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## CHEMICAL AND BIOLOGICAL STUDIES ON SOME THAI MEDICINAL PLANTS*

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### ABSTRACT

*Examination of the amino acid compositions and contents in ethanol extracts from 20 species of Thai medicinal plants revealed the significant differences. Several biological activities, including the antipyretic and antimicrobial activities of the aqueous extracts of these selected plants were also examined.*

### INTRODUCTION

A large number of medicinal plants are found all over Thailand which have been used to relieve diseases in the form of traditional medicines, or old-style preparations by native practitioners for a very long time. Therefore, it is quite interesting to study medicinal plants of Thailand owing to her rich heritage of effective plants whilst much works still remain to be done in this field. During our study on chemical constituents of medicinal plants in Thailand[1-4], we have investigated the amino acid compositions and contents and biological activities of some selected species. The present investigation deals with the preliminary chemical and biological studies, in the hope that it would contribute to expand our knowledge and prove the effectiveness of herbal medicines.

* Parts of this work were presented at the 105th Annual Meeting of the Pharmaceutical Society of Japan at Kanazawa, 5 April 1985 (Abstract p. 437).

## MATERIALS AND METHODS

### Plant materials.

The plant materials were collected in the vicinity of Bangkok, Thailand, in March 1986 and identified by Assoc. Prof. N. Ruangrungsi, Chulalongkorn University. The herbarium samples were kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

### Amino acid analysis

All plant materials (each 20 g) were air-dried and cut into small pieces. An extract of each plant was prepared by macerating the plant material with 75% EtOH. The extracted solution were filtered and evaporated to dryness *in vacuo* below 45°C and then dissolved in 50 ml of 0.02 NHCl (pH 1.7). Amino acid analyses of these samples were carried out with a Hitachi 835-10 automatic amino acid analyzer| using Li-citrate buffer system as described previously.[5]

### Biological activity assays

1) *Preparation of aqueous extracts.* Each of the dried plant materials (100 g) listed in Table 2, except *E. phaseoloides, M. collettii* and *A. pavonina,* was ground into a fine powder and boiled with water for 1 hr. The mixture was filtered through a cotton gauze and the residue was washed with a small amount of hot water. The remaining residue in the filtrate was further removed by centrifugation. The supernatants were combined and lyophilized to produce crude extracts for biological activity screening.

2) *Anti-platelet aggregatory activity.* Blood samples were obtained from healthy New Zealand rabbits, which had not taken any drugs for at least two weeks. The blood was withdrawn from the femoral artery into syringes containing 3.8% sodium citrate solution (citrate : blood = 1.9). Platelet rich plasma (PRP) was obtained from the citrated blood by centrifugation at 800 rpm for 10 min. After PRP was separated, the aqueous layer was further centrifuged at 3000 rpm for 15 min and platelet poor plastma (PPP) was obtained. A platelet counter (Coulter Counter T660) was used to adjust the number of platelets in PRP to a constant value ($3 \times 10$ platelets/$\mu$l) in each experiment with homologous PPP. Each sample (450 $\mu$l) was pre-warmed for 1 min at 37°C and stirred at 1000 rpm before addition of tested samples. Then, 50 $\mu$l of tested solution or vehicle was added to each sample, and 1 min later, 50 $\mu$l of ADP (an aggregating agent) was added. The aggregation curve was monitored by means of a blood platelet aggregometer (Payton Aggregation Module, Payton Associate). Inhibition of platelet aggregation was assessed by comparing maximal optical transmission change of the sample-treated PRP tested sample with the vehicle treated PRP sample, and this was expressed in percentage changes.

3) *Antimicrobial activity.* Antimicrobial activity of crude extracts were determined by the disc agar diffusion method. Tested microorganism were *Escherichia coli, Staphy-*

*lococcus aureus, Pseudomonas aeruginosa* and *Candida parapsilosis.* Heart infusion agar was used as culture medium. The crude extracts were tested at various concentrations of $10^{-2}-10^{-7}$ mg/ml and 50 $\mu$l of each was used per disc (diameter : 8 mm). Incubation was performed at 37°C for 24 hrs. Kanamycin (5, 10, 30 $\mu$g),tetracycline(5, 10, 30 $\mu$g)and gentamicin (2, 5, 10 $\mu$g) were used as reference standards.

4) *Antipyretic activity.*[6] Fever was induced in rats by using 20% dry yeast (Nissin Co.) as pyrogen. Eighteen hours after the yeast was injected, the animals that had a rectal temperature of 38.6°C or over were selected and then each of tested compounds was administered p.o. at adose of 2 g/kg body weight. The rectal temperature was measured by thermister every hour for 4 hrs after the administration of tested compounds. Aminopyrine at a dose of 100 mg/kg body weight was used as a standard reference.

