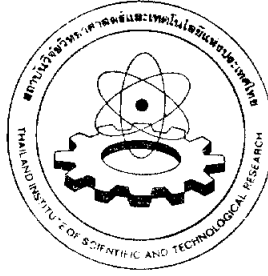


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THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

PHARMACOLOGICAL STUDY ON THE ANTIBACTERIAL  
AND ANTIFUNGAL ACTIVITIES OF ACTIVE PRINCIPLES FROM *ALPINIA CONCHIGERA* GRIFF.

BY

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ARUBOL CHOTIPONG

TISTR, BANGKOK 1986

not for publication

The publication of this report has been approved by  
the Governor of Thailand Institute of Scientific and Technological Research.

A handwritten signature in cursive script, reading "Smith Kampempool".

(Professor Dr. Smith Kampempool)

Governor

THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

RESEARCH PROJECT NO. 25-11  
THE PHARMACEUTICALS FROM MEDICINAL PLANTS

REPORT NO. 2

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## PREFACE

The study of antimicrobial activity of Alpinia spp., one of the sub-project of antimicrobial medicinal plant projects, has so far been performed by the Pharmaceuticals and Natural Products Department (PNPD), Thailand Institute of Scientific and Technological Research (TISTR) since 1981. Due to the fact that Thailand has imported more than 500 million baht (C.F.F. value) annually, especially for antimicrobials and antibiotics, PNPD therefore investigated antimicrobial medicinal plants for selection of the highly effective raw materials in the industrial production of antimicrobials. This will lessen some amounts of imported medicine from abroad.

The preliminary study on antimicrobial of Alpinia showed very interesting results of the 3 species, namely - Alpinia galanga, Alpinia spp. and Alpinia conchigera, among which Alpinia conchigera showing the highest antibacterial property. The research project was substantially enlarged in order to develop Alpinia conchigera into drug industries, thus resulting in the promotion of its cultivation beyond its uses for food preparation.

การศึกษาคุณสมบัติในการยับยั้งการเจริญของเชื้อแบคทีเรียและ  
เชื้อราของสารสกัดแสดงฤทธิ์จากสมุนไพรชาลิ้ง Alpinia conchigera Griff.

โดย ศศิธร วสุวัต, พุทธิรินทร์ วรรณิสสร, วิไลพร แซ่มซ่าง,  
ทวีศักดิ์ สุนทรชนศาสตร์, ปัทมา สุนทรสารทูล และ อรุบล โชติพงษ์

### บทคัดย่อ

สารสกัดจากชาลิ้งด้วย 70% ethanol ที่ความเข้มข้น (MIC) 20-50 มิลลิกรัม/มิลลิลิตร  
สามารถยับยั้งการเจริญของเชื้อแบคทีเรีย ชนิด Staphylococcus aureus ได้ และที่ความ  
เข้มข้น 15-25 mg/disc ยับยั้งเชื้อได้ 10-15 mm, เปรียบเทียบได้ใกล้เคียงกับ  
chloramphenicol 30 µg/disc และ ampicillin 10 µg/disc. สารสกัดนี้ไม่มีผล  
ต่อเชื้อรา.

น้ำมันชาลิ้งมีผลยับยั้งเชื้อรา Trichophyton mentagrophytes, Microsporum  
gypseum และ Epidermophyton floccosum โดยมีผลสูงสุดต่อเชื้อ E. floccosum  
ที่ความเข้มข้น (MIC) 0.07-0.1% และเปรียบเทียบประสิทธิภาพใกล้เคียงกับ Canesten  
(Bayer) ซึ่งใช้เป็นสารแสดงฤทธิ์เปรียบเทียบ.

น้ำมันชาลิ้งมีความเป็นพิษเฉียบพลันต่ำ, มีค่า approximate lethal dose  
10.7 กรัม/กิโลกรัมน้ำหนักตัว เมื่อทดลองโดยการให้รับประทานครั้งเดียว (single oral  
dose) ในหนูถีบจักร (mice).

ครีมน้ำมันชาลิ้ง 3% พัฒนาโดยใช้สูตรครีมเบส สวท. 1 พบว่าให้ผลในการทดลอง  
โดยวิธี Serial Dilution ดีกว่าการทดลองโดยวิธี Punch Test.

ครีมน้ำมันชาลิ้ง 3% นี้ไม่ก่อให้เกิดความระคายเคืองต่อผิวหนัง เมื่อทดสอบกับผิวหนัง  
กระต่าย.

PHARMACOLOGICAL STUDY ON THE ANTIBACTERIAL AND  
ANTIFUNGAL ACTIVITY OF ACTIVE PRINCIPLES FROM ALPINIA CONCHIGERA GRIFF.

