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Research Article

Chemical compositions, antimicrobial activity and mosquito larvicidal actions of essential oils from the leaves and fruits of two Rutaceae family of plants from Vietnam

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Abstract
The paper reports that the main constituent of essential oil from the leaves of *Macclurodendron oligophlebia* (Merr.) Hartl. growing in Vietnam was α-pinene (81.7%), while α-pinene (67.4%) and limonene (11.2%) were the major compounds in fruit essential oil. The composition of essential oils from the leaves and fruits of *Melicope ptelefolia* (Champ. ex Benth.) T.G. Hartley was characterized as germacrene D (34.0% and 31.1%, respectively), bicyclogermacrene (5.5% and 7.6%, respectively), d-elemene (9.9% and 9.0%, respectively) and α-pinene (6.5% and 9.8%, respectively). The fruit essential oil contains limonene (4.9%) and (*E*)-β-ocimene (8.1%). The fruit essential oils of *M. oligophlebia* and *M. ptelefolia* exhibited anti-candial activity towards *Candida albicans* ATCC 10231, with minimum inhibitory concentration (MIC) value of 8.0 µg/mL and 32.0 µg/mL, respectively. *M. oligophlebia* fruit oil also showed action against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579, each with MIC value of 32.0 µg/mL. Also, *M. oligophlebia* leaf showed higher activity against *Pseudomonas aeruginosa* ATCC 27853 (MIC, 32.0 µg/mL). The other oil samples exhibited varying degrees of antimicrobial activity with MIC values of either 64.0 µg/mL or 128. 0 µg/mL. The leaf essential oil of *M. oligophlebia* displayed larvicidal activity against *Aedes aegypti* larvae with minimum lethal concentration (LC_{50}) values of 16.71 µg/mL and 11.40 µg/mL, respectively at 24 h and 48 h. The fruit oil did not exhibit any larvicidal action. However, *M. ptelefolia* leaf and fruit showed activity at 24 h with LC_{50} values of 33.18 µg/mL and 35.06 µg/mL, respectively.

Keywords
Gram-positive bacteria, Gram-negative bacteria, *Candida albicans*, Terpenes, *Aedes aegypti*

Introduction

Plants are part of our daily life and essential oils have been extracted from several different species. Essential oils consist of a variety of compounds that are well known for their various biological and pharmacological effects. These activities are normally related to the chemical substances mostly terpenes that are present in them[1]. Several plant species of the Rutaceae family are medicinal and essential oil-bearing plants. To provide more information for utilization of some species of this family in Vũ Quang National Park, essential oils were obtained from the leaves and fruits of two species. *Macclurodendron oligophlebia* (Merr.) Hartl. is an evergreen tree that grows up to 16 m high and diameter of 30-40 cm[2]. The leaves are ovate while the bark is pale-grey. The bisexual flowers are white-bluish in colouration and flowering commences in June[2]. The authors are aware of only one piece of information on the chemical compounds of *M. oligophlebia* collected from Ben En National Park, Vietnam[3]. The main constituents

of the essential oils were α-pinene (17.5%), β-caryophyllene (15.5%) and caryophyllene oxide (10.6%) in the leaf, while the fruit oil was dominated by benzyl benzoate (16.8%), (*E, E*)-farnesol (8.3%) and β-caryophyllene (6.0%)[3].

Melicope ptelefolia (Champ. ex Benth.) T.G. Hartley is a well-known herb in Asian culture that has been touted for its medicinal benefits. The plant's native range is Southern China to Indo-China, Taiwan and Vietnam. It is known in Vietnamese name as Ba chạc. Extracts from *M. ptelefolia* are well known for medicinal benefits such as anticancer activity[4,5], anti-nociceptive and anti-inflammatory[6]. Extracts of *M. ptelefolia* increase sperm efficiency in rat model[7]. Recently, acyphloroglucinol, 2,4,6-trihydroxy-3-geranylacetophenone or tHGA, was described as the active principle of *M. ptelefolia* inhibiting soybean 15-LOX in anti-inflammatory model[8]. Also, tamarixetin 3-robinobioside isolated from the leaves of *M. ptelefolia* was found to posses neuraminidase inhibitory activities, while kaempferol 3-robinobioside, kaempferol 3-O-β-d-glucopyranosyl (1 → 2)-α-d-xylopyranoside and tamarixetin 3-robinobioside, also showed moderate reductions in H1N1-induced cytopathic effects on MDCK cells[9]. Moreover, melicoester, melicopeprenoate, and p-O-geranyl-7″-acetoxy coumaric acid in addition to kokusaginine, genistein, p-O-geranyl coumaric acid, 4-stigmasten-3-one, and 3β-hydroxystigma-5-en-7-one amongst others were also characterized from the plant[10]. Previous reports on essential oils of *M. pteleifolia* from Vietnam were documented. The major compounds in the essential oil of *M. pteleifolia* grown in Lam Dong Province, Vietnam are (+)-sabinene (34.73%) and *cis*-α-bergamotene (13.2%), while the essential oil also displayed antibacterial activity against *Streptococcus pyogenes* and *Eschetichia coli*[11]. In addition, 1,9-decadiyne (32.59%), patchoulene (10.04%) and viridlforene (9.40%) were described previously from samples collected from Binh Chau-Phuoc Buu Nature Reserve, Vietnam[12].

