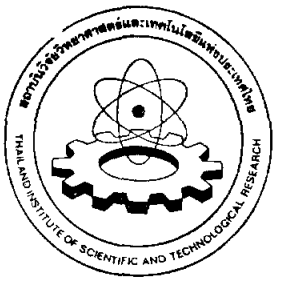

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THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH
FACULTY OF MEDICINE, SIRIRAJ HOSPITAL, MAHIDOL UNIVERSITY

CLINICAL STUDY ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *ALPINIA CONCHIGERA* GRIFF. IN SKIN INFECTION

BY

PATCHAREE SOONTHORNPALIN
SASITHORN WASUWAT
SOPHON KONGSAMRAN
MERANI THIANPRASIT

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(Professor Dr. Smith Kampempool)

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PHARMACEUTICALS FROM MEDICINAL PLANTS

REPORT NO. 2

STUDY ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *ALPINIA CONCHIGERA* GRIFF. IN SKIN INFECTION

BY
PAIYSAREE SOONTHORNPALIN
SASITHORN WASUWAT
SOPHON KONGSAMRAN
MERANI THIANPRASIT

TISTR, BANGKOK 1986

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โดย พัชรี สุนทรพะลิน, ศศิธร วสุวัต, โสภณ คงสำราญ
และ เมระนี เทียนประสิทธิ์

บทคัดย่อ

ได้ทำการศึกษาฤทธิ์ของสารที่สกัดจากรากข่าลิง Alpinia conchigera Griff. ต่อการติดเชื้อแบคทีเรียและเชื้อราของผิวหนัง โดยแบ่งขั้นตอนดังนี้:

1. ศึกษาการสกัดสารจาก ข่าลิง โดยใช้ 70 % ethyl alcohol และทำเป็นครีมโดยใช้ความเข้มข้น 4.4% ใน Carbopol 934 cream base เพื่อทาภายนอก ได้ครีมสีน้ำตาล มีกลิ่นข่าชัดเจน. ทายาบนผิวหนังผู้ป่วยที่มีการติดเชื้อแบคทีเรีย 13 แห่ง วันละ 3 ครั้ง. ปรากฏว่าได้ผลดี ผู้ป่วยหายภายใน 7 วัน 46.15%, เมื่อเปรียบเทียบกับ Gentamycin cream 1% ที่ใช้เป็น control 5 แห่ง ซึ่งทำให้หายได้ 60%.

2. การศึกษาสารสกัดจาก ข่าลิง โดยกลั่นด้วยไอน้ำ แยกเก็บส่วนน้ำมันนำมาทำเป็นครีม 3% โดยใช้ cream base PNP ได้ครีมสีขาว. ทายาบนผิวหนังผู้ป่วยโรคกลาก วันละ 3 ครั้ง 11 แห่ง ปรากฏว่าหายภายใน 3 อาทิตย์ 63.63%, เมื่อเปรียบเทียบกับ Tolnaftate 1% ซึ่งทำให้ผื่น 7 แห่ง หาย 100%.

เนื่องจากจำนวนผู้ป่วยในการทดลองนี้น้อยมาก ทำให้ไม่สามารถคำนวณเปรียบเทียบผลทางสถิติได้. แต่จากผลการทดลองมีแนวโน้มที่แสดงว่า สารสกัดจากข่าลิง มีผลต่อการติดเชื้อแบคทีเรียที่ผิวหนังน้อย ไม่สมควรที่จะนำมาผลิตเพื่อผลทางอุตสาหกรรม, แต่สารที่สกัดที่ใช้กับเชื้อราน่าจะมีการทดลองในขั้นต่อไปเพื่อหาประสิทธิภาพที่แท้จริงของยา.

STUDY OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *Alpinia conchigera* GRIFF. IN SKIN INFECTION

By Patcharee Soonthornpalin*, Sasithorn Wasuwat+,
Sophon Kongsamran* and Merani Thianprasit*

ABSTRACT

The rhizomes of Alpinia conchigera Griff. were extracted and prepared as two creams for the study of antibacterial and antifungal activities on human skin.

