## **CHAPTER 5**

## Conclusion

Preparation of botanical products from 33 plant species provided essential oils and ethanolic extracts by using steam distillation and ethanol solvent extraction, respectively, with varied yields, physical characteristics, and repellent activities against Aedes aegypti. Subsequent extractions of Angelica sinensis, which is the most effective plant, with different organic solvents of increasing polarity (hexane, acetone, methanol, and absolute ethanol) provided four extractants, with slight variations in chemical compositions and degrees of repellency against Ae. aegypti. Chemical analysis demonstrated phthalides (3-N-butylphthalide, butylidenephthalide, and ligustilide) and phthalates (di-iso-octyl phthalate) as the principal constituents in the A. sinensis solvent extracts by using gas chromatography/mass spectrometry. The highest repellency afforded by A. sinensis hexane extract (AHE), with a median complete-protection time of 7.5 (6.5-8.5) h, compared favorably to that of its essential oil (7.0, 6.0-7.5 h) and the standard synthetic repellent, N,N-diethyl-3-methylbenzamide (DEET: 6.25, 5.0-6.5 h). AHE- and DEET-based repellent products, including AHE-ethanol solution (AHE-ES), AHE-vanillin nanoemulsion gel (AHEv-NEG), DEET-ethanol solution (DEET-ES), and DEET-vanillin nanoemulsion gel (DEETv-NEG) were found to have remarkable repellency against mosquitoes under both laboratory and field conditions. AHE-ES and DEET-ES, with and without 5% vanillin supplementation, were effective in repelling laboratoty Ae. aegypti, with comparable protection times of 2.0-8.5 h and 2.25-8.25 h, respectively. Significant, commensurate repellency against laboratory strains of Ae.

aegypti, Culex quinquefasciatus, and Anopheles minimus also was observed in applications of 10% AHEv-NEG and 10% DEETv-NEG, with median protection times of 4.5 (4.0-6.0), 7.75 (6.5-11.5), and 11.0 (9.5-12.0) h, respectively, and 7.5 (6.5-9.0), 10.5 (9.5-16.0), and 12.0 (10.0-12.5) h, respectively. Field studies on the repellent activity of AHE- and DEET-based products discovered that 25% AHEv-ES and 25% DEETv-ES (Field I) as well as 10% AHEv-NEG and 10% DEETv-NEG (Field II) offered similarly strong repellency by minimizing bites with 99.9-100% protection against a wide range of natural mosquito populations. No local skin reaction such as rash, swelling, irritation or other allergic responses was recorded in either laboratory or field study periods. Furthermore, evaluation of skin irritation in 30 human volunteers also revealed no irritant potential from 25% AHE-ES or 10% AHEv-NEG. Determination of physical and biological stability of the AHE-based repellent products, AHE and 10% AHEv-NEG, demonstrated a relatively stable state and persistent repellency for a period of at least 6 months, when kept in various conditions. Consequently, AHE-based repellent products with proven repellent efficacy, no side effects on the skin, and relatively stable physical and biological performance could qualify for developing and registering a new natural alternative to DEET.

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