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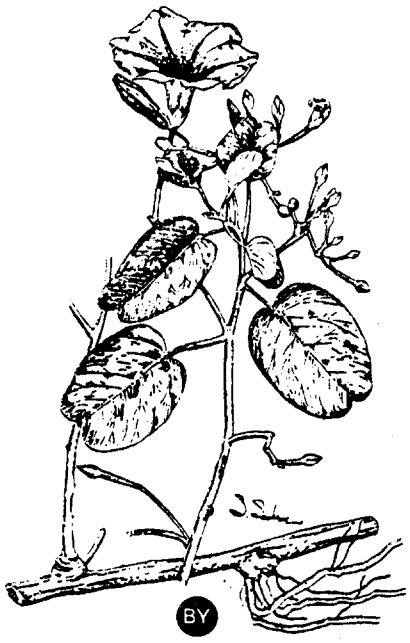
THE COMPARATIVE STUDIES ON PROPERTIES OF *Ipomoea pes-caprae* (L.) R. BR. OBTAINED FROM NATURAL SOURCES AND THE CULTIVATION ON INLAND AREA

THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH



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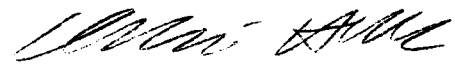

(Mr. Chalermchai Honark)

Governor

THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

RESEARCH PROJECT NO.17/8

STUDIES ON PHARMACOLOGICALLY ACTIVE PRINCIPLE (S) OF "PHAKBUNGTHA-LE", *Ipomoea pes-caprae* (Linn.) Roth

REPORT NO.4

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BY

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EDITORS

VALYALADA HONGTHONG

NARUMOL RUENWAI

TISTR, BANGKOK 1995

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The authors would like to express their thanks to Agro-Technology Department, TISTR, for cultivating *Ipomoea pes-caprae* (L.) R.Br. at TISTR Agricultural Experimental Station, Nakhon Ratchasima. We are also indebted to Mrs. Saisurang Chotipanich, Computer Division, TISTR, for her assistance in the performance of statistical analysis.

การศึกษาเปรียบเทียบคุณสมบัติต่าง ๆ ของผักบุ้งทะเลจากแหล่งธรรมชาติ และจากแปลงทดลอง

โดย ปัทมา สุนทรศารทูล, อุบลวรรณ พงศ์ประยูร, ศิริเพ็ญ จริเกษม, เตือนตา เสมาทอง, จักรพงษ์ ลิมปนุสสรณ์, ชุลีรัตน์ บรรจงลิขิตกุล, ประไพภัทร คลังทรัพย์, อัจฉราพร พันธุ์รักส์วงศ์, ทวีศักดิ์ สุนทรธนศาสตร์, รุ่งระวี เต็มศิริฤกษ์กุล, วงศ์สถิตย์ ฉั่วกุล และอุบลวรรณ บุญเปล่ง

บทคัดย่อ

การศึกษาเปรียบเทียบคุณสมบัติต่าง ๆ ของผักบุ้งทะเลที่เก็บจากแหล่งธรรมชาติ 2 แหล่ง คือ ชายทะเลจังหวัดชลบุรี และจังหวัดประจวบคีรีขันธ์ กับผักบุ้งทะเลในแปลงทดลองปลูกของสถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย (วท.) ที่จังหวัดนครราชสีมา พบว่าผักบุ้งทะเลทั้ง 3 แหล่ง มีลักษณะทางพฤกษ-อนุกรมวิธาน และกายวิภาคไม่แตกต่างกัน. สารสกัดแสดงฤทธิ์ (IPA) จากผักบุ้งทะเลทั้ง 3 แหล่ง มีประสิทธิผลทางเภสัชวิทยาใกล้เคียงกัน ได้แก่ ประสิทธิผลการลดการอักเสบ ซึ่งทดสอบด้วยวิธีการเหนี่ยวนำให้เกิดอาการบวมที่ใบหูของหนูขาว, ประสิทธิผลการป้องกันการทำลายโปรตีนของพิษแมงกะพรุนในหลอดทดลอง, และประสิทธิผลการป้อง-กันการเกาะกลุ่มของเกล็ดเลือด ซึ่งเหนี่ยวนำให้เกิดโดยพิษแมงกะพรุนในหลอดทดลอง.

ผลการศึกษานี้เป็นข้อมูลพื้นฐานสำคัญในการพัฒนาวัตถุดิบ อันจะเป็นประโยชน์ในอุตสาหกรรมการผลิตยาชนิดใหม่เพื่อใช้รักษาอาการอักเสบของผิวหนังที่เกิดจากพิษแมงกะพรุนต่อไปในอนาคต.

THE COMPARATIVE STUDIES ON PROPERTIES OF *Ipomoea pes-caprae* (L.) R.BR. OBTAINED FROM NATURAL SOURCES AND THE CULTIVATION ON INLAND AREA

By Pattama Soontornsaratune[*], Ubonwan Pongprayoon[*], Sasithorn Wasuwat[*], Siripen Jarikasem[*], Tuanta Sematong[*], Jakkrapong Limpanussorn[*], Chuleratana Banchongiikitkul[*], Prapaipat Klungsupya[*], Acharaporn Punruckvong[*], Taweesak Suntorntanasat[*], Rungravi Temsiririrkkul[**], Wongstit Chuokul[**] and Uboiwan Boonpieng[**]

ABSTRACT

The properties of *Ipomoea pes-caprae* (L.) R. Br. obtained from two natural sources, the seashore of Chon Buri and Prachuap Khiri Khan, were compared with that obtained from TISTR Agricultural Experimental Station at Nakhon Ratchasima. The taxonomy and anatomy studies indicated the same structures and descriptions. The active fractions (IPA) extracted from the plants of all sources showed similar pharmacological efficacy in ethyl phenylpropiolate induced ear oedema in rats, neutralization of proteolytic effects of jellyfish venoms *in vitro* and inhibition of platelet aggregation induced by jellyfish venoms *in vitro*.

The results revealed important information for raw material development of a new drug for the treatment of dermatitis caused by poisonous jellyfishes.

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* Pharmaceuticals and Natural Products Department, Thailand Institute of Scientific and Technological Research (TISTR)

** Faculty of Pharmacy, Mahidol University

INTRODUCTION

Ipomoea pes-caprae (L.) R. Br. has been used in Thai traditional medicine for the treatment of dermatitis caused by poisonous jellyfishes. This plant is found along sandy tropical seashores. The active fraction (IPA) extracted from the plant was proven to possess anti-inflammatory (Pongprayoon 1991a), anti-spasmodic (Pongprayoon 1992), prostaglandin synthesis inhibiting (Pongprayoon 1991b) and venom neutralizing activities (Pongprayoon 1991). IPA in a preparation of % cream exhibited satisfactory results in patients with various degrees of symptoms ranging from erythema and vesicular pruritus to burns, ulceration and necrosis due to dermatitis caused by jellyfishes(Soontornpalin 1987).

