APPLIED SCIENTIFIC RESEARCH CORPORATION OF THAILAND
DEPARTMENT OF MEDICAL SCIENCE, MINISTRY OF PUBLIC HEALTH
FACULTY OF MEDICAL SCIENCE, FACULTY OF PHARMACY, AND
FACULTY OF MEDICINE-SIRIRAJ HOSPITAL, UNIVERSITY OF MEDICAL SCIENCES

COOPERATIVE RESEARCH PROGRAMME NO. 17

PHARMACEUTICALS FROM INDIGENOUS MEDICINAL PLANTS OF THAILAND

RESEARCH PROJECT NO. 17/4

STUDIES ON HYPOTENSIVE ACTIVE PRINCIPLE (S) OF "KAFAK-MAMUANG", *LORANTHUS PENTANDRUS* L., LORANTHACEAE

REPORT NO. 1

EXTRACTION AND FRACTIONATION OF THE ACTIVE PRINCIPLE (S) OF *LORANTHUS PENTANDRUS* L. (KAFAK-MAMUANG)

BY

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EXTRACTION AND FRACTIONATION OF THE ACTIVE PRINCIPLE(S) OF LORANTHUS PENTANDRUS L. (KAFAK-MAMUANG)

By Lars Johansson* and Nitasana Pichitakul*

SUMMARY

About 1,000 g of stems and 250 g of leaves of Loranthus pentandrus L. have been minced and extracted separately with boiling water. Some of the aqueous extracts were further fractionated by shaking with chloroform to eliminate chloroform-soluble substances or passed through an aluminium oxide column. The precipitated tannin from some fractions was redissolved in a small amount of water and also tested for active compound(s). Acidic ion-exchanger was also used to fractionate the aqueous extracts. The different fractions so prepared, altogether twenty-two, were tested for hypotensive activity on dogs anaesthetized with barbiturates.

It was observed that the hypotensive activity was most pronounced in the basic fraction from the ion-exchanger column. The aqueous extracts from leaves had a more pronounced hypotensive effect than the extracts from the stems and this effect was not reduced by chloroform extraction.

INTRODUCTION

Loranthus pentandrus L. ('kafak-mamuang') is a semi-parasitic evergreen plant growing on the branches and trunks of other trees especially the mango tree, Mangifera indica L. This parasite resembles mistletoe, Viscum album L. and belongs to the same family, LORANTHACEAE. It has long been used in Thailand in the form of a decoction as a remedy for hypertension, and in the form of a poultice for the treatment of small sores, ulcers, and other skin lesions (Pengsritong, Limpinuntana, and Chungcharoen, unpublished report). This report concentrates on the method of extraction and fractionation of the active compound(s). It should be noted that the pharmacological results in detail are to be found in Report No. 2 on Research Project No. 17/4 "Studies on the activities of certain fractions of Loranthus pentandrus L.," by Dr. Komol Pengsritong.

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BACKGROUND INFORMATION

Several studies on mistletoes have been reported, especially on Viscum album. Winterfield and co-workers (Winterfield 1942; Winterfield and Doerle 1942; Winterfield and Kronenthaler 1942) were able to separate two fractions which exerted greater effect either on the blood pressure or on the cardiac muscle. The substance which depressed the blood pressure was believed to be acetylcholine. Mueller (1932) suspected that the extract of V. album contained propionylcholine, while Dressler and his colleagues (1933) thought that it was possibly choline, whose effect was increased by acetylation. Sajner and Veris (1955, 1957, 1958) believed that histamine was possibly one of the active principles. Paskov et al. (1958) found that the aqueous extract of V. album was strongly hypotensive, while the alcoholic extract and the aqueous acidified extracts were less effective. Sandberg and Samuelsson (1964) isolated viscotoxin (a peptide compound) and arginine by extracting V. album with aqueous ethanol (40 % v/v).

Hypotensive active compounds of L. pentandrus have not been isolated and identified. Mongkolsuk and Podimuang (unpublished paper 1966) have carried out the studies of the leaves of this parasite. They found quercetin in the methanolic extract but it was not certain that this compound showed any pharmacological effect. Recently Pengsritong, Limpinuntana and Chungcharoen (unpublished paper 1966) extracted L. pentandrus with different solvents either water, ethanol, chloroform, or ether. They tested these extracts pharmacologically on dogs anaesthetized with barbiturates, and on isolated perfused frog's hearts. They found that the aqueous extracts possess both the vasodepressant and cardiac toxic activities but neither ethanol, chloroform or ether shows any effect on the blood pressure of the animal.

MATERIALS AND METHODS

Two samples of L. pentandrus were supplied by Mrs. Chalao Limpinuntana and Mrs. Sasithorn Wasuwat. Both sources were in Bangkok, but more than ten kilometres apart. The samples were chopped into small pieces before they were minced through a 2-mm screen.

