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ELUCIDATING THE MECHANISM OF ACTION OF *Centella asiatica* ETHANOLIC EXTRACT AND TRITERPENOIDS AS A POTENTIAL ANTI-LEUKAEMIC AGENT THROUGH THE INTEGRATION OF *in silico* AND *in vitro* STUDIES

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ABSTRACT

Centella asiatica (C. asiatica), locally known as 'pegaga', is a herb used in traditional Chinese medicine to treat various diseases, including cancer. There is, however, no information on its potential anti-leukaemic activity. Thus, this study investigated the underlying mechanism of anti-leukaemic activity of *C. asiatica* ethanolic extract and triterpenoids (madecassoside; ME, asiaticoside; AE, madecassic acid; MA, asiatic acid; AA) on human CCRF-CEM T-acute lymphoblastic leukaemia (T-ALL) cells through *in silico and in vitro* methods. A ligand-based target prediction model comprising 26 leukaemia-related targets and 26,540 compounds was first built using a random forest algorithm. The model was validated through internal and external validation. Molecular docking was then performed on the targets predicted for the triterpenoids. The *in vitro* anti-proliferative activity was evaluated by assessing the cell apoptosis rate using annexin-V flowcytometry. The intracellular reactive oxygen species (ROS) level and mitochondrial membrane change (MMP) were measured using fluorescence analysis. At the molecular level, the gene expression profile was assessed by microarray, followed by an analysis of protein-protein interaction and gene enrichment functional analysis. In addition, the protein expression level of the apoptosis-related gene was determined using western blot analysis. The built prediction model, named the Leuk-RP model, showed an average sensitivity, specificity and accuracy of 87.4%, 95.0% and 91.8%, respectively. In the external validation, the model showed an average sensitivity, specificity and accuracy of 89.8%, 87.5% and 81.9%, respectively. The triterpenoids were predicted to bind to SLC29A1, TNFα, and NR3C1 with different scores. Docking results demonstrated that the triterpenoids could bind to TNFα active site, with binding energies ranging from -9.0 to -9.5 kcal/mol. An MTT assay demonstrated that *C. asiatica* extract (RECA) and triterpenoids effectively inhibited CCRF-CEM cell viability in a time and dose-dependent manner with IC_{50} values of 41.34 μg/ml for RECA and ranging from 13.15 to 95.49 μM for the triterpenoids. RECA and triterpenoids also induced cell apoptosis, where MA induced the highest early and late apoptosis by 5.08% and 16.43% at 48 h compared to others. Subsequently, activation of caspase-3, caspase-7, caspase-9 and cleavage of poly (ADP-ribose) polymerase (PARP) was observed in the treatment of triterpenoids in leukaemia cells. Increased ROS levels causing oxidative stress and attenuation of MMP were also observed in the triterpenoids. Leukaemia-related genes were differentially expressed in the MA-target-pathway networks, which include *EGR1, EGR2, JUND, JUNB, FOS, ATF3* and *JUN*. Significantly enriched pathways associated with potential target proteins include the TNFα and MAPK signalling pathways. Based on the results, several key observations can be deduced: (i) the triterpenoids may act primarily on the regulation of TNFα genes to induce apoptosis in leukaemia cells. Apoptosis through the TNFα signalling pathway by the triterpenoids may stimulate two downstream paths involving Caspases and/or AP-1 transcription factors, (ii) the triterpenoids exhibit anti-proliferative effect based on MTT assay and upregulation of *JUNB*, *JUND*, *JUN*, and *FOS* by MA and (iii) MA may exert an anti-inflammatory effect on leukaemia cells through NR3C1. In conclusion, these results revealed that *C. asiatica* constituents have potential anti-leukaemic activity via a "multi-component, multi-target, multi-pathway" regulatory network. Furthermore, this study also demonstrated that the combination of *in silico* and *in vitro* studies effectively discovers and elucidates the mechanism of action of *C. asiatica* constituents.

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