5) *Pharmacological action on the isolated guinea-pig ileum.*[7] Isolated muscle preparation used in this study was suspended in a 10 ml organ bath filled with Tyrode solution. Male guinea-pigs, weighting 300 g, were sacrificed by a blow on the head and the ileum of each was isolated and placed in Tyrode solution. Preparation (2-3 cm long) of ileum used were connected to an isotonic transducer and responses to drugs were recorded isotonically under a tension of 1.0 g. The tested samples (concentration $10^{-4}$ g/ml) were added in the organ bath to detect their effects on contractile force, and responses induced by these samples were registered with a pen-recorder. Dose response curves recorded were compared with those of post-administration of tested samples. In addition, effects of these samples on contraction induced by acetylcholine, histamine and barium chloride were also studied.

## RESULTS AND DISCUSSION

### Amino acid Compositions and Contents

The amino acid compositions and contents in ethanol extracts of 20 species of Thai medicinal plants were investigated by using automatic amino acid analyzer as shown in Table 1. A large amount of asparagine has been found in *C. macrophylla, C. buchanani, T. trilobatum* and *E. phaseoloides* : 185, 5.44, 7.24 and 99.3 $\mu$mol/g dry wt, respectively. *K. galanga* yielded a high concentration of arginine (7.26 $\mu$mol/g dry wt) while *E. sessiliflora, H. indicum, K. galanga, S. trilobatum* and *T. triandra* yielded a large amount of gamma-aminobutyric acid (GABA) : 7.01, 1.73, 2.36, 2.68 and 1.36 $\mu$mol/g dry wt, respectively. *M. collettii* also furnished a large amount of L-DOPA (62.5 $\mu$mol/g dry wt) which occupied about 83% of all free amino acid in this plant. Unknown acidic and neutral amino acids were also detected in different amounts in some plants. Since GABA and L-DOPA are both potentially effective compounds, those plants containing large amount of them might be worth from a therapeutic point of view. In addition, L-DOPA is known

to be effective for Parkinson's syndrome. Therefore, *M. collettii* might be most valuable as a medicine among these plants, because *M. pruriens* belonging to the same genus also contains L-DOPA[7] and is widely utilized as a folk remedies.

**Biological Activity**

The aqueous extracts of the plant samples listed in Table 2 were evaluated for their activity to inhibit adenosine 5'-diphosphate (ADP)-induced aggregation of rabbit platelets. Among the tested plants, *A. colorata* and *G. cochinchinense* showed some inhibitory effects on the ADP-induced platelet aggregation.

Preliminary screening tests for the antimicrobial activity of Thai medicinal plants were also carried out on aqueous extracts (Table 2). These extracts were individually tested by the disc agar diffusion method against 4 microorganisms: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida parapsilosis.* Kanamycin, tetracycline and gentamicin were employed as reference standards. Results of this study revealed that none of the tested plants had growth inhibitory effects on all tested micro-organisms.

The preliminary pharmacological screening for antipyretic activity was performed only on the medicinal plants used as antipyretics in a traditional medicine, such as *A. colorata, G. cochinchinense, K. galanga, L. rugosa* and *T. triandra,* according to the method of Tanaka *et al.* [6] The aqueous extracts of these plants were studies by the rectal temperature of rats using aminopyrine as a standard drug. It was found that all of the tested plants exhibited no significant antipyretic activity in rats. The 50% alcoholic extracts of these plants, except *A. colorata,* were evaluated for their antipyretic activities previously and reported to be devoid of antipyretic action in rabbits.[9] From this result, it may be concluded that these plants probably do not possess any antipyretic activity as suggested by the ancient inscriptions of traditional medicine.

Preliminary examination for the effects of the aqueous extracts of all plants, on the isolated guinea-pig ileum was also performed, following the method of Miure *et al.*[9] The direct effects, as well as effects on the contraction induced by some stimulants, acetylcholine, histamine and barium chloride, were studied (Table 2). The aqueous extracts of *A. colorata* and *G. cochninchinense* both contracted the smooth muscle of the ileum of guinea-pig directly at a dose of $10^{-4}$ g/ml. Contractile force induced by that of *G. cochinchinense* was weaker than that of *A. colorata.* Contraction induced by the extracts of *A. colorata* was not antagonized by chlorpheniramine (histamine $H_1$-receptor blocking agent and tetrodotoxin (neuron blocker), but it was antagonized by atropine sulphate at a dose of $7\times10^{-9}$ g/ml. These results suggested that the extract of *A. colorata* had a muscarinic activity. However, the contraction induced by the extract of *G. cochinchinense* was not antagonized by those three blockers. From the study of effects on the contraction induced by acetylcholine, histamine and barium chloride, it was found that the contraction induced by histamine was diminished by *B. abbreviata, S. trilobatum* and *T. triandra,* but it was enhanced by *E. sessiliflora, L. rugosa, P. pentandra* and *S. acmella. C. buchanani* showed the antagonistic effect on the contraction induced by barium chloride, while *E.*