Cream "A" for antibacterial activity was prepared by 70% ethyl alcohol extracted part mixed in cream base Carbopol 934 with the concentration of 4.4%. This cream was applied on 13 lesions of pyoderma, folliculitis and infected eczema. The results were effective 46.15% comparing with Gentamycin cream 1% which were effective 60% in 5 lesions.

Cream "B" for antifungal activity was prepared by water distillation part mixed in cream base PNP with the concentration of 3%. This cream was applied on 11 lesions of dermatophytosis. The results were effective 63.63% comparing with Tolnaftate cream 1% which cleared all of 7 lesions (100%).

In this study, the number of lesions were not adeguate for statistically study. The results of both creams should be studied with more patients to find out the exact action which will be useful in Primary Health Care; especially antifungal activity, which found to be effective.

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*Siriraj Hospital

+Pharmaceuticals and Natural Products Research Department, Thailand Institute of Scientific and Technological Research

I. INTRODUCTION

1. Significance and the causes of problems

Herbs which have been used as medicine for hundreds of years in Thailand are economical and easily obtainable. Many of them have been known for the treatment of skin diseases as stated by our ancestors and traditional doctors but no adequate evidences are supported for use in modern medicine.

Galangal, which belongs to Genus Alpinia comprising approximately 250 species, has pungent smell, red-brown rhizome and bitter taste. Essential oil about 0.04% is found in fresh rhizome. This oil is composed of 48% methylcinnamate, 20-30% cineol, camphor and d-pinene. Alpinia conchigera and Alpinia galanga are used for the treatment of Flatulency, Ringworm and Pediculosis as stated in the Medicinal Plant Books (Anon 1983; Boonrod 1965).

The most common skin diseases in Thailand are bacterial infections which may develop to pus formation, abscess, boil and acne. Such early stages when drug administered topically can cure or decrease degrees of severity. These diseases are common in the rural areas all over the country. The antibiotics imported from foreign countries are too expensive.

The study of extracts of Alpinia revealed that the alcoholic extracts of Alpinia galanga possessed antibacterial property against both gram-positive and gram-negative bacteria e.g. Staphylococcus aureus, Pseudomonas aeruginosa, E. coli in vitro. These extracts can be prepared as cream or tulle for convenient topically application. Such extract preparations can be absorbed through the skin.

In B.E. 2524 (1981) The Pharmaceuticals and Natural Products Research Department (PNPRD) of TISTR reported the study of antimicrobial properties of 3 kinds of Alpinia (Disyaboot et al. 1981):

Fresh galangal, Alpinia spp. were purchased from "Pak Khlong Talat" Market - Alpinia galanga (L.) Willd Syn., Languas galanga (L.) Stuntz, Alpinia Pyramidata BL; and Alpinia conchigera Griff. All of these species possess bactericidal properties against Bacillus subtilis ATCC 6633, Sarcina lutea ATCC 9341, Staphylococcus aureus ATCC 6538 p, Salmonella typhi (Siriraj Hospital), Salmonella typhimurium ATCC 13311, Escherichia coli ATCC 19536,

Pseudomonas aeruginosa (Siriraj Hospital), Shigella dysenteriae (Siriraj Hospital) and Lactobacillus buchneri from the Fermentation Technological Laboratory.

It revealed from the experiment that alcoholic extracted active constituents yield higher potency than any other solvent extracted active constituents; and Alpinia conchigera has higher antibacterial efficacy than the other two, but it is significantly less effective when compared with control antibiotics.

From literature survey, there are no report on the study of the properties of Alpinia conchigera but some are on Alpinia galanga, A. speciosa, A. katsumadai, A. flabellata, A. japonica, A. nutans, A. oxyphylla and A. fructus.

However, it is revealed that one fraction which inhibits growth of fungi (antifungal) is found during antibacterial extracting process, thus the concurrent study of antifungal property was performed.