By Sasithorn Masuwat<sup>\*</sup>, Puttarin Vannissorn<sup>\*</sup>, Wilaiporn Chamchaang<sup>\*</sup>,  
Taweesak Suntornritanasat<sup>\*</sup>, Pattama Soontornsarathune<sup>\*</sup> and  
Arubol Chotipong<sup>\*</sup>

ABSTRACT

The 70% ethanol Alpinia conchigera extract at the minimum inhibitory concentration (MIC) of 20-50 milligrams/millilitre inhibited Staphylococcus aureus and at the concentration of 15-25 milligrams/disc inhibited bacterial growth of 10-15 mm with similar effectiveness comparing with chloramphenicol (30 µg/disc) and ampicillin (10 µg/disc). This extract had no inhibitory effect on fungi.

Distilled conchigera oil possessed antifungal property against Trichophyton mentagrophytes, Microsporium gypseum and Epidermophyton floccosum, among which the maximum inhibition was to E. floccosum at the minimum inhibitory concentration (MIC) of 0.07-0.1% in similar effectiveness comparing with Canesten<sup>®</sup> (Bayer) as control.

Distilled conchigera oil had low acute toxicity with approximate lethal dose of 10.7 grams per kg body weight when administered in single-dose orally to mice.

The study of 3% distilled conchigera oil cream, formulated by using PNP-1 cream base, determined by Serial Dilution Method was better than Punch Test method.

It was noted that 3% distilled conchigera oil cream had no dermal irritation in rabbits.

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\* The Pharmaceuticals and Natural Products Department, Thailand Institute of Scientific and Technological Research.



## INTRODUCTION

The Pharmaceuticals and Natural Products Department, Thailand Institute of Scientific and Technological Research has studied the antimicrobial property of medicinal plants and the report in 1971 revealed 3 species of *Alpinia*, namely:

*Alpinia galanga* (L.) Willd Syn., *Languas galanga* (L.) Stuntz and *Alpinia pyramidata* Bl.

*Alpinia conchigera* Griff. possessed antibacterial activity against *Bacillus subtilis* ATCC 6633., *Sarcina lutea* ATCC 9341, *Staphylococcus aureus* ATCC 6538 F., *Salmonella typhi* Siriraj Hospital, *Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* Siriraj Hospital, *Shigella dysenteriae* Siriraj Hospital, and *Lactobacillus buchneri*.

The Fermentation Laboratory found that active constituents extracted by alcohol revealed higher potency than those extracted by other solvents. Moreover, when compared among the 3 species, *Alpinia conchigera* had more antibacterial activities than the other two, though it was much less effective than the control antibiotics. From the literature searching, there was no report of the clinical and chemical study on *Alpinia conchigera*; there were, however, only reports on *Alpinia galanga*, *A. speciosa*, *A. katsumadai*, *A. flabellata*, *A. japonica*, *A. nutans*, *A. oxyphylla* and *A. fructus*.

The research on this programme has continued since 1983. The study of antifungal properties in dermatophytosis of 3 types: *Trichophyton mentragrophytes* (T.m.), *Microsporum gypseum* (M.g.) and *Epidermophyton floccosum* (E.f.) has been conducted concomitant with the study of antibacterial properties on skin in 2 species of bacteria: *Staphylococcus aureus* and *Pseudomonas aeruginosa* under collaborative project of the Department of Medicine, Faculty of Medicine, Siriraj Hospital. The objective of the cooperation is to study active principles of *Alpinia conchigera* for the benefit of formulating antibacterial and antifungal cream for skin diseases.

## MATERIALS AND METHODS

### A. The extraction and test of effectiveness of the extracted constituents in the inhibition of various bacterial growth

#### 1. Materials

1.1 Alpinia conchigera from retailers in Bangkok Metropolis.

1.2 70% ethyl alcohol as a solvent from the Government Pharmaceutical Organization (GPO).

#### 1.3 Media

##### 1.3.1 Media for bacterial culture

1.3.1.1 Trypticase Soy broth, pH 7.5

1.3.1.2 Trypticase Soy agar, pH 7.5

##### 1.3.2 Media for fungal culture

1.3.2.1 Sabouraud dextrose broth

1.3.2.2 Sabouraud dextrose agar

#### 1.4 Bacteria

1.4.1 Staphylococcus aureus (S.a.)

1.4.2 Pseudomonas aeruginosa (P.a.)

#### 1.5 Fungi

1.5.1 Trichophyton mentagrophytes (T.m.)

1.5.2 Microsporum gypseum (M.g.)

1.5.3 Epidermophyton floccosum (E.f.)

1.6 Carbopol 934 cream base

1.7 Non-irritated emulsion cream base

1.8 Stainless steel percolator

1.9 Steam distillation apparatus

1.10 Rotavap, Büchi (Switzerland)

1.11 Canesten<sup>®</sup>, (Bayer)

1.12 Chloramphenicol, Difco (30 mg/disc)

## 2. Methods

### 2.1 Extraction of antibacterial active constituents

Using 70% ethyl alcohol by Percolator technique, then evaporating by Rotavap.

