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Preliminary studies on the analysis of fatty acids, essential oils and flavonoids in *Acalypha indica* L.

[Kajian awal analisis asid lemak, minyak pati dan flavonoid di dalam pokok kucing galak (*Acalypha indica* L.)]

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Key words: *Acalypha indica*, methylated fatty acids, volatile compounds, flavonoids

Abstract

The fatty acids and volatile oil in 'kucing galak' *(Acalypha indica)* plant were analysed using GCMS whereas flavonoids were identified using HPLC. Whole plant was extracted and fractionated using column chromatography using methanol. Among the fatty acids found in the 12th fraction were eicosatrienoic acid methyl ester (35.47 ± 2.40%), hexatriacontane (9.56 ± 0.71%), 2,6,10 trimethyl undecatriene (8.69 ± 0.59%) and trifluoroacetic acid, n-heptadecyl ester (8.92 ± 0.52%). Volatile essential oil was extracted using the modified Licken and Nickerson apparatus. The highest volatile component was phytol (38.85%). Among the flavonoids identified in *A. indica* leaf were naringin, quercitrin, hesperitin dan kaempferol. The highest flavonoid compound was naringin, which accounted for 234.21 ± 24.5 µg/g dwt. According to literature, most of the identified components in *A. indica* plant have their own medicinal properties.

Introduction

Acalypha indica L. (Eurphorbiaceae) or 'kuppai meni' or 'kucing galak' *(Plate 1)* is a weed plant and has been used in Indian medicine (Albert 1988). In India it is used for the prevention and reversal of the atherosclerotic disease (Shanmugasundaram et al. 1983). The petroleum ether and ethanol extracts of *A. indica* are most effective in causing significant anti-implantation (anti-fertility) activities (Hiremath et al. 1999).

Acalypha indica extract also possesses anti-bacterial activity against *Aeromonas hydrophilla* and *Bacillus cereus* (Perumal et al. 1999). According to Reddy et al. (2002), *A. indica and Plumbago zeylanicum* can be used for wound healing. The ethanol extract of *A. indica* at 100 and 200 mg/kg, has an anti-ulcer activity and does not produce any signs of toxicity up to a dose of 200 mg/kg (Satyanarayan and Purohit 2002). In contrast, Lamabadusuriya and Jayantha

Plate 1. ***Acalypha indica*** *leaves*

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(1994) reported that ingestion of a broth containing *A. indica* caused intravascular haemolysis.

Previous study on the identification of active compounds in *A. indica* using H NMR was also carried out. Nahrstedt et al. (1982) for instance, reported that a new cyanogenic glucoside called acalyphin, was isolated from the aerial parts of *A. indica*. Since not many studies have been conducted on the chemical analysis of *A. indica*, thus, the identifications of flavonoids, essential oils and fatty acids in *A. indica* need to be carried out. The information obtained would possibly help the herbal industries in Malaysia to diversify the use of this plant in their products.

Materials and methods

Fresh *A. indica* was obtained from Taman Seri Serdang, Selangor, Malaysia. Sample was selected then washed under running tap water before analyses. Samples for the analyses of flavonoids and fatty acids were dried using freeze dryer for 2 days, whereas sample for the analysis of essential oil was freshly prepared.

Extraction of volatile oil

Volatile oil from *A. indica* whole fresh plant was extracted using modified simultaneous distillation extraction technique (SDE) based on the Licken Nickerson apparatus (Suri et al. 2001). A total of 200 g *A. indica* leaf plus stem and root were mixed with 200 mL of distilled water and extracted with 40 mL pentane (HPLC grade, Fisher) (100%). The extraction process was carried out for 2 h. Approximately 5 g of sodium sulphate anhydrous was added to the extract. A Whatman filter paper no. 141 was used to separate sodium sulfate from the extract. The extract was concentrated using purified nitrogen. Identification of volatile components was carried out using gas chromatography mass spectrophotometry (GCMS) Shimadzu QP5050 and NIST library. The column used was BPX 5; 30 m x 0.25 mm i.d. and 0.25 mm thickness.