Aedes aegypti L. is an important vector of dengue and chikungunya fever, diseases that still cause high infant and pregnant mortalities in developing countries of the world[13]. However, the lack of effective treatment with vaccines for these ailments and complications and the increase in mosquito resistance of *A. aegypti* to known pesticides has led to the search for new approaches to control mosquito population. Essential oils have been recently observed and could be used as effective natural antimicrobial[14-17] and insecticides against *A. aegypti* larvae[13]. This prompted the present study aimed at the investigation into the chemical constituents, antimicrobial and larvicidal activities of essential oils from *M. oligophlebia* and *M. ptelefolia*. In addition, the chemotaxonomic implication of the data was also discussed.

Materials and methods

Collection of the leaves and fruit samples of M. oligophlebia and M. ptelefolia from Vũ Quang National Park

The plants were collected from Vũ Quang National Park (GPS 18°17′15″N, 105°21′39″E), north-central Vietnam, in October 2022, during daylight. Thereafter, the samples were identified by Dr. Dai, D.N. and voucher specimens viz. VNU 764 and VNU 769, respectively, were preserved for *M. oligophlebia* and *M. ptelefolia*. The voucher specimens were preserved in the plant specimen room, NgheAn College of Economics, NgheAn, Vietnam. In the preparation of the samples for hydrodistillation experiment, unwanted materials were completely removed thereby leaving a total of 2 kg of pulverized individual sample.

Hydrodistillation of essential oils from the leaves and fruits of M. oligophlebia and *M. ptelefolia*

The leaves and fruits of the studied plants were divided separately into three equal parts for the hydrodistillation experiments. Considering the procedures described in earlier studies[14-17], the required sample was packed separately into a 5 L flask, and distilled water was added to an acceptable level while the flask was connected to the Clevenger-type apparatus and the source of heat. The hydrodistillation experiment was allowed to run for 3 h at normal pressure

when essential oil was distilled off. Each of the distilled essential oils were collected separately inside sample bottles and weighed accordingly. The preservation of the essential oils was done inside refrigerator (4°C) before the instrumental and biological analyses were performed. The distillation of essential oils from each of the plant was conducted three times. The mass (g) of the essential oil was divided by the mass (g) of each plant to obtain the percentage yield of the essential oils.

Analysis of the essential oils

The use of Gas chromatography (GC) and Gas chromatography-mass spectrometry (GC/MS) as reported previously were also adopted in this study[14-17]. In both analyses, the GC operating conditions were the same. The Gas chromatograph used was a HP 7890A Plus manufactured by Agilent Technologies, USA. Each of the essential oil samples (1.0 µL) was injected separately into the GC mode by splitting method at an Inlet pressure of 6.1 kPa. The components of the GC include a HP-5MS column of dimension 30 m x 0.25 mm, and with film thickness of 0.25 µm; and a flame ionization detector (FID). The operating parameters were the carrier gas (H_e) at a flow rate of 1 mL/min; injector temperature of 250°C; and detector temperature of 260°C. Each of the essential oil was injected into the GC column by split method at a split ratio of 10:1. The column was temperature programmed from 40°C (held 2 min isothermally) to 220°C (10 min hold) at 4°C /min. Each of the essential oils was analysed three times. The relative amounts of individual components were calculated based on the GC peak area (FID response) as described in previous studies[14-17]. As each essential oil was obtained three times, so was the instrumental analysis as described in previous studies[14-17].

In the GC/MS analysis, the GC chromatograph was coupled with a mass spectrometer HP 5973 MSD. The GC components and operating parameters were similar as described above for the GC experiments. Some of the conditions are Helium was used as carrier gas (1 mL/min) and the column was also HP-5MS (30 m x 0.25 mm, and with film thickness of 0.25 µm). The operating MS conditions were the ionization voltage maintained at 70eV with the emission current of 40 mA. The acquisition mass range was scanned from 45 to 350 amu and at a sampling rate: 1.0 scan/s.

For the identification of the components present in the individual essential oils, the procedures described earlier were used, namely the comparison of GC retention indices with reference to a homologous series of *n*-alkanes (C_6-C_{40}), co-injection with known compounds under the same GC conditions, and correlation of the MS fragmentation patterns in the individual GC/MS spectra with known essential oil composition in literature[18] as described recently[14-17].

Study on antimicrobial activity

The source of the microbial strains used for the study is the laboratory stock of Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The microbial strains were described earlier[13-16] and these were *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076 and *Candida albicans* ATCC 10231.