Meanwhile, it is of considerable importance to have adequate research and data to develop Alpinia conchigera into drug industry. This has had a profound influence on enhancing Alpinia conchigera cultivation for drug materials.

2. Objective

The objective of this project is to study topically antibacterial and antifungal activities of Conchigera cream.

3. Benefit and expected results

1. These studies will be advantageous for confirming the effectiveness of the antibacterial and antifungal properties of Alpinia conchigera in patients for the benefit of medicinal plants in modern-herbal medicines in the future.

2. If it is effective, it will be introduced to the public in Primary Health Care because Alpinia conchigera is an easily-grown plant.

3. If the extraction of Alpinia conchigera is beneficial for drug industry, it will save our country's foreign currency because Alpinia conchigera is low in cost and easily obtainable.

II. METHODS

Methods consist of 4 phases:

1. Preparing cream from active extract of Alpinia conchigera.

2. Irritation and sensitivity tests on normal human skins.

3. Study of antibacterial activity of Conchigera Cream "A" on the skin.

4. Study of antifungal activity of Conchigera Cream "B" on the skin.

1. Preparing cream from active extract of Alpinia conchigera

Materials and methods

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

1.10 Rotavap, Benchi (Switzerland)

1.11 Canesten,(R) (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 934 cream base containing 4.4% Alpinia conchigera extract by 70% ethyl alcohol.

2.4 Formulation of antifungal Conchigera cream

One and three per cent extracted oil of Alpinia conchigera in non-irritated PNP-1 cream base formulated by the Pharmaceuticals and Natural Products Research Department.

2.5 Study of antimicrobial activity of Alpinia conchigera extract

Agar dilution method: The extracts were mixed with media to give the various required concentrations and poured into the 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 molfen 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 g 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°C for 24 hours. Cream base was used in the similar procedure as above. Using plate count technique to enumerate cells in each cream of Alpinia conchigera containing tube compared with the number of cells in cream base containing tubes (control).

2.6.2.2 Test for fungi

The same method as in 2.6.2 was performed, however, the fungal incubation was at 30°C for 10 days.

3. Irritation and sensitivity tests on normal human skins

Test with 5 cream preparations:

1. Antibacterial Conchigera cream "A".
2. Cream base for Conchigera cream "A".
3. 3% antifungal Conchigera cream "B".
4. Cream base for Conchigera cream "B".
5. White petrolatum as control.

The close patch test of 5 cream preparations were tested: the cream on Finn chambers were applied on human back without exposing to water. Result was evaluated after 48 hours according to direction of the International Contact Dermatitis Research Group (Wilkinson et al. 1970).

The test was performed on 80 normal human subjects divided into 2 groups:

Group 1 — 50 subjects were treated with antibacterial Conchigera cream "A", using cream base and white petrolatum as control.

Group 2 — 30 subjects were treated with antifungal Conchigera cream "B", using cream base and white petrolatum as control.

4. Study of antibacterial activity of Conchigera cream "A" on the skin

4.1 Selection of patients

11 volunteer patients - 1 man and 10 women, age between 15-75 years - with 1-3 areas of bacterial infections totaling to 18 areas were selected after explanation of drug application and clinical screening which included a non-serious abscess without signs of other systemic infections, however, some cases with many abscesses were hematological checked-up by leucocyte and erythrocyte counts. No abnormal finding was detected.

The selected patients who had no specific or chronic illness which would be interfered with treatment such as diabetes mellitus and no other drug administration and pregnancy were considered for clinical trials.

4.2 Method

The treatment of abscesses in more than one area was administered by Conchigera cream for 1 area and 1% Gentamycin cream as control for the other area. On the other hand, only Conchigera cream was treated in patients with one-area abscess. Thus 13 areas totally receiving Conchigera cream and 5 areas totally receiving Gentamycin cream. The characteristics of abscesses were carefully observed and photographed. The pus from infected skin were scraped for bacterial cultures before the treatment.

