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GOVERNMENT PHARMACEUTICAL ORGANIZATION

COOPERATIVE RESEARCH PROGRAMME NO. 17
PHARMACEUTICALS

RESEARCH PROJECT NO. 17/4
PHARMACEUTICALS FROM *LORANTHUS PENTANDRUS* L. (KEFAK–MAMUANG)

REPORT NO. 3
EXTRACTION AND FRACTIONATION OF THE ACTIVE PRINCIPLE (S) OF *LORANTHUS PENTANDRUS* L. (KAFAK–MAMUANG)

BY
SUPATRA MUNSAKUL
KAMOL SAWASDIMONGKOL

ASRCT, BANGKOK 1972
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F O R E W O R D

This is the third report on Research Project 17/4, Pharmaceuticals from Loranthus pentandrus L. (kafak-mamuang), of Cooperative Research Programme No. 17, Pharmaceuticals. The pharmacological work in this report was performed by Mr. Kamol Sawasdimongkol at the Department of Medical Sciences, Ministry of Public Health.

The aim of this project is to isolate and identify the active principles of Loranthus pentandrus L. by extraction and fractionation. The information in this report represents only a part of task 3(4) as assigned in the project outline, and the work was also done in a short period of time. This report is written to bring the work up-to-date and to transfer it to Miss Prakongsiri Chantarasomboon, who will continue the work of this project in Tokyo, Japan.

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

By Supatra Munsakul* and Kamol Sawasdimongkol+

SUMMARY

Crude extracts of Loranthus pentandrus L. leaves from Chon Buri were prepared and also dialyzed. The fractionation of dialyzate with silica gel (kieselgel 0.2-0.5 mm, E. Merck AG., Darmstadt, Germany) was attempted. Five different fractions were prepared and were tested for hypotensive activity on dogs. Two fractions showed significant effect on blood pressure.

INTRODUCTION

Previous works on the activities of Loranthus pentandrus L. (kafak-mamuang) have been reviewed in reports No. 1 and No. 2 on this project. It has been found that extraction is best performed with boiling water, the method involving dialysis is the most promising, and the method for fractionation of the dialyzate was tried. Work in this report was continued from the previous one. Attempts to isolate and identify hypotensive active compounds of Loranthus pentandrus L. have been made.

MATERIALS AND METHODS

1. The leaves from the parasitic Loranthus pentandrus L. growing on the mango tree were collected from Chon Buri in June 1970, dried, and minced.

2. Tubing for dialysis, diameter 28 mm (The Scientific Instrument Centre, Ltd., London).

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3. Silica gel für die Chromatographic (kieselgel . 0.2-0.5 mm, E. Merck AG. Darmstadt, Germany).

4. The methods used for extraction and fractionation are described in detail in EXPERIMENTAL AND RESULTS section.

EXPERIMENTAL AND RESULTS

Extraction

The aqueous extractions were carried out as described previously by Sunanta Jindaprasarn and Lars Johansson*. Five hundred grammes of dried and minced leaves of Loranthus pentandrus L. were added to 5 litres of boiling water and allowed to boil for 5 minutes with continuous stirring. The extract was allowed to cool and was then filtered through cotton cloth and centrifuged. The precipitate was rejected, and the supernate was evaporated to a volume of 500 ml, corresponding to a concentration of 1 g dry leaves /ml solution and was used as the starting material in this experiment. This solution was called "A" and its pharmacological activity is listed in Table 1.

Elimination of precipitated tannin

The crude extract was stored in refrigerator ($+5^{\circ}$) overnight. The precipitate of tannin which formed was separated from the solution by centrifugation (Martin Christ Osterode/Harz, Karl-Kolb, Germany) at 3000 rev/min for 3 minutes.

Dialysis

Dialysis was carried on as described previously by Sunanta Jindaprasarn and L. Johansson*. The crude extract (500 ml), free from tannin, was dialyzed in 5 litres of distilled water for 4 days. Three drops of xylene and three drops of chloroform were added daily as preservative. The solution left inside the tubing was filtered and evaporated in vacuo to a suitable concentration (1 g/ml). This solution

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*Report No. 1 on Research Project 17/4. ASRCT unpublished report.

was called "B" and the water solution outside the tubing, after evaporation to the same concentration, was designated "C". Solution B was used for the preparation of the column fractions.

