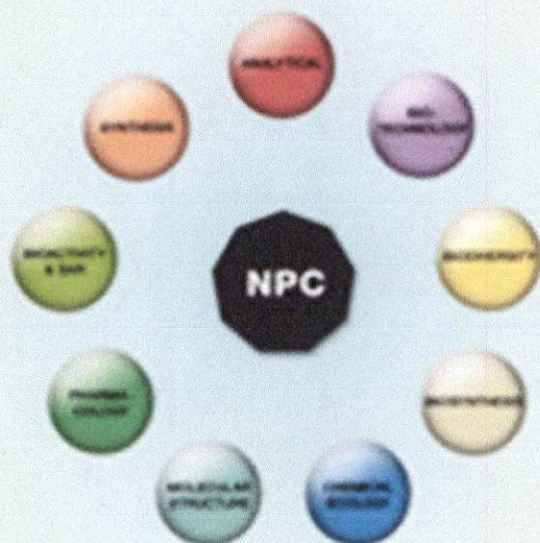


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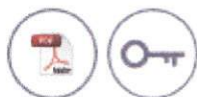


Alarm Pheromone Activity of Nymph-specific Geraniol in Chrysanthemum Lace Bug *Corythucha marmorata* against Adults and Nymphs

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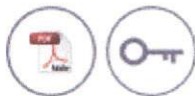
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Biological Activity and Chemical Constituents of Essential Oil and Extracts of *Murraya microphylla*

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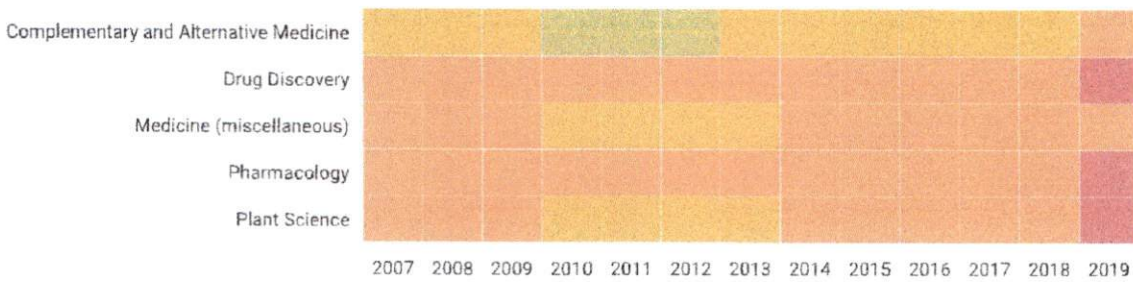
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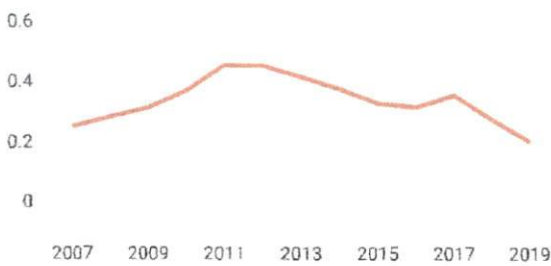
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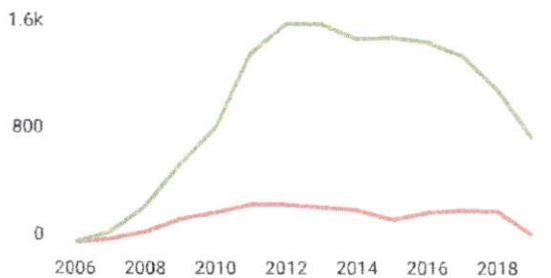
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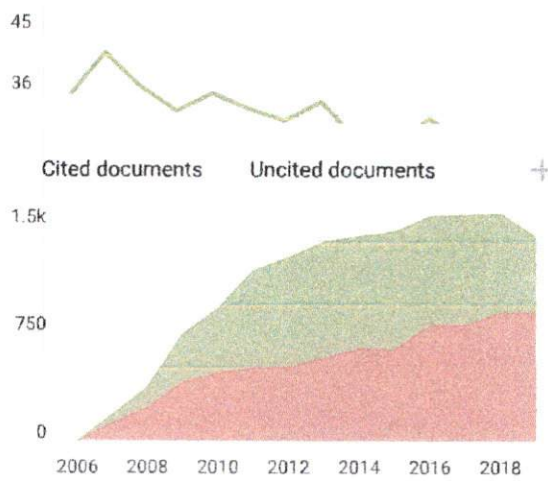
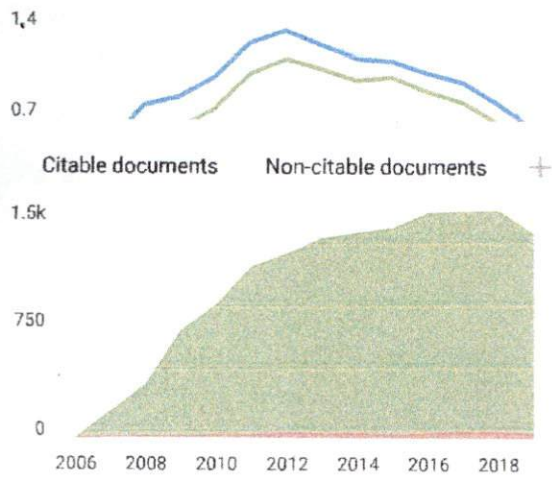
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Anti-malarial Activity of Isoquinoline Alkaloids from the Stem Bark of *Actinodaphne macrophylla*Mehran Fadaeinasab^{a*}, Hairin Taha^a, Putri Narrima Mohd Fauzi^b, Hapipah Mohd Ali^a and Aty Widyawaruyanti^c^aDepartment of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia^bCentre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603 Kuala Lumpur, Malaysia^cFaculty of Pharmacy, Universitas Airlangga, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

