DEVELOPMENT OF A HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) – DENSITOMETRIC METHOD FOR THE ESTIMATION OF THE BIOACTIVE CONSTITUENTS IN *Centella asiatica* (TAKIP-KUHOL) EXTRACT

HERLENE MAE BAHIAN CANTORES

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University of the Philippines Visayas

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ABSTRACT

A high-performance thin-layer chromatography – densitometric method was developed for the estimation of the amount of madecassoside, asiatioside, madecassic acid and asiatic acid in the aerial part of *Centella asiatica*. The developed method is useful in quantifying the compounds from different plant accessions and can be applied in routine analysis and quality assurance of related extracts, drugs and polyherbal formulations. The study involves the development of an extraction method by assessing four parameters; mobile phase, extraction solvent, extraction time and serial extraction. The repeatability and the linearity of the developed method were also assessed. Results showed madecassoside and asiaticoside can be separated using dichloromethane: methanol: water (14:6:1 v/v/v) and toluene: ethyl acetate: formic acid (5:5:1 v/v/v) yield well-separated bands for asiatic acid and madecassic acid. Moreover, high yield of the active compounds can be extracted using 80% ethanol as the extraction solvent; ten minutes as the extraction time; use of sonication in the extraction; until third extraction. The method developed was shown to be repeatable with inter- and intra-day precision (%RSD) lower than 1.089%. Furthermore, the method was shown to be linear within the given range having an R^2 not lower than 0.9988 and r value above 0.9990. The method was applied to the two plant accessions and it was obtained from the analyses that the compounds differ in the amount of the active constituents. However, madecassoside was found to be abundant in both plant samples (Panaad: 1291.9 (±1.2); TQP: 1104.60 (±0.20)).