Since the sources of the plant were less available due to the invading of construction along the beaches, TISTR by Pharmaceuticals and Natural Products Department (PNPD) has therefore undertaken to cultivate the plant inland at Nakhon Ratchasima. The studies were performed to decide whether IPA produced by the plant cultivated inland had the same characteristic in taxonomy, anatomy and biological properties as naturally occurring plant.

MATERIALS AND METHODS

MATERIALS

1. Specimens of *Ipomoea pes-caprae* (L.) R. Br. were collected along the seashores of Chon Buri and Prachuap Khiri Khan, during the period of July - August 1992 and from TISTR Agricultural Experimental Station at Nakhon Ratchasima.

2. Jellyfish venom genus *Mastigias* was captured in the Gulf of Siam outside the province of Prachuap Khiri Khan, Thailand. Fishing tentacles were cut from living specimens immediately after capture and then frozen.

3. Animals

   Healthy male Wistar rats of weight 55-60 g were purchased from National Laboratory Animal Centre, Mahidol University, Nakhon Pathom.

   Healthy albino rabbits, New Zealand white hybrid strain were purchased from Department of Animal Science, Faculty of Agriculture, Kasetsart University. Body weight range was 2-3 kilograms.

4. Rat feed, CP Mice Feed, Bangkok Feed Mill Co., Ltd.

5. Rabbit feed, Pokphand Animal Feed Co., Ltd. Thailand.

6. Equipment :

   - Microscope Olympus, model BHS-312, Japan.
   - Stereomicroscope Olympus, model VM-ILA-2, Japan.
   - Platelet Aggregometer, model 1010, Payton, USA.
   - Spectrophotometer, model UV-160 A, Shimadzu, Japan.
   - Oditest callipers, Mitutoyo, Japan.
   - Semiautomated hematology analyzer, model F-800,
   - Sysmex, TOA Medical Electronics Co., Ltd., Japan.
   - Micropipette, Gilson Medical Electronics, France.

- Semiautomatic analyzer, Hitachi photometer, model 4020, Boehringer Mannheim,Germany.
- Centrifuge, model J2-21, Beckman, USA.

7. Chemicals :

- Elhyl phenylpropiolate (EPP), Aldrich Chemical Co., Ltd, USA.
- Oxyphenbutazone, Sigma Chemical Company, USA.
- Azure, Calbiochem Behring Corporation, USA.
- Sodium hydroxide, Merck, E.Merck, Germany.
- Sodium chloride, Merck, E.Merck, Germany.
- Calcium chloride, Merck, E.Merck, Germany.

METHODS

1. Taxonomy and anatomy studies

1.1 Study of taxonomy

The plant specimens were collected from three sources and were identified by comparing with Flora Malesiana (Van Steenis 1954). Voucher specimens were deposited at Faculty of Pharmacy, Mahidol University.

1.2 Study of anatomy

Leaves were cut into thin sections using a sharp razor. The issue sections were mounted onto a slide in glycerine water and then examined under the microscope. Both sides of blade of leaves were peeled off in order to study the stomata type and to determine the stomatal index.

2. Extraction of active fraction, IPA

The active fractions, IPA of plant specimens from three sources were obtained by steam distillation of dried leaves followed by extraction of the water distillate with petroleum ether (Wasuwat 1970).

3. Biological activities studies

3.1 Study of topical anti-inflammatory activity

The experiment was performed according to the ethyl phenylpropiolate (EPP) induced ear oedema model in rats (Brattsand 1982). Male Wistar rats (55-60g) in groups of 5-13 animals were used. The thickness of ear was measured with Oditest Callipers just prior to the experimentation. Solutions containing 5% EPP plus various concentrations of IPA from each source dissolved in acetone were applied to the inner and outer surfaces of both ears (20µl/ear). The ear thickness was determined again at 30 and 60 minutes post application. The increase in ear thickness was compared to the vehicle treated group and the percent inhibition calculated. The ID_{50} was

calculated 30 minutes after induction of oedema, Oxyphenbutazone (1mg/ear) was used as a reference compound.

Statistical analysis was performed using analysis of variance and a p-value of 0.05 or less was considered to be significant. Regression analysis was used to calculate ID_{50}s.

3.2 Study of neutralization activity on jellyfish venom

Preparation of jellyfish venom

Specimens of the jellyfish genus *Mastigias* were used. The crude nematocysts were obtained by grinding the tentacles in a small amount of sea water which liberated nematocysts into the solution. The supernatant was decanted and then lyophilized. The venom was obtained by homogenizing the crude nematocysts in normal saline.

Proteolytic assay

A non specific chromogenic substrate, Azure, was used to assay proteolytic activity of the crude venom. When Azure, which is an insoluble dye-protein complex, is digested by a proteolytic enzyme, the bound dye will be released into the solution which can be measured photometrically.

Crude jellyfish venom solution (in 0.9% NaCl) of various concentrations was incubated with 16 mg of Azure at 37°C for 4 hours in a shaking water bath. The incubation volume was 2 ml. The assay was performed in duplicate. The reactions were terminated by rapid filtering through glass wool packed columns. The filtrates were centrifuged to remove any insoluble Azure that was left. Absorbance of the supernatant was read at 578 nm on a spectrophotometer. The concentration of venom required to produce a change in the absorbance of 0.5 units was defined as the minimum proteolytic concentration.

To determine the neutralization activity of IPA from different sources, the jellyfish venom at the minimum proteolytic concentration was used. IPA was pre-incubated with the venom at doses of 2, 4, 8 nd 16 µg IPA/mg venom. After 10 minutes, 16 mg of Azure (8 mg/ml) was added, the mixture was then incubated at 37°C for 4 hours. The rest of the experiment was continued as

described above. The results were expressed as percentage of inhibition. The absorbance of the supernatant in the tube containing venom and substrate (without IPA) was defined as zero percent inhibition. Regression analysis was used to calculate IC_{50}.

3.3 Study of platelet aggregation inhibiting activity

Collection of blood samples

Blood samples were drawn from the central ear artery of adult albino rabbits. Aliquots (9ml) of blood samples were placed in polystyrene tubes containing 1 ml of 3.2% (w/v) sodium citrate and mixed well by gentle inversion.