Drug administration

Applied Conchigera cream "A" on infected skin three times a day and examinations were taken every 1 to 3 days for 7-10 days until recovery. During treatment only Conchigera cream "A" was given and no other drug was administered. Photography was taken for each attending.

Bacterial examination

By bacterial culture 3 and 7 days before and after the treatment.

Procedure for examination (Lennette et al.1980; Finegold and Martin 1982)

1. Smeared on the slide and stained with Gram - stain and examined under the light microscope.

2. Inoculated the culture on Blood - agar, then kept in the container filled with 5% carbon dioxide and incubated at 35-37^{o}C.

Examined the growth of the culture at 24 and 48 hours. If there were colonies on the plates, identified the bacteria according to bacteriological methods. Moreover, drug efficacy had to be tested at many intervals in order to prove its quality in the laboratory.

4.3 Termination of research

4.3.1 When abscesses were healed or negative to microbial tests.

4.3.2 Patient noncompliance.

4.3.3 When adverse or side effects occurred.

4.3.4 After 7-day course, the abscesses were still not healed.

4.4 Assessment

Healing process was determined by comparing the duration of both groups by 50 and 100% of the original and by comparing the duration of bacterial cultures. The comparison was determined by the Chi Square test.

5. Study of antifungal activity of Conchigera cream "B" on the skin

5.1 Selection of patients

Ten patients were selected and diagnosed as dermatophytosis. Only 8 out of 10 dermatophytosis were collected at the end of the clinical trial comprising of 6 men and 2 women, age 15-27 years, screened from patients with more than 1 area skin rashes totaling to 18 areas. These volunteers had no other diseases, concurrent drug administration and pregnancy.

5.2 Method

Scraped the edge of the areas to examine under the light microscope using 20% KOH as solvent and the left scab or crust were cultured in Sabouraud dextrose agar and examined within three weeks.

The total 18 areas by KOH examination were divided into 2 groups - the 11 areas of dermatophytosis treated with Conchigera cream and the remaining 7 areas treated with 1% Tolnaftate as control. Patient who had more than 1 area of dermatophytosis would be given 2 drugs applying locally on separate lesions as a control in the same patient.

Drug administration

Applied the white Conchigera cream "B" or 1% Tolnaftate three times a day at the selected areas according to the schedule.

The follow-up and microbial tests

Patients were appointed to be seen every 7 day until recovery or until 3-week course. The follow-up examination on each visit would be concentrated on the character of the rashes, size, extent, scab, infection, papule, and itching and were photographed and scraped to be tested with KOH and cultured for fungal detection.

5.3 Termination of research

5.3.1 When rashes disappeared or negative to microbial tests.

5.3.2 Patient noncompliance.

5.3.3 When adverse or side effects occurred.

5.3.4 After 3-week course, rashes or fungi were still found.

5.4 Assessment

The rash disappearance comparison in both groups and the duration of time for negative fungal detection were determined to evaluate the results compared with the Chi Square test.

III. RESULTS

1. Alpinia conchigera extraction

Table 1 shows constituent analysis of Alpinia conchigera extraction with alcohol 15.3% of dry weight of extractive part.

Table 2 showing products of Alpinia conchigera extraction of which the 1.3% Conchigera oil was found.

Tables 3, 4, 5, 6 can be summarized as follows:

1. Active constituent extracted from Alpinia conchigera by 70% ethyl alcohol inhibited the growth of S. aureus better than P. aeruginosa at the minimum inhibitory concentration (MIC) of 20-50 mg/cc. Furthermore, it was found that there was no inhibitory effect on the growth of the three fungi at the concentration of 20-50 mg/cc by dilution method, and this experiment could not carried out over the concentration of 50 mg/cc because the ethyl alcohol serving as a solvent caused an antifungal activity.

2. Distilled Conchigera oil showed no antibacterial activity on test organisms, but could inhibit the growth of T. mentagrophytes, E. floccosum and M. gypseum at the concentration of 0.07-0.1% (MIC value).

3. The development of pharmaceutical products revealed that Alpinia conchigera could be prepared by 70% ethyl alcohol extract part successively mixed in cream base Carbopol 934 with the concentration of 4.4%.