The antimicrobial analysis involves the determination of the measurement of the minimum inhibitory concentration (MIC) and the measurement of the median inhibitory concentration (IC_{50}) values by using the microdilution broth susceptibility assay. The concentration of the essential oils was prepared by two-fold dilution from 1.6384 x 10^4 mg/mL to 2 mg/mL, as described earlier[14-17]. Both the Gram-positive and Gram-negative bacteria described above and employed in the determination of the antimicrobial activity were maintained in double-strength Mueller-Hinton broth and were standardized to 5 × 10^5 CFU/mL. The fungus strength was 1 × 10^3 CFU/mL, and grown in double-strength Sabouraud dextrose broth. Dilute solutions of essential oils in sterile distilled water and microorganisms were transferred to 96-well microtiter plates. The solutions were allowed to incubate for 24 h, and at temperature of 37°C.

Afterwards, the MIC values were evaluated from the well with the lowest concentration of essential oils which completely inhibited the growth of microorganisms. On the other hand, the IC_{50} values were measured by considering the percentage of microorganisms that inhibited growth based on the turbidity measurement data of the EPOCH2C spectrophotometer. For positive control, standard antimicrobial drugs of streptomycin (antibacterial), as well as nystatin and cycloheximide (anticandidal) were used. The last row of the microtiter plates containing only the serial dilutions of the essential oils without microorganisms was used as the negative (no growth) control. The data obtained were calculated as described previously[14-17].

Study of mosquito larvicidal action

The adults of *Aedes aegypti* used in the study of larvicidal activities of the essential oils were collected from Hoa Khanh Nam ward, Lien Chieu District, Da Nang city (16°03'14.9"N, 108°09'31.2"E), Vietnam. The tests were conducted at the Center for Entomology and Parasitology Research, Duy Tan University. The mosquito vectors were maintained under identical conditions as described previously[19,20]. Briefly, *A. aegypti* were kept in cages (40 x 40 x 40 cm). The adult mosquito vectors sustained on 10% sucrose solution were fed with blood of mice. Tap water was used to induce hatching of eggs. The resulting larvae were rare in plastic trays (24 x 35 x 5 cm) built for this purpose with temperature condition sustained at 25 ± 2°C and relative humidity of 65-75%. There was equal 12:12 h light: dark cycle through the duration of the study. Feeds for the larvae included dog biscuits and yeast powder in the ratio of 3:1 as reported earlier[19,20].

The larvicidal activities of the essential oils from *M. oligophlebia* and *M. ptelefolia* were evaluated as previously described[19,20]. Four different concentrations of the essential oils (12.5, 25, 50, and 100 µg/mL) were used in the experiment. With ethanol (EtOH) used as a negative control, permethrin, a larvicidal drug, was used as a positive control. Prior to analysis, 200 mg of each of the essential oils was dissolved in 20 mL of ethanol which was transferred into different beaker stocked with 20 fourth instar larvae of *A. aegypti*. The mortality of larvae of *A. aegypti* was recorded after 24 h and 48 h of exposure to the different concentrations of the essential oils and repeated four times.

The mortality rate of *A. aegypti* was calculated according to the formula described previously[19,20];

$$MC = \frac{Mo-Mt}{100-Mt} \times 100$$

Mo = mortality in the treated groups, Mt = mortality in the control group and Mc = calculated mortality.

Statistical analysis

The LC_{50} values, LC_{90} values, and 95% confidence limits were determined by log-probit analysis using Minitab® 19 (Minitab, LLC, State College, PA, USA). Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD, ±) of three (chemical constituents and antimicrobial analysis) and four independent measurements for the larvicidal test, using Microsoft Excel program 2003.

Results and discussion

Chemical constituents of the studied essential oils

Analysis of the compositional patterns of the essential oils of M. oligophlebia

The yields of the essential oils were 0.42% (w/w) and % (w/w) for the leaf and fruit respectively. Both essential oils were light yellow coloured. Moreover, the identified constituents amounted to 99.9% of the total oil content for both samples. Monoterpene hydrocarbons were the abundant class of compounds in both the leaf (90.0%) and fruits (85.9%). The leaf sample contained 7.7% of sesquiterpene hydrocarbons, while the fruit sample had 7.4% of oxygenated monoterpenes and 4.4% of sesquiterpene hydrocarbons (Table 1). α-Pinene (81.7%) was the most singly abundant constituent of the leaf essential oil. Other compounds identified in amount ≥ 1% were β-caryophyllene (3.2%), limonene (3.0%), myrcene (2.6%), α-santalene (1.1%), and aromadendrene (1.0%). The content of α-pinene

Table 1. Data on the chemical compositions of essential oils of from the leaves and fruits of *M. oligophlebia* and *M. ptelefolia*