Preparation of platelet suspension

Platelet rich plasma (PRP) was prepared by centrifuging citrated blood at 900 g for 10 minutes at room temperature. PRP was in the supernatant fraction. The blood remaining in the centrifuge tube was further centrifuged at 10,000 g for 10 minutes to prepare platelet poor plasma (PPP). The PRP from 3-4 rabbits were pooled together. The pooled platelet was determined using a microcell counter and subsequently adjusted with autologous PPP to obtain the final platelet suspension containing 300,000-500,000 platelet/ml. Throughout the experiment, the PRP and PPP were kept at room temperature in closed polystyrene containers. Platelet aggregation tests were performed between 30 minutes and 3 hours after the blood had been drawn.

Platelet aggregation in PRP

Aggregating agent used was jellyfish venom genus *Mastigias*. The venom was obtained by grinding the tentacles in a small amount of sea water and then centrifuged at 5,000 g to remove debris. Each IPA extract of 20 mg was dissolved in 500 µl normal saline solution. Ten µl of 2N NaOH was added.

PRP (450 µl) were placed in the siliconized cuvette glass and incubated at 36± 0.5°C in the incubator chamber of platelet aggregometor. Calcium chloride solution (1mM) was added into PRP sample which was being continuously stirred at 900 rpm and further incubated for 1 minute. Different doses of jellyfish venom were added into the stirred platelet suspension to induce platelet aggregation. The extent of platelet aggregation was shown as percentage of changes in light transmission through the cuvette comparing with that of PPP solution. Subminimum dose was determined and used throughout the experiments.

Various doses of IPA extracted from *Ipomoea pes-caprae* (L.) R. Br. of each source was added into the PRP incubating medium 3 minutes before addition of the venom. The changes in the light transmission were recorded. The inhibitory effect was observed as the decrease in the light transmission comparing with that observed in the aggregation without IPA. Absence of platelet aggregation was defined by having no changes in the light transmission after the period of 5 minutes. Platelet inhibition was expressed as percentage of change in light transmission which was calculated by using the following equation:

% inhibition = (Lc-Li)/Lcx100

Lc = Light transmission change in the absence of IPA

Li = Light transmission change in the presence of IPA

RESULTS

Study of taxonomy

After investigation, the description was as follows : a twining herb, stem creeping or sometimes twining at apex; perennial: leaves simple, coriaceous, broadly ovate-oval-orbicular, base cordate-truncate-rounded-cuneate; tip emarginate, 7-11 cm wide, 5-8 cm long; inflorescences in 1-10 flowered cymes; calyx ovate-oval with a rounded-truncate-emarginate apex; corolla red-purple, rarely white, actinomorphic; stamens 4, adnate to the corolla; ovary, superior, 2-celled; styll 1; stigmas 2; fruit a broadly ovoid-globose capsule; seed tomentose.

Plant specimens from three different sources; Chon Buri (Figure 1), Prachuap Khiri Khan (Figure 2) and Nakhon Ratchasima (Figure 3) have been identified as *Ipomoea pes-caprae* (L.) R.Br. The description of them were the same as described above.

Study of anatomy

Surface and sectional views of *Ipomoea pes-caprae* (L.) R.Br. leaves from three sources exhibited the following structures (Figure 4).

Epidermis of both surfaces were alike (Figures 5,6 and 7). In surface view, they were composed of layer of polygonal cells with paracytic stomata and glandular trichomes. Striated cutin was found coated both sides of the epidermises. The stomatal indices of the upper and lower epidermises were shown in Table 1.

Palisade mesophyll presented beneath both epidermises. Each tissue comprises 3-4 layers of short columnar cells.

Spongy mesophyll comprised several layers of rather loosely arranged round cells. The rosette crystals of calcium oxalate were scattered in the cells and crowed beneath the palisade layers. The vascular bundles were also scattered and surrounded by large parenchyma and bundle sheath.

Midrib was composed of the collateral type of vascular bundles at the centre and 5-6 layers of collenchyma beneath both epidermises. The latex ducts were found in groups around the vascular bundles of either the midrib or the veins.

Extraction of active fraction, IPA

The yield of IPA extracted from the plants of these three sources are shown in Table 2.

Topical anti-inflammatory activity

The topical application of IPA extracted from *Ipomoea pes-caprae* (L.) R.Br. of all sources significantly inhibited swelling in a dose-dependent manner (Table 3 and Figure 8). Regarding the comparing on potency of IPA of those sources, the oedema thickness of the same dose treated was statistically analysed. No significant difference was observed among the three sources. The ID_{50}s calculated 30 minutes after induction of oedema which revealed the same range were 0.91, 0.53 and 1.46 mg/ear for IPA extracted from *Ipomoea pes-caprae* (L.) R.Br. of Chon Buri, Prachuap Khiri Khan and Nakhon Ratchasima respectively (Figure 9).

Neutralization activity on jellyfish venom

IPA extracted from *Ipomoea pes-caprae* (L.) R.Br. of the three sources were able to neutralize the proteolytic activity of the venom in a concentration-dependent manner (Table 4). The IC50 values were 3.12, 5.28 and 1.57 µg/mg venom for IPA of Chon Buri, Prachuap Khiri Khan and Nakhon Ratchasima respectively, which revealed the same range (Figure 10).

Platelet aggregation inhibiting activity

Platelet aggregation curves obtained in response to the action of jellyfish venom in the absence or presence of IPA extracted from *Ipomoea pes-caprae* of Chon Buri, Prachuap Khiri Khan, and Nakhon Ratchasima, respectively were shown in Figure 11.

A concentration-related aggregation was observed in all samples of IPA at the end of the lag period. Percentage of inhibition was shown in Table 5 which revealed the same range of inhibition.

TABLE 1. THE STOMATAL INDICES OF THE UPPER AND LOWER EPIDERMIS OF THE LEAVES OF *Ipomoea pes-caprae* (L.) R. BR. FROM THREE DIFFERENT SOURCES

Sources	Stomatal index	
	Upper epidermis	Lower epidermis
Natural :		
Chon Buri	11.34	11.48
Prachuap Khiri Khan	10.29	14.29
Cultivated:		
Nakhon Ratchasima	8.62	10.94

TABLE 2. THE PERCENTAGE YIELD OF IPA EXTRACTED FROM *Ipomoea pes-caprae* (L.) R. BR. OF THREE SOURCES

Sources	Yield (%)
Natural :	
Chon Buri	0.019
Prachuap Khiri Khan	0.007
Cultivated:	
Nakhon Ratchasima	0.007

TABLE 3. EFFECT OF TOPICAL APPLICATION OF IPA EXTRACTED FROM *Ipomoea pes-caprae* (L.) R. BR. OF THREE SOURCES ON ETHYL PHENYLPROPIOLATE (EPP)-INDUCED EAR OEDEMA IN RATS

