification and identified using accurate mass, isotopic distribution, retention time and comparison with spectral databases and published reports. Compounds not previously described in *M. oleifera* LC-MS datasets were flagged as novel or underreported. **Results:** The dual-volume LC-MS approach yielded 83 reproducible molecular features spanning flavonoids, alkaloids, triterpenoids, coumarins, anthraquinones, glycosides and phenolic acids. Chromatographic peaks appeared between 0.5 and 11 minutes of retention time, with most detected in the 2-9 minutes range. Flavonoids dominated the profile with high-intensity peaks for quercetin, kaempferol derivatives and rutin as well as several structurally distinct flavonoid acetates. Alkaloids and triterpenoids formed the second-largest groups which are rarely reported in the *M. oleifera* literature. Two high-abundance constituents were identified for the first time in this species: Picrasidine S which is an indole alkaloid and Epigallocatechin hexaacetate which is an acetylated flavonoid. Both appeared among the top ten LC-MS peaks. Their absence from earlier phytochemical reports shows the value of high-resolution profiling for detecting structurally uncommon metabolites. Other important identifications were Lucidenic acid A, Scopolin, Emodin-8-O-sophoroside and Kuwanon A which expand the known chemical diversity of *M. oleifera.* Retention times and peak intensities were reproducible across injection volumes which confirmed the stability of the extract composition and the robustness of the method. **Conclusion:** This study provides one of the most detailed LC-MS-based phytochemical profiles of *M. oleifera* to date, capturing both abundant and trace-level metabolites with high analytical confidence. The discovery of previously unreported compounds including Picrasidine S which is an indole alkaloid and Epigallocatechin hexaacetate which is an acetylated flavonoid that also enriches the chemotaxonomic understanding of the species and shows the value of high-resolution mass spectrometry in natural product research. These findings establish a validated reference dataset for future pharmacological, metabolomic and quality-control studies on *M. oleifera*.

Keywords: *Moringa oleifera*, LC-MS profiling, Phytochemistry, secondary metabolites, novel compounds