GCMS conditions for the analysis of essential oil

The GCMS conditions were as follows: injection volume (1 mL), initial temperature, 80 °C; final temperature (200 °C); interface temperature (300 °C); column flow = 1 mL/min; linear velocity = 36 cm/s; split = 11; total flow = 12.6 mL/min.

Analysis of fatty acids

A sample of 7 g dried *A. indica* whole plant was extracted using petroleum ether using Soxhlet apparatus. The extract was then dried using rotary evaporator and fractionated using column chromatography [silica gel-mesh 60 (Merck)] and washed using methanol (100% - HPLC grade-Fisher). About 20 mL of solvent with more than 40 bottles of fractions were collected. Every fraction was dried at room temperature and the fraction with high lipid content was subjected for further analysis. A 0.2 g of extracted lipid was mixed with 1.0 mL hexane and 0.2 mL sodium methoxide (Supelco). The mixture was stirred for 10 s and let to stand for 30 min. The upper layer was injected into the GCMS (Shimadzu QP 5050).

GCMS conditions for the analysis of fatty acids

The GCMS conditions were injection volume (1 mL), initial temperature, 280 °C; final temperature (300 °C); interface temperature (300 °C); column flow = 1 mL/min; linear velocity = 41.1 cm/s; split ratio = 4; total flow = 9.6 mL/min. All compounds were detected using NIST library.

Extraction and identification of flavonoids

The extraction and identification of flavonoids were carried out according to the method by Suri et al. (2002) and Crozier et al. (1997). Blended samples were dried using freeze dryer (Lab Conco, USA) for 2 days. Dried samples (10 g) were extracted with 100 mL of 60% (v/v) methanol with 20 mM Na-DEDTC (Fisher), filtered with

Whatman no. 1. Then, 2.0 mL of the extract (glycosides) was separated and filtered with 0.45 mm PTFE membrane filter (Whatman). The other portion of the extract was mixed with 20 mL of 6 M HCl solution (AR grade). Each extract was separately refluxed at 90 °C for 2 h. A 50 mL hydrolysed extract containing aglycone was filtered with 0.45 mm PTFE membrane filter (Whatman)]. The extract was then mixed with filtered glucosides at the ratio of 1:1 prior to the injection into the HPLC.

HPLC conditions

Flavonoids were analysed using Suri et al. (2002) with slight modification. The extracts were injected into the HPLC (Beckman, USA) equipped with Novapak C18 (3.9 mm x 150 mm) (Waters, USA). The elution solvents were solvent A [(water adjusted to pH 2.5 with trifluoroacetic acid (TFA)] and solvent B (100% methanol). The HPLC was operated under gradient for 20 min [A:B (from 80:20 to 60:40)] for 20 min at 1.0 mL/min. Detection of flavonoids was performed at 370 nm using UV-DAD detector and absorbtion spectrum was recorded at 350 nm to 450 nm.

Preparation of standards

All standards (naringin, rutin, quercitrin, hesperitin and kaempferol) were obtained from Sigma and weighed. Mixed and individual standards (0.01 g to 1.0 g) were dissolved in methanol (100%). A 20 µL of the mixed and individual standards were then injected into the HPLC.

Results and discussion

Analysis of fatty acids in whole plant extract

Since there is no report on the analysis of fatty acids in *A. indica,* the study on the fatty acids composition in *A. indica* extract has to be carried out. Among the 40 fractions that were collected through column chromatography, only fraction 12 was analysed because it contained high lipid (0.015 g). All the compounds identified are shown in *Table 1* and *Figure 1*.