*Sessiliflora* showed the synergistic effect. It can be seen that *E. sessiliflora* enhanced the spasmogenic response of both histamine and barium chloride. Contraction induced by acetylchlorine was increased by *K. galanga* but was not antagonized by any of the other tested plants. In this study, 6 out of 17 species of medicinal plants used, *B. monieri, B. ovata, C. macrophylla, H. indicum, H. anthelminticus* and *M. hortensis,* did not show any effect on the isolated guinea-pig ileum.

Our previous works showed that *E. phaseoloides* contained sulphur-containing amides, entadamide A, B and C, which has inhibitory effects on the 5-lipoxygenase activity of RBL-1 cells (4). This finding suggests that *E. phaseoloides* can be used to treat the inflammatory diseases such as bronchial asthma, while the seeds of this plant are utilized as folk medicine to treat skin diseases and as a soap plant in Thailand and other tropical countries. In addition, the saponin isolated from the seed kernels of this plant was found to have antitumor activity.[10]

Thus, the chemical constituents and biological activity screenings of these Thai medicinal plants are preliminary investigations. The results from this screening, however, showed that some plants such as *T. triandra, G. cochinchinense* and *M. collettii* might be effective and valuable from a therapeutic point of view. Moreover, these observations suggested that to continue the study on these plants should be made to find out the active constituents, which would explain their uses in traditional medicine.

## REFERENCES :


1. Ikegami, F., Shibasaki, I., Ruangrungsi, N. and Murakoshi, I. (1985). *Chem. Pharm. Bull.* **33,** 5151.
2. Ikegami, F., Ohmiya, S., Ruangrungsi, N., Sakai, S. and Murakoshi, I (1987). *Phytochemistry* **26,** 1525.
3. Ikegami, F., Sekine, T., Duangteraprecha, S., Matsushita, N., Matsuda, N., Ruangrungsi, N. and Murakoshi, I. (1989). *Phytochemistry* **28,** 881.
4. Ikegami, F., Sekine, T., Aburada, M., Fujii, Y., Komatsu, Y. and Murakoshi, I. (1989). *Chem. Pharm. Bull.* **37,** in press.
5. Murakoshi, I., Ikegami, F., Hama, T. and Nishino, K. (1984). *Shoyakugaku Zasshi* **38,** 355.
6. Tanaka, Y., Maeda, M. and Nakamura, K. (1981). *Folia Pharmacol. Japan* **77,** 531.
7. Miura, T., Mikami, H., Tokumoto, J., Ohshima, K., Matsumoto, K. and Go, K. (1984). *Pharmacometrics* **28,** 411.
8. Damodaran, M. and Ramaswamy, R. (1973). *Biochem. J.* **31,** 2149.
9. Mokkhasmit, M., Ngarmwathana, W., Sawasdimongkol, K. and Permphiphat, U. (1971). *J. Med. Ass. Thailand* **54,** 490.
10. Liu, W. C., Kugelman, M., Wilson, R. A. and Rao, K. V. (1972). *Phytochemistry* **11,** 171.


## บทคัดย่อ


จากการตรวจสอบชนิดและปริมาณ ของกรดอะมิโนในสิ่งสกัดด้วย เอทานอล ของสมุนไพรไทย 20 ชนิด ได้ผลแตกต่างกันอย่างมีนัยสำคัญ และได้ทำการทดสอบฤทธิ์ทางชีวภาพ เช่น ฤทธิ์แก้ไข้ และต้านจุลชีพ ของสิ่งสกัดด้วยน้ำจากสมุนไพรดังกล่าวนี้ด้วย