### 2.2 Extraction of antifungal active constituents

Using Steam Distillation Method and separating oil fraction.

### 2.3 Formulation of antibacterial conchigera cream

With Carbopol 534 cream base containing 4.4%, Alpinia conchigera extract by 70% ethyl alcohol.

### 2.4 Formulation of antifungal conchigera cream

1% and 3% extracted oil of Alpinia conchigera in non-irritated PIP-1 cream base formulated by the Pharmaceuticals and Natural Products Department.

### 2.5 Study on antimicrobial activity of Alpinia conchigera extract

#### Agar Dilution Method

The extracts were mixed with media to give the required various concentrations and poured into plates and inoculated with the test organisms. The plates were incubated at 37°C for 24 hours in case of bacteria and at 30°C for 10 days in case of fungi.

The growth of test organisms on the extract-containing plates was compared with those on free extract-containing plates (control). The lowest concentration which caused complete inhibition of the growth was taken as the inhibitory concentration.

### 2.6 Study of antimicrobial activity of extracted cream

#### 2.6.1 Punch Test Method

##### Preparation of seeded plates

Poured 25 ml of molten agar media into each plate, allowed to be hardened. Added 0.1 ml of cell suspension to 5 ml of agar media, and mixed properly. Dispersed seeded media over the surface of the media in proceeding

plates. Allowed to be hardened again. Made a hole in each plate. Introduced the extracts diluted to various concentrations into the holes. Incubated all plates in the condition as mentioned in 2.5. After the incubation period, measured the diameter of inhibition zone around the hole of each plate.

## 2.6.2 Dilution Method

### 2.6.2.1 Antibacterial activity

Added 1 gram of conchigera cream to each sterile tube and mixed with 1 ml liquid media. Inoculated 0.1 ml known concentration of bacterial suspension into each tube, then agitated thoroughly to insure evenly bacterial distribution. Incubated at 37<sup>0</sup>C for 24 hours. Inspection was done afterwards.

Cream base was weighed as control and did the same as the mentioned method.

Examined the bacterial count in each tube by Plate Count Method comparing with control.

Did the same as 2.6.2 but dropped in each kind of fungi and incubated at 30<sup>0</sup>C for 10 days. Then inspected the result.

### Chemical study

The study of gas chromatography by Perkin-Elmer Sigma 3 comparing among the oil extracted from Alpinia galanga, Alpinia spp. and Alpinia conchigera was conducted.

## B. Assay of primary irritation of 3% conchigera oil cream

### 1. Materials

- 6 white rabbits about 2-3 kg body weight.
- patches consisted of absorbent gauze as industrial standard, absorbent gauze and gauze bandage as industrial standard (UDC.251), absorbent gauze type V with 25.4 mm/19 threads and 25.4 mm/23 threads with average weight 27.42 g/m<sup>2</sup> and 2.54 × 2.54 cm<sup>2</sup> of 10 sheets thick, sterilized by autoclave.
- electric clipper
- 3% conchigera oil cream
- cream base of PNP-1

2. Methods: modified from J.H. Draize's.

Six white rabbits were clipped free of hair without abrasion at areas below shoulder closing to back bones on both sides. Divided into 4 areas of prepared skin about 2.5 x 2.5 cm<sup>2</sup> of each. Abraded the skin avoiding bleeding with needle guage no. 18 in cross pattern # of 2.5 cm long on 2 areas of prepared skin. Each of 0.5 gram of conchigera oil cream on patch was applied on both abraded and non-abraded area.

Cream base was applied on the skin of each area as control. The entire trunk of rabbit was wrapped with elastic bandage. After 24 hours exposure periods, patches were removed.

Readings were made as follows:

Assessment

Erythema	<u>Abnormal sign</u>	<u>Value</u>
	- negative	0
	- very slight (barely perceptible)	1
	- well-defined	2
	- moderate to severe	3
	- severe to slight eschar formulation	4
Edema Formation	<u>Abnormal sign</u>	<u>Value</u>
	- negative	0
	- very slight (barely perceptible)	1
	- slight (definite raising edge)	2
	- moderate (raised approximately 1 mm)	3
	- severe (raised > 1 mm and extending beyond the area of exposure)	4

Determination of Primary Irritation Index.

$$= \frac{\text{means value of assayed rabbits}}{\text{number of rabbits}}$$

Indicative Value of Primary Irritation Index:

not more than 1 was considered to pass the test.