Extraction.--The aqueous extractions were carried out as described previously by Pengsritong, Limpinuntana and Chungchuroen with some modification. Two samples of leaves (100 g and 188 g) and two samples of stems (335 g and 665 g) were added into boiling water separately in four Erlenmeyer flasks. The proportions of water were about 100 g of sample to 1,000 ml of distilled water. After two hours of heating (30° 95°C) on the water bath, the aqueous extracts were cooled and allowed to stand at room temperature for 48 hours. The decoction was then filtered, and the filtrate was evaporated under reduced pressure. The concentrated extracts were made up to 100 g of the original weight of each sample to 100 ml of distilled water. Ten millimetres of each sample was sent for pharmacological studies.

Elimination of precipitated tannin.--On standing in the refrigerator, the aqueous solutions obtained showed some precipitation of tannin, which was centrifuged off at 3,000 r.p.m. for 30 minutes. Some of these precipitates were redissolved in a small amount of distilled water and sent for pharmacological tests.

Partitioning with chloroform.--In certain instances, 10 ml of the aqueous solution from each sample was shaken up with several portions of chloroform to remove any chloroform-soluble substances. The water phase from each sample was then filtered into an Erlenmeyer flask for hypotensive studies.

Fractionation with aluminum oxide column.--Twenty five millimetres of aqueous extract from stems of L. pentandrus was passed through a column of 90-ml capacity filled with neutral Al_2O_3. The first, coloured fraction was collected and evaporated to a volume of 10 ml under reduced pressure. The second, colourless fraction was obtained in the same manner as the first one, but after a further dilution with 250 ml of water. The fractions were sent for active principle tests.

Fractionation with ion-exchange resin.--Bio-rex 40, 20-50 mesh in hydrogen form was evacuated in water until no air bubbles appeared. Then the slurry was loaded into a column for chromatography. The column was 1 cm in diameter and 30 cm long. Twenty millimetres of an equeous extract was pussed through this ion-exchange column. Then about 20 ml of distilled water was used to rinse the column. The dark coloured

fraction so obtained was evaporated in vacuo to the volume of 10 ml. The second fraction, mainly alkaloids, amino acids, and peptides together with some very polar neutral compounds was eluted out with 40 ml 2N HCl. The solution was adjusted to pH 4-5 with 2N NaOH solution and evaporated in vacuo to a small volume (~5 ml). The precipitated white crystals of NaCl was filtered off before the volume was made up to 10 ml. Other samples of aqueous extracts have been worked up in the same manner.

TABLE 1

TREATMENTS OF AQUEOUS EXTRACTS IN THE FRACTIONATION

Samples	Leaves	Stems	Extracted with chloroform	Redissolved tannin	Al_2O_3 column		Ion-exchanger column	
					Coloured fraction	Colourless fraction	First fraction	Second fraction
I-A-1	✓		✓					
I-A-2	✓		✓	✓				
I-A-12	✓		✓				✓	
I-A-13	✓		✓					✓
II-A-1		✓	✓					
II-A-2		✓	✓	✓				
II-B-1		✓						
II-B-2		✓		✓				
II-B-13		✓					✓	
II-B-14		✓						✓
II-B-25		✓		✓			✓	
II-B-26		✓		✓				✓
III-A	✓		✓					
III-B	✓							
III-B-1	✓						✓	
III-B-2	✓							✓
IV-A		✓	✓					
IV-B-1		✓						
IV-B-2		✓			✓			
IV-B-3		✓				✓		
IV-B-4		✓					✓	
IV-B-5		✓					✓	✓

(From Record Book No. 133, pages 2-6)

Table 1 shows in what way the different fractions were treated. The table is also the key to the code designations used by Dr. Komol Pengsritong in his Report No. 2 on Research Project 17/4 "Studies on the activities of certain fractions of Loranthus pentandrus L." The different fractions so prepared were stored in the refrigerator before and after the pharmacological tests. These tests were carried out by Mrs. Chalao Limpinuntana and her assistants at the School of Pharmacy 2 to 4 weeks after the preparation. Recording of the blood pressure and respiratory rate of dogs were prepared on kymographic papers, the animals being under barbiturate anaesthesia. The fractions were diluted ten times with distilled water before using, and different doses of the diluted solutions were administered intravenously into the animals.

RESULTS, DISCUSSION, AND RECOMMENDATIONS

1. These results are consistent with and confirm those obtained in an experiment reported previously (Pengsritong, Limpinuntana and Chungcharoen, unpublished paper 1966).

2. The activity of aqueous extract of stems is much lower than that of the leaves.

3. The active compound(s) is (are) not chloroform-soluble.

4. From the use of the acidic ion-exchange column, it is learned that the second fractions are much more active than the first fractions which do not give the same abrupt fall in blood pressure.

5. The tannin precipitate obtained from the aqueous extract possesses no active principle(s).

6. There seem to be no active principles in the fractions from the Al_2O_3 column. This result may be due to the original aqueous extract from the stems used, which possesses already low activity. This step should be repeated by using high activity extract from leaves for fractionation.

7. Fractionation and pharmacological studies should be further directed towards the isolation and identification of the active compound(s).

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