Abstract: Essential oils have emerged as viable alternatives to synthetic insecticides for control of mosquito-borne pathogens. The leaf essential oils of eight species of *Premna* (Lamiaceae) growing in central Vietnam have been obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry. Sesquiterpene hydrocarbons dominated most of the *Premna* essential oils, with the notable exception of *Premna mekongensis* from Ngoc Linh Nature Reserve, which had α-pinene as the major component. Larvicidal activities against *Aedes aegypti* have been determined and all of the *Premna* essential oils showed larvicidal activity with 24-h LC₅₀ < 65 µg/mL. The leaf essential oils of *Premna cambodiana* from Chu Mom Ray National Park and *Premna mekongensis* from Ngoc Linh Nature Reserve showed the best larvicidal activities with 24-h LC₅₀ of 16.8 and 18.0 µg/mL, respectively. The essential oil compositions and larvicidal activities of *P. cambodiana*, *Premna flavescens*, *Premna maclurei*, *P. mekongensis*, and *Premna puberula* are reported for the first time. Although the larvicidal activities of *Premna* leaf essential oils are promising, the essential oil yields are relatively low (0.10–0.25%).

Keywords: Lamiaceae; *Aedes aegypti*; sesquiterpene hydrocarbons

1. Introduction

Mosquito-borne infectious diseases have been a persistent problem in Vietnam. Dengue fever and dengue hemorrhagic fever are especially problematic and chikungunya fever is an emerging threat in the country [1,2]. *Aedes aegypti* (L.) (Diptera: Culicidae), the yellow fever mosquito, and *Aedes albopictus* (Skuse) (Diptera: Culicidae), the Asian tiger mosquito, are important vectors of several viral pathogens, including dengue fever virus [3], yellow fever virus [4], chikungunya fever virus [5], and possibly Zika virus [6]. *Culex quinquefasciatus* Say (Diptera: Culicidae), the southern house mosquito, is a vector of...
lymphatic filariasis [7] as well as several arboviruses such as West Nile virus and St. Louis encephalitis virus [8], and possibly Zika virus [9].

Insecticide resistance has been emerging in many insect disease vectors, including mosquitoes [10–14]. Furthermore, the environmental impacts of synthetic insecticides have been felt for many years [15,16]. It has been reported that insecticide use has detrimental effects on non-target organisms, for example imidacloprid on honey bee (Apis mellifera) [17], damselfly (Ischnura senegalensis) [18], fathead minnow (Pimephales promelas), or the amphipod (Hyalella azteca) [19]. Thus, there is a need for new and complementary methods for controlling insect vectors, and essential oils have shown promise as renewable and environmentally-safe alternatives to the use of synthetic insecticides [20–25].

The Lamiaceae has been an important family in terms of biologically active essential oils. Essential oils from members of this family have demonstrated potential as natural insect pest control agents [24,26–32]. The genus Premna L. was formerly included in the family Verbenaceae, but has been reassigned to the Lamiaceae [33]. The genus is distributed in tropical regions of the Old World, from Africa, eastward through China, Southeast Asia and Malesia, to Australia and islands in the Pacific [34]. The number of species has been estimated to be as few as 50, or as many as 200 [34]. The ethnopharmacology, pharmacognosy, and phytochemistry of the genus have been reviewed [33,35–37]. As part of our ongoing efforts in identifying readily-available essential oils for mosquito control, we have examined the leaf essential oils of eight species of Premna (Table 1) found growing wild in central Vietnam for larvicidal activity against Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus. Several of these Premna species have been used traditionally in Vietnam (Table 1).

### Table 1. Premna species examined in this study.

| Premna Species | Native Range | Ethnobotanical Use in Vietnam |
|---------------|--------------|-------------------------------|
| *Premna cambodiana* Dop (Vietnamese name Cach cam bô) | Laos, Cambodia and Vietnam (Kon Tum, Gia Lai, and Đắk Nông provinces) [38–40]. | Used to treat spermatorrhoea and gynecological diseases [40]. |
| *Premna chevalieri* Dop (syn. *Premna acuminata*) Merr. (Vietnamese name Cach vâng) | Thailand, Laos, Vietnam, China (Hainan, Yunnan) [41]. In Vietnam, the plant has been recorded in Thái Nguyên, Phú Thọ, Bắc Giang, Hà Nội, Hòa Bình, Nghệ An, Hà Tĩnh, and Quảng Nam provinces [39,40]. | The plant is used to treat polio, jaundice [40]. |
| *Premna corymbosa* Rottler & Wild. (syn. *Premna integrifolia* L., *Cornutia corymbosa* Burm. f., *Premna integrifolia* L., *Gumira corymbosa* (Rottler & Willd.)) (Vietnamese name Cach biên) | Ranges from Madagascar, through tropical and subtropical Asia, to Australia and Pacific islands [38]. In Vietnam, *P. corymbosa* has been found in Quảng Ninh, Hà Nội, Hải Phòng, Hà Nam, Ninh Bình, Thanh Hóa, Thừa Thiên Huế, Đà Nẵng, Quảng Nám, Khánh Hòa, Kon Tum, Đắk Nông, Đồng Nai, Hồ Chí Minh, Bảo Rá-Vũng Tàu, Long An, and Khánh Giang provinces [39,40]. | The plant is used to treat skin diseases. Additionally, the leaves are used as culinary additives [40]. |
| *Premna flavescens* Buch.-Ham. ex C.B. Clarke (syn. *Premna lucidula*) | Southern China (Guangdong, Guangxi), and southern Yunnan), India, Indonesia, Malaysia, and Vietnam [41]. In Vietnam, *P. flavescens* has been recorded in Vĩnh Phúc, Nghệ An, Quảng Nam, Kon Tum, Gia Lai, Đắk Nông, and Đồng Nai provinces [39,40]. | A species commonly grown in Vietnam; a decoction of the leaves is taken daily as a tonic [40]. |
| *Premna maclurei* Merr. (Vietnamese name Cach machura) | China (Hainan) [41] as well as the provinces of Nghệ An and Quảng Nam, Vietnam [39,40]. | |
| *Premna mekongensis* W.W. Sm. (Vietnamese name Cach me cóng) | China (northeastern and western Yunnan province) [41] and in Vietnam (Hà Giang and Quảng Nam Provinces) [39,40]. | |
| *Premna puberula* Pamp. (syn. *Premna martini* H.Lév.) (Vietnamese name Cach hùn phùn) | China (Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hunan, southern Shanxi, Sichuan, and Yunnan) [41] as well as Vietnam (Hà Giang, Bạc Giang, and Nghệ An) [39,40]. | Used in traditional medicine [40]. |
| *Premna tomentosa* Willd. (syn. *Premna cordata* Blanco) (Vietnamese name Cach lòng to) | Ranges from China (Guangdong), through tropical Asia, to North Queensland, Australia [38]. In Vietnam, the plant has been recorded in Nghệ An province and South Vietnam [39,40]. | Leaves, roots as medicine [40]. |

A perusal of the literature has revealed no previous phytochemical reports on *P. cambodiana*, *P. flavescens*, *P. maclurei*, *P. mekongensis*, or *P. puberula*.