Eicosatrienoic acid methyl ester was highest at 35.47 ± 2.40% *(Figures 1* and *2)*. Other compounds were hexatriacontane (9.56 ± 0.71%), 2,6,10 trimethyl undecatriene (8.69 ± 0.59%) and n-heptadecyl ester, trifluoroacetic acid (8.92 ± 0.52%). The 2-methyl-pentadecane, dimethyl-docosane and 7-hexyl-eicosane have also been detected in *A. indica* and

Table 1. List of compounds found in fraction 12 of column chromatography after methylation (n = 3)

Peak	Retention time	Compounds	Percentage
1	5.042	Tetradecen-1-ol	0.805 ± 0.19
2	5.967	Hexadecanoic acid methyl ester	5.02 ± 0.37
3	7.925	Eicosatrienoic acid methyl ester	35.47 ± 2.40
4	10.008	Ethyl tetradecane	0.43 ± 0.01
5	10.392	Methyl arachate	0.65 ± 0.05
6	11.183	2-methyl tricosane	0.32 ± 0.01
7	12.167	Octadecane	1.54 ± 0.09
8	12.497	2-methyl pentadecane	4.64 ± 0.36
9	13.967	7 hexyl eicosane	0.63 ± 0.18
10	15.233	Trifluoro acetic acid, n-heptadecyl ester	8.92 ± 0.52
11	15.617	7-butyl docosane	2.97 ± 0.14
12	17.742	2,6,10 trimethyl undecatriene	8.69 ± 0.59
13	19.017	Trifluoro acetic acid, n-octadecyl ester	0.73 ± 0.34
14	19.492	Hexatriacontane	9.56 ± 0.71
15	24.55	7-hexyl eicosane	6.21 ± 0.66
16	24.908	Hexadecamethyl-heptasiloxane	2.89 ± 0.74

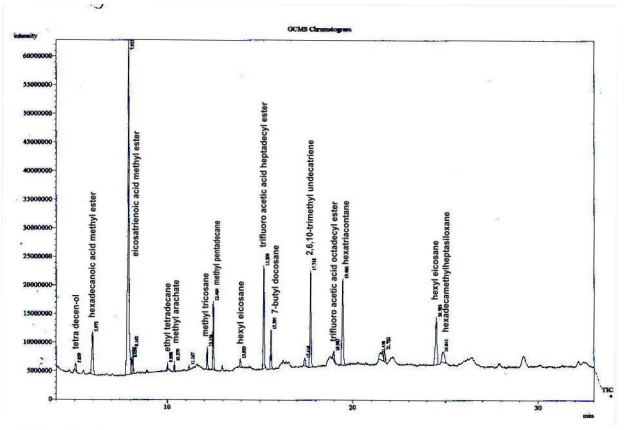

Figure 1. GCMS chromatogram of fatty acids in ***Acalypha indica***

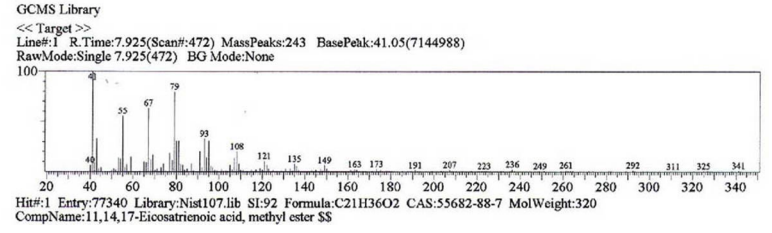

Figure 2. Mass-spectrum of eicosatrienoic acid methyl ester found in ***Acalypha indica*** *fatty acid*

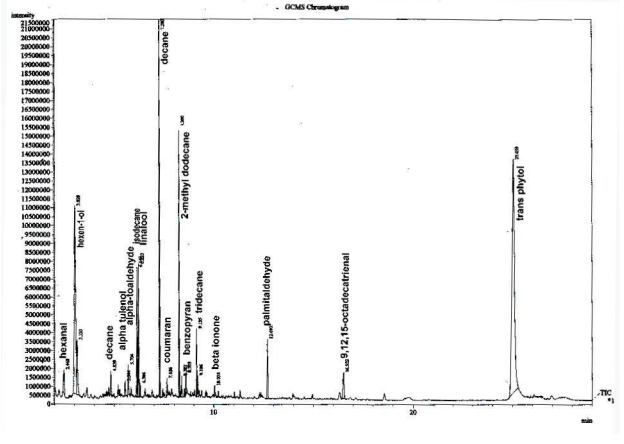

Figure 3. GCMS chromatogram of the volatile oil in ***Acalypha indica***

according to Linley and Carlson (1978), these compounds can be used as sex pheromone to attract male and female insects.

