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Chemical constituents of lawang root oil

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ABSTRACT

Spectroscopic analysis of lawang root oil identified methyl salicylate as the major component. This result contrasts with the known composition of bark-derived lawang oil, in which eugenol is the major component. The validity of the compositional analysis was confirmed by extraction of an authentic sample of plant root tissue, and comparison with the commercially available essential oil.

Keywords: chemical composition, culitlawan oil, essential oils, lawang root oil, mass spectrometry, methyl salicylate, paramao oil, structure elucidation.

The United Nation's Comtrade database reported that the global market for essential oils was valued at almost US$35 billion in 2021.[1] One of the major challenges facing this international industry is quality assurance. For both regulatory purposes and customer satisfaction, a detailed knowledge of the chemical constituents of essential oils is necessary. The process for ensuring that essential oils are free from adulteration is called authentication, and employs many analytical techniques.[2] To be effective, these comparative analysis methods require knowledge of the chemical constituents of a pristine sample of the essential oil in question.[3–7] In this communication, we report for the first time the chemical composition of lawang root oil.

Lawang oil (also called paramao oil and culitlawan oil) is used in traditional Indonesian medicine for the topical treatment of pain associated with swollen joints. In recent years, it has found increasing use in Australia as a natural remedy for muscle pain, and is available from a number of commercial sources. To the best of our knowledge, only one pharmacological study of lawang oil has been published. In 1968, McMillan reported the use of lawang oil as a topical treatment for lymphatic filariasis, resulting from nematode infection.[8]

Lawang oil is traditionally isolated by steam distillation of the bark of *Cinnamomum culitlawan* (Lauraceae) that occurs in East Java.[9] Initially there was some confusion between lawang oil and massoi oil, but Meijer conclusively demonstrated that these two essential oils were distinct.[10] Meijer noted that lawang oil smelled of cloves as the result of its high eugenol concentration, whereas massoi oil contained no eugenol. Indeed, recent analysis of lawang oil by Arifin and co-workers revealed that the major components were eugenol (**1**) (38%), followed by safrole (**2**) (18%), eucalyptol (7%), linalool (**3**) (5%), α-terpineol (**4**) (4%) and α-pinene (**5**) (1%) (Fig. 1).[11] In contrast to the work on bark-derived lawang oil, until now there have been no investigations into the chemical composition of lawang root oil. Despite this, root-derived lawang oil is also used as a topical analgesic for the relief of muscle pain. Given that secondary metabolites are unequally distributed throughout plant tissues,[12] we thought that an investigation into the chemical constituents of lawang root oil was warranted.

Our investigation began with CGMS analysis of commercial lawang root oil, and a commercial lawang root oil infusion (in which the plant tissue is present). As shown in Fig. 2, both essential oils contained one major component. Comparison with an authentic sample of eugenol (**1**) conclusively demonstrated that lawang root oil does not contain this compound. Comparison of the mass spectrum fragmentation pattern of the major component against an MS library suggested that the compound was in fact methyl salicylate (**7**).

The relative homogeneity of the lawang root oil facilitated detailed NMR analysis. The ^{1}H NMR spectrum featured a 3 H singlet at 3.95 ppm indicative of a methyl ester, and four resonances in the aromatic region that indicated an *ortho*-substituted benzene ring. ^{13}C NMR spectroscopy confirmed the presence of a methyl benzoate with a carbonyl resonance at 170 ppm and a methyl resonance at 52 ppm. The identity of the *ortho*-substituent was ascertained as a phenol on the basis of the signal at 162 ppm in the ^{13}C NMR spectrum. Together, these supported the assignment of the major component of lawang root oil as methyl salicylate (**7**) (Fig. 3).

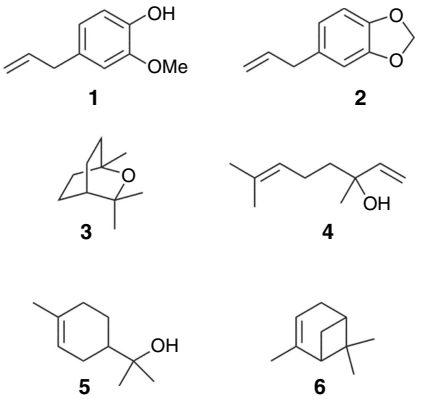

Fig. 1. Chemical constituents of bark-derived lawang oil.