Sr. No	Rt (min)	Compounds	RI (Cal.)	RI (Lit)	*Mo*L[a]	*Mo*F[a]	*Mp*L[a]	*Mp*F[a]
1	10.17	α-Pinene	940	932	81.7	67.4	6.5	9.8
2	10.62	Camphene	955	946	0.5	0.5	-	-
3	11.33	Sabinene	978	969	-	-	0.2	0.1
4	11.50	β-Pinene	984	976	1.6	1.9	0.2	0.3
5	11.73	Myrcene	992	988	2.6	3.3	0.3	0.5
6	12.19	(*Z*)-Hex-3-enyl acetate	1006	1001	0.2	-	-	-
7	12.34	α-Phellandrene	1008	1004	-	0.1	-	-
8	13.15	Limonene	1034	1032	3.0	11.2	2.6	4.9
9	13.21	β-Phellandrene	1036	1034	-	0.4	-	0.2
10	13.28	(*Z*)-β-Ocimene	1038	0134	0.1	0.3	0.4	1.1
11	13.66	(*E*)-β-Ocimene	1049	1044	0.3	0.5	3.6	8.1
12	14.15	γ-Terpinene	1063	1056	0.2	0.1	-	-
13	15.21	Terpinolene	1094	1093	-	0.2	-	-
14	15.50	Linalool	1103	1101	0.5	5.0	0.2	0.5
15	16.04	(*E*)-4,8-Dimethylnona-1,3,5-triene	1118	1117	-	-	-	0.3
16	17.09	*trans*-Tagetone	1148	1143	-	-	0.2	0.1
17	17.39	*cis*-Tagetone	1157	1153	-	-	0.2	1.0
18	18.12	Borneol	1177	1177	-	0.1	-	-
19	18.92	α-Terpineol	1200	1197	-	0.2	-	-
20	20.20	*cis*-Ocimenone	1237	1234	-	-	1.6	-
21	20.50	*trans*-Ocimenone	1246	1246	-	-	0.8	-
22	23.96	δ-Elemene	1348	1343	-	-	9.9	9.0
23	24.35	α-Cubebene	1360	1357	-	-	-	0.1
24	24.48	Eugenol	1367	1363	0.5	2.1	-	-
25	25.31	α-Copaene	1389	1387	0.3	0.1	0.2	0.4
26	25.67	β-Bourbonene	1400	1397	-	-	1.8	0.2
27	25.73	β-Cubebene	1402	1401	-	-	-	0.2
28	25.78	*cis*-β-Elemene	1403	1399	-	0.1	1.1	0.9
29	26.46	*trans*-α-Bergamotene	1425	1427	0.3	-	-	-
30	26.65	α-Santalene	1431	1431	1.1	0.2	-	-
31	26.75	β-Copaene	1434	1435	-	-	0.5	0.2
32	26.83	β-Caryophyllene	1437	1437	3.2	1.3	0.5	0.6
33	27.09	β-Gurjunene	1445	1443	-	-	1.0	-
34	27.11	γ-Elemene	1447	1450	-	-	-	0.8
35	27.45	Guaia-6,9-diene	1455	1454	-	-	0.9	0.7
36	27.47	Aromadendrene	1456	1457	1.0	0.3	-	-
37	27.57	*cis*-Muurola-4(14),5-diene	1460	1457	-	-	0.3	-

table 1. (continued).

Sr. No	Rt (min)	Compounds	RI (Cal.)	RI (Lit)	*Mo*L[a]	*Mo*F[a]	*Mp*L[a]	*Mp*F[a]
38	27.58	(*Z*)-β-Farnesene	1463	1461	-	-	-	0.2
39	27.91	α-Humulene	1471	1471	0.3	0.1	0.3	0.4
40	28.14	9-epi-(*E*)-Caryophyllene	1478	1477	0.1	-	-	-
41	28.56	γ-Muurolene	1490	1489	-	-	1.0	1.0
42	28.75	α-Amorphene	1495	1493	-	-	0.3	0.3
43	28.75	Germacrene D	1498	1497	-	0.1	34.0	31.1
44	28.95	β-Selinene	1504	1501	0.4	0.2	-	-
45	28.97	δ-Selinene	1505	1503	-	-	0.3	0.3
46	29.12	γ-Amorphene	1508	1509	-	-	-	0.2
47	29.16	Viridiflorene	1510	1510	0.1	0.2	-	-
48	29.17	(*E,E*)-β-Farnesene	1511	1511	-	-	-	0.5
49	29.19	α-Selinene	1512	1513	0.4	-	-	-
50	29.23	Bicyclogermacrene	1514	1515	0.4	1.0	5.5	7.6
51	29.72	δ-Amorphene	1521	1519	-	-	-	0.2
52	29.46	γ-Cadinene	1530	1525	-	-	0.2	0.4
53	29.80	Eugenol acetate	1533	1534	0.3	1.2	0.2	0.5
54	29.91	δ-Cadinene	1536	1535	0.1	0.2	0.6	1.3
55	30.25	*trans*-Cadina-1,4-diene	1548	1545	-	-	-	0.1
56	30.74	Elemol	1564	1563	-	-	0.3	0.3
57	30.95	(*E*)-Nerolidol	1571	1573	-	-	13.5	2.5
58	31.14	Germacrene B	1577	1577	-	-	2.1	2.3
59	31.74	Spathulenol	1598	1598	0.1	0.4	0.5	0.7
60	31.94	Caryophyllene oxide	1605	1603	0.6	-	0.3	-
61	31.95	Viridiflorol	1608	1605	-	0.6	-	0.4
62	32.17	Guiaol	1613	1615	-	-	0.1	0.3
63	33.90	α-Cadinol	1678	1677	-	-	0.3	0.6
Total					99.9	99.9	91.5	91.2
Monoterpene hydrocarbons (Sr. No. 1-5, 7-13)					90.0	85.9	13.8	25.5
Oxygenated monoterpenes (Sr. No. 14, 16-21, 24)					1.0	7.4	3.0	1.1
Sesquiterpene hydrocarbons (Sr. No. 22, 23, 25-52, 54, 55, 58)					7.7	4.4	59.5	46.6
Oxygenated ssquiterpenes (Sr. No. 53, 56,57, 58-63)					1.0	2.2	15.2	5.3
Non-terpenes (Sr. No. 6, 15)					0.2	-	-	0.3

