RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2014.40.414

*ScienceAsia* 40 (2014): **414–419**

# Determination of gallic acid and rutin in extracts *Cassia alata* and *Andrographis paniculata*

**Buran Phansawan**[a,*], **Supakorn Pongsabangpho**[b]

[a] School of Energy and Environment, University of Phayao, Phayao 56000 Thailand
[b] Faculty of Medical Science, University of Phayao, Phayao 56000 Thailand

*Corresponding author, e-mail: buranphan@hotmail.com

*Received 28 Jul 2014*
*Accepted 16 Nov 2014*

**ABSTRACT**: This study determines gallic acid and rutin in ethanol, methanol, and acetic acid extracts of *Cassia alata* and *Andrographis paniculata*. A preliminary study using thin layer chromatography and 0.2% DPPH (2,2-diphenyl-1-picrylhydrazyl) spraying found that gallic acid was present in all three solvent extracts. Antioxidant components of all extractions were then analysed using high performance liquid chromatography with a $C_{18}$ reverse-phase column at a flow rate of 0.50 ml/min and 25 °C. The results showed high components of gallic acid and rutin in all extracts of *C. alata*. The amounts of gallic acid found in *C. alata* extracted with ethanol, methanol, and acetic acid were 366, 410, and 258 mg/g crude extract, respectively. Amounts of gallic acid found in *A. paniculata* extracted with ethanol, methanol, and acetic acid were 309.4, 351.2, and 309.1 mg/g crude extract, respectively. Rutin concentrations found in *C. alata* extracted with ethanol, methanol and acetic acid were 194.4, 193.6, and 120.0 mg/g crude extract, respectively. The highest gallic acid level was found in *C. alata* extracted with methanol, while the highest rutin levels were found in *C. alata* extracted with ethanol and methanol.

**KEYWORDS**: antioxidant, DPPH, TLC, HPLC

## INTRODUCTION

Reactive oxygen species (ROS), including superoxide radical anion ($O_2^{\cdot -}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($O^{\cdot}$), are generated during normal physiological activities of the human body. ROS have been linked with ageing and several diseases such as cancer, inflammation, immune system decline, cardiovascular disease, neurological disease, and atherosclerosis. Since synthetic antioxidants have potential health hazards, natural free-radical scavengers have become more interesting to scientists. Antioxidants occurring in fruits and vegetables are believed to prevent and even repair oxidative stress which is thought to damage body cells and to be linked to diseases such as cancer, heart disease, Alzheimer's disease, and Parkinson's disease. Vitamin C and vitamin E, carotenoids, and selenium are the principal antioxidants available in the body. Clinical trials using supplements of vitamin C, vitamin E, and carotenoids have shown inconsistent results and have led to the belief that whole fruits and vegetables rather than the individual compounds they contain are likely to give more positive results in disease prevention [1].

Rutin or quercetin-3-rhamnosyl-glucoside, is a natural flavone derivative with significant scavenging properties on oxidizing species such as $OH^{\cdot}$, $O_2^{\cdot -}$, and the peroxyl radical. Due to its biological effects, it has been widely used in treating disease. Rutin pharmacological properties include: antiallergic, anti-inflammatory and vasoactive, antitumour, antibacterial, antiviral, and antiprotozoal, in addition to hypolipidaemic, cytoprotective, antispasmodic, and anticarcinogenic properties [2].

Gallic acid or 3,4,5-trihydroxybenzoic acid, $C_6H_2(OH)_3CO_2H$, is a polyphenyl natural product found in gallnuts, sumac, tea leaves, oak bark, and many other plants, both as a free state and as part of the tannin molecule. Since gallic acid has hydroxyl groups and a carboxylic acid group in the same molecule, two of its molecules can react with one another to form an ester, digallic acid. Gallic acid is obtained by the hydrolysis of tannic acid with $H_2SO_4$. It has radical scavenging, anti-oxidative, anti-inflammatory, anti-fungal, anti-cancer, and chemoprotective properties. Since these properties are of great importance, they provide motivation for the isolation of these compounds from natural sources [3,4].

