

CHARACTERIZATION, QUANTIFICATION AND BIOACTIVITIES OF POTENTIAL NEUROPROTECTIVE PHYTOCHEMICALS OF *Centella asiatica* (GOTU KOLA)

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Natural ethnic foods with traditionally known health benefits offer a substantive foundation for the discovery of functional foods with therapeutic potentials. *Centella asiatica* (Gotu Kola) is a green leafy vegetable and a medicinal herb in few countries of the world. Complementing its reputation in traditional medicine as a memory booster, research backed multifunctional properties make it a promising potential agent to target multiple pathways in neurodegenerative diseases such as Alzheimer's. Been rich with diverse phytochemicals, the occurrence of chemotype variations of this plant are reported in the production of these metabolites due to origin and growth conditions. Current study intended to optimize extraction, chemical profiling, targeted quantification and providing an insight into bioactivities of potential neuroprotective phytochemicals in *C. asiatica* extracts.

This study characterized the phytochemical profile of *C. asiatica* leaf extract; liquid and volatile fractions and quantified four major bioactive triterpenes and chlorogenic acid, of a variety found in New Zealand. Among the tested extraction techniques, ultrasound assisted extraction and steam distillation were chosen to extract liquid and volatile fractions. Q-extractive orbitrap high-resolution mass spectrometry and gas chromatography-mass spectrometry enabled profiling of phytochemicals in liquid and volatile fractions respectively. Initial HPLC-DAD quantification was followed by a multiple reaction monitored (MRM), UHPLC-ESI-triple quadrupole tandem mass spectrometry to further confirm and to enable more specific and sensitive determination of major saponins and saponogenins together with chlorogenic acid. Simultaneously, in-silico, in-vitro and in-vivo bioactivity testing; acetylcholine esterase inhibition, anti-amyloid aggregation and anti-oxidant potential, evaluated the possible target pathways of bio-activities.

The liquid fraction comprised of pentacyclic triterpenoid compounds; mainly the ursane and oleanane subtypes, and flavonoids. Sesquiterpenoids and monoterpenoids included as the significant compounds in essential oil fraction. *C. asiatica* leaf extracts contained high amounts of triterpenic glycoside asiaticoside, followed by madecassoside and chlorogenic acid. However, insignificant quantities of aglycone levels, compared to those reported elsewhere, indicated the occurrence of distinct chemotype of *C. asiatica* in New Zealand. Bioactivities indicated the potential of *Centella asiatica* as a functional brain food in promoting general neuroprotection as well as arresting multiple disease pathways in neurodegenerative disorders.

Keywords: *Centella asiatica*, Neuroprotective, Ultrasound-assisted, Mass spectrometry, Anti-amyloid