[a] Percentage composition, means of three replicates, SD ± standard deviation were insignificant and excluded to avoid congestion; RI (Cal.), Experimental retention indices; RI (Lit.) Literature retention indices from Reference[18]; *Mo*L, *M. oligophlebia* leaf; *Mo*F, *M. oligophlebia* fruits; *Mp*L, *M. ptelefolia*; *Mp*F, *M.ptelefolia* fruits; - Not identified

(81.7%) in the present investigated sample from Vu Quang Nature Reserve, Vietnam, was much higher than previously reported from the leaf of *M. oligophlebia* collected from Ben En National Park, Vietnam (17.5%)[3]. In addition, the amounts of β-caryophyllene (15.5%) and caryophyllene oxide (10.6%) in the previously analysed essential oil sample from Ben En National Park, Vietnam was higher when compared with data in the present study (3.2% vs 0.6%, respectively).

On the other hand, α-pinene (67.4%) and limonene (11.2%) were the major compounds

identified in the fruit essential oil of *M. oligophlebia* under investigation. In addition, linalool (5.0%), myrcene (3.3%), eugenol (2.1%), β-pinene (1.9%), β-caryophyllene (1.3%), eugenol acetate (1.2%) and bicyclogermacrene (1.0%) were present in amount ≥ 1%. It is interesting to note that benzyl benzoate and (*E, E*)-farnesol, which are the main compounds of previously analysed fruit essential oil of *M. oligophlebia* collected from Ben En National Park, Vietnam[3], are conspicuously absent in the present study. Moreover, the present oil sample contained lower amount of β-caryophyllene than previously reported[3]. It can be concluded that essential oils from different organs of *M. oligophlebia* exhibited chemical variability. Information is scanty on the chemical compositions of essential oils from any species of *Macclurodendron,* as such the results cannot be compared further.

Compositional patterns of essential oils of M. pteleifolia

From Table 1, essential oils from the leaves and fruits of *M. ptelefolia* were identified in 91.5% and 91.2% of the total oil contents, respectively. The main classes of compounds in the essential oils were monoterpene hydrocarbons (13.8% and 25.5%, respectively), sesquiterpene hydrocarbons (59.5% and 46.6, respectively) and oxygenated sesquiterpenes (15.3% and 5.35, respectively). The oxygenated monoterpenes were present in amount of 3.0% and 1.1%, respectively. The essential oils were characterized by germacrene D (34.0% and 31.1%, respectively), bicyclogermacrene (5.5% and 7.6%, respectively), δ-elemene (9.9% and 9.0%, respectively) and α-pinene (6.5% and 9.8%, respectively). Two previous reports on essential oils of *M. pteleifolia* from different provinces of Vietnam were documented. The main compounds in the essential oil of *M. pteleifolia* from Lam Dong Province which are (+)-sabinene and *cis*-α-bergamotene[11], as well as 1,9-decadiyne, patchoulene and viridlforene, present in samples collected from Binh Chau-Phuoc Buu Nature Reserve, Vietnam[12], were not identified in the samples under investigation. Conversely, germacrene D, bicyclogermacrene, and δ-elemene that were identified in higher amounts in the samples under investigation, were also absent from previously analysed samples[11,12]. The α-pinene content of the present study was higher than reported from Lam Dong Province[11]. This study shows that the chemical compositions of the essential oils of *M. pteleifolia* from the different regions of Vietnam are very variable.

Moreover, the composition of the present studied *M. pteleifolia* essential oils were compared with data available for the essential oils of other *Melicope* plants analysed in Vietnam and the rest of the world. Varieties of terpenoids have been reported from *Melicope* species (Table 2). The results indicated that the chemical compositions of the essential oils from the different *Melicope* species are very variable. Both intra-specific and inter-specific variations have been reported. For example, the leaf essential oils of *M. micrococca* from Australia was found to exist in two chemical forms of α-pinene/(*E*)-β-ocimene β-caryophyllene and bicyclogermacrene/ caryophyllene oxide/spathulenol[22]. The leaf essential oils from *M. melanophloia* from Australia indicate the existence of three chemical forms, namely, methyl chavicol/methyl eugenol form, α-pinene/1,8-cineole form and (*Z*)-β-ocimene/(*E*)-β-ocimene form[24]. The leaf[26] and flower[27] essential oils of *M. lunu-ankenda* from India, as well as essential oil from the flowers of *M. hortensis* forma hortensis from Australia[29] were dominated by evodione, whereas the leaves of *M. hortensis* forma hortensis contained high content of menthofuran[29]. The leaf essential oils of both *M. bonwickii* and *M. elleryana* from Australia[22] and *M. denhamii* from India[25] contained a high amount of zierone, while β-caryophyllene and α-humulene were the characteristic compounds of both *M. fellii* and *M. peninsularis* from Australia[22].