4. The development of cream of Conchigera oil revealed that cream base PNP-1 could be effectively used with 1 and 3% distilled Conchigera oil.

5. The antimicrobial action of cream of 70% ethanol extract of Alpinia conchigera showed little action against bacteria and fungi, but the comparison with cream of Conchigera oil showed that cream of 70% ethanol

extract was marked less effective. The study in 1971 revealed inhibition zone to S. aureus only 10-15 mg/disc, 15-25 mg/disc of Alpinia conchigera comparing with 30 ug/disc of Chloramphenicol and 10 ug/disc of Ampicillin.

6. The antifungal Conchigera cream containing 3% oil showed inhibitory effect on 3 types of fungi and on E. floccosum, the most sensitive organism, in equivalent to Canesten cream (Bayer).

From the previous study, the Pharmaceuticals and Natural Products Research Department of the Thailand Institute of Scientific and Technological Research had prepared the listed cream for clinical trial tests:

1. 4.4% antibacterial Conchigera cream "A", brownish color and natural pungent of Conchigera.

2. 3% antifungal Conchigera cream "B", white color and less smell of its natural odor.

3. Cream base for preparing Conchigera cream "A".

4. Cream base for preparing Conchigera cream "B".

The above mentioned cream in various formulae were filled in tight - closure tubes at dose of 20 grams each for 50 tubes.

2. Study of Patch test on normal human skins

Group 1 50 patients received antibacterial Conchigera cream "A", cream base and white petrolatum.

Result:-

- 8 treated episodes (16%) were positive to Conchigera cream "A".

- 4 treated episodes (8%) were positive to cream base.

- All were negative to white petrolatum.

Though 4 treated episodes, who were positive to Conchigera cream, were positive to cream base, the remaining 4 episodes, who were positive to Conchigera cream, were negative to cream base.

Group 2 30 patients received antifungal Conchigera cream "B", cream base and white petrolatum.

Result:-

All were negative to the three creams.

3. The study of effects on bacterial skin infection

In Table 7, the patients who were examined are shown; the diagnosis of folliculitis in 2 patients (No. 1, 10) revealing multiple pustules at one area. The patient No. 1 had recurrent infections for many months. The second group, 5 patients, had pyoderma. The third group, 4 patients, had infected eczema. The pustular areas varied from 3 mm to 10 mm in diameter, but the eczematous processed about 40 mm in diameter and scattered pustules were found on inflamed skin.

The examination of bacteria revealed Staphylococcus areas to be highest infected for 12 areas, Beta - hemolytic streptococci group A for 5 areas, Micrococcus spp. for 1 area at the head, and after treatment Streptococcus pyogenes infections were observed in 3 areas.

In Table 8, the effects of Conchigera cream A comparing with Gentamycin cream were shown.

Conchigera cream A was used in 13 areas and found healing in 6 areas, i. e., 46.15%.

Gentamycin cream was used in 5 areas and found healing in 3 areas, i. e., 60%.

According to the clinical trial, the patients were very small in number, the accurate statistic comparison could not be evaluated. It could only be interpreted that the effect was less than Gentamycin cream.

The untoward effects of Conchigera cream "A" were red-brown in color, smell of its characteristic of natural product, and biting pain sensation if applying to wide area of inflammation or infected eczema.

4. Results of antifungal skin infection test

From Table 9, the patients with many areas of infections were tested by 2 drugs in the same patient for comparison. The duration of infections ranged from 4 days to the recurrent infection of 5 months, and the sizes varied from 4x4 sq cm to 4x20 sq cm. The numbers of the infected areas were noted, and revealed as follows:

Tricophyton rubrum 9 areas

Tricophyton mentagrophyte 4 areas

The examinations revealed positive by KOH but 5 areas of contamination were found by culture. The results of treatment after applying 3 times daily, determined from the process of healing within 3 weeks, in some cases hyperpigmentation still remained but cultures showed negative.