*Andrographis paniculata* (Burm.f.) Nees., known in Thai as 'fa-thalai-chon' (Fig. 1), is an important medicinal plant in India, China, Thailand, and Scandinavia. The aerial parts of the plant (leaves and stems) are normally used for the extraction of the

**Fig. 1** *Andrographis paniculata* (Burm.f.) Nees.

**Fig. 2** *Cassia alata* L.

active phytochemicals. Extracts of the plant and their constituents have been reported to exhibit a wide spectrum of biological activities of therapeutic importance including antibacterial, antiviral, anti-inflammatory, antimalarial, immunostimulant, hepatoprotective, antithrombotic, anticancer, hypoglycaemic, and hypotensive properties[5].

Where *Cassia alata* L., known in Thai as 'chum-het-thet' (Fig. 2), is recommended for primary health care in Thailand to treat ringworm, constipation, and skin disease. Several reports have described antimicrobial substances from *C. alata*[6,7].

The objectives of this study were to determine whether the antioxidant compounds, gallic acid and rutin, are present in *C. alata* and *A. paniculata* extracts using HPLC.

## MATERIALS AND METHODS

### Materials

The samples of *A. paniculata* were collected from Chiang Mai Province and the samples of *C. alata* were collected from Phitsanulok Province, between May and November 2011.

The HPLC grade gallic acid, catechin, tannic acid, and rutin were from Sigma (St. Louis, MO, USA). Acetonitrile, distilled water, 99% acetic acid, 99% methanol, 99% ethanol, chloroform, and silica gel $60\,F_{254}$ TLC plate were purchased from Merck (Darmstadt, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl) was from Sigma (St. Louis, MO, USA).

### Sample preparation

Plants were cleaned and cut into small pieces before being dried at room temperature and subjected to extraction with three solvents; 99% acetic acid, 99% methanol, and 99% ethanol. Each extraction step was completed within 24 h. The extracts were filtered (Whatman No. 1) and concentrated in a rotavapor apparatus at approximately 40 °C. Finally, the concentrated extracts were dried using a freeze-vacuum dryer (FTS System, Flexi-Dry) and stored at −40 °C for the measurement of antioxidant compounds.

### Thin layer chromatography analysis

Crude extracts were spotted on aluminium plates of silica gel $60\,F_{254}$ thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany) and were developed in a mobile phase consisting of chloroform, acetic acid, and methanol solution (80:16:4 v/v/v).

After development, TLC plates were dried at room temperature. The resulting bands were sprayed with 0.2% (w/v) DPPH solution in methanol. Gallic acid, catechin, tannic acid, and rutin were used as reference compounds (standards)[8]. Free radical scavenging zones and $R_f$ values were identified immediately as yellow areas against a purple background. High-performance liquid chromatography (HPLC) was then used in the next step to quantify antioxidant compounds.

### High-performance liquid chromatography analysis

HPLC analysis was performed using a Varian Series PS 240, PS 325, PS 400 system equipped with an automatic injector and UV detector. The column used was a 5 µm Varian $C_{18}$ column (250 mm × 4.6 mm). The temperature was maintained at 25 °C, with an injection volume of 20 µl, a flow rate of 0.5 ml/min, and a run time of 7 min. The sample and standards (gallic acid, catechin, and rutin) were eluted using a reverse-phase Varian $C_{18}$ column with distilled water-acetonitrile (50:50 v/v) mobile phase and detected at 210 nm. The calibration graph was obtained by plotting the peak area versus concentration and the calibration curve was linear from 10–50 µg/ml.

### Statistical analysis

Three replicate values for the antioxidant compounds from each sample were used for the statistical analysis. The ANOVA and means were carried out using a computer-based statistical program. Data were subjected to ANOVA and means ± SD compared by Duncan methods, which differences at $p \leqslant 0.05$ considered to be significant.

## RESULTS

### Dry weight extracts

Dry weight of *C. alata* and *A. paniculata* were obtained from three solvent extraction methods, i.e. ethanol, methanol, and acetic acid (Table 1). The results showed that acetic acid was the best solvent for *C. alata* (37.2 mg/g fresh) where methanol was the best solvent extraction for *A. paniculata* (59.6 mg/g fresh).

### TLC analysis

Thin layer chromatography screening using chloroform, acetic acid and methanol (80:16:4 v/v/v) separated many compounds (Fig. 3). The compounds were identified by comparison with the standard used in the study. There were about two to four compounds presented in the *C. alata* and *A. paniculata* extracts.