Sources	Treatment	n	Oedema thickness (μm)		Inhibition (%)	
			30 min	1 hr	30 min	1hr
Natural : Chon Buri	IPA 0.4 mg/ear	22	105.0± 7.0	147.3±7.6	39	30
	1.0 mg/ear	24	85.0±7.2	124.2±6.5	50	41
	2.0 mg/ear	24	65.0± 7.0	125.8±6.8	62	40
Prachuap Khiri Khan	IPA 0.4 mg/ear	12	89.2± 15.5	156.7± 10.7	48	25
	1.0 mg/ear	12	78.3± 5.0	131.7± 8.2	54	37
	2.0 mg/ear	12	45.8± 4.2	116.7± 7.7	73	44
Cultivated : Nakhon Ratchasima	IPA 0.4 mg/ear	12	121.7± 11.1	160.0±10.1	29	24
	1.0 mg/ear	12	105.0±8.2	130.8±10.7	39	38
	2.0 mg/ear	10	72.0±10.5	142.0±21.4	58	32
	Actone	16	171.9±9.1	210.4±8.6		
	Oxyphenbutazon 1 mg/ear	26	13.8±3.0	93.7±9.0		

Results are means ± SEM

TABLE 4. INHIBITION EFFECT OF IPA EXTRACTED FROM *Ipomoea pes-caprae (L.)* R. BR. OF THREE SOURCES ON JELLYFISH VENOM-INDUCED PROTEOLYSIS

Dose (ug IPA/mg venom)	Inhibition		
	Chon Buri	Prachuap Khiri Khan	Nakhon Ratchasima
2	28	20	-
4	69	30	67
8	87	70	79
16	84	-	92

TABLE 5. INHIBITION EFFECT OF IPA EXTRACTED FROM *Ipomoea pes-cparae* (L.) R. BR. OF THREE SOURCES ON JELLYFISH VENOM-INDUCED PLATELET AGGREGATION

IPA (mg/ml)	Inhibition (%)		
	Chon Buri	Prachuap Khiri Khan	Nakhon Ratchasima
0.8	23	22	18
1.6	36	26	17
3.2	65	52	42

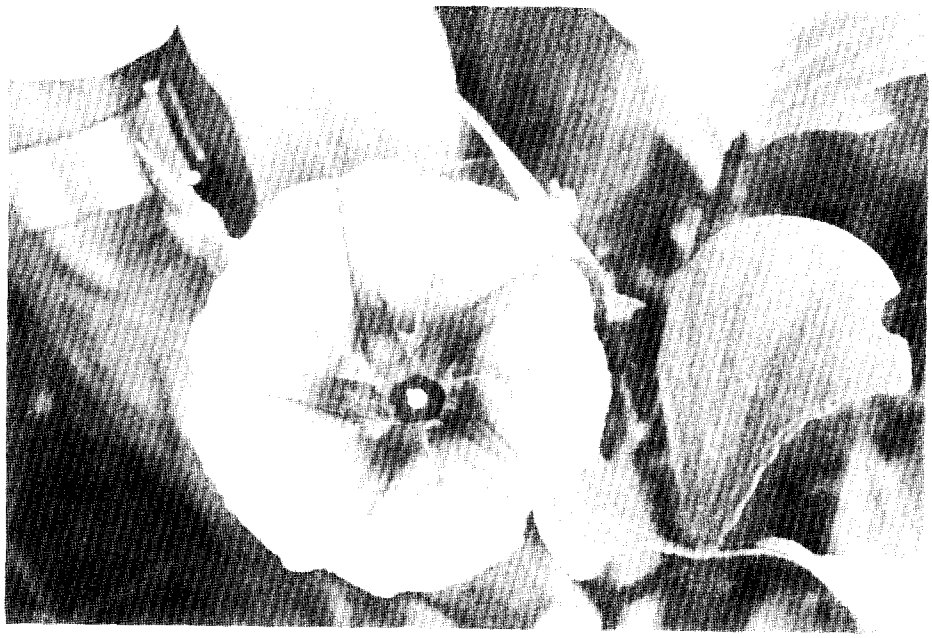

Figure 1. *Ipomoea pes-caprae* (L.) R. Br. growing on sandy seashore, Chon Buri.

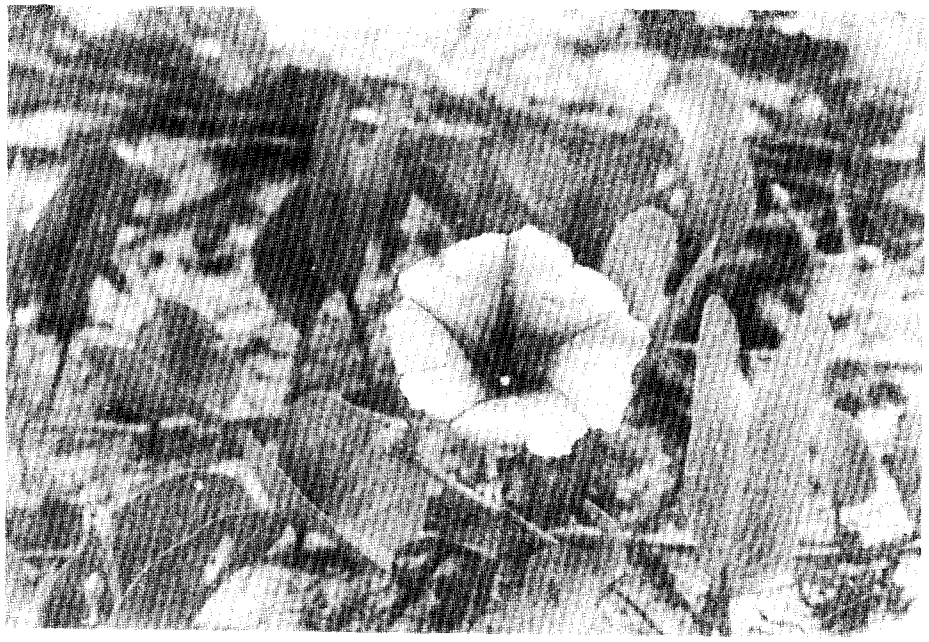

Figure 2. *Ipomoea pes-caprae* (L.) R. Br. growing on sandy seashore, Prachuap Khiri Khan.