**TABLE 1.** Amino Acid Compositions and Contents of Thai Medicinal Plants

| Scientific name | Family | Part examined | Amino acids (μmol/g of dry weight) | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Asp | Thr | Ser | Asn | Glu | Gln | Pro | Gly | Ala | Val | Cyt | Met | Ile | Leu | Tyr | Phe | Orn | Trp | Lys | His | Arg | DOPA | GABA |
| 1. *Adenanthera pavonina* | Leguminosae | Seed | 0.09 | 0.03 | 0.14 | 0.58 | 0.34 | –* | 0.22 | 0.36 | 0.47 | 0.09 | 0.19 | – | 0.04 | 0.03 | 0.05 | 0.12 | 0.01 | 0.09 | 0.05 | 0.05 | 0.42 | – | 0.11 |
| 2. *Ardisia colorata* | Myrsinaceae | Fruit | 0.58 | 0.16 | 0.27 | 3.83 | 0.55 | – | 1.60 | 0.11 | 0.72 | 0.16 | – | – | 0.05 | 0.06 | 0.06 | 0.06 | 0.01 | – | 0.02 | 0.01 | 0.02 | – | 0.70 |
| 3. *Bacopa monnieri* | Scrophulariaceae | Whole | 0.03 | 0.01 | 0.05 | – | 0.03 | – | 0.01 | 0.03 | 0.03 | 0.01 | – | – | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | – | 0.01 | tc | 0.01 | – | 0.06 |
| 4. *Balanophora abbreviata* | Balanophoraceae | Whole | 0.03 | 0.01 | 0.06 | – | 0.03 | – | 0.01 | 0.05 | 0.04 | 0.01 | – | tc* | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | – | 0.01 | 0.01 | tc | – | 0.08 |
| 5. *Bridelia ovata* | Euphorbiaceae | Leaf | 0.18 | 0.21 | 0.23 | 0.32 | 0.67 | +* | 6.03 | 0.07 | 0.93 | 0.40 | 0.10 | – | 0.19 | 0.15 | 0.11 | 0.13 | – | – | 0.50 | – | 0.28 | – | 1.23 |
| 6. *Clitoria macrophylla* | Leguminosae | Root | 4.64 | 0.98 | 58.7 | 185 | 0.94 | + | 8.82 | 0.90 | 5.17 | 1.11 | 0.34 | – | 0.21 | 0.23 | 0.21 | 0.45 | 0.07 | + | 0.22 | 0.42 | 0.35 | – | 4.01 |
| 7. *Cryptolepis buchanani* | Asclepiadaceae | Stem | 0.39 | 0.04 | 0.13 | 5.44 | 0.37 | + | 0.11 | 0.10 | 0.23 | 0.07 | 0.06 | – | 0.03 | 0.03 | – | 0.02 | 0.01 | – | 0.03 | 0.01 | 0.40 | – | 0.76 |
| 8. *Entada phaseoloides* | Leguminosae | Seed | 3.01 | 2.45 | 2.10 | 99.3 | 0.33 | + | 2.72 | 0.71 | 4.20 | 6.24 | 1.44 | – | 2.53 | 2.03 | 0.67 | 5.05 | – | – | 0.14 | 0.52 | – | – | 4.34 |
| 9. *Euphorbia sessiliflora* | Euphorbiaceae | Root | 10.2 | 1.59 | 6.33 | 0.97 | 3.47 | + | 1.18 | 0.67 | 6.97 | 0.95 | 0.39 | – | 0.29 | 0.22 | 1.28 | 0.36 | 0.77 | – | 0.27 | 0.47 | 1.94 | – | 7.01 |
| 10. *Gymnopetalum cochinchinense* | Cucurbitaceae | Fruit | 0.36 | 0.06 | 0.10 | – | 0.96 | – | 0.13 | 0.16 | 0.44 | 0.23 | 0.12 | 0.05 | 0.17 | 0.54 | 0.04 | 0.11 | 0.05 | – | 0.11 | – | 0.02 | – | 0.58 |
| 11. *Heliotropium indicum* | Boraginaceae | Whole | 0.28 | 0.06 | 0.08 | 0.08 | 0.47 | + | 2.49 | 0.04 | 0.45 | 0.38 | 0.14 | – | 0.18 | 0.07 | 0.05 | 0.05 | 0.01 | – | 0.03 | – | 0.02 | – | 1.73 |
| 12. *Hydnocarpus anthelminticus* | Flacourtiaceae | Seed | 0.04 | 0.10 | 0.05 | – | 0.09 | – | 1.11 | 0.13 | 0.60 | 0.09 | 0.12 | – | 0.05 | 0.03 | 0.01 | 0.03 | 0.01 | + | 0.03 | tc | 0.01 | – | 0.44 |
| 13. *Kaempferia galanga* | Zingiberaceae | Rhizome | 1.49 | 0.56 | 0.70 | 3.34 | 2.65 | + | 0.55 | 0.47 | 2.91 | 0.46 | 0.49 | – | 0.25 | 0.45 | 0.29 | 0.27 | 0.07 | – | 0.68 | 0.27 | 7.26 | – | 2.36 |
| 14. *Limnophila rugosa* | Scrophulariaceae | Whole | 0.12 | 0.01 | 0.06 | – | 0.11 | + | 0.05 | 0.13 | 0.22 | 0.04 | – | – | 0.03 | 0.02 | 0.21 | 0.02 | 0.02 | – | 0.03 | 0.01 | 0.03 | – | 0.26 |
| 15. *Millingtonia hortensis* | Bignoniaceae | Flower | 0.16 | 0.03 | 0.13 | – | 0.71 | – | 0.30 | 0.15 | 0.42 | 0.11 | 0.40 | 0.01 | 0.07 | 0.06 | 0.03 | 0.04 | 0.04 | – | 0.03 | 0.01 | 0.01 | – | 0.19 |
| 16. *Mucuna collettii* | Leguminosae | Seed | 1.59 | 0.14 | 0.34 | tc | 1.64 | – | 6.24 | 1.02 | tc | 0.28 | – | – | 0.24 | 0.23 | 0.41 | 0.24 | – | – | 0.08 | 0.03 | 0.07 | 62.5 | 0.15 |
| 17. *Pouzolzia pentandra* | Urticaceae | Whole | 0.10 | 0.04 | 0.08 | 0.47 | 0.19 | + | 0.09 | 0.02 | 0.28 | 0.07 | 0.06 | – | 0.04 | 0.04 | 0.02 | 0.03 | 0.01 | + | 0.01 | 0.01 | 0.55 | – | 0.93 |
| 18. *Solanum trilobatum* | Solanaceae | Fruit | 2.28 | 0.16 | 0.30 | 5.86 | 1.66 | + | 2.28 | 0.33 | 0.81 | 1.16 | 0.58 | – | 0.80 | 0.86 | 0.54 | 0.79 | 0.04 | – | 0.56 | 0.10 | 0.06 | – | 2.68 |
| 19. *Spilanthes acmella* | Compositae | Whole | 2.16 | 0.23 | 0.32 | 2.65 | 0.77 | – | 2.72 | 0.24 | 0.31 | 0.56 | 0.07 | 0.02 | 0.21 | 0.14 | 0.10 | 0.22 | 0.07 | – | 0.18 | 0.02 | 0.03 | – | 0.66 |
| 20. *Tiliacora triandra* | Menispermaceae | Stem | 0.41 | 0.06 | 0.16 | 7.24 | 0.18 | + | 0.13 | 0.07 | 0.54 | 0.11 | 0.03 | – | 0.06 | 0.10 | 0.04 | 0.04 | 0.01 | – | 0.04 | 0.04 | 0.05 | – | 1.36 |