**Table 1** Dry weight of *C. alata* and *A. paniculata* extracts.

| Solvents | Dry weight (mg/g fresh) | |
|---|---|---|
| | *C. alata* | *A. paniculata* |
| ethanol | 31.2 | 30.5 |
| methanol | 37.2 | 34.6 |
| acetic acid | 26.0 | 59.6 |

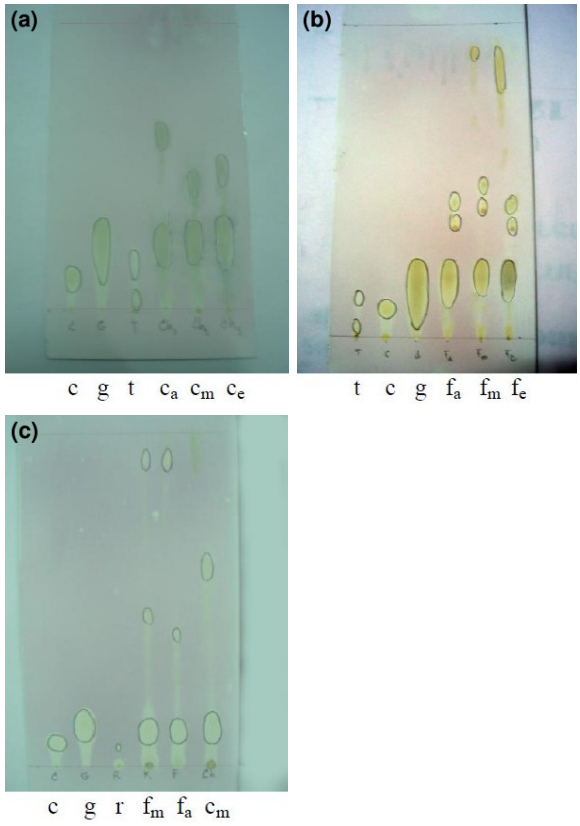


**Fig. 3** TLC profiles: (a) antioxidant compounds in *C. alata* extracts extracted with acetic acid ($c_a$), methanol ($c_m$), and ethanol ($c_e$); (b) antioxidant compounds in *A. paniculata* extracts extracted with acetic acid ($f_a$), methanol ($f_m$), and ethanol ($f_e$); (c) standard of antioxidant compound: tannic acid (t), gallic acid (g), catechin (c), and rutin (r).

The $R_f$ values (ratio of the distance travelled by the solute to the distance travelled by mobile phase) of antioxidant compounds in the sample extracts and the standard are listed in Table 2.

Qualitative detection of antioxidant compounds in *C. alata* extract revealed compounds with $R_f$ values of 0.25, 0.27, and 0.25 extracted with ethanol, methanol, and acetic acid, respectively, (Fig. 3a), the first of which represented gallic acid ($R_f = 0.24$) where the other compound found at $R_f$ 0.41, 0.45, and 0.56

**Table 2** $R_f$ values of *C. alata* and *A. paniculata* extracts and standards.

| Solvents | $R_f$ | |
|---|---|---|
| | *C. alata* | *A. paniculata* |
| ethanol | 0.25, 0.41 | 0.23, 0.41, 0.47, 0.92 |
| methanol | 0.27, 0.45 | 0.22, 0.35, 0.41, 0.92 |
| acetic acid | 0.25, 0.56 | 0.23, 0.40, 0.43 |
| catechin | 0.11 | 0.11 |
| gallic acid | 0.24 | 0.24 |
| Rutin | 0.083 | 0.083 |
| Tannic acid | 0.15 | 0.15 |

**Table 3** Total gallic acid and rutin contents of *C. alata* and *A. paniculata* extracted with ethanol, methanol, and acetic acid (mg/g crude extracts).

| Solvent | *C. alata* | | *A. paniculata* | |
|---|---|---|---|---|
| | Gallic acid | Rutin | Gallic acid | Rutin |
| ethanol | $366.3 \pm 6.1^{b}$ | $194 \pm 29^{a}$ | $309 \pm 13^{b}$ | N/A |
| methanol | $410.0 \pm 7.8^{a}$ | $193.6 \pm 9.5^{a}$ | $351.2 \pm 5.6^{a}$ | N/A |
| acetic acid | $257.6 \pm 3.1^{c}$ | $120.0 \pm 3.0^{b}$ | $309.1 \pm 4.7^{b}$ | N/A |