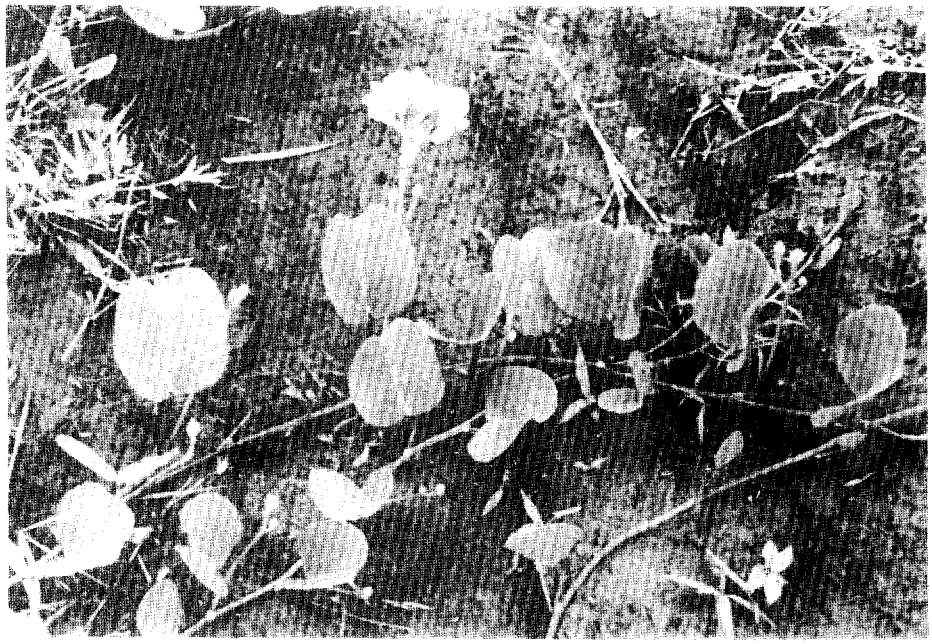

Figure 3. *Ipomoea pes-caprae* (L.) R. Br. growing at TISTR Agricultural Experimental Station, Nakhon Ratchasima.

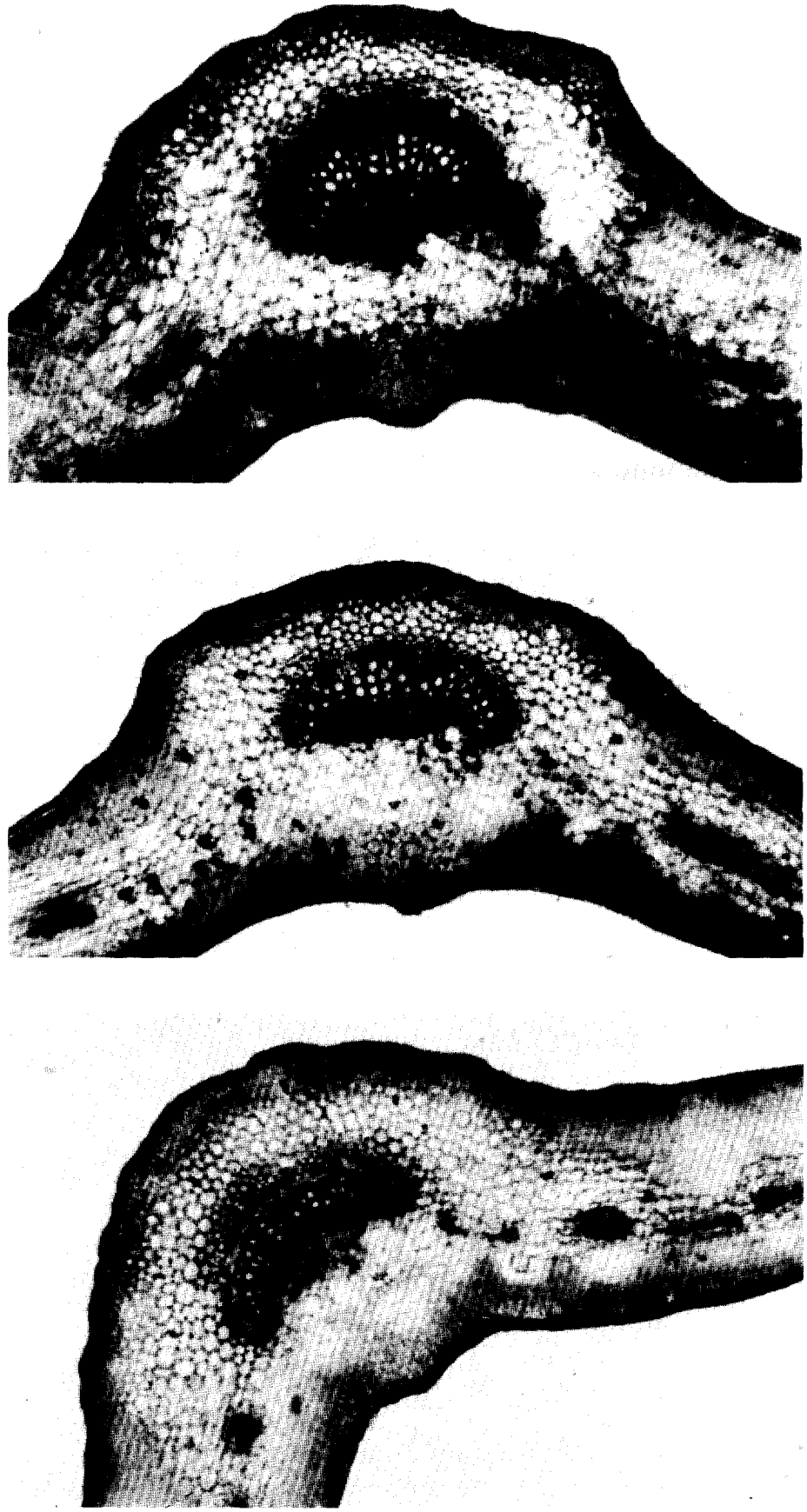

Figure 4. Sectional view of *Ipomoea pes-caprae* (L.) R. Br. leaves from Chon Buri (a), Prachuap Khiri Khan (b) and Nakhon Ratchasima (C).