mehranfadaei@um.edu.my

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Seven isoquinoline alkaloids isolated from the bark of *Actinodaphne macrophylla* in this study demonstrated *in vitro* antiplasmodial activities against *Plasmodium falciparum* 3D7 with IC₅₀ values of 0.08 μM, 0.05 μM, 1.18 μM, 3.11 μM, 0.65 μM, 0.26 μM, and 1.38 μM for cycleanine, 10-demethylxylolinine, reticuline, laurotetanine, bicuculine, α-hydrastine and anolobine, respectively, which are comparable with the reference standard, chloroquine. 10-Demethylxylolinine was found to be the most active of these compounds.

Keywords: *Actinodaphne macrophylla*, Lauraceae, Isoquinoline alkaloids, Antiplasmodial activity, *Plasmodium falciparum* 3D7.

Malaria is a disease caused by a parasite, transmitted by the bite of infected mosquitoes. Malaria produces recurrent attacks of fever and kills an estimated 1 million people each year worldwide [1a]. Plants of *Actinodaphne* are widely used traditionally for treating stomach-ache, rheumatism, inflammation and disorders of the urinary tract in many countries from South East Asia, India and China [1b]. In Malaysia, apart from treating general ailments, the leaves are also used as a mosquito repellent due to their fragrant smell [1c]. The main chemical constituents of these plants are isoquinoline alkaloids, which are clinically responsible for their pharmacological activities such as the well-known narcotic analgesics, morphine and codeine, apomorphine (a derivative of morphine) used in Parkinson's disease, the muscle relaxant papaverine, and the antimicrobial agents sanguinarine and berberine [1d]. Most interestingly, several plant extracts and isolated compounds from the Lauraceae family have been reported to have anti-malarial activity [1e]. In fact, several species in tropical regions have been identified to be potent against human malaria parasites [2a]. Malaysia is known for its vast tropical forest and green vegetation. Its diverse nature and multiple uses include medicinal values. The Malaysians also utilize traditional and herbal remedies as an alternative choice of treating malarial infection [2b].