N/A = not detected. Superscript letters a, b, and c indicate significant differences at $p < 0.05$.

of ethanol, methanol, and acetic acid, respectively, have not been identified. In *A. paniculata* extracts, compounds appeared with $R_f$ values of 0.23, 0.22, and 0.23 when extracted with ethanol, methanol, and acetic acid, respectively, (Fig. 3b) which represented gallic acid (standard $R_f = 0.24$). Rutin was not found in *C. alata* or *A. paniculata* (Fig. 3c). The identification and quantification of some compounds in the sample extracts were also confirmed by HPLC analysis.

## High performance liquid chromatography analysis

The qualitative and quantitative analyses of *C. alata* and *A. paniculata* extracts were carried out using HPLC with a UV detector (Table 3 and Fig. 4). The components catechin, gallic acid, and rutin were identified by comparing retention times, with the retention times of catechin, gallic acid, and rutin standards being 4.659, 3.307, and 3.795, respectively (Fig. 5). Quantitative data for the sample extracts were calcu-

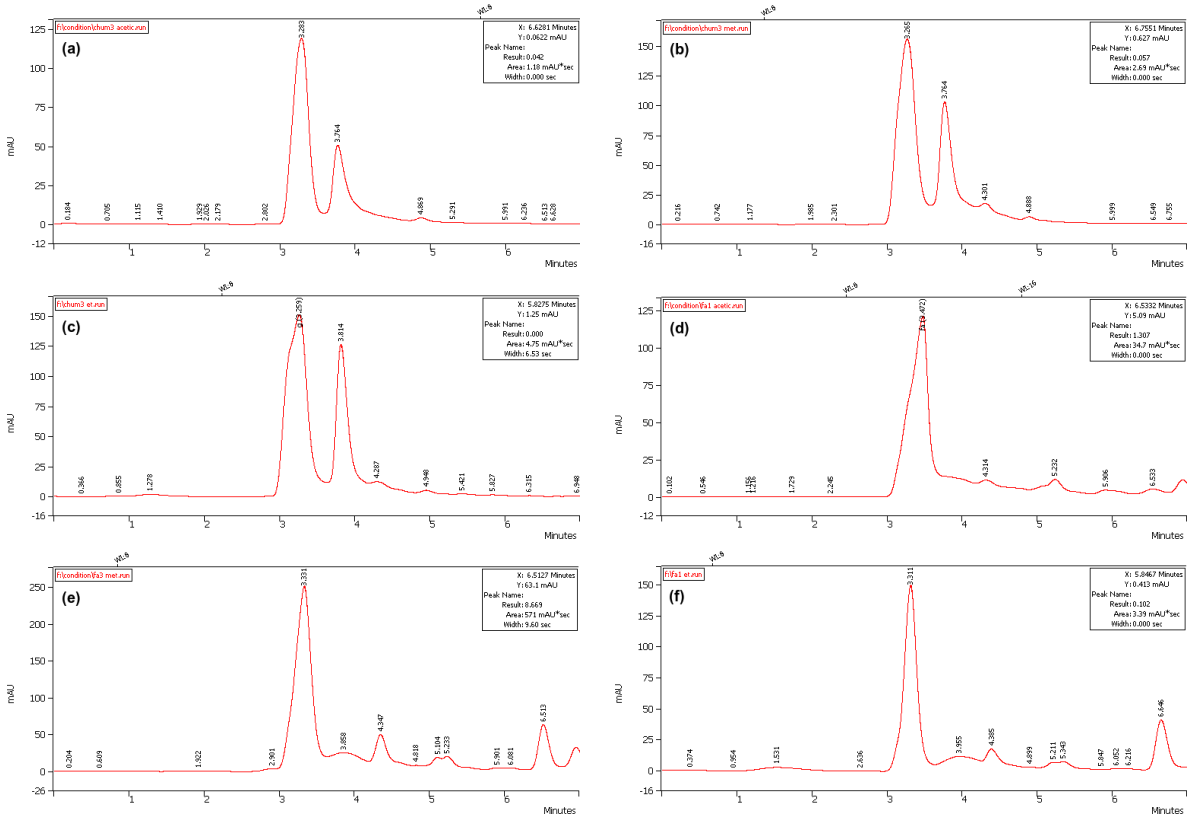


**Fig. 4** HPLC chromatogram of *C. alata* extracted with (a) acetic acid, (b) methanol, and (c) ethanol and *A. paniculata* extracted with (d) acetic acid, (e) methanol, and (f) ethanol.