C. Study on the acute toxic effect and approximate lethal dose of conchigera oil

1. Materials

1.1 Animals - Swiss mice JCL/ICR Strain of both sexes from the National Animal Center; female weight 21-22 grams, male weight 25-26 grams were fasted overnight before testing.

1.2 Tested material - Distilled conchigera oil in the form of emulsion with 20% w/w concentration.

2. Methods - Followed the methods of Wm.B. Deichmann and T.J. Le Blanch.

The tested material was given to the animals orally by special oral feeding needles.

Observed and recorded the abnormal signs in details for 1-2 hours and then at an interval.

After 3 days, if the animals survived, it was considered that no acute toxic effect of the tested material appeared.

TABLE 1. THE CONSTITUENT ANALYSIS OF ALPINIA CONCHIGERA\* (% IN GRAM OF DRIED WEIGHT)

Description	Dried weight (%)
Water and volatile matter	12.7
Fat	1.3
Ash	9.0
Fibre	21.7
Protein	2.7
Carbohydrate	65.4
Water soluble ash	4.0
Acid insoluble ash	4.3
Chloroform soluble extractive	3.9
Alcohol soluble extractive	15.3

\* By the Analytical Chemistry Laboratory, Thailand Institute of Scientific and Technological Research

TABLE 2. PRODUCTS OF ALPINIA CONCHIGERA EXTRACTION

	(%)
70% ethanol extract	9
80% ethanol extract	10.5
Conchigera oil	1.3

TABLE 3. EFFECTS OF VARIOUS CONCENTRATIONS OF  
ALPINIA CONCHIGERA RHIZOME EXTRACTS ON MICROBIAL GROWTH TESTS

Conc. (mg/ml)	<u>S. aureus</u>			<u>P. aeruginosa</u>		
	A	B	C	A	B	C
Control	+4	+4	+4	+4	+4	+4
5	a	+4	+4	+4	+4	+4
10	a	a	+4	+4	+4	+4
20	-	-	+3	-*	+4	+4
50	-	-	-	-*	-	-
70	-	-	-	-*	-	-

Remark A = less water-soluble fraction  
 B = total fractions  
 C = highly water-soluble fraction  
 +4 = maximal growth  
 +3 = moderately growth  
 a = minimal growth  
 - = no growth  
 \* = solvent inhibitory effect on growth

TABLE 4. EFFECTS OF VARIOUS CONCENTRATIONS OF CONDENSATE ON MICROBIAL GROWTH TESTS

Conc. % (w/v)	<u>Trichophyton</u> <u>mentagrophytes</u>	<u>Microsporium</u> <u>gypseum</u>	<u>E. floccosum</u>	<u>S. aureus</u>	<u>P. aeruginosa</u>
Control	+4	+4	+4	+4	+4
10	+2	+2	+2	+4	+4
50	+1	+1	+1	+2	+2
90	+1	+1	+1	a	a
100	+1	+1	+1	a	a

Remark +4 = maximal growth  
 +2 = moderately growth  
 +1 = minimal growth  
 a = very slightly growth



TABLE 5. DISC SENSITIVITY SHOWING INHIBITION ZONE  
OF BACTERIAL GROWTH BY CREAM OF ALPINIA CONCHIGERA

Regimens	<u>T. mentagrophytes</u>	<u>M. gypseum</u>	<u>E. floccosum</u>	<u>S. aureus</u>	<u>P. aeruginosa</u>
Conchigera oil					
0.001 g	1.5	0.5	2.3	0.7	0
0.003 g	2.3	1.4	3.2	0.7	0
Conchigera oil cream 1%	0.4	0	0	0	0
Conchigera oil cream 3%	0.6	0.1	1.3	0.4	0
Conchigera 70% ethanol extract cream	0.75	0.5	0.65	0.75	0.5
Canesten	1.2	1.2	2.0	not test	not test
Chloramphenicol	not test	not test	not test	0.7	0
Control cream for conchigera oil cream	0	0	0	0	0
Control carbopol cream for conchigera 70% ethanol extract cream	0	0	0	0	0