From the foregoing analysis, the leaf essential oil of *M. pteleifolia* had compositional pattern quite different from other *Melicope* species so far analysed in the literature (Table 2). Although, germacrene D was the second abundant compound found in the leaf essential oil of *M. rubra*[22] and *M. hayessii*[22] from Australia,

Table 2. Essential oils of *Melicope* species in literature

Species	Parts	Origin	Main constituents	References
M. contermina	Leaf	Australia	limonene (33%) and elemol (23%)	21
M. polybotrya	‘’	‘’	geijerene (41%), pregeijerene (38%)	21
M. affinis	‘’	‘’	bicyclogermacrene (7-18%) and β-bisabolene (t-9%)	22
M. bonwickii	‘’	‘’	Isomers of zierone (A, B each 5-10% and C 13-20%)	22
M. broadbentiana	‘’	‘’	α-pinene (21-76%) and limonene (0.6-28%).	22
M. elleryana	‘’	‘’	zierone (26-42%), allo-evodione (4-10%) and evodione (10-22%)	22
M. fellii	‘’	‘’	β-caryophyllene (9.9%), α-humulene (8.4%) and caryophyllene oxide (7.4%)	22
M. hayesii	‘’	‘’	bicyclogermacrene (22.8%), germacrene D (13.9%), (*E*, *E*)-α-farnesene (9.2%) and globulol (10.6%)	22
M. micrococca	‘’	‘’	α-pinene (1-46%), (*E*)-β-ocimene (t-10%), β-caryophyllene (0.4-15%), bicyclogermacrene (t-11%), caryophyllene oxide (0.3-23%) and spathulenol (1-12%)	22
M. peninsularis	‘’	‘’	β-caryophyllene (30-49%) and α-humulene (26-35%)	22
M. rubra	‘’	‘’	sabinene (31.1%), and germacrene D (22.6%)	22
M. vitiflora	‘’	‘’	sabinene (0.1-54%) and limonene (1-47%)	22
M. xanthoxyloides	‘’	‘’	β-caryophyllene (13-47%), spathulenol (1-18%) and α-pinene (t-15%)	22
M. belahe	‘’	Madagascar	α-pinene (42.6%), linalool (6.2%) and (*E*)-β-caryophyllene (5.2%)	23
M. melanophloia	‘’	Australia	methyl chavicol (5-13%) and methyl eugenol (51-67%)	24
	‘’	‘’	α-pinene (34-37%), and 1,8-cineole (4-12%)	24
	‘’’	‘’	limonene (1-8%), (*Z*)-β-ocimene (12-18%) and (*E*)-β-ocimene (23-56%)	24
M. denhamii	‘’	India	zierone (22.49%) and α-gurjunene (19.96%)	25
M. lunu-ankenda	‘’	‘’	evodione (20.2%) and leptonol (22.5%)	26
‘’	flower	‘’	evodione (38.9%), (*E*)-β-ocimene (12.4%), isolycodolin (11.7%) and alloevodionol (10.6%)	27

table 2. (continued).

Species	Parts	Origin	Main constituents	References
M. obscura	leaf	Re-union Island	linalool (25.2%-36.5%), geraniol (9.7%-18.7%), melicopenol (8.6-30.1%)	28
M. hortensis forma *hortensis*	‘’	Australia	menthofuran (64.0%) and evodone (27.0%)	29
	flower	Australia	evodone (44.0%), α-copaene (8.0%), and menthofuran (7.0%)	29
M. rutaecarpa	fruits	Italy	limonene (33.79%), β-elemene (10.78%) and linalool (8.15%)	30
M. rutaecarpa var. *officinalis*	‘’	‘’	myrcene (32.79%), limonene (18.36%), and β-caryophyllene (9.92%)	30

sabinene, the main compound of *M. rubra*[22] was identified in insignificant quantity in the studied *M. pteleifolia*. Also, bicyclogermacrene the main constituent of *M. hayessii*[22] was present in much lower quantity in *M. pteleifolia* from Vietnam which also does not contain globulol and (*E,E*)-farnesene as seen in *M. hayessii*. It may be substantiated that *M. pteleifolia* essential oils from Vietnam exists in three chemical forms viz. (+)-sabinene/*cis*-α-bergamotene form[11], 1,9-decadiyne/patchoulene/viridlforene[12] and germacrene D/d-elemene (this study). These variations in the compositional patterns of *Melicope* essential oils may be explained by the nature of the plant parts, differences in the ecological and climatic conditions at the points of collection among others.