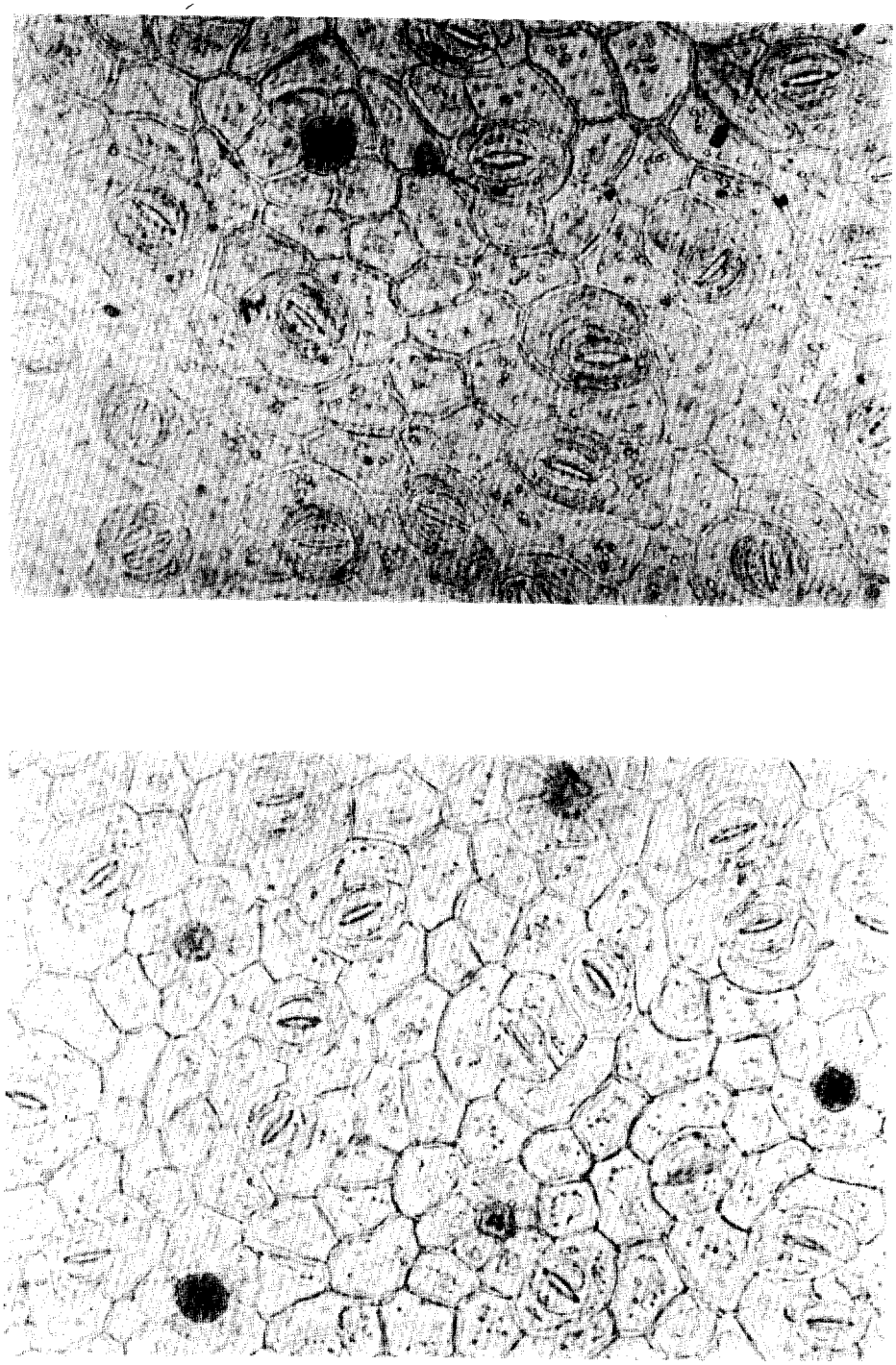

Figure 5. Upper (above) and lower (below) epidermis of *Ipomoea pes-caprae* (L.) R. Br. leaves from Chon Buri.

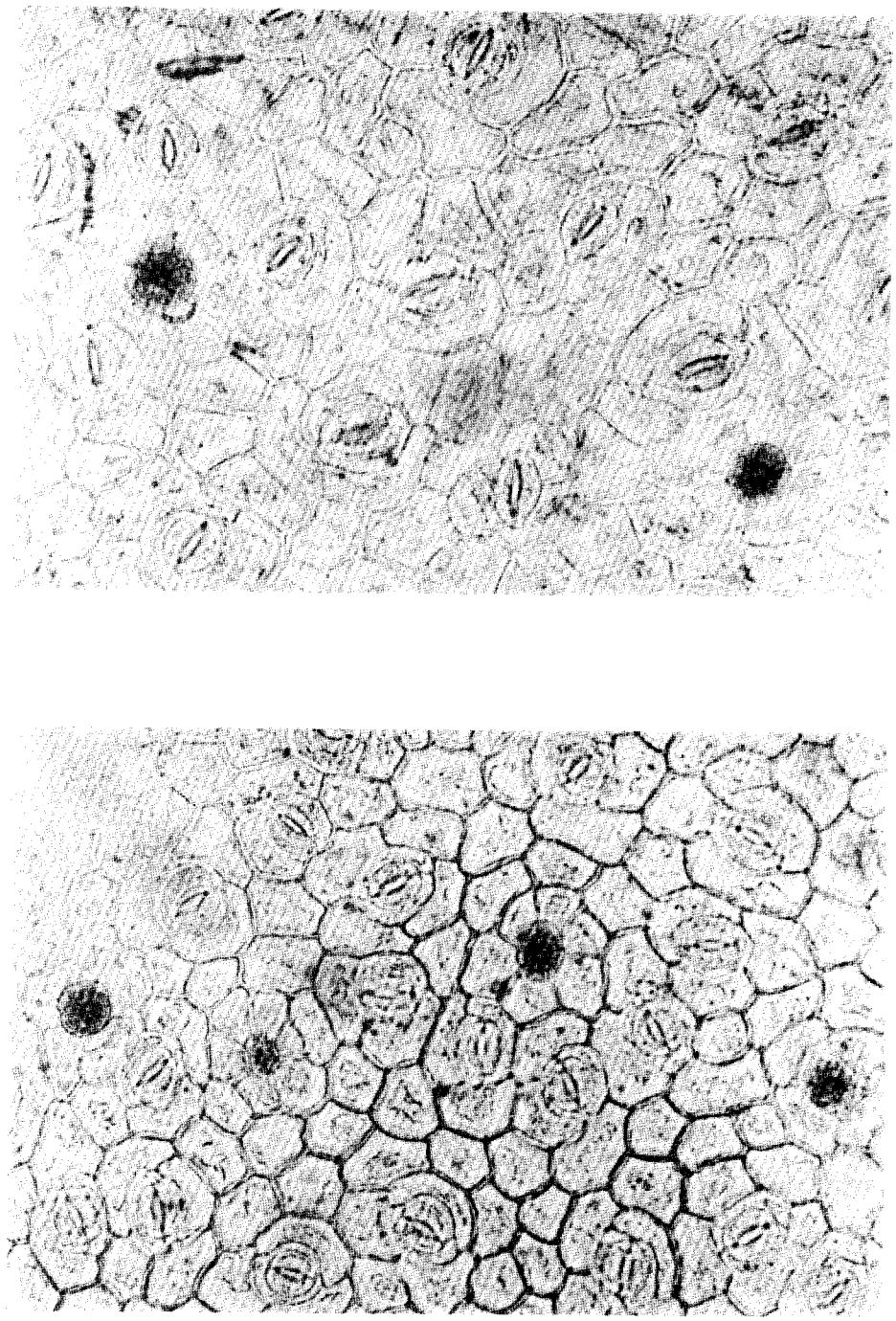

Figure 6. Upper (above) and lower (below) epidermis of *Ipomoea pes-caprae* (L.) R. Br. leaves from Prachuap Khiri Khan.

