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Analysis of cardiac glycosides in "suicide tree" kernel

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Objective: *Cerbera odollam* (also known the suicide tree, pong-pong tree, and othalanga among other names) is an evergreen tree that bears fruits resembling mango. It is widely distributed around India and Southeast Asia. Since this tree contains several highly toxic cardiac glycosides, its kernel is sometimes used to commit suicide, and the plant is therefore known as the "suicide tree" in some regions. Gaillard *et al*. reported that between 1989 and 1999, in a single Indian state, approximately 537 people died after ingesting the kernel of the tree's fruit. *C. odollam* has possibly been used for homicide; however, ingestion of its poisonous cardiac glycosides is difficult to prove. There are insufficient data regarding aspects such as detection methods, toxicokinetics, and metabolism of these poisonous compounds. Therefore, we attempted to develop a detection method for cardiac glycosides derived from these kernels.

Methods: Dried fruits of *C. odollam* were purchased via the online shop. Fruit kernels were cut into small pieces before adding ethanol. The mixture was then homogenized in a bead beater-type homogenizer to extract cardiac glycosides into the ethanol. The extract was directly analyzed by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS, SCIEX Triple TOF 5600) for comprehensive data acquisition.

Results: We devised an analytical method for the detection of cardiac glycosides using LC-QTOF-MS and successfully detected the known cardenolide-type cardiac monoglycosides, cerberin, neriifolin, tanghinin, and deacetyltanghinin, from *C. odollam* kernel extract. From the comprehensive data acquired by LC-QTOF-MS, we detected possible diglycoside-, triglycoside-, and tetraglycoside-type compounds, and found that the triglycoside-type compounds produced the most abundant peaks. We also detected acetylated and dehydrogenated forms of these compounds.

Conclusion: We successfully devised a method for the detection of cardiac glycosides using LC-QTOF-MS, and detected several cardenolide-type cardiac glycosides in *C. odollam* kernel extract. We also found that these compounds mainly exist as triglycosides in the kernel. In future, this method will be applied to the detection of cardiac glycosides in victims' biological materials.