Antimicrobial activity of M. oligophlebia

The essential oil from the fruits of *M. oligophlebia* displayed higher antibacterial activity against *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 and *B. cereus* ATCC 14579, with MIC value of 32.0 mg/mL (Table 3). The corresponding IC_{50} value was 15.67 mg/mL. Conversely, the leaf essential oil showed activity against the above microorganisms at MIC values of 64.0 mg/mL., 128.0 g/mL, and 64.0 mg/mL, respectively. However, the leaf essential oil exhibited more microbial activity towards *P. aeruginosa* ATCC 27853 than the fruit essential oil, at MIC value of 32.0 mg/mL. Only the fruit essential oil showed more potent anti-candidal action against *C. albican*s ATCC 10231, with MIC value of 8.0 mg/mL, with IC_{50} value was 4.67 mg/mL. Both essential oils, however, did not exhibit any activity towards *E. coli* ATCC 25922 and *S. enterica* ATCC 13076. Overall, essential oil from the fruit of *M. oligophlebia* showed greater antimicrobial activity than the leaf essential oil. This is the first report on the antimicrobial activity of any other species of *Macclurodendron* and in fact *M. oligophlebia*.

Antimicrobial activity of essential oil of M. ptelefolia

From Table 3, the leaf essential oil of *M. ptelefolia* showed similar antimicrobial pattern to five of the tested microorganisms namely *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *B. cereus* ATCC 14579, and *P. aeruginosa* ATCC 27853, with MIC value of 64.0 µg/mL. The same MIC value was shown in the anti-candidal test towards *C. albican*s ATCC 10231. The corresponding IC_{50} values were 32.66 µg/mL, 32.67 µg/mL, 32.66 µg/mL and 33.33 µg/mL, respectively for the antibacterial testing; and 33.33 µg/mL for the anti-candidal activity. On the other hand, the fruit essential oil displayed activity against *S. aureus* ATCC 25923and *B. cereus* ATCC 14579, with same MIC value of 64.0 µg/mL, with IC_{50} value of 32.56 µg/mL. The fruit oil did exhibit good anti-candidal activity at MIC value of 32.0 µg/mL and IC_{50} value of 15.67 µg/mL. Also, both essential oils, however, did not exhibit any activity towards *E. coli* ATCC 25922

Table 3. Results of the antimicrobial activity of essential oils of from the leaves and fruits of *M. oligophlebia* and *M. ptelefolia*

Microorganisms	MIC µg/mL[a]				IC_{50} µg/mL[a]			
	***Mo*L**	***Mo*F**	***Mp*L**	***Mp*F**	***Mo*L**	***Mo*F**	***Mp*L**	***Mp*F**
Enterococcus faecalis ATCC 29212	64.0	32.0	64.0	128.0	33.56	15.67	32.66	65.22
Staphylococcus aureus ATCC 25923	128.0	32.0	64.0	64.0	65.78	15.67	32.67	32.56
Bacillus cereus ATCC 14579	64.0	32.0	64.0	64.0	33.45	15.67	32.66	32.56
Pseudomonas aeruginosa ATCC 27853	32.0	64.0	64.0	Na	15.67	32.99	33.33	Nt
*Candida albican*s ATCC 10231	128.0	8.0	64.0	32.0	64.56	4.67	33.22	15.67
Escherichia coli ATCC 25922	Na	Na	Na	Na	Nt	Nt	Nt	Nt
Salmonella enterica ATCC 13076	Na	Na	Na	Na	Nt	Nt	Nt	Nt

[a] SD within the range of 0.001 and excluded to avoid congestion in the Table; Na, No activity; Nt, Not tested; *Mo*L, *M. oligophlebia* leaf; *Mo*F, *M. oligophlebia* fruits; *Mp*L, *M. ptelefolia*; *Mp*F, *M. ptelefolia* fruits

and *S. enterica* ATCC 13076. Overall, the leaf essential oil of *M. ptelefolia* had broad-spectrum of antimicrobial activity. Previous information has shown that both the extracts[31] and essential oils from *Melicope* plants have shown varying degree and selective antimicrobial activities. In a previous study, *M. ptelefolia* essential oil showed significant activities against *Streptococcus pyogenes* and *E. coli* [11]. The present oil samples did not display any activity towards *E. coli.* In addition, *M. denhamii* leaf oil like *M. ptelefolia* tested against Gram-positive and Gram-negative bacteria showed significant activity against *B. subtilis*[25]. However, unlike *M. ptelefolia,* the essential oil also exhibited activity against *E. coli.* This shows *Melicope* essential oils showed selective antimicrobial activities.

Quite expected, the studied essential oils from Rutaceae family of plants showed different chemical compositional patterns and invariably would also have influence on the antimicrobial potential of each of the studied plant samples. It is well known that the major compounds of an essential oil have direct influence on its observed biological activities. However, most often, the synergistic effects of some other compounds have been given consideration. The monoterpene, α-pinene is widely tested against many micro-organisms and found to possess potent activity against variety of microorganisms[32], while limonene displayed antimicrobial activity towards *E. faecalis, S. aureus, B. cereus* and *C. albicans*[33]. Other compounds including δ-cadinene[34] and germacrene D[35] were previously considered as antibacterial agents. Likewise, bicyclogermacrene is an important agent *S. aureus* and *E. coli*[36].