### 2. Results and Discussion

#### 2.1. Plant Collection and Essential Oils

The leaves of eight species of *Premna* were collected from several sites in Vietnam. The collection sites, voucher numbers, and essential oil yields are summarized in Table 2.
Table 2. Collection details and yields for *Premna* leaf essential oils from central Vietnam.

| *Premna* Species | Collection Site | Voucher Numbers | Essential Oil Yield (% v/w) |
|------------------|-----------------|----------------|-----------------------------|
| *Premna cambodiana* | Chu Mom Ray National Park, 14°25'33.5" N, 107°43'15.6" E, 672 m elevation | DND 88 | 0.14 |
| *Premna chevalieri* | Tuy Giang District, Quang Nam Province, 15°49'59" N, 107°21'10" E, 962 m elevation | DND 101 | 0.10 |
| *Premna corymbosa* | Nam Giai Commune, Quê Phong district, Pu Hoat Nature Reserve, Nghe An province, 19°41'40" N, 104°49'29" E, 670 m elevation | DND 788 | 0.22 |
| *Premna flavescens* | Dong Van Commune, Quê Phong District, Pu Hoat Nature Reserve, Nghe An province, 19°50'45" N, 105°06'09" E, 511 m elevation | DND 711 | 0.11 |
| *Premna maclurei* | Nam Giai Commune, Quê Phong district, Pu Hoat Nature Reserve, Nghe An province, 19°41'40" N, 104°49'29" E, 670 m elevation | DND 747 | 0.12 |
| *Premna mekongensis* | Ngoc Linh Nature Reserve, Quang Nam Province, 15°50'16.0" N, 107°22'54.7" E, 1341 m elevation | DND 102 | 0.19 |
| *Premna puberula* | Chu Mom Ray National Park, 14°25'33.5" N, 107°43'15.6" E, 672 m elevation | DND 84 | 0.21 |
| *Premna tomentosa* | Nghia Dan District, Nghe An province, 19°20'23" N, 105°25'18" E, 49 elevation | DND 23 | 0.12 |

2.2. Essential Oil Compositions

The *Premna* leaf essential oils were analyzed by gas chromatography–mass spectrometry and the chemical compositions are summarized in Table 3.

2.2.1. *Premna cambodiana*

A total of 72 compounds were tentatively identified in the leaf essential oil of *P. cambodiana*, accounting for 97.4% of the total composition (Table 3). Sesquiterpene hydrocarbons dominated *P. cambodiana* leaf essential oil with α-copaene (23.3%), α-gurjunene (11.3%), (E)-caryophyllene (12.8%), and δ-cadinene (5.5%) as the major sesquiterpene components. There have been no previous phytochemical investigations on *P. cambodiana* reported in the literature; this is the first report on its essential oil composition.

2.2.2. *Premna chevalieri*

Eighty-five components (99.8% of the composition) were tentatively identified in *P. chevalieri* essential oil. The major components in the leaf essential oil of *P. chevalieri* were the sesquiterpenes (E)-caryophyllene (31.5%) and α-humulene (7.5%) and the monoterpenes α-pinene (12.2%) and β-pinene (16.8%) (Table 3). There have been no previous phytochemical investigations on *P. chevalieri* reported in the literature; this is the first report on the leaf essential oil composition of this plant.

2.2.3. *Premna corymbosa* (syn. *P. integrifolia, P. serratifolia*)

Leaves of *P. corymbosa* were collected from two different sites (i.e., Nam Giai Commune, Quê Phong district, Pu Hoat Nature Reserve, Nghe An province, and Son Tra Peninsula, Da Nang province). Although the two essential oil compositions are qualitatively similar, there are notable quantitative differences (Table 3). The sample from Nghe An province was rich in oxygenated sesquiterpenoids, e.g., spathulenol (17.3%) and caryophyllene oxide (16.8%), while the sample from Da Nang was dominated by sesquiterpene hydrocarbons, including allo-aromadendrene (39.7%), (E)-caryophyllene (13.3%), and α-copaene (8.1%).
Table 3. Chemical compositions of leaf essential oils of *Premna* species from central Vietnam.

| RI<sup>a</sup> | RI<sup>b</sup> | Compound<sup>c</sup> | *P. cambodioides* | *P. chevalieri* | *P. corymbosa* | *P. flavescens* | *P. flavescens* | *P. maculata* | *P. mekongensis* | *P. mekongensis* | *P. puberula* | *P. tomentosa* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 922 | 923 | Tricyclene | — | — | — | — | — | — | — | — | — | — |
| 925 | 927 | α-Thujene | 0.1 | 0.3 | — | — | — | — | — | — | — | — |
| 931 | 933 | α-Pinene | 1.9 | 12.2 | 0.5 | 0.5 | 0.2 | 0.1 | 0.4 | 66.9 | 1.5 | 1.3 | 3.0 |
| 947 | 948 | α-Fenchene | — | — | — | — | — | — | — | — | — | — | — |
| 949 | 951 | Camphene | 0.1 | 0.7 | 0.2 | 0.2 | 0.1 | — | 0.1 | 0.1 | — | — | — |
| 952 | 953 | Thuja-2,4(10)-diene | tr | tr | — | — | — | — | — | — | — | — | — |
| 971 | 971 | Sabine | — | tr | 0.3 | — | — | tr | 0.9 | 0.1 | — | 2.0 | — |
| 976 | 978 | β-Pinene | 1.7 | 16.8 | 0.6 | 0.1 | 0.1 | 0.2 | 0.5 | 0.7 | 0.1 | — | 0.7 |
| 978 | 978 | 1-Octen-3-ol | — | — | — | — | — | — | — | — | — | — | — |
| 983 | 986 | 6-Methylhept-5-en-2-one | — | — | — | — | — | — | — | — | — | — | — |
| 987 | 989 | Myrtenol | 0.1 | — | — | — | — | — | — | — | — | — | — |
| 1001 | 1004 | p-Mentha-1(7),8-diene | — | — | — | — | — | — | — | — | — | — | — |
| 1005 | 1005 | α-Thujene | 0.1 | — | — | — | — | — | — | — | — | — | — |
| 1017 | 1018 | α-Thujene | 0.1 | — | — | — | — | — | — | — | — | — | — |
| 1029 | 1031 | β-Pinene | 0.2 | — | — | — | — | — | — | — | — | — | — |
| 1032 | 1032 | β-Caryophyllene | — | — | — | — | — | — | — | — | — | — | — |
| 1034 | 1034 | (Z)-β-D-pinene | — | — | — | — | — | — | — | — | — | — | — |
| 1045 | 1045 | (E)-β-D-pinene | — | — | — | — | — | — | — | — | — | — | — |
| 1057 | 1057 | γ-Terpine | — | — | — | — | — | — | — | — | — | — | — |
| 1085 | 1086 | Terpinolene | — | — | — | — | — | — | — | — | — | — | — |
| 1089 | 1090 | trans-Pinocarveol | — | — | — | — | — | — | — | — | — | — | — |
| 1103 | 1104 | 2-Methylbutyl-2-methylbutanoate | — | — | — | — | — | — | — | — | — | — | — |
| 1107 | 1108 | p-Mentha-2,6-dien-1-ol | — | — | — | — | — | — | — | — | — | — | — |
| 1111 | 1111 | (3E,4,8-Dimethyl-1,3,7-nonatriene | — | — | — | — | — | — | — | — | — | — | — |
| 1125 | 1126 | α-Campholenol | — | — | — | — | — | — | — | — | — | — | — |
| 1136 | 1138 | Benzeneacetonitrile | — | — | — | — | — | — | — | — | — | — | — |
| 1154 | 1154 | trans-Pinocarveol | tr | — | — | — | — | — | — | — | — | — | — |
| 1156 | 1156 | p-Anisylisole | — | — | — | — | — | — | — | — | — | — | — |
| 1161 | 1164 | Pinocarveol | — | — | — | — | — | — | — | — | — | — | — |
| 1162 | 1163 | 2,4-Dimethyl-3-(3-methoxyphenyl)butanol | — | — | — | — | — | — | — | — | — | — | — |
| 1166 | 1166 | 3-Methylfarnesol | — | — | — | — | — | — | — | — | — | — | — |
| 1170 | 1170 | 1,8-Cineole | — | — | — | — | — | — | — | — | — | — | — |
| 1179 | 1180 | Methanol | — | — | — | — | — | — | — | — | — | — | — |
| 1182 | 1183 | p-Cymene | tr | — | — | — | — | — | — | — | — | — | — |
| 1183 | 1183 | α-Terpinene | — | — | — | — | — | — | — | — | — | — | — |
| 1188 | 1190 | 3-Octen-2-one | — | — | — | — | — | — | — | — | — | — | — |
| 1192 | 1194 | 4,8-Dimethyl-1,3,7-trien-7-ol | — | — | — | — | — | — | — | — | — | — | — |
| 1198 | 1199 | α-Methylsalicylate | 0.1 | — | — | — | — | — | — | — | — | — | — |
| 1202 | 1203 | α-Phellandrene | — | — | — | — | — | — | — | — | — | — | — |
| 1212 | 1213 | 3-Octanol | 0.1 | — | — | — | — | — | — | — | — | — | — |
| 1213 | 1216 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |
| 1225 | 1227 | 3-Octen-2-one | — | — | — | — | — | — | — | — | — | — | — |
| 1227 | 1229 | 2,4-Dimethyl-3-(3-methoxyphenyl)butanol | — | — | — | — | — | — | — | — | — | — | — |
| 1229 | 1230 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |
| 1234 | 1235 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |
| 1237 | 1238 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |
| 1238 | 1239 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |
| 1247 | 1248 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |
| 1250 | 1251 | trans-Verbenol | — | — | — | — | — | — | — | — | — | — | — |

