



Research Report

**Preparation of asiatic acid and madecasic acid
enriched *Centella asiatica* extract for formulation of
anti-oral mucositis preparation**

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ABSTRACT

Centella asiatica is a folk medicine that possesses a wide range of pharmacological properties, especially for wound healing and brain enrichment. Pentacyclic triterpenes; asiatic acid, madecassic acid, asiaticoside, and madecassoside were reported as the active compounds. The present studies also evaluated a microwave-assisted extraction (MAE) for the four pentacyclic triterpenes as well as developing a method for preparing a pentacyclic triterpenes-rich extract from *C. asiatica*. MAE was capable of increasing the yield of the pentacyclic triterpenes up to twice that produced by the heat reflux method, and it was also much less time consuming. The optimal conditions of MAE employed were: extraction with absolute ethanol as solvent, an irradiation power of 600 W, at 75° C, with four irradiation cycles, and four extraction times. Here we also provide a simple method for preparation of the pentacyclic triterpenes-rich *C. asiatica* extracts (PRE), which contained not less than 65% w/w total pentacyclic triterpenes. The method involved a macroporous resin (Diaion® HP-20) column eluted with ethanol, and a decolourisation step with activated charcoal. Biological activity evaluations of the standardized PRE revealed that the extracts possessed antibacterial activity against *Streptococcus mutans*, *S. mitis* and *S. pyogenes*, with its potency almost equal to those of madecassic acid; anti-inflammatory activity *via* inhibition of nitric oxide production by murine macrophage-like RAW264.7 cells, with an IC₅₀ value of 64.6 µg/mL; tyrosinase inhibitory activity with an IC₅₀ value of 104.8 µg/mL. Unfortunately, the PRE showed weak antioxidant activity *via* DPPH radical scavenging assay, with an IC₅₀ value of 348.3 µg/mL. The biological activities of the PRE suggest the potential use of the PRE as a natural active ingredient for medicines, nutraceuticals and cosmeceuticals.

Stability evaluations of the PRE through a period of 4 months found that the PRE were most stable when kept in a well-closed container protected from light and stored at 4 °C. The PRE that were exposed to light were not stable after 2 weeks of storage. Under accelerated conditions at 45°C with 75% relative humidity, the PRE were not stable after 2 weeks of storage. Study on the effect of pH on the stability of the aqueous alcoholic solution of the PRE found that the solutions were stable at pH 5.8 and 7.0, but not stable at pH 8.2. Thus, preparation of the PRE in an aqueous solution should be performed in weak acidic or neutral conditions.

Distribution of pentacyclic triterpenes accumulated in various parts of *C. asiatica* as well as effects of five different places for cultivation and three different harvesting periods on pentacyclic triterpene content were determined. The separate plant parts selected were; leaves,

stolons, petioles, flowers, fruits and roots. Among the various parts of *C. asiatica*, the leaves contained the highest amount of pentacyclic triterpenes with a total content of pentacyclic triterpenes of 19.5 ± 0.9 mg/g dry powder. The contents of the pentacyclic triterpenes in *C. asiatica* also varied according to the place of cultivation and the harvesting period. *C. asiatic* collected from Trang gave the highest content of total pentacyclic triterpenes (37.2 ± 0.5 mg/g dry powder) when harvested in March, while those collected from Songkhla gave the highest value (37.4 ± 0.3 mg/g dry powder) when collected in December. *C. asiatic* collected from Nakornsrihammarat and Ratchaburi gave the lowest content of total pentacyclic triterpenes in all experimental harvesting periods.