Silica gel column

The dialyzate (2 ml) was loaded on a silica gel (0.2-0.5 mm) column (8 cm x 20 cm) and eluted with n-butanol-acetic acid-water (4:1:2) and distilled water.

From n-butanol-acetic acid-water (4:1:2) solution, three bands were obtained; the first one was dark brown, the second one yellow, and the third one brown. The first fraction was acidified with 2 M HCl and then extracted several times with ether. The two layers were separated; the ether layer was collected and called "H"; the aqueous layer was neutralized with 4 M sodium hydroxide. The solid sodium acetate and sodium chloride formed on standing were filtered off, and the remaining filtrate was then made up to a concentration of 1 g/ml with distilled water and called fraction "D".

The second and third fractions were similarly treated, resulting in fraction "E" and fraction "F". The ether layer solution was also called fraction "H".

All portions of fraction H were combined, evaporated to small volume, and then neutralized with 4 M sodium hydroxide. Crystals of sodium acetate precipitated out and the remaining liquid was evaporated to dryness and then made up to a concentration of 1 g/ml with distilled water.

The column was next eluted with distilled water. A pale yellow solution was obtained. This solution was shaken several times with ether. The ether layer was collected and treated as fraction H and was combined with previous ones. The aqueous layer was neutralized with sodium hydroxide before its evaporation to a small volume. The solid sodium acetate and sodium chloride formed on standing was filtered off, and the remaining filtrate was made up to a concentration of 1 g/ml and called fraction "G".

Pharmacological tests

Fractions A-G were tested for active principle. Their pharmacological activities are listed in Table 1.

TABLE 1
HYPOTENSIVE EFFECT IN DOG OF EACH FRACTION INJECTED INTRAVENOUSLY

Sample	Action	Remarks
Fraction A	Active	A blood pressure drop of $\geq$ 18 but $\leq$ 36 mm Hg.
Fraction B	Active	A blood pressure drop of $\geq$ 18 but $\leq$ 36 mm Hg.
Fraction C	Inactive	
Fraction D	Inactive	
Fraction E	Inactive	
Fraction F	Inactive	
Fraction G	Active	A blood pressure drop of $\geq$ 30 but $\leq$ 60 mm Hg.
Fraction H	Active	A blood pressure drop of $\geq$ 20 but $\leq$ 50 mm Hg.

The pharmacological tests were performed at the Pharmacological-toxicological Laboratory, Department of Medical Sciences, Ministry of Public Health, on dogs under narcosis with a mixture of chloralose (8 g %), urethane (25 g %) and borax (5 g %). Tubocurarine hydrochloride 0.1 mg/kg dog was infused at the beginning of each experiment, and artificial respiration was then applied. The tested sample was infused via the femoral vein at the rate of 1 ml/min to doses of 100 and 1000 mg/kg dog. Blood pressure, heart rate, and ECG were recorded simultaneously, using a multipurpose polygraph and its transducers.

Results

Fractions C, D, E, and F did not show any significant effects on blood pressure, heart rate, and ECG of the narcotised dogs when they were infused up to the dose of 1000 mg per kg dog.

Fractions A, B, G and H, when infused to the dose of 200 mg per kg dog, started to decrease the blood pressure. The maximal decrease was obtained when the samples were infused to a point of 600-800 mg per kg. No more effect occurred with continued infusion from this

point up to 1000 mg per kg dog. The effect of these substances on heart rate was inconsistent. There was no significant change on ECG.

DISCUSSION AND CONCLUSIONS

The activities of crude aqueous extract of the dry leaves of Loranthus pentandrus L. and the dialyzability of the crude aqueous extract have been confirmed. The crude aqueous extract showed a moderate degree of hypotensive activity. When the crude extract was subjected to dialysis in distilled water, the pharmacological activity goes completely with the dialyzate after 4 days. The active compounds, which passed through a cellophane membrane, had molecular weights of not more than 6,000. Therefore proteins could be excluded as active compounds.

The results on fractions from the column indicated that the hypotensive principle of Loranthus pentandrus L. could be highly concentrated in fractions G and H. More work of this kind on the isolation and purification of the active compound(s) should be performed. As this report is written, Miss Prakongsiri is continuing the work in Japan. Another purification method—preparative thin-layer chromatography using impregnated plates and hydrophilic eluent system according to Kirchner (1967)—was also being tried, but not enough materials have yet been obtained for pharmacological study.

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