**An Integrated *In-Silico* and *In-Vitro* Approach for Screening and Optimizing Medicinal Plant Extracts Against Dengue Virus: Case Studies of *Phyllanthus amarus* and *Euphorbia thymifolia***

**Hoa Hoang Quynh1,** Giang Pham Thi Linh**1**

Department of Botany, Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy1, Hanoi, Vietnam

**Background and aims.** This study aimed to screen and optimize anti-dengue agents from Vietnamese medicinal plants by systematically combining *in-silico* screening, *in-vitro* testing, and extract optimization. Traditional knowledge guided the selection of plant species used for treating *Dengue*-like symptoms.

**Methods.** Fifty-five traditional plants were reviewed for known phytochemicals (n = 2445), filtered using oral bioavailability and Lipinski’s rule. Compounds (n=407) were screened against *Dengue* virus NS2B/NS3 (PDB: 2FOM) and NS5 RdRp (PDB: 6IZZ) using AutoDock 4.2.6. Compounds with binding energy ≤ -7 kcal/mol were shortlisted. Five species were selected for *in-vitro* PRNT assays. Extract optimization studies were implemented by Response Surface Methodology (RSM) in case studies.

**Results.** From 2445 phytochemicals (in 55 species), 407 compounds met oral bioavailability and Lipinski’s criteria, and 94 compounds showed strong binding affinity (ΔG ≤ -7 kcal/mol). Among selected plants that contain the most potential compounds, extracts from *E. thymifolia* and *P. amarus* showed the strongest inhibition across all DENV serotypes, with PRNT50 values ranging from 19.5 to 78.0 µg/mL.

*Phyllanthus amarus*. Fifty-two in over 100 known compounds were docked; geraniin had the strongest docking scores (-11.93 and -11.08 kcal/mol). Extraction was optimized via RSM (70.3oC, 1.25h, 32.7% ethanol), yielding 15.2% geraniin. PRNT50 values against DENV1-4 were 9.8, 9.8, 4.9, and 9.8 µg/mL, respectively.

*Euphorbia thymifolia*. Of 71 known compounds, 45 had 3D structures suitable for docking. Quercetin was selected as the marker compound (-7.2 to -7.9 kcal/mol). Extraction was optimized via RSM (73.0oC, 58.5 minutes, 90% ethanol, 20 ml/g solvent ratio), yielding 0.0482% quercetin. PRNT50 values were 19.5, 4.9, 9.8, and 9.8 µg/mL (DENV1-4).

**Conclusion/Discussion.** This integrative approach enabled the discovery and optimisation of anti-*Dengue* phytochemicals from traditional plants. It leads a practical and ethical strategy that contributes to the replacement of animal models in early-stage screening, aligning with the trends of animal-free research.

**References:**

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