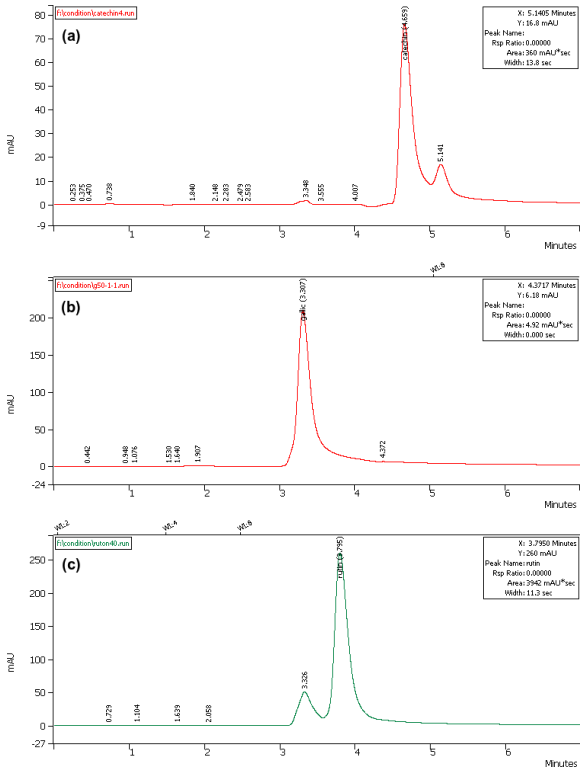


**Fig. 5** HPLC chromatogram of standard solutions: (a) catechin, (b) gallic acid, and (c) rutin. Eluent was distilled water:acetonitrile (50:50 v/v); detection was done at 210 nm.

lated from their calibration curves for gallic acid and rutin. Values (mg/g) are expressed as mean ± standard error. Gallic acid was found to be the main identified component in the ethanol, methanol, and acetic acid in the *C. alata* and *A. paniculata* extracts. Gallic acid in the ethanol, methanol and acetic acid extracts of *C. alata* was found to be $366.3 \pm 6.1$, $410.0 \pm 7.8$, and $257.6 \pm 3.1$ mg/g crude extract, respectively, while gallic acid in the ethanol, methanol and acetic acid extracts of *A. paniculata* was found to be $309 \pm 13$, $351.2 \pm 5.6$, and $309.1 \pm 4.7$ mg/g crude extract, respectively. The rutin in the ethanol, methanol and acetic acid extracts of the *C. alata* was found to be $194 \pm 29$, $193.6 \pm 9.5$, and $120.0 \pm 3.0$ mg/g crude extract, respectively. However, rutin was not found in the three extracts of *A. paniculata*. Thus it was observed that the levels of gallic acid in the methanol extract of *C. alata* and *A. paniculata* were higher than that of the ethanol and acetic acid extracts, while the rutin content in the ethanol and methanol extracts of *C. alata* was comparable with others but was higher than that of the acetic acid extract.

## DISCUSSION

This study found that methanol was the best solvent for the extraction of *C. alata* and *A. paniculata* since it produced the highest gallic acid levels. A similar observation has been reported by Phongpaichit et al[7] who found antifungal activity in leaf extracts of *C. alata*, *C. fistula*, and *C. tora*. In addition, Panichayupakaranant and Kaewsuwan[9], who used DPPH radical scavenging assay to investigate the antioxidant activity of crude methanol extracts from leaves, flowers, and pods of *C. alata* found that the leaf extract exhibited a stronger antioxidant activity than the flowers and pods. The highest gallic acid content was found in *A. paniculata* extracted with methanol, while rutin was not found in any of the three extracts. This finding agrees with the previous report of Akowuah et al[10] on the free radical scavenging activity of methanol extracts compared to water extract of *A. paniculata*.

*Acknowledgements*: The authors gratefully thank the University of Phayao for providing a research grant and all department colleagues for making equipment available.

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