–* : not detected.

+* : not calculated.

tc* : trace (less than 0.01 μmol/g of dry wt).

**TABLE 2. Biological Activities of Thai Medicinal Plants**

| Plant samples | Biological activities | | | |
|---|---|---|---|---|
| | Antipyretic activity | Antimicrobial activity | Anti-platelet aggregatory activity | Effect on isolated ileum* |
| 1. *A. pavonina* | n.d.** | n.d. | n.d. | n.d. |
| 2. *A. colorata* | – | – | + | A |
| 3. *B. monnieri* | n.d. | – | – | – |
| 4. *B. abbreviata* | n.d. | – | – | B– |
| 5. *B. ovata* | n.d. | – | – | – |
| 6. *C. macrophylla* | n.d. | – | – | – |
| 7. *C. buchanani* | n.d. | – | – | D– |
| 8. *E. phaseoloides* | n.d. | – | n.d. | n.d. |
| 9. *E. sessiliflora* | n.d. | – | – | B+, D+ |
| 10. *G. cochinchinense* | – | – | + | A |
| 11. *H. indicum* | n.d. | – | – | – |
| 12. *H. anthelminticus* | n.d. | – | – | – |
| 13. *K. galanga* | – | – | – | C+ |
| 14. *L. rugosa* | – | – | – | B+ |
| 15. *M. hortensis* | n.d. | – | – | – |
| 16. *M. collettii* | n.d. | – | n.d. | n.d. |
| 17. *P. pentandra* | n.d. | – | – | B+ |
| 18. *S. trilobatum* | n.d. | – | – | B– |
| 19. *S. acmella* | n.d. | – | – | B+ |
| 20. *T. triandra* | – | – | – | B– |

* : A; contracted the smooth muscle of the isolated ileum directly, B+ or B-; enhanced or diminished the contraction induced by histamine, C+ or C–; enhanced or diminished the contraction induced by acetylcholine, D+ or D–; enhanced or diminished the contraction induced by barium chloride. ** : not determined.