TABLE 6. EFFECTS OF VARIOUS DILUTIONS OF CONCHIGERA CREAM ON BACTERIAL GROWTH

Materials	Bacterial Count	
	<u>S. aureus</u> <sup>1/</sup>	<u>P. aeruginosa</u> <sup>2/</sup>
1. Control	$1.56 \times 10^{11}$	$1.01 \times 10^{11}$
2. Oil cream 1%	$2.9 \times 10^5$	$2.0 \times 10^8$
3. Oil cream 3%	1.4	$1.8 \times 10^6$
4. Cream 70% ext.	<100	<100

<sup>1/</sup>S. aureus initial number =  $4.17 \times 10^7$  cells

<sup>2/</sup>P. aeruginosa initial number =  $2.18 \times 10^7$  cells

TABLE 7. DISC SENSITIVITY SHOWING INHIBITION ZONE  
OF FUNGAL GROWTH BY CREAM OF ALPINIA CONCHIGERA

Regimens	Fungal inhibition by Dilution Method		
	Diameter of colony (cm)		
	<u>Trichophyton</u> <u>mentagrophytes</u>	<u>Microsporium</u> <u>gypseum</u>	<u>Epidermophyton</u> <u>floccosum</u>
Conchigera oil cream 1%	0	0	0
Conchigera oil cream 3%	0	0	0
Control cream base for conchigera oil cream	7	8	7
70% ethanol extract conchigera cream	0	0	0
Control cream base for 70% ethanol extract cream	0	0	0

TABLE 8. RESULTS OF THE STUDY ON PRIMARY IRRITATION OF 3% CONCHIGERA CREAM ON RABBITS' SKIN

Rabbit number	3% Conchigera oil cream				Cream base PNP-1			
	normal skin		scratched skin		normal skin		scratched skin	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	1	0	0	0	1	0
2	1	0	2	0	1	0	1	0
3	0	0	1	0	0	0	1	0
4	0	0	1	0	0	0	1	0
5	0	0	1	0	0	0	1	0
6	0	0	1	0	0	0	1	0

Primary Irritation Index of 3% conchigera oil cream was 0.33.

Primary Irritation Index of cream base PNP-1 was 0.29.

The study of acute toxic effect and approximate lethal dose of conchigera oil revealed acute toxicity to be in 3 phases:

1. Skeletal muscle relaxation

After the administration of assayed material 3-5 minutes, ataxia of animals were observed as well as drowsiness and weakness of muscle extremities. Levels of muscle relaxation were in relation with the dosage given. The skeletal muscle relaxation and drowsiness prolonged for 3 hours and 5 hours respectively, afterwards the animals recovered to normal.

2. Loss of righting reflex

The animals not recovered in phase I were lapsed into phase II. Animals with high dosage given lost their righting reflex immediately in 20-30 minutes after receiving dosage (7.1 g/kg-10.7 g/kg). The recovered animals were in phase II for 6-24 hours, and if they were still not recovered, they were lapsed into phase III.

3. Respiration arrest

The animals breathed slowly for some time until the breathing finally stopped though their hearts were still beating for a little while longer.

No abnormal sign found in the experimented animals which control solvent was administered. The approximate lethal dose of conchigera oil was 10.7 g/kg in both male and female mice. This is considered to be practically nontoxic substance.

## CONCLUSION AND RECOMMENDATION

1. The extract of Alpinia conchigera by 70% alcohol yielded a substance that inhibited growth of bacteria with better result for S. aureus as compared with Pseudomonas aeruginosa. The minimum inhibitory concentration (MIC) of 20-50 mg/ml by Dilution Method resulted in no growth inhibition of 3 kinds of fungi, and the concentration above 50 mg/ml could not be obtained because the ethanol itself used in the experiment caused inhibition of fungal growth.

2. Essential oil from Alpinia conchigera caused no inhibition of experimented bacterial growth but inhibited all 3 kinds of fungal growth at the minimum inhibitory concentration (0.07-0.1%).

3. The development of pharmaceutical products as cream from 3 species of Alpinia extracted by 70% ethyl alcohol using 7% of Carbopol 934 cream base.

4. The development of cream from essential oil of Alpinia conchigera revealed PNP-1 cream base being effective both 1 and 3% of Conchigera oil.

5. The inhibition of bacterial growth of cream developed from 70% alcohol extracted Alpinia conchigera revealed less effective for bacterial and fungal growth. When compared with cream from essential oil of Alpinia conchigera, cream from 70% ethanol extracted conchigera was much less effective. The experimental results in 1981 reported that 15-25 mg/disc concentration of conchigera showed inhibition zone to S. aureus only 10-15 mm as compared with chloramphenicol 30 µg/disc and ampicillin 10 µg/disc.

6. The study on inhibition effect of fungal growth by cream developed from essential oil of conchigera revealed that 3% conchigera oil cream inhibited all 3 kinds of fungi and had most effective inhibition to Epidermophyton floccosum equivalent to Canesten<sup>®</sup> (Bayer).