<sup>a</sup> Relative retention time (RI<sub>top</sub>)

<sup>b</sup> Relative retention time (RI<sub>bot</sub>)

<sup>c</sup> Chemical compound
Table 3. Cont.

| Compound | P. cambodiana | P. chevalieri | P. corimbosa (Ngh An) | P. corimbosa (Da Nang) | P. flavescens (Nam Giâi) | P. flavescens (Đông Văn) | P. maclurei | P. mekongensis (Ngoc Linh) | P. mekongensis (Chu Mom Ray) | P. puberula | P. tomentosa |
|----------|---------------|---------------|------------------------|--------------------------|--------------------------|--------------------------|------------|--------------------------|-------------------------------|------------|------------|
| Bornyl acetate | — | — | — | — | — | — | — | — | 3.9 | — | — |
| Dihydroedulan IA | — | — | — | — | 0.8 | — | tr | 0.4 | — | 0.2 | — |
| Dihydroedulan IIA | — | — | 0.1 | — | — | — | 1.2 | — | — | 3.4 | — |
| Theaspirane A | — | — | — | — | — | — | — | — | — | — | — |
| Tridecane | — | — | — | — | — | — | — | — | — | — | — |
| Isoascaridole | — | — | — | — | — | — | — | — | — | — | — |
| Unidentified | — | — | — | — | — | — | 1.2 | — | — | 5.3 | — |
| Theaspirane B | — | — | — | — | — | — | — | — | — | — | — |
| 3-Hydroxycineole | — | — | — | — | — | — | — | — | — | — | — |
| Bicycloelemene | — | — | — | 0.1 | 0.6 | 0.1 | — | — | 2.5 | 0.2 | — |
| δ-Elemene | 0.2 | — | — | — | — | — | — | — | 0.1 | 0.6 | 0.2 |
| α-Cubebene | 0.7 | 0.1 | 0.5 | 0.3 | 0.2 | 0.5 | — | — | 0.4 | 0.5 | 0.2 |
| α-Terpinyl acetate | — | — | — | — | — | — | — | — | — | — | — |
| Decanoic acid | — | — | — | — | — | — | — | — | — | — | — |
| Isoascaridole | — | — | — | — | — | — | — | — | — | — | — |
| α-Ylangene | 0.3 | — | 0.6 | 0.1 | 0.6 | 0.1 | — | — | 1.1 | — | 0.2 |
| Cycloartene | tr | — | — | — | — | — | — | — | — | — | — |
| Gerosyl acetate | 0.1 | — | — | — | — | — | — | — | — | — | — |
| α-Copaene | 23.3 | 0.9 | 6.8 | 8.1 | 0.1 | 2.9 | 2.6 | — | 1.6 | 3.3 | 3.1 |
| (E)-Damascone | — | — | — | 0.1 | — | — | — | — | — | — | — |
| (Z)-Hexyl hexanoate | — | — | — | — | — | — | — | — | — | — | — |
| cis-β-Elemene | 0.2 | tr | — | 0.5 | 0.5 | 0.1 | — | — | 0.1 | — | 0.3 |
| β-Beurenene | tr | tr | — | 0.1 | 0.1 | 0.1 | — | — | 0.1 | — | — |
| Hexyl hexanoate | — | — | — | — | — | — | — | — | 2.1 | — | — |
| β-Cubebene | 1.1 | 0.2 | 1.8 | 0.9 | — | 0.6 | 1.6 | — | 0.3 | 1.5 | 0.7 |
| trans-β-Elemene | 3.5 | 0.3 | 1.5 | 1.0 | 0.9 | 8.7 | 1.8 | 0.5 | 1.3 | 1.9 | 0.8 |
| (Z)-Carophyllene | — | 0.1 | — | 0.2 | — | — | — | — | — | — | — |
| 9,10-Dihydroisocamphene | — | — | — | — | — | — | — | — | 0.8 | — | — |
| Cyperene | — | — | — | — | — | — | — | — | — | 0.1 | — |
| α-Gurjunene | 11.3 | — | 0.6 | 0.1 | 0.1 | 19.6 | — | — | 0.3 | 1.3 | 5.2 |
| β-Maaliene | 0.2 | — | — | 0.4 | — | — | — | — | — | — | 2.8 |
| β-Copaene | — | tr | — | — | — | — | — | 0.1 | 1.5 | — | — |
| β-Beurenene | tr | tr | — | — | 0.1 | 0.1 | — | — | — | — | — |
| Hexyl hexanoate | — | — | — | — | — | — | — | — | 2.1 | — | — |
| β-Cubebe | 1.1 | 0.2 | 1.8 | 0.9 | — | 0.6 | 1.6 | — | 0.3 | 1.5 | 0.7 |
| trans-β-Elemene | 3.5 | 0.3 | 1.5 | 1.0 | 0.9 | 8.7 | 1.8 | 0.5 | 1.3 | 1.9 | 0.8 |
| (Z)-Carophyllene | — | 0.1 | — | 0.2 | — | — | — | — | — | — | — |
| 9,10-Dihydroisocamphene | — | — | — | — | — | — | — | — | 0.8 | — | — |
| Cyperene | — | — | — | — | — | — | — | — | — | 0.1 | — |
| α-Gurjunene | 11.3 | — | 0.6 | 0.1 | 0.1 | 19.6 | — | — | 0.3 | 1.3 | 5.2 |
| β-Maaliene | 0.2 | — | — | 0.4 | — | — | — | — | — | — | 2.8 |
| (E)-Carophyllene | 12.8 | 31.5 | 6.9 | 13.3 | 41.0 | 11.8 | 30.7 | 14.7 | 3.9 | 0.6 | 22.0 |
| γ-Elemene | 0.7 | tr | — | — | — | — | — | — | — | 2.4 | — |
| γ-Maaliene | — | — | — | — | — | — | — | — | — | 0.1 | 3.1 |
| β-Copaene | — | tr | 0.2 | 0.2 | — | 0.1 | 0.4 | — | 1.2 | — | — |
| trans-α-Bergamotene | — | — | — | 0.1 | 0.7 | — | — | — | — | — | — |
| β-Gurjunene (= Calarene) | — | — | — | — | — | — | — | — | — | — | — |
| α-Graiene | 0.1 | — | — | — | 0.1 | 0.1 | 0.4 | 0.5 | 0.4 | 0.3 | 0.4 |
| β-Maaliene | 0.1 | — | — | — | — | 0.1 | 0.1 | — | 0.1 | — | — |
| Aromadendrene | 0.1 | tr | 0.5 | 0.1 | 0.4 | 0.3 | 0.3 | — | 1.0 | — | 5.4 |
| Guai-6,9-diene | 0.1 | — | — | — | — | 1.1 | — | — | — | — | — |
| Selina-5,11-diene | 0.1 | — | — | — | — | — | 1.1 | tr | — | — | — |
| (E)-β-Farnesene | — | — | — | 0.4 | — | — | — | — | — | — | — |
| α-Humulene | 3.7 | 7.5 | 2.6 | 3.7 | 2.4 | 3.1 | 5.3 | 2.5 | 2.0 | 0.4 | 3.9 |
| α-Aromadendrene | 1.6 | 0.1 | 7.7 | 39.7 | 0.1 | 3.0 | 0.2 | — | 1.0 | 4.1 | 1.0 |
| (E)-β-Farnesene | — | — | — | — | — | — | — | — | — | — | — |
| α-Humulene | 3.7 | 7.5 | 2.6 | 3.7 | 2.4 | 3.1 | 5.3 | 2.5 | 2.0 | 0.4 | 3.9 |
| α-Aromadendrene | 1.6 | 0.1 | 7.7 | 39.7 | 0.1 | 3.0 | 0.2 | — | 1.0 | 4.1 | 1.0 |
| (E)-β-Farnesene | — | — | — | — | — | — | — | — | — | — | — |
| α-Humulene | 3.7 | 7.5 | 2.6 | 3.7 | 2.4 | 3.1 | 5.3 | 2.5 | 2.0 | 0.4 | 3.9 |
| α-Aromadendrene | 1.6 | 0.1 | 7.7 | 39.7 | 0.1 | 3.0 | 0.2 | — | 1.0 | 4.1 | 1.0 |
### Table 3. Cont.

