

Inhibition of Heinz Body Induction of Six Common Thai Medicinal Leaves and Creeping Stems in In Vitro Antioxidant Study Model

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Keywords: herbs, traditional medicine

Abstract

Six common Thai plant species were examined for antioxidant activities: two creeping stems *Derris scandens* Benth and *Cryptolepis buchanani* Roe et Sch; and four leaves *Tamarindus indica* Linn, *Acacia concinna* DC, *Bauhinia malabarica* Roxb and *Andrographis paniculata* Wall Ex Ness. *T. indica*, *A. concinna*, and *B. malabarica* presented the most potent inhibition effect.

INTRODUCTION

In Thailand, herbs have been used for food and medicinal purposes for centuries and known to cure the common ailment and conditions such as anemia, cardiovascular diseases, anxiety, arthritis, colds, coughs, constipation, fever, headaches, infections, insomnia, intestinal disorders, premenstrual syndrome, stress, and weakness (Craij, 1999). Flavonoids are a large group of polyphenolic antioxidants that occur naturally in vegetables and fruits. The most important groups of flavonoids are anthocyanins, flavonols, catechins, and flavanones. Flavonoids are scavengers of superoxide anions (Robak and Gryglewski, 1988), singlet oxygen (Husain et al., 1987), and lipid peroxy radicals (Sorata et al., 1982). Flavonoids in regularly consumed foods may reduce the risk of death from coronary heart disease in elderly men (Hertog et al., 1993a) and may protect against stroke (Keli et al., 1996). Various herbs possess hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer (Craij, 1999). In this study, six traditional Thai herbs (*Derris scandens* Benth, *Cryptolepis buchanani* Roe et Sch, *Tamarindus indica* Linn, *Acacia concinna* DC, *Bauhinia malabarica* Roxb, and *Andrographis paniculata* Wall Ex Ness.) were examined for antioxidant activities. Antioxidant activity of extracts from these herbs was demonstrated by inhibition of hemoglobin precipitation caused by oxidants.

MATERIALS AND METHODS

Buffer solution consisted of 1.3 parts of M/15 KH_2PO_4 (Merck, Germany) (9.1 g of KH_2PO_4 dissolved in 1 L distilled water) mixed with 8.7 parts of M/15 Na_2HPO_4 (Merck, Germany) (9.5 g Na_2HPO_4 dissolved in 1 L of distilled water). 200 mg glucose was added to 100 mL buffer solution.

Acetyl phenylhydrazine (BDH Chemicals, England) was prepared by dissolving 100 mg of Acetyl phenylhydrazine in 100 mL of buffer solution.

The herbal extract was prepared by boiling herbal leaves and stems in water with a ratio of herb to water at 1:20 by weight. The mixture was then shaken intermittently. After boiling, the mixture was cooled to room temperature, centrifuged at 2,500 rpm for 15 min, and the supernatant frozen at -20°C .

Crystal violet solution prepared by dissolving 2 g of crystal violet powder (Merck, Germany) in 100 mL 0.73% NaCl at room temperature. The solution was shaken for 5 min, then filtered through Whatman no. 3 filter paper. Working solution was prepared by using equal volume of 0.73% NaCl added to the filtered solution.

A positive control system, following the method described by Reinhart et al. (1986) was used. Two mL acetylphenylhydrazine was added to 0.1 mL of blood incubated at 37°C for 2 h. Heinz bodies were counted under microscope (1,000 x). A negative control was prepared by adding 2 mL of buffer solution into 0.1 mL of blood incubated at 37°C for 2 h, then the Heinz body was observed. In the test system, each herb was tested, for the following two tests:

Test 1

2 mL extract (original 1:20) was mixed with 0.1 mL blood and incubated for 2 h, then 2 mg acetyl phenylhydrazine was added. The mixture was incubated for another 2 h then observed and the Heinz bodies counted. If this test was positive, the extract was diluted twice and tested again.

Test 2

2 mL extract mixed with 0.1 mL blood and incubated for 2 h was observed for Heinz body. It was incubated for another 2 h and the Heinz body observed again.

We performed the counter stain of Heinz body by transferring solutions from each test mixed with crystal violet solution with equal volume. The mixture was left at room temperature for 5 min. A thin smear was performed and the Heinz body was observed in 100 red cells under the microscope (1,000 x).

RESULTS AND DISCUSSION

D. scandens, *C. buchanani*, and the creat (*A. paniculata*) could not inhibit the formation of Heinz body by acetyl phenylhydrazine. *B. malabarica* Roxb and tamarind leaves could inhibit the formation of Heinz body at the dilution of 1:20 but *A. concinna* could inhibit up to 1:40 (Table 1). We investigated the antioxidant activity of six common Thai herbs by boiling these herbs in water so as to mimic Thai traditional medicine, which was extracted by boiling herb for a certain period of time. The antioxidant activity of the studied herbs could be probably due to the flavonoids, which were the largest group of polyphenolic antioxidants that occur naturally in vegetables and fruits, and in beverages such as tea and wine (Kuhnau, 1976; Hertog et al., 1993b). The creat showed no antioxidant activity, but was used as herbal medicine for periodontitis caused by *Porphyromonas gingivalis* in the form of gel (MU Researchers, 2002). The creeping stems had no antioxidant activity, although the leaves were antioxidants (Kuhnau, 1976; Reinhart et al., 1986).

ACKNOWLEDGEMENTS

The author thanks all staff who helped in collecting herbs and the research fund from Faculty of Allied Health Sciences, Chulalongkorn University.

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Tables

Table 1. Inhibition of Heinz body formation by the herbal extracts.

Herbs	% of Heinz body formation in extract dilutions			
	1:20	1:40	1:80	1:160
<i>Derris scandens</i> Benth	100	ND	ND	ND
<i>Cryptolepis buchanani</i> Roem et Sch	100	ND	ND	ND
<i>Tamarindus indica</i> Linn	0	100	ND	ND
<i>Acacia concinna</i> DC	0	0	100	100
<i>Bauhinia malabarica</i> Roxb	0	100	100	100
<i>Andrographis paniculata</i> Ex Nees	100	ND	ND	ND

ND = not done