The present study examined *Actinodaphne macrophylla* (Blume) from Lauraceae family to determine its anti-malarial activity by focusing on isoquinoline alkaloids: cycleanine (1), 10-demethylxylolinine (2), reticuline (3), laurotetanine (4), bicuculine (5), α-hydrastine (6), parfumine (7) (which could not be obtained as a pure compound) and anolobine (8) (Figure 1). These isolated compounds were elucidated by spectroscopic methods [3-10] and the seven pure alkaloids were used to study their *in vitro* activity against *Plasmodium falciparum*. Isoquinoline alkaloids from different plant families have been used widely for anti-malarial activity, such as protopine and coreximine from *Corydalis crispera* [11]. Results showed that the alkaloid crude extract has an IC₅₀ value of 0.5 ppm, which is considered active if less than 50 ppm [12a]. Table 1 shows the *in vitro* antiplasmodial activity against *P. falciparum* 3D7 of the isolated isoquinoline alkaloids. According to

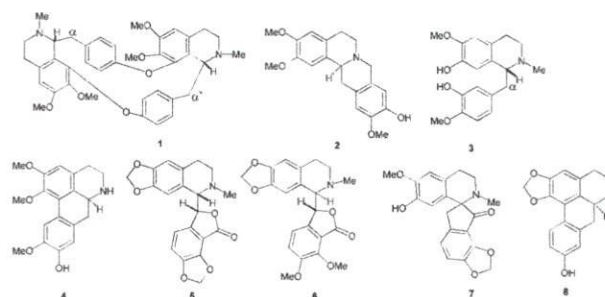


Figure 1: Anti-malarial isoquinoline alkaloids isolated from the stem bark of *Actinodaphne macrophylla*

the antimalarial activity criteria [12b], all of the tested compounds were active. In comparison with chloroquine, 10-demethylxylolinine (2), a protoberberine type alkaloid, was found to be the most active (IC₅₀ 0.05 ± 0.04 μM), followed by cycleanine (1), α-hydrastine (6), bicuculine (5), reticuline (3), anolobine (8) and laurotetanine (4). The potential of isoquinoline alkaloids as antimalarial agents has gained interest among natural product scientists for further study of these compounds [12c]. A previous report demonstrated that crude alkaloids of Brazilian plant species containing isoquinoline alkaloids had anti-malarial activity, but there was no further evaluation of the isolated alkaloids [13]. A recent study also reported the antimalarial activity of the crude alkaloid extract of *A. macrophylla* with an IC₅₀ of 0.1 ppm, but the compounds responsible for the bioactivity were not determined [12b].

Experimental

Plant materials: The stem bark of *Actinodaphne macrophylla* was collected from Johor in 2012. The botanical identification (voucher specimen (KL 4940) was made by Mr Teo Leong Eng, Faculty of Science, University of Malaya.

Extraction and isolation: The dichloromethane (CH₂Cl₂) alkaloid crude extract of *A. macrophylla* bark (2.7 kg) was obtained by using

an acid and base extraction method. The crude extract (6.2 g) was fractionated on a silica gel column (CH₂Cl₂/MeOH, 100:0 → 50:50), followed by a Si-amine silica gel column (CH₂Cl₂/MeOH, 100:0 → 50:50) and finally purified by preparative HPLC (50-100% MeOH-H₂O with detection at 248 and 283 nm, and a flow rate of 7 mL/min C18 Column) to yield cycleanine (1) (13 mg, 0.00048 %); 10-demethylxyloripine (2) (8 mg, 0.00029 %); reticuline (3) (11 mg, 0.0004 %); laurotetanine (4) (10 mg, 0.00037 %); bicuculine (5) (8 mg, 0.00029 %), α-hydrastine (6) (10 mg, 0.00037 %); parfumine (7), (7 mg, 0.00025 %); and anolobine (8) (8 mg, 0.00029 %).