Mosquito larvicidal activity of the essential oils

The leaf essential oil of *M. oligophlebia* displayed larvicidal activity against *Ae. aegypti* larvae with minimum lethal concentration (LC_{50}) values of 16.71 µg/mL and 11.40 µg/mL, respectively at 24 h and 48 h (Table 4). The observed LC_{90} values were recorded at 26.08 µg/mL and 20.76 µg/mL, respectively. However, the fruit essential oil did not exhibit any larvicidal action. On the other hand, *M. ptelefolia* leaf essential oil displayed larvicidal activity towards *Ae. Aegypti* with LC_{50} values of 33.18 µg/mL (24 h) and 30.32 µg/mL (48 h). The LC_{90} larvicidal activity were 47.09 µg/mL (24 h) and 44.50 µg/mL (48 h). In addition, the fruit essential oil of *M. ptelefolia* also showed activity at 24 h with LC_{50} and LC_{90} values of 35.06 µg/mL and 60.34 µg/mL, respectively. However, at 48 h, the larvicidal activity was determined to

Table 4. Larvicidal action (µg/mL) of *M. oligophlebia* and *M. ptelefolia* against *Aedes aegypti*

Minimum lethal concentration (µg/mL)				
	LC_{50}	LC_{90}	$\chi 2$	*p*
M. oligophlebia leaf				
24 h	16.71 (15.34-18.25)	26.08 (23.30-30.51)	1.4189	0.701
48 h	11.40 (10.31-12.56)	20.76 (18.17-25.06)	0.5242	0.914
M. ptelefolia leaf				
24 h	33.18 (30.64-36.12)	47.09 (42.45-54.50)	0.2952	0.961
48 h	30.32 (28.07-33.01)	44.50 (39.82-52.50)	0.7923	0.851
M. ptelefolia fruit				
24 h	35.06 (31.93-38.53)	60.34 (53.23-71.67)	4.6216	0.202
48 h	23.94 (21.61-26.51)	46.48 (40.35-56.34)	3.0551	0.383

[c] Permethrin, the standard drug used as positive control displayed larvicidal activity against *C. quinquefasciatus* and *A. aegypti* with LC_{50} values in the range of 2.19 - 3.43 µg/mL

be 23.94 µg/mL (LC_{50}) and 46.48 µg/mL (LC_{90}). Overall, the essential oils displayed promising larvicidal activity towards *A. aegypti* with most LC_{50} and LC_{90} values at tested periods less than 50 µg/mL.

This is the first report on the larvicidal activity of essential oils from *M. oligophlebia* and *M. ptelefolia* essential oil. In addition, the authors have found no report on the mortality and larvicidal activity of essential oils from other *Macclurodendron* and *Melicope* species. However, extracts and compounds isolated from some *Melicope* plants have shown insect-deterrent and larvicidal activities. For example, meliternatin isolated from *M. subunifoliolata* from Malaysia showed strong feeding deterrent activity against *Sitophilus zeamais* and very good larvicidal activity against *Aedes aegypti*[37]. The compounds, dictamnine and evolitrine were responsible for the antifeedant activities of *Evodia lunu-ankenda* against tobacco caterpillar IV instar larvae *Spodoptera litura*[38]. Moreover, *p*-O-geranylcoumaric acid described from *M. lunu-ankenda* leaf extract was found to be strongly larvicidal with LC_{50} values below 20 µg m/L[39]. It can be concluded that both the volatile and non-volatile extracts and constituents of *Melicope* exudates insect mortality and larvicidal activities. But the compounds elucidating these activities are quite different between the two extracts. Therefore, *M. ptelefolia* essential oil could be exploited further as larvicides.

The compounds present in the essential oils have proven to eb active against *A. aegypti*. Limonene and α-pinene showed larvicidal activity with mortality between 90 and 100%[40]. Germacrene D has also demonstrated larvicidal activity towards *A. aegypti*[41]. Moreover, bicyclogermacrene has shown larvicidal action against several strains of *Aedes* species[42]. The results of larvicidal evaluation suggest the existence of a synergistic effect of minor components present in the studied essential oils.

Conclusion

The major constituents found in *M. oligophlebia* leaf was α-pinene (81.7%), while α-pinene (67.4%) and limonene (11.2%) were the major compounds in fruit essential oil. The composition of essential oils from the leaves and fruits of *M. ptelefolia* comprised mainly of germacrene D (34.0% and 31.1%, respectively), bicyclogermacrene (5.5% and 7.6%, respectively), and δ-elemene (9.9% and 9.0%, respectively). The essential oils demonstrated varying degree of antimicrobial activity, as well as larvicidal actions towards *A. aegypti*. The studied essential oils may have potential for use in the control of infectious diseases and malaria mosquitoes.

Competing interests

The authors declare that no competing interest exists.

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