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Free fatty acids from the crude hexane extract of the aerial parts of Heliotropium indicum Linn. Growing in Phitsanulok, Thailand

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Abstract

Sixteen free fatty acids from the crude hexane extract of the aerial parts of *Heliotropium indicum* Linn. growing in Phitsanulok, Thailand, have been identified after conversion to their methyl esters with boron trifluoride-methanol followed by quantification by GC-FID and identification by GC-MS analysis. They accounted for 95% of the chromatographable components, with 9,12-octadecadienoic acid, (39.7%), 9-octadecenoic acid (32.4%), hexadecanoic acid (14.2%) and octadecanoic acid (5.1%), as the major constituents. A small amount of 6,10,14-trimethyl-2-pentadecanone and 3,7,11,15-tetramethyl-2-hexadecen-1-ol as well as a homologous series of *n*-alkanes present at trace level and ranging from C_{25} to C_{31} was also found (see Table 1). The crude hexane extract has been shown to have modest antituberculosis activity (MIC of 100 mg/mL) against *Mycobacterium tuberculosis* H37Ra.

Keywords

Free, Fatty, Acids, from, Crude, Hexane, Extract, Aerial, Parts, Heliotropium, indicum, Linn, Growing, Phitsanulok, Thailand, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Free Fatty Acids from the Crude Hexane Extract of the Aerial Parts of *Heliotropium indicum* Linn. Growing in Phitsanulok, Thailand

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Abtract

Sixteen free fatty acids from the crude hexane extract of the aerial parts of *Heliotropium indicum* Linn. growing in Phitsanulok, Thailand, have been identified after conversion to their methyl esters with boron trifluoride-methanol followed by quantification by GC-FID and identification by GC-MS analysis. They accounted for 95% of the chromatographable components, with 9,12-octadecadienoic acid, (39.7%), 9-octadecenoic acid (32.4%), hexadecanoic acid (14.2%) and octadecanoic acid (5.1%), as the major constituents. A small amount of 6,10,14-trimethyl-2-pentadecanone and 3,7,11,15-tetramethyl-2-hexadecen-1-ol as well as a homologous series of *n*-alkanes present at trace level and ranging from C_{25} to C_{31} was also found (see Table 1). The crude hexane extract has been shown to have modest antituberculosis activity (MIC of 100 μg/mL) against *Mycobacterium tuberculosis* H37Ra.

Keywords*:* Fatty acid; *Heliotropium indicum*; methylation; fatty acid methyl ester; GC-FID; GC-MS.

Introduction

The genus *Heliotropium* (Boraginaceae) is distributed throughout the world and several species have been used as ingredients in traditional medicines of many countries for treatment of asthma,[1] wounds, ulcers, venereal diseases[2] and dysmenorrhea and as a diuretic.[1,2] The plant *Heliotropium indicum* Linn., the one species found in Thailand and commonly known as "Yaa Nguang Chaang", is used in Thai folk medicine as a treatment for asthma and as a diuretic.[1] There have been several studies reported on the chemical composition of *H. indicum*, including the characterization of some bioactive pyrrolizidine alkaloids,[3-9] triterpenes,[10] steroids,[7,11] and flavonoids.[12] While an analysis of the cyanolipids of the light petroleum extracts of the seeds of *H. indicum* has been reported[13] there have been no reports on the chemical constituents of the non-polar solvent extracts of the dried aerial part of this plant. We report here the chemical composition of the crude hexane extract of this plant that was identified by first methylation and then GC-FID and GC-MS analysis. Our identification shows that the crude hexane extract consists primarily of 16 fatty acids and has been shown to have modest antituberculosis activity against *Mycobacterium tuberculosis* H37Ra.