Volatile oil

Only 0.01% of volatile oil was found in the *A. indica* whole plant. Analysis using GCMS showed the presence of 20 components *(Figure 3* and *Table 1)*. At present, there is no available report on the analysis of volatile oil in *A. indica*. Among the components identified were 2-dimethyl dodecane, palmitaldehyde, octadecatrienal and trans phytol (38.85%) *(Table 2* and *Figure 4)*. According to Sung et al. (1999), trans-phytol is one of the three components in *Solidago virga-aurea* var gigantea, which has cytotoxic activity. Schlüter et al. (2002) reported that phytanic acid, which is a derivative of phytol, is a side-chain of chlorophyll that may act as a natural rexinoid in adipose cells, which can be used to treat human type 2 diabetes and obesity.

Table 2. Volatile compounds in ***Acalypha indica*** (n = 3)

Peak No	Ret. Time	Compounds	Percentage
1	2.468	Hexanal	1.46 ± 0.17
2	3.028	Hexenol	17.5 ± 1.99
3	4.839	Decane	1.04 ± 0.13
4	5.544	α-tulenol	0.68 ± 0.02
5	5.704	α-toaldehyde	1.09 ± 0.04
6	6.147	Isodecane	3.94 ± 0.17
7	6.223	Linalool	4.13 ± 0.11
8	7.280	Decane	10.29 ± 0.22
9	7.636	Coumaran	0.70 ± 0.15
10	8.260	2-dimethyl dodecane	6.83 ± 0.79
11	8.383	Benzopyran	1.09 ± 0.39
12	9.133	Tridecane	1.69 ± 0.05
13	10.058	β-ionone	0.62 ± 0.04
14	12.692	Palmitaldehyde	2.55 ± 0.23
15	16.525	9,12,15 octadecatrienal	1.94 ± 0.11
16	25.058	Trans phytol	38.64 ± 2.74

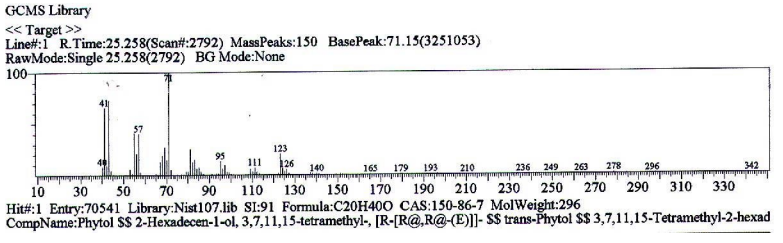

*Figure 4. Mass-spectrum of trans-phytol found in **Acalypha indica** essential oil*

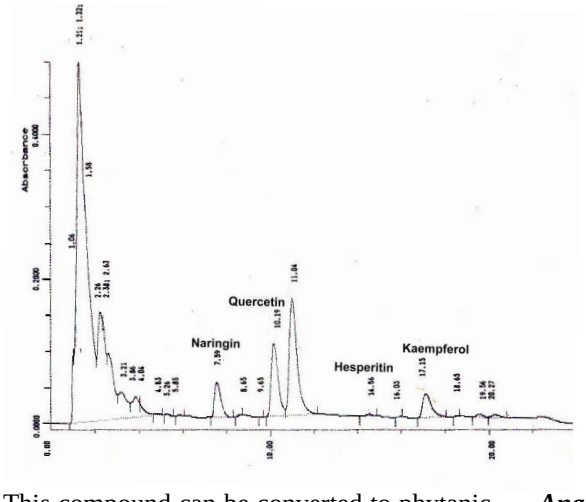

*Figure 5. HPLC chromatogram of the flavonoids in leaf of **Acalypha indica***

This compound can be converted to phytanic acid in rat liver and the conversion rate is in the range of 2–3% (Muralidharan and Muralidharan 1985).