Antiplasmodial activity: Continuous *in vitro* cultures of asexual erythrocytic stages of *Plasmodium falciparum* 3D7, a chloroquine sensitive strain, were maintained following the methods of Trager and Jensen [14], on glucose-enriched RPMI 1640 medium, supplemented with 10% human serum at 37°C. The alkaloid crude extracts and pure compounds were tested against *P. falciparum* 3D7 [15]. The antimalarial activity of the isolated compounds and crude extracts was determined by the procedure described as follows. In brief, 1 mg of each sample was separately dissolved in DMSO and kept at -20°C until used. The stock samples were further diluted ten-

fold as required with RPMI 1640 medium. The final concentrations of samples were: 10, 1, 0.1, 0.01 and 0.001 µg/mL, respectively. The malarial parasite *P. falciparum* 3D7 was propagated in a 24-well culture plate with a wide range of concentrations for each sample. The growth of the parasite was monitored by performing blood smears. The percentage of growth inhibition was expressed according to the following equation: Growth inhibition % = 100 - (test parasitaemia/control parasitemia) × 100. Antimalarial activity of each sample was expressed as an IC₅₀ value, defined as the concentration of the sample causing 50% inhibition of parasite relative to an untreated control. Probit analysis with SPSS was used as statistical analysis to determine the IC₅₀ value [16]. Compounds or isolates that had an IC₅₀ value < 1.5 µM were considered active [12b].

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Table 1: Inhibition growth percentage of *Plasmodium falciparum* and probit analysis with SPSS 11.5 (isoquinoline alkaloids of *Actinodaphne macrophylla*).

| Alkaloids | % Inhibition at concentration (µg/mL) ± SD | | | | | IC ₅₀ ± SD (µM) |
|---------------------------|--|-------------|-------------|--------------|-------------|----------------------------|
| | 10 | 1 | 0.1 | 0.01 | 0.001 | |
| Cycleanine (1) | 100 ± 0.03 | 100 ± 0.1 | 98.8 ± 0.05 | 51.9 ± 0.1 | 27.0 ± 0.09 | 0.08 ± 0.06 |
| 10-Demethylxyloripine (2) | 99.0 ± 0.08 | 96.1 ± 0.02 | 90.0 ± 0.04 | 60.8 ± 0.1 | 45.6 ± 0.1 | 0.05 ± 0.04 |
| Reticuline (3) | 94.8 ± 0.02 | 85.0 ± 0.04 | 52.8 ± 0.08 | 35.76 ± 0.09 | 17.1 ± 0.1 | 1.18 ± 0.08 |
| Laurotetanine (4) | 99.3 ± 0.1 | 65.1 ± 0.06 | 40.7 ± 0.09 | 29.8 ± 0.03 | 11.9 ± 0.05 | 3.11 ± 0.15 |
| Bicuculine (5) | 98.3 ± 0.09 | 70.8 ± 0.2 | 61.7 ± 0.06 | 44.0 ± 0.05 | 25.2 ± 0.04 | 0.65 ± 0.06 |
| α-Hydrastine (6) | 98.4 ± 0.1 | 93.1 ± 0.1 | 77.8 ± 0.07 | 50.5 ± 0.02 | 22.1 ± 0.2 | 0.26 ± 0.02 |
| Anolobine (8) | 96.9 ± 0.2 | 91.3 ± 0.09 | 55.7 ± 0.03 | 28.5 ± 0.06 | 14.5 ± 0.1 | 1.38 ± 0.02 |
| Chloroquine | 87.3 ± 0.1 | 76.4 ± 0.03 | 54.4 ± 0.04 | 31.1 ± 0.02 | 19.2 ± 0.09 | 0.002 ± 0.09 |

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