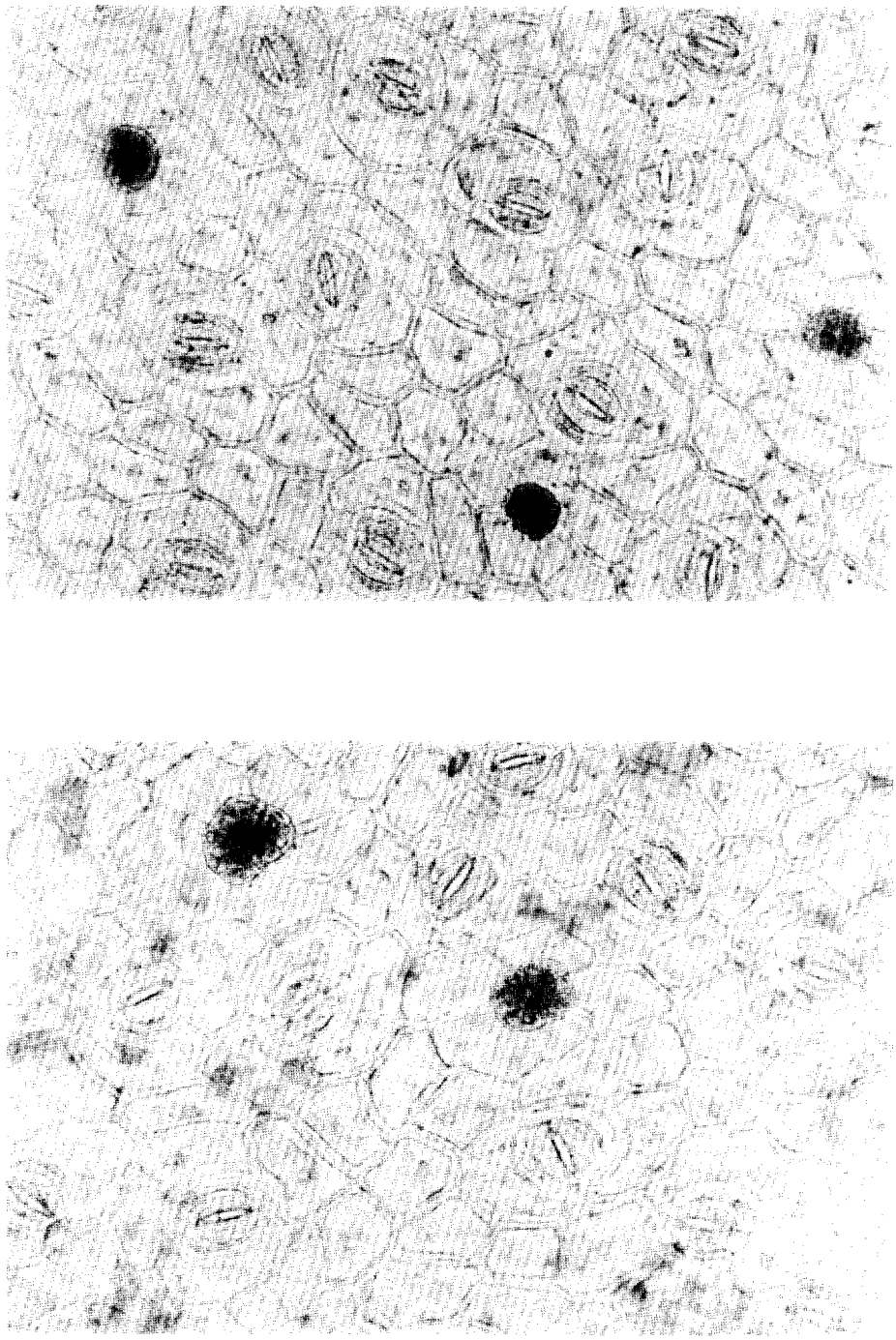

Figure 7. Upper (above) and lower (below) epidermis of *Ipomoea pes-caprae* (L.) R. Br. leaves from Nakhon Ratchasima.

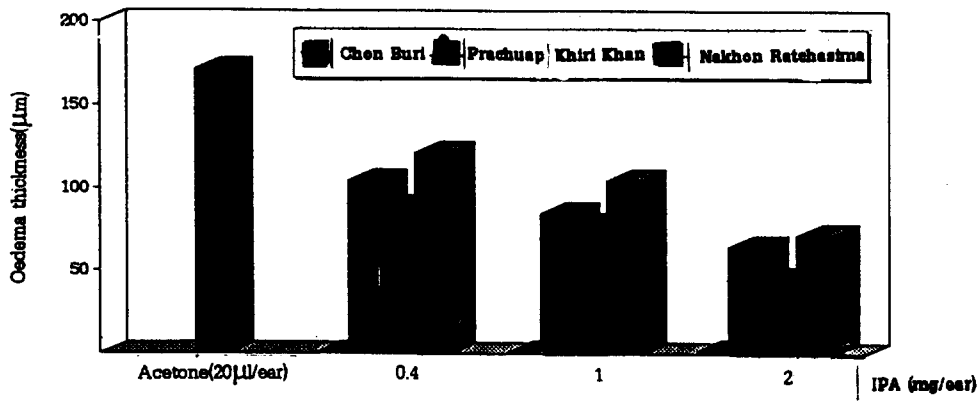

Figure 8. Effect of topical application of IPA extracted from *Ipomoea pes-caprae* (L.) R. Br. of three sources on EPP-induced ear oedema in rats (observed at 30 min).