Comparative GCMS analysis of the two samples of lawang root oil against an authentic sample of methyl benzoate (**7**) unambiguously confirmed that **7** was the major component of this essential oil (Fig. 4).

Given the previous uncertainties surrounding lawang oil, we investigated the chemical extracts from a sample of the plant material that is used in the commercial preparation. The plant material was generously donated by J.G. Davidson and A.G Holland (Directors of Gahn Elements). The root

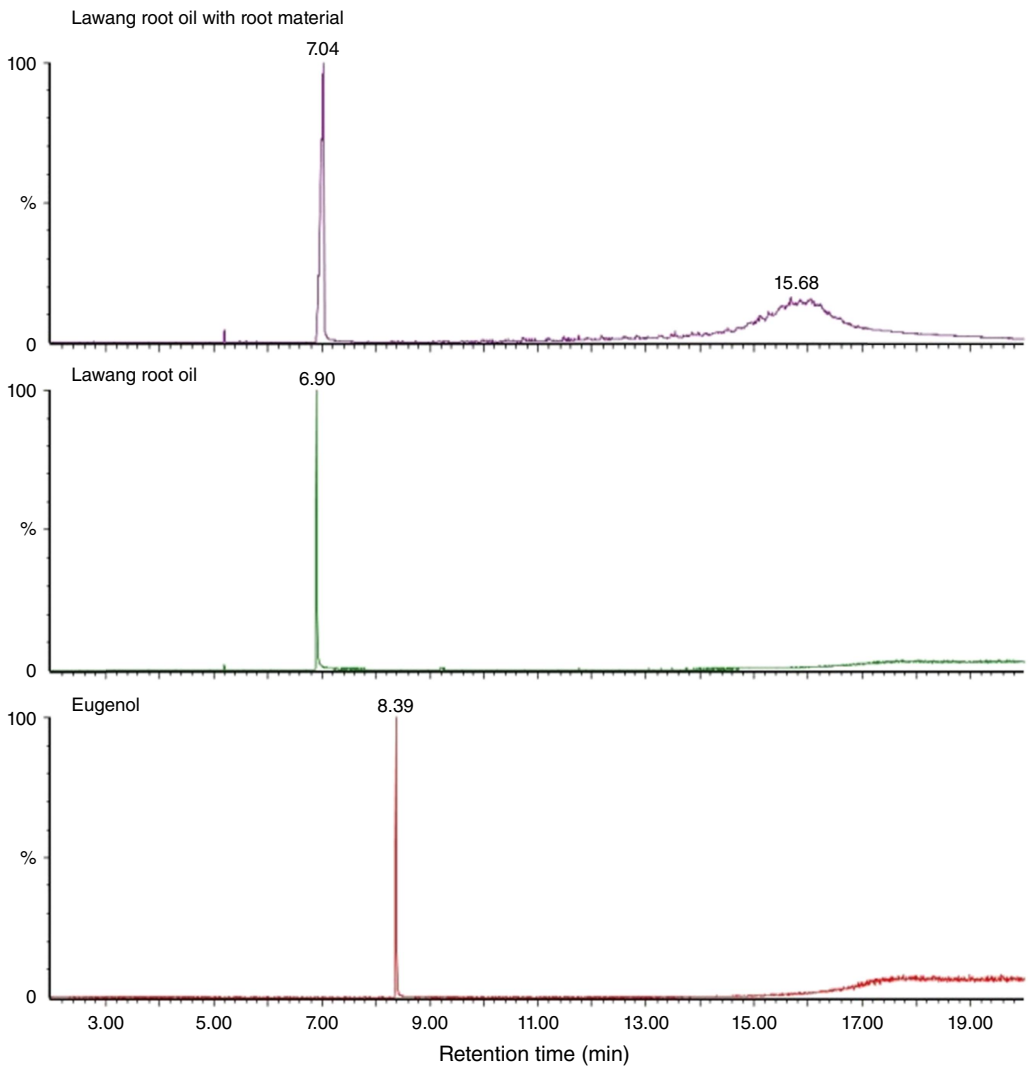

Fig. 2. GCMS analysis of lawang root oil, with and without plant tissue. Top, lawang root oil with root material; middle, lawang root oil; bottom, eugenol (**1**).