7. The acute toxic effect on rats revealed that the approximate lethal dose of conchigera oil administered via oral was 10.7 g/kg. It was classified in a practically nontoxic group.

8. The irritation test of rabbits' skin obtained Primary Irritation Index of 0.33; it was considered to pass the experimented trial.

9. The results of these experiments indicate that a feasibility study of conchigera oil should be carried on to evaluate a benefit of industrial pharmaceutical preparation as topical antifungal agents.

10. The methodology of growth inhibition study revealed the Serial Dilution Method being better than Punch Test Method, because of the well-spreading of the test agent.

11. The differential extraction of chemical composition of conchigera oil is now in progressing process at the Pharmaceuticals and Natural Products Department to investigate the pure substance in parallel with conchigera planting project for studying the cost benefit.

12. The clinical study was performed by Dr. Patcharee Soonthornpalin et al. and the result was in a separated report.

13. The semi pilot plant project is being carried on together with the pharmacological study, toxicological investigation, economic investment and production of agricultural raw material by TISTR for the development of new pharmaceutical products from natural Thai medicinal plants to become industrialized production according to the Project UNIDO/THA/82/006.

#### ACKNOWLEDGEMENTS

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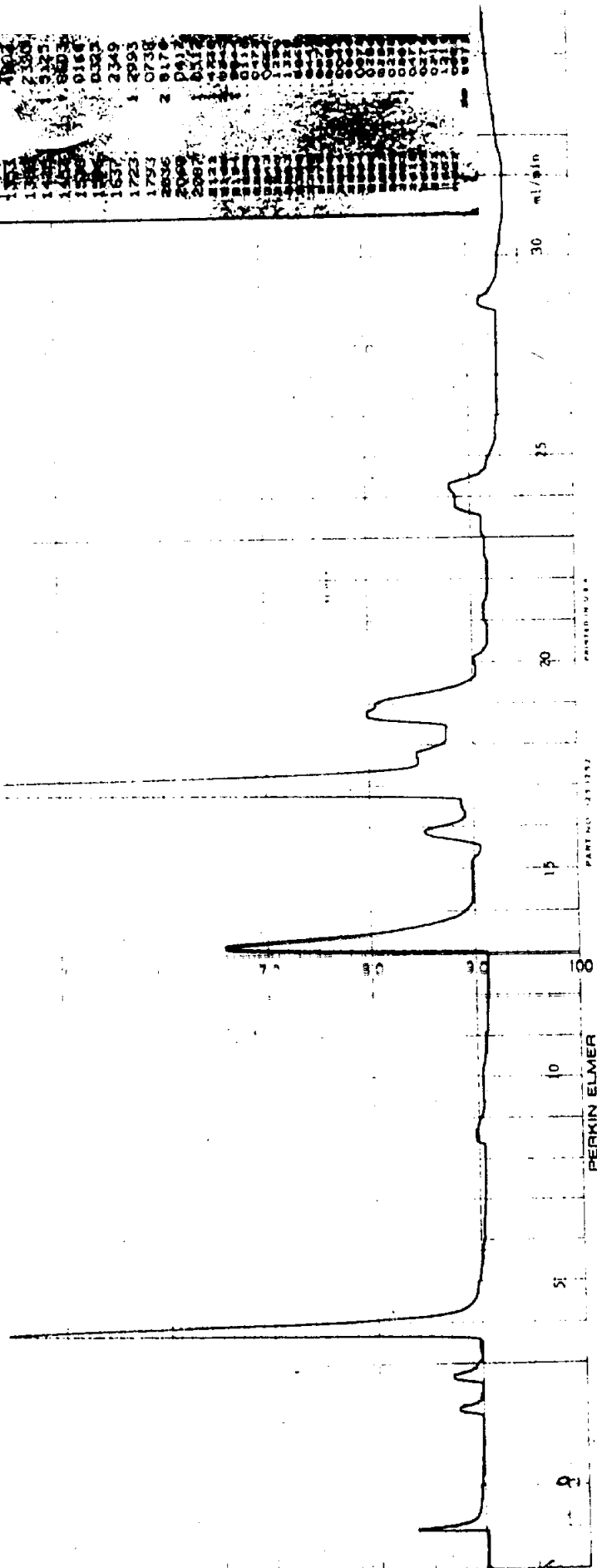
Gratitude is also given to Miss Vannah Kovitaya of the PNPd for her translation this report into English, and to Prof. Dr. Sunthorn Tandhanand for his editing.