| Compound | P. cambodiana | P. chualier | P. corombose (Ngãi An) | P. corombose (Da Nang) | P. flavescens (Nam Giãi) | P. flavescens (Đông Văn) | P. maclurei | P. mekongensis (Ngo Linh) | P. mekongensis (Chu Mom Ray) | P. puberula | P. tomentosa |
|----------|---------------|-------------|-------------------------|------------------------|---------------------------|--------------------------|-------------|--------------------------|-------------------------------|-------------|-------------|
| 1469 1473 | 4,5-di-epi-Aristolochene | — | 0.1 | 1.0 | 0.1 | — | — | — | — | — | — | — |
| 1471 1477 | γ-Murolene | 0.6 | 0.4 | 0.4 | 0.1 | 1.2 | 1.7 | 0.3 | — | 0.3 | — | — |
| 1473 1475 | Salsola-4,11-diene | — | 2.0 | — | 0.2 | 0.1 | 0.5 | 0.7 | — | — | — | — |
| 1476 1477 | β-Chaminigrene | — | tr | — | 0.2 | 1.3 | 0.1 | — | — | — | — | — |
| 1476 1479 | α-Amorphene | 0.3 | — | — | 0.2 | 0.6 | — | 0.6 | — | — | — | — |
| 1479 1480 | Germacrene D | 2.2 | 0.3 | 0.2 | 3.0 | 0.1 | 5.6 | — | — | 11.4 | — | — |
| 1479 1482 | γ-Himachalene | 0.1 | — | — | 0.2 | — | — | — | — | — | — | — |
| 1480 1483 | truns-β-Bergamotene | — | — | — | 0.2 | — | — | — | — | — | — | — |
| 1484 1491 | Eremophillene | — | — | — | 0.1 | — | — | — | — | — | — | — |
| 1486 1498 | β-Selinene | — | — | — | 0.2 | — | — | — | — | — | — | — |
| 1487 1497 | β-Selinene | 1.4 | 1.2 | 4.0 | 0.2 | 1.3 | 9.7 | 0.3 | 0.4 | 0.7 | 0.7 | 4.3 |
| 1491 1492 | trans-Murola-6(14),5-diene | — | — | — | 0.2 | — | — | — | 0.2 | — | — | — |
| 1491 1499 | Valencene | — | — | — | 0.2 | — | — | — | — | — | — | — |
| 1493 1495 | γ-Muurolene | 0.6 | — | 0.4 | 0.4 | — | 1.7 | 0.3 | 0.4 | 1.2 | — | 0.9 |
| 1497 1498 | α-Muurolene | 0.5 | — | 0.2 | 0.3 | 0.2 | 0.4 | — | — | — | — | — |
| 1495 1497 | α-Selinene | 1.0 | 1.5 | 2.7 | — | 8.7 | — | 0.4 | — | — | — | 5.5 |
| 1496 1497 | Bicyclogermacrene | — | — | — | 1.1 | 7.8 | — | 1.9 | — | — | — | 11.9 |
| 1497 1500 | a-Aphoromene | — | 0.1 | — | — | — | — | — | — | — | — | — |
| 1499 1505 | α-Bulnesene | — | 0.1 | — | 4.1 | 0.2 | 5.4 | 0.3 | 0.1 | — | 0.6 | 0.1 |
| 1502 1503 | (E,E)-α-Farnesene | — | 3.4 | — | 0.3 | — | 0.1 | — | — | — | — | 1.0 |
| 1505 1506 | β-Bisabolole | — | — | — | 0.4 | 0.5 | 0.2 | — | — | — | — | — |
| 1508 1511 | Germacrene A | — | — | — | 0.2 | — | — | — | — | — | — | — |
| 1511 1512 | γ-Cadinene | 0.4 | tr | 0.2 | 0.3 | 0.1 | 0.4 | 0.2 | 0.4 | 1.1 | — | 0.2 |
| 1513 1515 | Cubebol | 0.1 | — | 0.6 | — | — | — | 0.2 | — | 0.3 | 0.6 | 1.6 |
| 1516 1518 | γ-Cadinene | 5.5 | 0.3 | 0.4 | 2.0 | 0.1 | 8.4 | — | 3.2 | — | — | 1.8 |
| 1518 1520 | β-Caracorene | 0.5 | 0.1 | 0.4 | 0.3 | 0.2 | 0.4 | — | — | — | — | — |
| 1518 1521 | a-Paraisinsen | — | — | — | 0.1 | — | — | — | — | — | — | — |
| 1519 1522 | trans-Calamenene | 0.5 | 0.1 | 0.4 | 0.3 | 0.3 | 0.6 | — | 1.0 | — | — | 0.1 |
| 1522 1524 | Zonane | — | — | — | — | — | — | — | — | — | — | — |
| 1532 1538 | α-Cadinene | 0.2 | — | 0.1 | — | — | — | 0.1 | 0.3 | — | — | — |
| 1534 1536 | trans-Cadinene-1,4-diene | — | — | — | — | — | — | — | — | — | — | — |
| 1535 1539 | cis-Calamenene | — | — | — | — | — | — | — | — | — | — | — |
| 1536 1540 | (E)-α-Bisabolene | — | — | — | — | — | — | — | — | — | — | — |
| 1536 1540 | Selena-4(15),7(11)-diene | — | — | — | — | — | — | — | — | — | — | — |
| 1539 1540 | selena-Sesquisabinene hydrate | — | — | — | — | — | — | — | — | — | — | — |
| 1539 1544 | α-Calacorene | 0.9 | — | 0.6 | 0.1 | — | 0.6 | — | 0.2 | 3.4 | — | — |
| 1546 1547 | α-Elemol | 0.1 | — | 0.4 | 0.1 | — | 0.4 | — | 0.2 | — | — | — |
| 1547 1551 | (Z)-Caryophyllene oxide | 0.4 | — | — | 0.1 | 0.6 | — | — | — | 1.5 | — | — |
| 1550 1554 | cis-Murolol-5-m-en-4(3)-ol | — | — | — | — | — | — | — | — | — | — | — |
| 1551 1557 | Germacrene B | 0.6 | — | 0.1 | — | 0.1 | 0.5 | 0.5 | 3.5 | — | — | — |
| 1559 1560 | (E)-Nerolidol | — | — | — | — | — | — | — | — | 11.1 | — | — |
| 1560 1563 | β-Calacorene | 0.4 | — | — | 0.7 | 0.1 | 0.8 | — | — | — | — | — |
| 1563 1566 | 1,10-Tetradecadiene | — | 0.3 | — | — | — | — | — | — | — | — | — |
| 1567 1568 | 1,5-Epoxyval-4(14)-ene | 0.3 | — | 0.4 | — | 6.8 | 1.7 | 3.9 | — | — | — | — |
| 1568 1571 | Palustrol | — | — | — | 0.1 | — | — | — | — | — | — | — |
| 1572 1574 | Dendrolasin | — | 2.0 | — | — | — | — | — | — | — | — | — |
| 1574 1575 | Farnesolactone | — | — | — | 0.2 | — | — | — | — | — | — | — |