Results and discussion

When the crude hexane extract from the aerial parts of *H. indicum* was analyzed by direct insertion mass spectrometry (DI-MS) the presence of at least 11 fatty acids was indicated based upon fragment-ion (EI mode) and molecular weight (CI mode) information. Determination of the percentage composition of the crude hexane extract and identification of the fatty acids was performed by conversion to their methyl esters using the methylation protocol of Bannon *et al.*[14] followed by GC-FID and GC-

MS analysis. The methyl esters were identified by their RI values and a comparison of their mass spectra with literature data (NIST, NISTREP). Table 1 lists 16 fatty acids, identified as their methyl esters, in the order of their elution on the capillary column used for the GC-MS analysis. Percent composition values were obtained from GC-FID integrator data, assuming equal relative FID response, and showed that the fatty acids comprise 95% of the chromatographable components of the crude extract with 9,12-octadecadienoic acid, (39.7%), 9-octadecenoic acid (32.4%), hexadecanoic acid (14.2%) and octadecanoic acid (5.1%), as the major constituents. The GC-MS total-ion current (TIC) profile of the methylated fatty acids from *H. indicum* is presented in Figure 1 and shows 46 compounds detected during the mass-spectrometric analysis. Twenty six of these compounds account for 90% of the TIC. The major components comprise fatty acids with a small amount of 6,10,14-trimethyl-2-pentadecanone and 3,7,11,15-tetramethyl-2-hexadecen-1-ol, as well as a homologous series of *n*-alkanes present at trace level and ranging from C_{25} to C_{31} also found (see Table 1). In Table 1 the TIC integrator raw peak area for each component is expressed as a percentage of the total TIC raw area for the chromatographable components of the methylated extract. A number of components (with collective peak area accounting for ca. 10%) could not be identified in the TIC due to the lack of reference spectra and/or their relatively low abundance and are not listed in Table 1.

Although the GC-FID analysis showed peaks in the chromatogram for only the relatively abundant fatty acids, the presence of other *n*-alkanoic acids, some present at trace level, was inferred from minor peaks in the TIC and their coincidence with a plot of MS fragment ions characteristic for their methyl esters (*m/z*: 74 and 87). Their presence was confirmed and their identity verified by further mass-spectral evidence and the fact that their calculated RI values corresponded to those obtained

for the appropriate methyl ester homologue from the least-squares equation of the RI values generated from the more abundant acids.

The MS fragment ion plot for all of the *n*-alkanoic acid esters shows a profile extending over the carbon number range C_{14} to C_{28} with pronounced even over odd carbon number preference (CPI) characteristic of epicuticular waxes derived from the leaves of terrestrial higher plants.[15]

Preliminary biological testing showed that the crude hexane extract of *H. indicum* had modest antituberculosis activity against *Mycobacterium tuberculosis* H37Ra in alamar blue assay system with a MIC of 100 μg/mL.

In conclusion, the major free fatty acids contained in the crude hexane extract of *H. indicum* comprise 95 % of the total chromatographable components. They have been quantified by GC-FID and identified by GC-MS analysis of their methyl esters.

Experimental

Plant material

Complete fresh aerial parts of *H. indicum* were collected from Teng Nam Village, Muang District, Phitsanulok, Thailand in February 2003. A voucher specimen (No. 003463) was deposited in the herbarium of the Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Thailand.

Extraction and identification of fatty acids

The complete fresh aerial parts (20 kg) of *H. indicum* were air-dried and ground giving a powdered material (2.5 kg). This material was extracted by soaking in hexane at room temperature for 2 weeks with three changes of hexane (1 × 3 L, 2 × 2 L). The resultant extracts were combined and concentrated under a vacuum to yield a

dark green gum (27.67 g). Initial analysis of this extract by DI-MS using both chemical ionisation (CI: *iso*-butane reagent gas) and electron impact (EI) modes indicated that the crude hexane extract consisted primarily of free fatty acids.

Methylation of fatty acids

The methylation procedure was based on the protocol of Bannon.[14] A solution of the crude hexane extract (50 mg) in 50% boron trifluoride-methanol complex in methanol (1 mL) was stirred and heated at reflux for 5 minutes. The flask was removed from the heat source, isooctane (2 mL) and saturated sodium chloride solution (approximately 15 mL) were added and the flask stoppered and shaken vigorously for a minute. The phases were allowed to separate and 1 μL of the upper layer was analyzed by GC-FID and GC-MS.