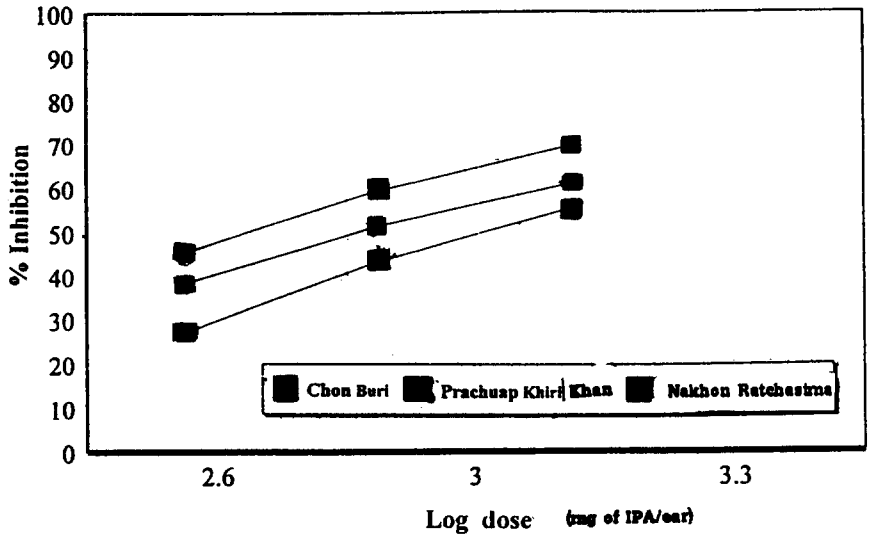

Figure 9. Inhibitory effect of IPA extracted from *Ipomoea pes-caprae* (L.) R. Br. of three sources on EPP-induced ear oedema (observed at 30 min).

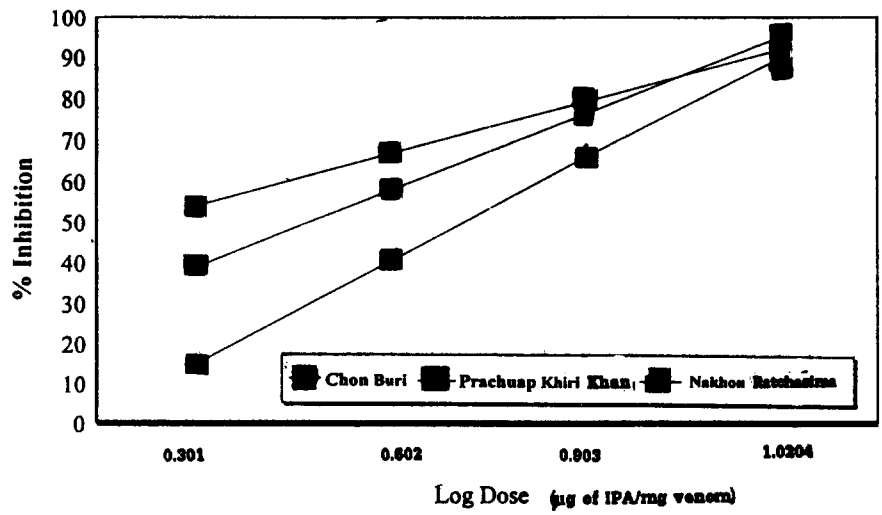

Figure 10. Neutralization of jellyfish venom-induced proteolysis by IPA extracted from *Ipomoea pes-caprae* (L.) R. Br. of three sources.

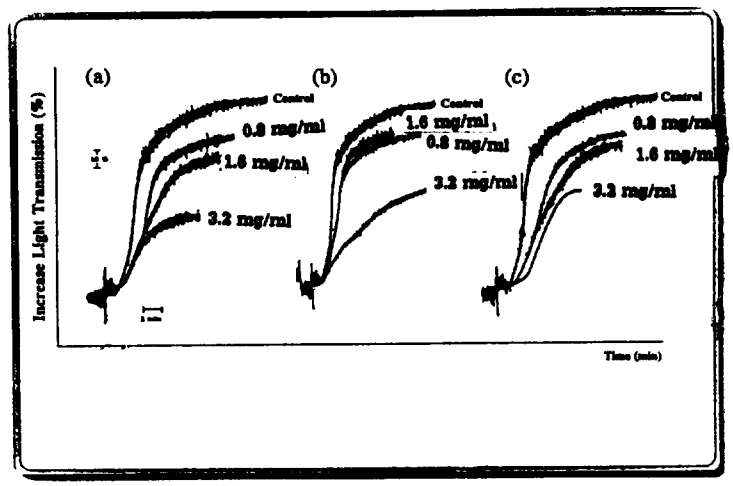

Figure 11. Tracings showing platelet aggregation induced by jellyfish venom (control) and inhibiting activity of various doses of IPA extracted from Chon Buri (a), Prachuap Khiri Khan (b) and Nakhon Ratchasima (c).

DISCUSSION

The taxonomy study revealed that plant specimens from three sources had the same description and were identified as *Ipomoea pes-caprae* (L.) R. Br. The anatomy study also showed the same structures.

Since stomatal index figure was fairly constant for a particular species (Wallis 1976), although some variation could be accepted depending on the location of the plant and environmental conditions (Wilson *et al.* 1971), the figures of stomatal index obtained from plant specimens of all sources which were in the same range confirmed the above results.

The percentage yield of IPA extracted from the specimens collected from Chon Buri was about 2-3 times higher than those obtained from Prachuap Khiri Khan and Nakhon Ratchasima. However these figures were resulted from one batch of extraction. To obtain the exact comparative yield, a few batches of each specimen should have been extracted and statistically analyzed.

It was, at the moment, absolutely impossible to use chemical constituent data to compare the properties of IPA extracted from different sources. The gas chromatography study of IPA extracted from Chon Buri exhibited almost a hundred main chemical components (unpublished data). At present, only seven compounds have been isolated and identified. The study of biological activities of these compounds indicated that all of them interfered with the process of inflammation in different ways (Pongprayoon 1991a; Pongprayoon 1991b; Pongprayoon 1992).

Thus, biological study of one experimental model *in vivo* and two of *in vitro* were performed. The EPP-induced ear oedema model was used to demonstrate the topical anti-inflammatory activity. The study of neutralization activity against jellyfish venom was carried out to indicate the antagonistic potency of IPA on proteolytic activity of the venom. Moreover, vascular effect has been reported to be involved in the dermatitis caused by jellyfish sting, leading

to local vascular insufficiency and gangrene. Agents with direct vasodilating activity, e.g. papaverine has been recommended for the treatment (Williamson et al. 1988) of such toxin-induced dermatitis. The study of inhibiting activity of IPA on platelet aggregation induced by jellyfish venom was then performed.

The results showed very interesting information that IPA extracted from the plant specimen cultivated on inland possessed the activities with the same range of potency in all experimental models compared to those obtained from natural sources.

Since the availability of *Ipomoea pes-caprae* (L.) R. Br. has become difficult as most of the area along the seashore has become tourist area and the plant has been eradicated, the plant cultivated on inland could thus be the compensating sources of IPA to be used as raw material of a new drug for the treatment of dermatitis caused by poisonous jellyfishes.

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