**Notes:** RI = Relative Intensity, DBB = Dibutyl Phthalate Baseline Breakpoint.
Table 3. Cont.

| RLc,c | RLd,d | Compound | P. cambodia | P. chuarlier | P. coromobosa (Ngh An) | P. coromobosa (Da Nang) | P. flavescens (Nam Giai) | P. flavescens (Đồng Văn) | P. mekongensis (Ngoc Linh) | P. mekongensis (Chu Mom Ray) | P. puberula | P. tomentosa |
|-------|-------|----------|-------------|-------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------|-------------|
| 1577  | 1576  | Spathulene | 0.8         | 0.6         | 17.3                   | 1.4                    | 1.8                    | 1.0                    | —                      | —                      | —           | 2.8         |
| 1580  | 1577  | (E)-Caryophyllene oxide | 4.3       | 5.3         | 16.8                   | 3.0                    | 1.7                    | 1.3                    | 13.3                   | 0.8                    | 0.3         | 21.2        |
| 1584  | 1590  | Globulol | 0.5         | 0.1         | 1.7                    | 0.3                    | 0.3                    | 0.2                    | 0.3                    | —                      | 1.2         | 2.0         |
| 1590  | 1596  | Cubeban-1-ol | —           | —           | —                      | 0.1                    | —                      | —                      | —                      | —                      | —           | —           |
| 1593  | 1595  | Verticiferol | —           | —           | 1.8                    | 0.3                    | 0.2                    | 0.1                    | —                      | 5.6                    | 0.7         | —           |
| 1595  | 1593  | Guaiol | —           | 1.2         | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1600  | 1603  | Guaiotone | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1602  | 1605  | Ledol | 0.2         | —           | 0.3                    | 0.3                    | —                      | 0.3                    | —                      | 1.2                    | 0.6         | 6.1         |
| 1607  | 1613  | Humulene epoxide II | 0.8       | 1.0         | 3.4                    | 0.4                    | 0.1                    | 0.2                    | 1.0                    | 0.1                    | —           | 4.7         |
| 1612  | 1615  | Rosidial | —           | 1.1         | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1614  | 1616  | 1,10-di-β-Cubeno| —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1622  | 1632  | Muurocol-4,10(14)-dien-1β-ol | 0.1       | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1624  | 1627  | 1-β-Cubeno| 0.4         | —           | 0.4                    | 0.2                    | —                      | 0.1                    | —                      | 1.0                    | —           | —           |
| 1629  | 1629  | iso-Spathulene | —           | —           | 1.0                    | 0.1                    | 0.4                    | —                      | 0.5                    | —                      | —           | 0.1         |
| 1633  | 1635  | Caryophylla-4(12),8(15)-dien-5β-ol | 0.1      | —           | —                      | 0.2                    | —                      | 0.6                    | 0.1                    | —                      | —           | —           |
| 1634  | 1634  | cis-Cadin-8-en-7-ol | 0.2       | —           | —                      | —                      | —                      | —                      | 0.9                    | —                      | —           | —           |
| 1641  | 1641  | alli-Aromadendrene epoxide | 0.6      | —           | —                      | 0.3                    | —                      | 0.1                    | —                      | 0.1                    | —           | 0.2         |
| 1643  | 1643  | γ-Cadinol | —           | —           | 0.4                    | 0.2                    | —                      | —                      | —                      | 1.5                    | —           | —           |
| 1644  | 1643  | Cubenol | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1644  | 1643  | Hexylcadinol | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1643  | 1643  | α-Muurolol (+-β-Cadinol) | 0.3       | —           | 0.3                    | 0.1                    | 0.1                    | —                      | 0.4                    | —                      | 0.1         | —           |
| 1645  | 1645  | β-Muurolol | 0.2         | —           | 0.4                    | 0.2                    | —                      | —                      | —                      | —                      | —           | 0.1         |
| 1647  | 1649  | β-Eudesmol | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1653  | 1652  | α-Cadinol | 0.6         | 0.3         | 1.9                    | 0.3                    | 0.3                    | 0.1                    | 0.3                    | —                      | 1.6         | 0.6         |
| 1654  | 1663  | cis-Calamenen-10-ol | 0.4       | —           | —                      | —                      | —                      | —                      | 0.9                    | —                      | —           | 0.8         |
| 1654  | 1653  | Pegisal | —           | 0.3         | 0.3                    | —                      | —                      | —                      | 0.8                    | 1.4                    | —           | 1.2         |
| 1658  | 1658  | Selin-11-en-4α-ol | 0.5       | —           | 0.9                    | 0.4                    | 0.2                    | —                      | 0.3                    | —                      | —           | —           |
| 1659  | 1664  | α-Turmerone | —           | —           | —                      | —                      | —                      | —                      | 0.1                    | —                      | —           | —           |
| 1667  | 1667  | trans-Calamenen-10-ol | 0.6       | —           | —                      | —                      | —                      | —                      | 0.7                    | —                      | —           | 1.0         |
| 1668  | 1664  | Caladene | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1669  | 1668  | 14-Hydroxy-9-α-epi-(E)-caryophyllene | —           | —           | 0.5                    | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1671  | 1681  | Mustakone | —           | —           | —                      | —                      | —                      | —                      | 3.6                    | —                      | —           | 2.6         |
| 1674  | 1676  | Aepide | —           | —           | —                      | —                      | —                      | —                      | 1.6                    | —                      | —           | 0.6         |
| 1682  | 1683  | Germacre-4(15),5,10(14)-trien-1α-ol | —           | —           | 1.0                    | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1685  | 1688  | α-Isobasedol | —           | 0.1         | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1700  | 1708  | 15α Calamenen-10-one | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1700  | 1708  | 5-Dodecalactone | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1702  | 1727  | Zerumbone | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1744  | 1746  | α-Cyperone | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1746  | 1748  | Geranyl hexanoate | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1748  | 1763  | β-Costol | —           | —           | —                      | —                      | —                      | —                      | 0.4                    | —                      | —           | —           |
| 1761  | 1761  | 10-nor-Calamenen-10-one | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1886  | 1848  | Corymbolone | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |
| 1848  | 1848  | - Unidentified | —           | —           | —                      | —                      | —                      | —                      | —                      | —                      | —           | —           |