GC-FID and GC-MS analysis

GC-FID analysis was carried out using a Varian 3700 gas chromatograph coupled to a Shimadsu C-R3A integrator. Separation was achieved using H_2 as carrier gas (ca. 1 mL/min) with a fused silica capillary column (25QC/BP5) obtained from SGE, Australia (25 m × 0.25 mm i.d., 0.25 μm film thickness). The capillary column was connected to a Z guard column (2 m) of deactivated fused silica supplied by Phenomenex. Injector and detector temperatures were 260°C and 280°C, respectively; oven temperature programme, 2 min isothermal at 40°C, then at 4°C/min to 280°C (10 min isothermal).

GC-MS analysis was performed in electron impact mode (EI, 70 eV) with a Shimadzu QP5050A system, using the same temperature programme, with He as the carrier gas (1 mL/min) and a capillary column (BP1) supplied by SGE, Australia (30

m × 0.32 mm i.d., 0.25 μm film thickness). For each analysis, programmed-temperature Kováts retention indices (RI) were obtained by analysis of an aliquot of the methylated extract spiked with an *n*-alkane mixture containing each homologue from *n*-C_8 to *n*-C_{30}.

Biological activity

The antituberculous activity of the crude hexane extract of *H. indicum* was determined against *Mycobacterium tuberculosis* H37Ra in the alamar blue assay system and isoniazide and kanamycin were used as the standard drug. This screening test was performed at BIOTEC Central Reserch Unit, Bangkok, Thailand.

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Table 1. Chemical components found in the crude hexane extract of *H. indicum*. after methylation. Peak numbers refer to TIC of Figure 1.

Peak number	MS library match	MW	RI [a] (exp)	RI[b] (lit.)	RA[c] (%)	RA[d] (%)
2	tetradecanoic acid, methyl ester	228	1708	1707	0.3	0.5
3	pentadecanoic acid, methyl ester	242	1808		0.2	Tr
4	6,4,10-trimethyl-2-pentadecanone	268	1828		1.0	ND
6	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	1878		0.3	ND
7	9-hexadecenoic acid, methyl ester	268	1883	1886	0.4	ND
10	hexadecanoic acid, methyl ester	270	1910	1909	16.1	14.2
11	hexadecanoic acid	256	1947*	1939	1.5	ND
14	heptadecanoic acid, methyl ester	284	2009		0.4	Tr
17	9,12-octadecadienoic acid, methyl ester	294	2073	2074	29.8	39.7
18	9-octadecenoic acid, methyl ester	296	2082	2082	16.3	32.4
20	octadecanoic acid, methyl ester	298	2110	2109	9.2	5.1
21	octadecenoic acid	282	2116		2.0	ND
23	nonadecanoic acid, methyl ester	312	2209		0.2	Tr
27	eicosanoic acid, methyl ester	326	2311	2312	2.4	0.7
30	*n*-pentaeicosane	352	2500		0.4	ND
31	docosanoic acid, methyl ester	354	2512		2.6	0.5
32	*n*-hexaeicosane	366	2600		0.2	Tr
33	tricosanoic acid, methyl ester	368	2612		0.5	Tr
35	*n*-heptaeicosane	380	2700		1.0	Tr
36	tetracosanoic acid, methyl ester	382	2714		1.5	0.5
38	*n*-octaeicosane	394	2800		0.3	Tr
40	pentacosanoic acid, methyl ester	396	2809		0.2	Tr
41	*n*-nonaeicosane	408	2900		1.7	Tr
43	hexacosanoic acid, methyl ester	410	2908		0.6	0.6
45	hentriacontane	437	3100		0.4	Tr
46	octacosanoic acid, methyl ester	438	3107		0.5	0.4

[a] RI(exp): programmed temperature retention indices as determined on BP-1 column using a homologous series of *n*-alkanes (C_8-C_{30}) as internal standard and He as carrier gas.

[b] RI(lit.): programmed temperature retention indices from the literature[16] using He as carrier gas.

[c] RA: % TIC area (raw peak area relative to total TIC area).

[d] RA: % GC-FID area (raw peak area relative to total peak area); Tr : Trace (<0.1%); ND : Not detected.

* Confirmed by comparison with hexadecanoic acid standard analysed under identical conditions.