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APPENDIX

Alpinia conchiformis oil  
 SUBSTANCE *Alpinia conchiformis* SIZE 0.1 µl  
 VOLUME 1 µl ST 30' on Chromosorb W HMDS 60/80, 1/8" x 100' long  
 INSTRUMENT PERKIN ELMER SIGMA-3 RECORDER PERKIN ELMER 023  
 DETECTOR PID 10 mV  
 ATTENUATION RANGE 8 x 10 CHART SPEED 1 ml/min.  
 ZONE: DETECTOR, INJECTOR 230°C AIR 30 psi  
 HYDROGEN 20 psi  
 N<sub>2</sub> 31 ml/min  
 TEMPERATURE PROGRAM OBSERVATION 37 psi  
 ISOTHERMAL INITIAL 70°C OPERATOR A. CHATTERJEE  
 RAMP RATE 4°C/min DATE 11 March 86  
 ISOTHERMAL FINAL 200°C NOTE

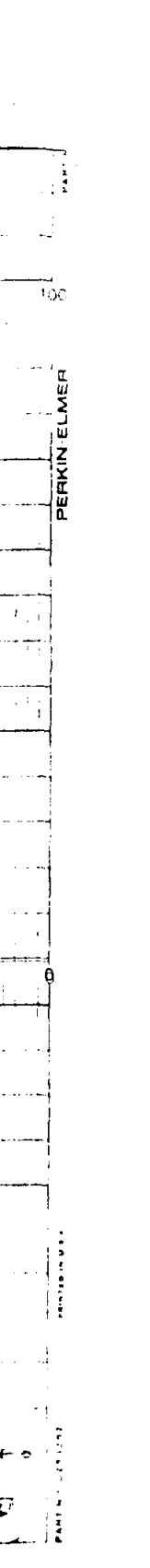


114	0.03
151	1.0671
199	1.789
230	15.3371
373	0.196
454	0.076
466	0.253
508	0.691
568	4.879
769	0.843
791	13.5207
867	67.87
896	3336
946	3.3658
987	1.3306
1019	39.1423
1061	6283
1096	6.895
1173	5.5402
1190	0.043
1196	4.779
1251	6254
1303	4.804
1308	2.380
1345	5.225
1403	1.803
1508	0.160
1571	0.323
1637	2.349
1723	1.2993
1793	0.738
2009	0.912
2087	0.512
2122	1.220
2171	0.973
2203	0.023
2213	0.023
2219	0.023
2220	0.023
2221	0.023
2222	0.023
2223	0.023
2224	0.023
2225	0.023
2226	0.023
2227	0.023
2228	0.023
2229	0.023
2230	0.023
2231	0.023
2232	0.023
2233	0.023
2234	0.023
2235	0.023
2236	0.023
2237	0.023
2238	0.023
2239	0.023
2240	0.023
2241	0.023
2242	0.023
2243	0.023
2244	0.023
2245	0.023
2246	0.023
2247	0.023
2248	0.023
2249	0.023
2250	0.023

SUBSTRATE: Alipha Alkanol-ol  
 COLUMN: 5x SE 30 pm Chromosorb M RSDS 60/80, 11 feet long  
 INSTRUMENT: PERKIN-ELMER 580A-1  
 DETECTOR: FID  
 ATTENUATION: 8 x 10  
 ZONE: DETECTOR INJECTOR 200°C  
 CHART SPEED: 1 cm/min.  
 HYDROGEN: 20 psi  
 N<sub>2</sub>: 31 ml./min.  
 OBSERVATION: 37 psi  
 OPERATOR: A. CHOTIRONG  
 DATE: 11 March 86  
 NOTE:

TEMPERATURE PROGRAM  
 ISOTHERMAL INITIAL: 50°C  
 RAMP RATE: 4°C/min  
 ISOTHERMAL FINAL: 200°C

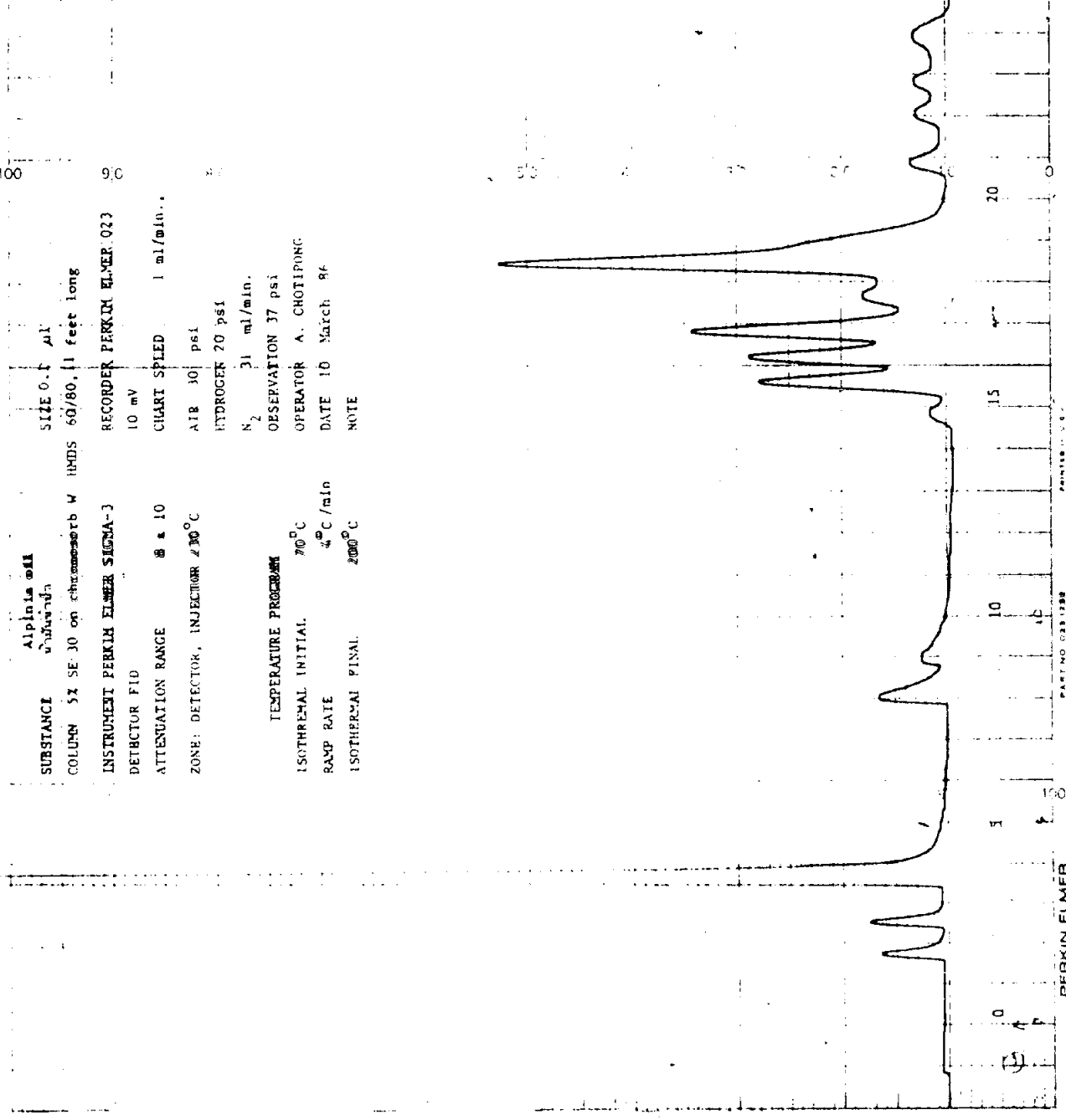
1.961	49.0902
2.26	2.323
3.38	0.479
4.24	0.054
4.52	1.103
4.95	1.6716
5.55	3.538
6.48	0.099
7.33	0.0610
7.68	0.0619
7.92	8.2534
8.58	0.030
8.94	2.6162
9.46	9.0777
10.22	6.5666
10.71	10.8688
11.18	4.7753
11.95	1.3395
12.64	1.325
13.03	0.665
13.48	0.782
13.90	50.68
14.44	17.13
14.88	1.042
15.38	0.042
15.88	0.021
16.35	0.021
16.41	0.026
16.76	0.073
	99.9904



PERKIN-ELMER



SUBSTANCE: Alpina oil  
 COLUMN: 5x SE 30 on Chromasorb W HMDS 60/80, 11 feet long  
 INSTRUMENT: PERKIN ELMER SIGMA-3  
 DETECTOR: FID  
 ATTENUATION RANGE: 8 & 10  
 ZONE: DETECTOR, INJECTOR 200°C  
 RECORDER: PERKIN ELMER 023  
 CHART SPEED: 1 ml/min.  
 HYDROGEN: 20 psi  
 AIR: 30 psi  
 N<sub>2</sub>: 31 ml/min.  
 TEMPERATURE PROGRAM:  
 ISOTHERMAL INITIAL: 70°C  
 RAMP RATE: 4°C/min  
 ISOTHERMAL FINAL: 200°C  
 OPERATOR: A. CHOTIPONG  
 DATE: 10 March 86  
 NOTE:



PERKIN ELMER

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