For the compound "Unidentified f", the following values are noted: 3.6, 2.6, 0.6.
Table 3. Cont.

| RI<sub>calc</sub> | RI<sub>db</sub> | Compound | P. cambodiana | P. chevalieri | P. corombose (Nghe An) | P. corombose (Da Nang) | P. flavescens (Nâm Giã) | P. flavescens (Đồng Văn) | P. maclorei | P. mekongensis (Ngoc Linh) | P. mekongensis (Chu Mom Ray) | P. puberula | P. tomentosa |
|-----------------|----------------|-----------|----------------|----------------|------------------------|------------------------|------------------------|------------------------|------------|------------------------|------------------------|-------------|-------------|
| 1939 1947       |                | iso-Phytol | —              | —              | —                      | —                      | —                      | —                      | —          | —                      | —                      | —           | —           |
| 1958 1958       |                | (Z,Z)-Geranyl linalool | —              | 0.1            | —                      | —                      | —                      | —                      | —          | —                      | —                      | —           | —           |
| 1983 1995       |                | Mammol oxide | —              | —              | —                      | —                      | —                      | 0.4                    | —          | —                      | —                      | —           | —           |
| 2019 2022       |                | (E,E)-Geranyl linalool | —              | 0.1            | —                      | —                      | —                      | —                      | —          | —                      | —                      | —           | —           |
| 2022 2022       |                | Geranyl linalool | 4.3            | 2.2            | 0.3                    | 4.9                    | —                      | 2.4                    | 2.2        | —                      | —                      | 0.7         | —           |
| 2102 2131       |                | P. puberula | 0.6            | —              | —                      | —                      | 0.4                    | —                      | —          | —                      | —                      | —           | —           |
| 2132 2138       |                | P. tomentosa | 0.6            | 2.0            | 1.6                    | tr                     | 1.4                    | 0.6                    | 1.1        | 0.3                    | 3.2         | 0.1         | 0.3         |

Monoterpene hydrocarbons 3.8 31.8 2.7 5.3 0.5 0.6 0.7 72.5 12.5 1.8 7.0
Oxygenated monoterpensoids 0.1 1.0 1.0 0.2 0.2 0.4 0.2 2.5 6.0 1.4 0.5
Sesquiterpene hydrocarbons 76.1 49.9 40.2 85.4 69.9 92.2 62.5 59.6 46.9 22.4 81.0
Oxygenated sesquiterpenoids 12.3 12.6 50.6 8.2 6.0 4.8 30.1 2.5 27.4 58.2 10.2
Diterpenoids 4.5 2.4 0.0 0.0 5.2 0.0 3.1 2.2 0.0 1.9 0.7
Others 0.6 2.0 1.6 1.8 1.7 1.4 0.6 1.1 0.3 3.2 0.1 0.3
Total Identified 97.4 99.8 96.1 99.6 83.3 98.6 97.7 97.0 96.0 85.7 99.8

<sup>a</sup> RI<sub>calc</sub> = Retention indices determined with respect to a homologous series of n-alkanes on a ZB-5ms column. <sup>b</sup> RI<sub>db</sub> = Retention indices from the databases [42–45]. <sup>c</sup> Tentative identification based on RI and MS fragmentation agreement. <sup>d</sup> tr = Trace (<0.05%). <sup>e</sup> MS: 162(42%), 147(54%), 133(24%), 120(24%), 119(36%), 105(100%), 91(79%), 79(37%), 77(26%), 65(14%), 55(2%), and 41(3%). <sup>f</sup> MS: 206(28%), 147(5%), 134(12%), 133(100%), 120(45%), 107(41%), 105(16%), 91(8%), 77(11%), 55(7%), and 41(6%). <sup>g</sup> MS: 206(10%), 107(100%), 77(6%), and 41(3%). Concentrations of major components are highlighted in bold.
The major components of the leaf essential oil of *P. corymbosa* (reported as *P. integrifolia*) from Bangladesh were phytol (27.3%), α-humulene (14.2%), spathulenol (12.1%), 1-octen-3-ol (8.2%), eugenol (6.7%), and phenylethyl alcohol (5.8%) [46]. Neither 1-octen-3-ol, phenylethyl alcohol, nor eugenol were detected in the samples from Vietnam. Likewise, neither α-copaene nor *allo*-aromadendrene were reported from the Bangladeshi sample. In contrast, *P. corymbosa* leaf essential oil (reported as *P. serratifolia*) displayed a very simple composition of eugenol (47.9%), eugenyl acetate (9.1%), massoialactone (32.9%), and a compound identified as cis-2-oxabicyclo[4.4.0]decane (12.4%) (likely incorrect based on relative retention times) [47]. Thus, there is wide variation in the essential oil compositions of this plant, which suggests different chemotypes are possible or these three plants represent different species.

### 2.2.4. *Premna flavescens*

Leaves of *P. flavescens* were collected from two different sites (i.e., N'am Giả Commune, Quế Phong district, Pu Hoat Nature Reserve, Nghe An province, and Đồng Văn Commune, Quế Phong District, Pu Hoat Nature Reserve, Nghe An province). The leaf essential oils from the two sites showed notable differences in compositions (Table 3). (E)-Caryophyllene was abundant in both samples (41.0% and 11.8% in the N'am Giả and Đồng Văn samples, respectively), as was trans-β-elemene (9.9% and 8.7%, respectively). The sample from Đồng Văn was rich in α-gurjunene (19.6%), but only a minor component (0.1%) in the sample from N'am Giả. Likewise, α-guaiene and α-bulnesene were relatively abundant in the Đồng Văn sample (6.1% and 5.4%), but minor in the sample from N'am Giả (0.5% and 0.2%, respectively). Interestingly, bicyclogermacrene (7.8%) and an unidentified component (RI 1759, 14.7%) in the sample from N'am Giả, were not detected in the sample from Đồng Văn. Conversely, α-selinene, 8.7% in the sample from Đồng Văn, was not detected in the sample from N'am Giả. As far as we are aware, there have been no previous reports on the essential oil chemistry of *P. flavescens*.

### 2.2.5. *Premna maclurei*

The leaf essential oil composition of *P. maclurei* is shown in Table 3. The essential oil was dominated by sesquiterpene hydrocarbons (62.5%) and oxygenated sesquiterpenoids (30.1%) with (E)-caryophyllene (30.7%), α-humulene (5.3%), δ-cadinene (8.4%), spathulenol (6.8%), and caryophyllene oxide (12.3%) as the major components. To our knowledge, there have been no previous reports on the essential oil composition of *P. maclurei*.

### 2.2.6. *Premna mekongensis*

Essential oils were obtained from leaves of *P. mekongensis* from two different locations, Ngoc Linh Nature Reserve in Quang Nam Province, and Chu Mom Ray National Park. The leaf essential oil compositions are listed in Table 3. The two samples showed very different compositions. The Ngoc Linh sample was dominated by α-pinene (66.9%) and (E)-caryophyllene (14.7%). The leaf essential oil from Chu Mom Ray, on the other hand, had relatively low concentrations of α-pinene (1.5%) and (E)-caryophyllene (3.9%). In addition, the Chu Mom Ray essential oil was much more complex with 95 identified components compared to only 37 in the Ngoc Linh sample. The high concentration of α-pinene in *P. mekongensis* leaf essential oil from Ngoc Linh was unexpected and uncharacteristic of *Premna* leaf essential oils, which are generally low in monoterpenic hydrocarbon concentrations (see below). To our knowledge, there have been no previous studies on the essential oil composition of *P. mekongensis*.