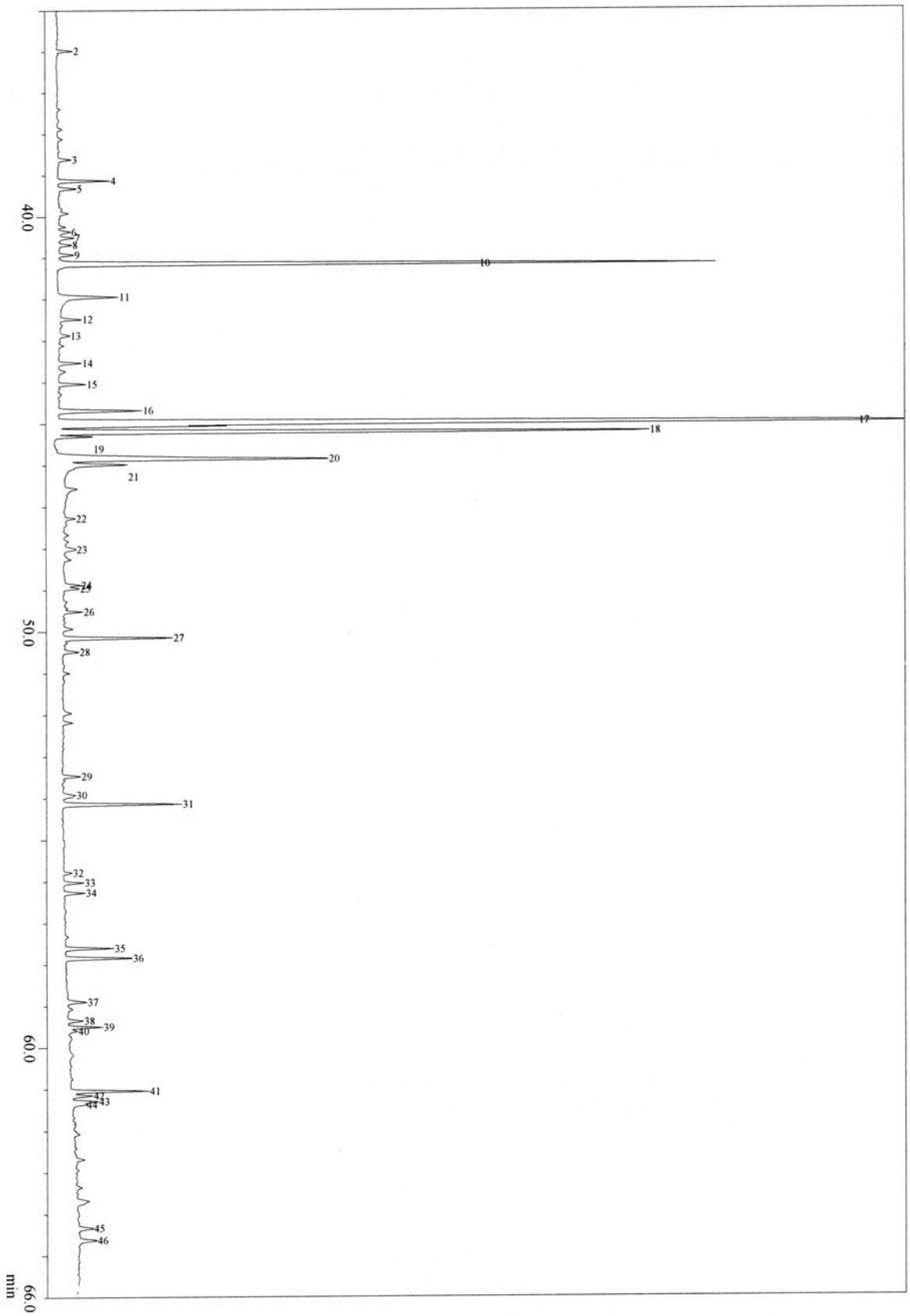

Figure 1. TIC profile of the methylated crude hexane extract from the aerial parts of *H. indicum*. See experiment for GC-MS conditions and Table 1 for peak identifications.