Group 1, Conchigera cream "B" caused healing in 7 out of 11 areas, i.e.,63.63%.

Groups 2, 1% Tolnaftate cream caused healing in all 7 applied areas, i.e.,100%.

The test showed no side effect. The cream was white color.

IV. DISCUSSION

Galangal is a plant in the family of Zingiberaceae which is used in the treatment of many diseases. There are many reports that reveal the effectiveness of galangal for antibacterial and antifungal activities. This study group is intended to verify the clinical drug evaluation for the benefit of Primary Health Care.

From the report by Wasuwat et al. (1986) the study of active ingredients of Alpinia galanga, Alpinia conchigera and Alpinia sp. by using gas chromatography revealed that antibacterial and antifungal constituents were in Alpinia conchigera more than in other species. Thus, extract of Alpinia conchigera was used instead of Alpinia galanga which was initially intended to be used. Moreover, Alpinia conchigera had been used in ancient herbal medicine ("traditional medicine") for abdominal and intestinal distression with gas as well as Alpinia galanga. A. conchigera is found to be harmless for human use. The same report also showed the study of toxicity of Alpinia conchigera oil at approximate high lethal dose of 10.7 grams per 1 kilogram body weight of mice. The irritation test on rabbits disclosed the primary irritation index 0.33 of Conchigera cream and 0.29 of base cream, thus meaning no irritating effect.

Alpinia conchigera cream is prepared from the whole plant extract. The isolation of purified substance will be performed in the future program of TISTR. Though the cream has natural smell, this cream is accepted by the patient.

It is of special interest to note that 8% of the patients are sensitized to Conchigera cream "A" while the other 8% to both Conchigera cream "A" and its cream base, which may due to cross sensitization with galangal taken in food. There is burning pain if the cream is applied in wide area. Whereas the Conchigera cream "B" has no irritation and sensitization effect. Thus, both creams are topically administered.

The clinical outcomes of the Conchigera cream treatment are as follows:

(1) 46.15% antibacterial efficacy comparing with 60% of that of Gentamycin cream.

(2) 64% antifungal efficacy comparing with 100% of that of 1% Tolnaftate cream.

Though the number of patients were very small and being not justified for statistical evaluation, but the result inclined to be less effective than the drug used as control. This study stressed on selecting the patients suffering for more than 2 areas, so that biological variation would be excluded.

Possibly the therapeutic effect of the Conchigera cream for bacterial infection was not effective or the patients did not follow the proper application of the cream, because the patients had suffered from wide area of inflammation and complained of burning pain especially with the infected eczema. However, the small infected areas without eczematous process were healed.

From the examination of bacterial infection of infected area or pustules, it appeared that the healing process in 7 days duration was only infected by S. aureus (area 3, 4, 5, 6) and the sizes of areas were small; at area No. 14 S. aureus with Beta hemolytic Strep. gr. A were found and also infected area was small in size.

The area which improved rapidly was No. 16, healing within 3 days. It was infected by Micrococcus spp. It is most likely that Conchigera cream is suitable for small infected area caused by S. aureus. This is the same result obtained from the investigation that the extraction of A. conchigera is only effective on S. aureus. Because the infected area or pustules are caused by mixed infection i.e. S. aureus and Streptococcus gr., hence Conchigera cream is non effective or less effective. The results were illustrated in healing process of infected area and pustules, area No. 11, 12 and 17. Therefore, the infected areas or infected eczema caused by mixed infection are not suitable for using Conchigera cream.

The effect of 1% Gentamycin cream is good for S. aureus and Streptococcus infection. The result was not effective in the whole number of infected area because the assessment duration was only 7 days. The reason for using 7-day period is that, if the medicine is not effective it should be discontinued due to the wide spread of infection which may be harmful to the patient.

Although the study was taken with a small number of patients, the effectiveness of Conchigera cream "A" is relatively less than the well-known antibiotics which are so inexpensive. The practical use of Alpinia spp. in Primary Health Care can be used in the case of small pustules caused by primary infection and not secondary infection superimposed on eczema.