### 2.2.7. *Premna puberula*

The chemical composition of the leaf essential oil of *P. puberula* is shown in Table 3. The major chemical classes present in the essential oil were sesquiterpene hydrocarbons (22.4%), with α-copaene (5.3%) and *allo*-aromadendrene (4.1%) as major components, and oxygenated sesquiterpenoids (58.2%),
dominated by (E)-caryophyllene oxide (21.2%) along with spathulenol (7.7%) and humulene epoxide II (4.7%). There have been no previous reports on the essential oil of *P. puberula*.

### 2.2.8. Premna tomentosa

The leaf essential oil composition of *P. tomentosa* is shown in Table 3. A total of 82 compounds were tentatively identified in the essential oil accounting for 99.8% of the composition, which was dominated by sesquiterpene hydrocarbons, especially (E)-caryophyllene (22.0%) and germacrene D (11.4%). The only previous examination of the essential oil of *P. tomentosa* is a relatively old work by Narayan and Muthana in 1953 [48]. These workers identified limonene (57.8%), (E)-caryophyllene (17.2%), an unidentified cadinane sesquiterpene (7.8%), an unidentified sesquiterpene alcohol (5.6%), and an unidentified diterpene hydrocarbon (5.5%) in the leaf essential oil from southern India.

### 2.2.9. Species Composition Comparison

Analogous to most of the *Premna* essential oil compositions observed in this study, leaf essential oils of other *Premna* species have shown compositions dominated by sesquiterpene hydrocarbons, e.g., *Premna coriacea* (55.2%) [49], *Premna latifolia* (76.4%) [50], *Premna quadrifolia* (65.5%) [51], and *Premna odorata* (62.3%) [52]. On the other hand, other *Premna* species are particularly rich in low molecular weight alcohols such as 1-octen-3-ol in *Premna barbata* (37.3%) [33], *P. latifolia* (35.7%) [54], and *Premna angolensis* (28.0%) [51]. In contrast, the essential oil of *Premna microphylla* was dominated by the sesquiterpenoid derivative blumenol C (49.7%) [55]. Compounds common to all eight of the *Premna* leaf essential oils in this study were α-pinene, β-pinene, p-cymene, limonene, linalool, *trans*-β-elemene, (E)-caryophyllene, α-humulene, β-selinene, and caryophyllene oxide. These are all relatively common essential oil constituents, and therefore cannot be considered as key compounds defining the genus. Furthermore, leaf essential oils of other *Premna* species were missing several of these components. The leaf essential oil of *P. coriacea* from Karnataka, India, was devoid of α-pinene, β-pinene, linalool, and β-selinene [49]. Likewise, the leaf oil from *P. microphylla* from Zhejiang Province, China, contained no α-pinene, β-pinene, linalool, (E)-caryophyllene, or α-humulene [55]. *Premna integrifolia* leaf essential oil from Bangladesh did not show p-cymene, limonene, linalool, β-elemene, or β-selinene [46]; *P. odorata* leaf oil from Giza, Egypt, showed no α-pinene, β-pinene, p-cymene, limonene, β-elemene, or β-selinene [52]; *P. angolensis* leaf oil from Comé, Benin, contained no β-pinene or caryophyllene oxide, and *P. quadrifolia* from Comé, Benin, contained no α-pinene, limonene, or linalool [51].

### 2.3. Mosquito Larvicidal Activity

The *Premna* leaf essential oils have been screened for mosquito larvicidal activity against *Aedes aegypti* and, if sufficient mosquito larvae were available, also against *Ae. albopictus* and *Culex quinquefasciatus*. The 24-h and 48-h larvicidal activities are shown in Tables 4 and 5, respectively. Considering larvicidal activities against *Ae. aegypti*, the most active *Premna* leaf essential oils were *P. cambodiana* (24-h LC$_{50}$ = 16.8 µg/mL) and *P. mekongensis* from Nghe An (24-h LC$_{50}$ = 16.8 µg/mL). The pronounced larvicidal activity of *P. mekongensis* (Ngoc Linh) against *Ae. aegypti* can be attributed to the high concentration of α-pinene. This monoterpane has shown larvicidal activity against *Ae. aegypti* with LC$_{50}$ values ranging from 15.4 µg/mL to 79.1 µg/mL [56]. Interestingly, the larvicidal activity of *P. mekongensis* (Ngoc Linh) against *Cx. quinquefasciatus* was less (24-h LC$_{50}$ = 42.7 µg/mL), consistent with the reduced activity of α-pinene against this mosquito larva (LC$_{50}$ = 95 µg/mL) [57].
For example, the essential oil from the inflorescences of *Piper marginatum* α-copaene and 13.1% (α-copaene (23.3%), α-caryophyllene) showed larvicidal activity against *Ae. aegypti* or *P. mekongensis* (Ngoc Linh) 35.81 (31.76–42.26) 4.14 0.126

*P. mekongensis* (Chu Mom Ray) 41.63 (38.79–44.49) 55.94 (52.45–60.49) 35.0 0.000

*P. puberula* (Nghe An) 50.88 (46.25–56.36) 80.60 (72.74–91.86) 12.7 0.002

*P. tonentosa* (Nghe An) 34.21 (31.02–37.67) 54.36 (49.42–61.35) 0.225 0.893

Permethrin (control) 0.0094 (0.0082–0.0107) 0.0211 (0.0185–0.0249) 57.6 0.000

### Culex quinquefasciatus

*P. chevalieri* (Quang Nam) 75.68 (68.51–84.52) 129.8 (115.9–150.0) 6.94 0.031

*P. mekongensis* (Ngoc Linh) 42.66 (38.71–47.43) 69.35 (62.21–79.95) 1.68 0.431

*P. mekongensis* (Chu Mom Ray) 33.16 (30.30–36.25) 52.01 (47.55–58.29) 11.8 0.003

*P. puberula* (Nghe An) 60.59 (55.77–66.33) 87.68 (80.11–98.09) 12.4 0.002

Permethrin (control) 0.0188 (0.0173–0.0206) 0.0294 (0.0270–0.0326) 24.1 0.000

### Table 5. Forty-eight-hour mosquito larvicidal activities of *Premna* leaf essential oils.

| Premna Species (Collection Site) | LC50 (95% Limits), µg/mL | LC90 (95% Limits), µg/mL | χ² | p |
|----------------------------------|---------------------------|---------------------------|-----|-----|
| *Aedes aegypti*                  |                           |                           |     |     |
| *P. cambodiama* (Chu Mom Ray)    | 13.68 (10.72–15.77)       | 25.62 (22.82–30.59)       | 0.00399 | 0.998 |
| *P. chevalieri* (Quang Nam)      | 30.23 (27.75–32.92)       | 45.11 (41.41–50.23)       | 4.59 | 0.101 |
| *P. corymbosa* (Nghe An)         | 33.59 (28.68–38.65)       | 71.64 (62.98–84.86)       | 2.98 | 0.225 |
| *P. corymbosa* (Da Nang)         | 60.43 (55.81–66.17)       | 83.54 (76.24–94.13)       | 8.07 | 0.018 |
| *P. flavescens* (Đồng Văn)        | 62.42 (56.58–69.12)       | 105.9 (96.5–119.0)        | 2.33 | 0.312 |
| *P. macheri* (Nghe An)           | 41.63 (38.85–44.63)       | 57.07 (53.07–62.68)       | 0.922 | 0.631 |
| *P. mekongensis* (Ngoc Linh)     | 17.62 (15.37–19.67)       | 30.00 (26.76–35.65)       | 0.0564 | 0.982 |
| *P. mekongensis* (Chu Mom Ray)   | 38.70 (36.18–41.21)       | 49.94 (47.01–53.73)       | 0.130 | 0.937 |
| *P. puberula* (Nghe An)          | 45.71 (41.21–50.97)       | 76.15 (68.30–87.56)       | 3.40 | 0.182 |
| *P. tonentosa* (Nghe An)         | 31.4 (28.32–34.69)        | 50.80 (46.13–57.36)       | 0.0878 | 0.957 |
| Permethrin (control)             | 0.0087 (0.0074–0.0102)    | 0.0204 (0.0181–0.0236)    | 39.6 | 0.000 |

