

Executive Summary

Traditional knowledge of herbal medicines plays an important role in the search for novel chemotherapeutic agents. Recently, due to the adverse effects of synthetic drugs, there has been a rapid growth of interest from the western population in herbal remedies which are safe and effective. Traditional and herbal remedies offer a valuable alternative treatment in developing countries where traditional therapies are considered cheap and readily available. The studies of medicinal plants have been receiving attention in various areas of health research; much information has been documented as regards their various biological effects. The usage of herbal medicine for primary health care needs by people in local communities due to limited availability and affordability of pharmaceutical products is still occupying a prominent position. Therefore, the awareness of local communities should be enhanced by incorporating the traditional knowledge with scientific findings in order to promote cautious use of herbal medicine. *Albizia myriophylla* Benth is a tree, widely distributed in southeastern Asian countries. In Thailand, it is commonly called “Cha-am Thai”. This plant species is used in Thai traditional medicine as antitussive (root), expectorant (fruit and root), demulcent (root), and tonic (wood). *A. myriophylla* is among the most important medicinal plant which is used alone or in combination with other medicinal plants in various Thai herbal formulas, especially those for remedy of oral diseases including dental caries and aphthous ulcer. In our previous work, the herbal formula comprising four different medicinal plants including *Albizia myriophylla* Benth. (Leguminosae), *Alpinia galanga* (L.) Willd. (Zingiberaceae), *Avicennia marina* (Forssk.) Vierh. (Acanthaceae), and *Ocimum sanctum* L. (Lamiaceae) was the most active against *Streptococcus mutans*, responsible for dental caries, with MIC of 250 µg/ml as compared with the other formulas tested. Subsequent MIC determination of these medicinal plants revealed that the wood extract of *A. myriophylla* exhibited the strongest antibacterial activity against *S. mutans* ATCC 25175 with MIC value of 3.9 µg/mL. This result suggests the presence of phytochemical components with good antibacterial potency in this plant extract. Up to now, only one phytochemical investigation from its wood extract has been reported while no biological activity data, especially anticariogenic activity, is currently available. This work was carried out in order to investigate the chemical compositions from the wood of *A. myriophylla* as well as the cytotoxic and antibacterial activities of its isolated compounds. Phytochemical investigation of *A. myriophylla* wood has led to the isolation of five flavonoids 3,4,7,3'-tetrahydroxyflavan (1), 7,3',4'-trihydroxyflavanone (2), 8-methoxy-7,3',4'-trihydroxyflavone (3), 7,8,3',4'-

tetrahydroxyflavone (4), lupinifolin (5), a triterpenoid lupeol (6) as well as two set of mixtures belonging to the class of sterols including a mixture of β -sitosterone (7) and stigmasta-5,22-dien-3-one (8) and a mixture of β -sitosterol (9) and stigmasterol (10) were isolated from the wood of this plant species. The structures of all these compounds were determined by extensive spectroscopic studies, including comparisons of their UV, IR, MS, and NMR data with those previously reported. Some of the isolated compounds, particularly those belonging to flavonoid group were evaluated for their antibacterial activity against *S. mutans* ATCC 25175, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 29213 using broth microdilution method as well as cytotoxicity against oral cavity cancer (KB) cell line using resazurin microplate assay. All the tested compounds, except for compound 1 and 2, exhibited antibacterial activity against *S. mutans* ATCC 25175 with minimum inhibitory concentration (MIC) ranging from 0.98–250 $\mu\text{g/ml}$. Among the compounds tested, lupinifolin (5) showed the most potent anti-*S. mutans* activity with MIC of 0.98 $\mu\text{g/ml}$ comparable with the reference standard chlorhexidine with MIC of 0.5 $\mu\text{g/ml}$. Compound 5 also displayed marked antibacterial activity against *B. cereus* ATCC 11778 and *S. aureus* ATCC 29213 with the same MIC of 15.6 $\mu\text{g/ml}$. Compounds 3 and 4 showed moderate activity against the three tested bacterial strains with MIC values ranging from 62.5–250 $\mu\text{g/ml}$, whereas compounds 1 and 2 exhibited no antibacterial activity against the tested pathogens at the highest concentration tested of 250 $\mu\text{g/ml}$. Regarding the cytotoxicity, lupinifolin (5) was found to have potent anticancer activity against KB cell with IC_{50} of 4.9 $\mu\text{g/ml}$, whereas the other tested compounds at the highest concentration of 50 $\mu\text{g/ml}$ did not exert cytotoxic effect against cancer cell tested. These results could be used as scientific evidences for the traditional use of *A. myriophylla* as a remedy for dental caries. Our study also reported the bioactive ingredients of *A. myriophylla*, which support its ethnomedical claims as well. Lupinifolin may have a potential to be a natural anticariogenic agent. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains. The study on the quality control of *A. myriophylla* extract is important for further development of this plant species as herbal oral care product as well.

We described herein the isolation and structure identification of chemical substances from the wood of *A. myriophylla* as well as the cytotoxic and antibacterial activities of the semi-purified fractions and the isolated compounds. The results of this study could be used as the scientific evidences for the traditional uses of *A. myriophylla*. Besides, our finding may contribute to the increase of knowledge of the chemotaxonomy and biological activity in *Albizia* species. In addition, if such very promising bioactive molecules are isolated, they will be used for the development of novel therapeutic agents in the future.