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.)]

R. Suri*, H. Abu Bakar*, A. Noor Rehan*, O. Rosnah* and A. Normah*

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

*Food Technology Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
Authors’ full names: Suri Roowi, Abu Bakar Hussin, Noor Rehan Abdullah, Rosnah Othman and Normah Ahamad
E-mail: suri@mardi.my
©Malaysian Agricultural Research and Development Institute 2004
Chemical constituents in *Acalypha indica* L.

(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
1. 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.

2. 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.

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

Table 1. List of compounds found in fraction 12 of column chromatography after methylation (n = 3)
Chemical constituents in *Acalypha indica* L.

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.
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 3-1-
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, gallicatechin-(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.

**Acknowledgement**

The authors thank Mr Arif Zaidi Jusoh for providing technical help.

**References**


Muralidharan, F.N. and Muralidharan, V.B. (1985). In vitro conversion of phytol to phytanic acid in rat liver: sub-cellular distribution of activity and chemical characterization of


---

**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 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.

*Accepted for publication on 20 May 2004*