| *Culex quinquefasciatus*         |                           |                           |     |     |
| *P. corymbosa* (Da Nang)         | 35.13 (31.93–38.74)       | 56.97 (51.54–64.86)       | 0.148 | 0.929 |
| *P. flavescens* (Đồng Văn)        | 74.14 (66.55–81.95)       | 133.2 (121.1–149.9)       | 9.87 | 0.007 |
| *P. puberula* (Nghe An)          | 98.1 (91.0–105.7)         | 151.1 (140.3–165.0)       | 37.2 | 0.000 |

The major components in *P. cambodiama* leaf essential oil were the sesquiterpene hydrocarbons α-copaene (23.3%), (E)-caryophyllene (12.8%), and α-gurjene (11.3%). (E)-Caryophyllene has shown larvicidal activity against *Ae. aegypti* with reported LC50 values of 38.6 µg/mL [58] and 88.3 µg/mL [59]. As far as we are aware, there have been no reports on the larvicidal activities of either α-copaene or α-gurjene. However, essential oils rich in α-copaene have shown notable larvicidal activity. For example, the essential oil from the inflorescences of *Piper marginatum* Jacq. (Piperaceae) (9.4% α-copaene and 13.1% (E)-caryophyllene) showed larvicidal activity against *Ae. aegypti* with LC50 of 19.9 µg/mL [60]; the ripe peel essential oil of *Hymenaea courbaril* L. (Fabaceae) (11.1% α-copaene) had an LC50 of 14.8 µg/mL on *Ae. aegypti* [61]. Note, however, that the leaf essential oil of *P. corymbosa* from Da Nang was also rich in α-copaene (8.1%) and (E)-caryophyllene (13.3%), but the larvicidal activity against *Ae. aegypti* was weaker (LC50 = 6.18 µg/mL). Similarly, the leaf essential oil of *P. flavescens* from...
Dong Van had α-gurjunene (19.6%) and (E)-caryophyllene (11.8%) as major components, but also showed weak larvicidal activity against *Ae. aegypti* (*LC*₅₀ = 64.7 μg/mL). The mere presence of the sesquiterpene hydrocarbons α-copaene, α-gurjunene, and (E)-caryophyllene is not sufficient to impart good larvicidal activity; there are likely synergistic effects of these compounds with minor components that account for the activities.

3. Materials and Methods

3.1. Plant Material

Leaves of *Premna* species were collected from several different locations in central Vietnam (Table 1). The plants were identified by Dr. Do Ngoc Dai, and voucher specimens (see Table 2) have been deposited in the plant specimen room, Faculty Agriculture, Forestry, and Fishery, Nghe An, College of Economics. The fresh leaves (2.0 kg each), immediately after collection, were shredded and hydrodistilled for 4 h using a Clevenger type apparatus (Witeg Labortechnik, Wertheim, Germany). Essential oil yields are summarized in Table 2. Essential oils were dried over anhydrous Na₂SO₄ and stored in sealed glass vials at 4 °C until analyzed.

3.2. Gas Chromatography–Mass Spectrometry

The *Premna* leaf essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS) as described previously [56]: Shimadzu GCMS-QP2010 Ultra, electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s; ZB-5ms column (30 m length × 0.25 mm inner diameter × 0.25 μm film thickness); He carrier gas, head pressure of 552 kPa, flow rate of 1.37 mL/min; injector temperature was 250 °C, ion source temperature was 200 °C; GC oven temperature program: 50 °C initial temperature, increased 2 °C/min to 260 °C; 5% solution of essential oil in CH₂Cl₂, 0.1 μL injection, splitting ratio 30:1. Putative identification of the essential oil components was based on their calculated retention indices (RI), based on a homologous series of *n*-alkanes (C₈-C₄₀), and their mass spectral fragmentation patterns compared with those reported in the databases [42–45], with RI values within ±10 units and with matching factors >80%. The concentrations of the essential oil components were calculated from raw peak areas, normalized to 100%, without standardization.

3.3. Mosquito Larvicidal Assay

Eggs of *Aedes aegypti* were purchased from Institute of Biotechnology, Vietnam Academy of Science and Technology and maintained at the Laboratory of Department of Pharmacy of Duy Tan University, Da Nang, Vietnam. Adults of *Culex quinquefasciatus* and *Aedes albopictus* collected in Hoa Khanh Nam ward, Lien Chieu district, Da Nang city (16°03′14.9″ N, 108°09′31.2″ E) and were identified by National institute of Malariology, Parasitology, and Entomology, Ho Chi Minh City. Adult mosquitoes were maintained in entomological cages (40 × 40 × 40 cm) and fed a 10% sucrose solution and were allowed to blood feed on 1-week-old chicks and mice, respectively. Egg hatchings were induced with tap water. Larvae were reared in plastic trays (24 × 35 × 5 cm). The larvae were fed on Koi fish food. All developmental stages were maintained at 25 ± 2 °C, 65–75% relative humidity and a 12:12 h light:dark cycle at the Laboratory of the Faculty of Environmental and Chemical Engineering of Duy Tan University, Da Nang, Vietnam.

Larvicidal activities of the *Premna* essential oils were determined following the protocol previously reported [62]: 250-mL beakers, 150 mL of water, and 20 larvae (fourth instar), aliquots of the *Premna* essential oils dissolved in EtOH (1% stock solution) were added to give final concentrations of 100, 50, 25, 12.5, and 6 μg/mL; EtOH only (negative control) and permethrin (positive control), mortality recorded after 24 h and 48 h of exposure, experiments were carried out at 25 ± 2 °C, each test was conducted with four replicates. The data obtained were subjected to log-probit analysis [63] to obtain
LC_{50} values, LC_{90} values and 95% confidence limits using Minitab® 19 (Minitab, LLC, State College, PA, USA).

All experimental procedures that involved animals (mice, mosquitoes, chicks, and non-target organisms) were conducted in accordance with the “Guideline for the Care and Use of Laboratory Animals” which was approved by the Medical-Biological Research Ethics Committee of Duy Tan University (DTU/REC2020/NHH01), Vietnam.

4. Conclusions

The leaf essential oils of eight species of Premna have been obtained in yields ranging from 0.10% to 0.25%. The mosquito larvicidal activities of these species have been determined for the first time and this is the first report of the essential oil compositions of *P. cambodiana*, *P. flavescens*, *P. maclurei*, *P. mekongensis*, and *P. puberula*. The essential oil compositions were largely dominated by sesquiterpene hydrocarbons and oxygenated sesquiterpenoids. The larvicidal activities against *Aedes aegypti* (LC_{50} < 65 µg/mL) are promising and can probably be attributed to these components. The essential oil yields, however, are low and likely preclude their consideration as viable alternatives to other essential oils for control of mosquito vectors. However, potential utility of these essential oils will necessitate exploration of cultivation, including plant breeding aimed at increasing oil yield and/or larvicidal activity, potential detrimental effects of the essential oils on the environment, as well as field experiments on application of the essential oils, effects of environmental conditions and potential formulations on essential oil evaporation rates.

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