material was subjected to continuous extraction with methanol. GCMS comparison of the extract with the commercial lawang root oil confirmed that the methyl salicylate (**7**) originated from the root tissues.

Finally, although the commercial lawang oil consisted predominantly of methyl salicylate (**7**), both extraction of the commercial lawang root oil infusion and the extracted plant tissue also resulted in the isolation of waxy components that resisted separation and characterisation (see Supplementary Material).

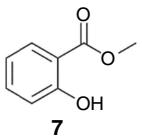

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Fig. 3. Major component of lawang root oil.

In summary, we report the first investigation into the chemical composition of lawang root oil. In contrast to the bark-derived material, which contains eugenol, the major component of the root material is methyl salicylate. Given the long history of methyl salicylate (oil of wintergreen) as a treatment for muscular pain and its efficacy in the treatment of acute pain,[13] it is likely that this compound is responsible for the reported beneficial effects of topical lawang root oil application.

Experimental

All solvents and reagents were used as received from commercial sources. Lawang root oil (paramao root oil) was generously donated by Gahn Elements. Infrared spectra were acquired neat on a Bruker Alpha-E ATR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE

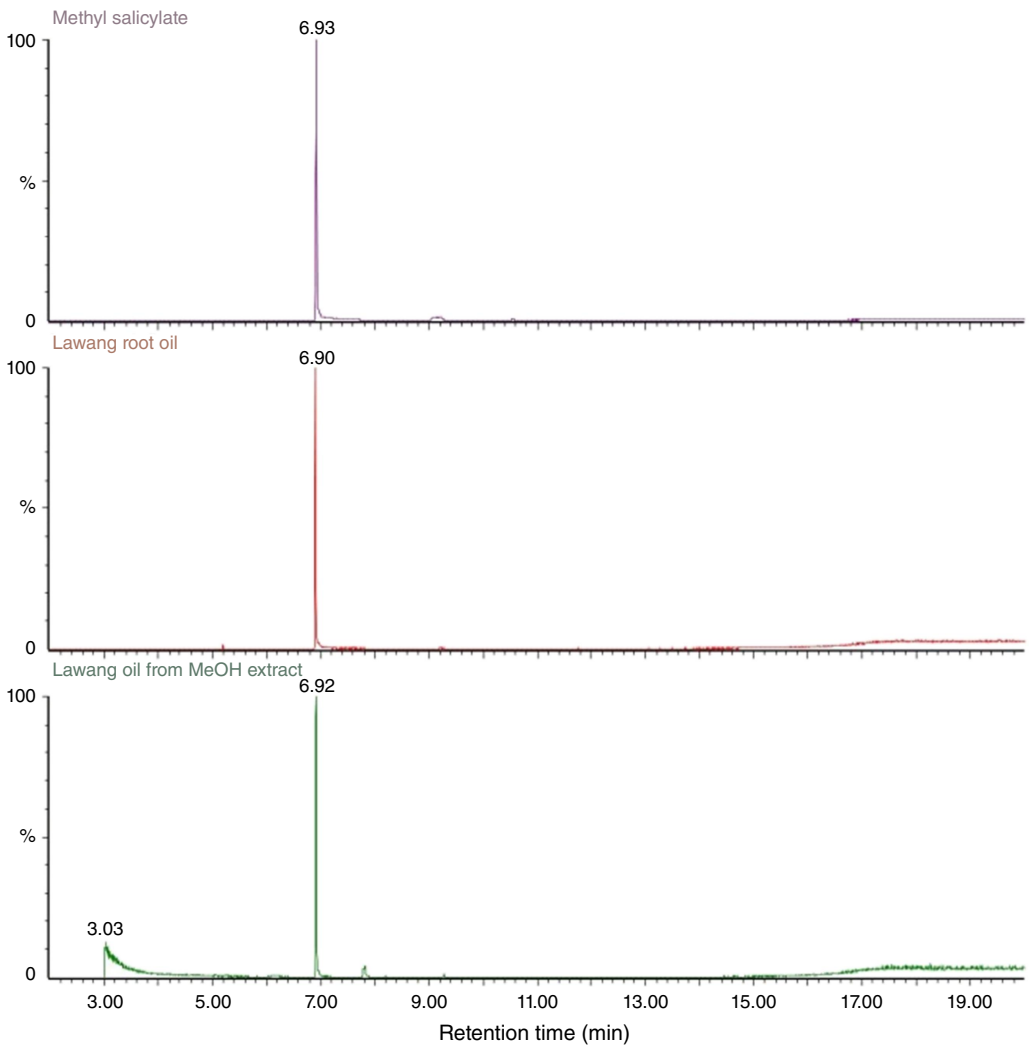

Fig. 4. GCMS analysis of lawang root oil. Top, methyl salicylate (**7**); middle, lawang root oil; bottom, methanol extract of plant tissue.

DPX300 (^{1}H frequency 300 MHz; ^{13}C frequency 75 MHz). ^{1}H chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 7.26) as reference and are reported as chemical shift (δ_H); relative integral; multiplicity (s, singlet; t, triplet; dd, doublet of doublets; td, triplet of doublets) and coupling constants (J) reported in hertz. ^{13}C NMR chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 77.1) as internal reference and are reported as