Analysis of flavonoids in leaf

More than 10 flavonoids were separated using HPLC. However, only four types of flavonoids i.e. 4',5,7,trihydroxy flavanone 7-rhamnoglucoside (naringin), rutin, 3,3',4',5 pentahydroxy flavone 3-l-

rhamnopyranoside (quercitrin), 3'5,7, trihydroxy-4-methoxy-flavone (hesperitin) and kaempferol *(Figure 5)* have been identified in *A. indica* leaf, [naringin (234.21 ± 24.5 µg/g dwt.), rutin (29.73 ± 2.98 5 µg/g dwt), quercitrin (0.83 + 0.01 µg/g dwt.), hesperitin (27.09 + 8.05 5 µg/g dwt.), and kaempferol (2.879 + 0.93 5 µg/g dwt.)]. Only quercitrin was detected in stem.

To date, not many studies have been conducted on the analyses of flavonoids in *A. indica*. Satyanarayan and Purohit (2002) have isolated flavonoid (5,7-dihydroxyflavone or chrysin) from different *A. indica* extracts. *Bridelia ferruginea* Benth. which belongs to the same family as *A. indica* also contains various flavonoids and flavonoid glycosides quercetin derivatives such as rutin, myricetin derivatives, gallocatechin-(4'-0-7)-epigallocatechin, 3,5-dicaffeoylquinic acid and 1,3,4,5-tetracaffeoylquinic acid (Addae-Mensah and Achenbach 1985; De Bruyne et al. 1997).

According to Chul et al. (2001), naringin can lower blood and hepatic cholesterol, inhibit intestinal cancer cell line (Kuntz et al. 1999), has higher antioxidant activities than phenolic acid (Robards et al. 1999), protect against ulceration (Parmar and Ghosh 1980), inhibit human breast cancer cell-line (Chul et al. 2001) and inhibit development of tumors induced by DMBA (Diane et al. 2001). Quercetin and kaempferol can be used as antiulcer, antioxidant, antineoplasm and antivirus (Raj Narayana et al. 2001). These compounds are also able to control the level of human estrogen and androgen (Raj Narayana et al. 2001).

Conclusion

Various types of fatty acids, volatile compounds and flavonoids were successfully detected in different extracts of *Acalypha indica*. Although these compounds were reported for their beneficial properties, further experiments on the efficacy, safety and toxicity of the locally grown *A. indica* are still needed to be carried out to support the medicinal properties of this plant.

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Abstrak

Kandungan asid lemak dan minyak meruap di dalam pokok kucing galak *(Acalypha indica)* telah dianalisis menggunakan GCMS manakala flavonoid telah dikenal pasti menggunakan HPLC. Keseluruhan pokok kucing galak telah diekstrak menggunakan kromatografi turus dengan pelarut metanol. Antara asid lemak yang dikenal pasti dalam pecahan ke-12 ialah asid eikosatrienoik metil ester (35.47 ± 2.40%), heksatriakontan (9.56 ± 0.71%), 2,6,10 trimetil undekatrien (8.69 ± 0.59%) dan asid trifluoroasetik, n-heptadesil ester (8.92 ± 0.52 %). Minyak meruap telah diekstrak menggunakan kaedah 'Licken dan Nickerson' yang diubah-suai. Komponen minyak meruap tertinggi ialah fitol (38.85%). Antara bahan flavonoid yang dikenal pasti di dalam daun kucing galak ialah naringin, quercitrin, hesperitin dan kaempferol. Bahan flavonoid yang tertinggi ialah naringin (234.21 ± 24.5 μg/g berat kering). Menurut ulasan kajian terdahulu, hampir kesemua komponen yang dikenal pasti di dalam pokok kucing galak mempunyai nilai perubatan tersendiri.

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