chemical shift (δ_C); multiplicity (assigned from DEPT experiments). GCMS spectra were recorded on a Perkin Elmer Clarus 680 with Clarus SQ 8 C electron ionisation (EI) quadrupole mass spectrometer, using a Perkin Elmer DB5 stationary phase (30 m × 250 µm × 25 µm film thickness). Injection port: 250°C; carrier gas: helium, 1.3 mL/min; split injection with 100:1; oven program 50°C for 1 min, then 15°C/min to 290°C, hold for 2 min; MS transfer line: 200°C; MS temperature: 150°C; EI voltage: 70 eV; MS solvent delay: 4 min; scan range: 45–600 m/z. High resolution mass spectra were recorded on a Thermo Velos Pro Orbitrap using the standard electrospray ionisation source and inbuilt syringe pump.

Sample preparation

A sample (0.5 µL) of lawang root oil, lawang root oil infusion, eugenol (**1**) or methyl salicylate (**7**) was diluted to 1:1000 with hexane and subjected to GCMS analysis.

A root sample of *Cinnamomum culitlawan* (1.10 g) was placed in a Soxhlet apparatus and continuously extracted with methanol overnight. The solvent was evaporated and the crude residue was subjected to column chromatography, eluting with pentane, to give methyl salicylate (**7**), 10 mg, 1% w/w as a colourless oil, and wax, 96 mg, 8.7% w/w.

Methyl salicylate (7)

^{1}H NMR (300 MHz, $CDCl_3$): 3.95 (3H, s, Me), 6.88 (1H, t, J = 8.1 Hz, ArH), 6.98 (1H, d, J = 8.7 Hz, ArH), 7.45 (1H, td, J = 8.7, 1.8 Hz, ArH), 7.83 (1H, dd, J = 8.1, 1.8 Hz, ArH) 10.75 (1H, s, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 52.3 (CH_3), 112.5 (C),117.7 (CH), 119.3 (CH), 130.0 (CH), 135.8 (CH), 161.7 (C), 170.7 (C); MS (EI, 70 eV) m/z (%) 152 [M^+], 121, 120 (100), 92, 65; HRMS: m/z [M + H^+] calcd for $C_8H_9O_3$: 153.05462; found: 153.05451.

Supplementary material

^{1}H, ^{13}C NMR spectra and MS. Supplementary material is available online.

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Data availability. The data that support this study are available in the article and accompanying online Supplementary Material.

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