The treatment results of ringworm by Conchigera cream "B" showed 63.63% effectiveness comparing with 100% by 1% Tolnaftate, as follows:

1. Type of fungi - the infected area by T. rubrum, was effective in 3 areas out of 5 areas i.e. 60% effectiveness. The infected areas by T. mentagrophytes were effective in 1 area out of 3, i.e., 30% effectiveness. This is in accordance with laboratory test that Conchigera cream "B" is able to control the growth of T. rubrum.

2. The size of area is rather not significant. For example, one patient had two infected areas at the same time, the one with 4x20 cm area (No. 16) was healed but the 5x5 cm area (No. 17) was not healed, though the fungi was the same type. The other patients suffered from rather the same size of infected areas.

3. The application of Conchigera cream in the non-effective patient revealed that the application was irregular. Some of them applied the cream once a day and the amount used was insufficient, though there was no side effect i.e. no burning sensation. It was found that area No. 11 was healed by daily applying the cream once for a continuous period of one month, and the culture of fungi was negative.

From this primary investigation it was found that Conchigera cream "B", though less effective than 1% Tolnaftate cream, can be used in Primary Health Care beneficially with cheaper cost. It will be further investigated with a larger number of patients for determination the real effectiveness of Alpinia conchigera.

V. CONCLUSION

It can be summarized that Conchigera cream "A" at 4.4% concentration was effective 46.15% when applied to pyoderma, folliculitis and infected eczema, comparing with 60% effectiveness of 1% Gentamycin cream. As for Conchigera cream "B" at 3% concentration was effective 63.63% when applied to dermatophytosis, comparing with 100% effectiveness of 1% Tolnaftate cream.

In this study, the number of lesions was not adequate for statistically study. The results of both creams should be studied with more patients. This will be further studied from the prepared material.

VI. RECOMMENDATION

In this study, the number of patients was below expectation. This may due to the patients at Siriraj Hospital was limited by a small number of patients suffering from pyoderma and dermatophytosis. Patients, age 12 or more at the Department of Medicine were screened and those with more than 2 infected areas and could attend the whole study were selected. Therefore, the number of patients was inadequate for statistical study. Being a pilot study, some suggestions are as follows:

1. This study should be further conducted in the aspect of Conchigera use for antifungal effect for the economic and Primary Health Care purposes which would benefit the people, despite its relatively less efficiency than other synthetic antifungal drugs.

As for Conchigera, though containing much less antibacterial efficacy than other synthetic antibiotics, it should be further studied.

2. Considerations in the pharmaceutical preparations of these drugs need to be followed more clinical study.

The drugs which had been prepared will be further investigated and it will be reported later.

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VIII REFERENCES

ANON 1983 "Handbook of Medicinal Plants No. 1 --Projects of Medicinal Plant and Primary Health Care." p. 43, 1st Edition (H-N Press : Bangkok.) (In Thai.)

BOONROD, Phong 1965. "Thai Medicinal Plants." p. 94. (Kasembarnakit Press : Bangkok.) (In Thai.)

CHOPRA, L.C., KHAJURIA, B.N. and CHOPRA, C.L. 1957 Antibacterial properties volatile principles form Alpinia galanga and Acorus calamus. Antibio. Chemother. 7 (7) : 378-383

DISYABOOT, Pornsawan, WITHAMYO, Pranom and KHUNAJUK, Nongpayha. 1983. The study of the properties of Alpinia galanga at various duration of growth for inhibition of bacterial growth and the primary investigation of botanical chemistry of Alpinia galanga. Report in the "Annual Meeting of Pharmacy" February 8-10. (Bangkok.)

DISYABOOT, Pornsawan, WASUWAT, Sasithorn, THEPSITHAR, Pattama and SRICHANT, Siripen. 1981. The study of inhibition of bacterial growth of medicinal plants and galangal. TISTR : Bangkok (Report no. 1, Research Project no. 21/10.)

FINEGOLD, S.M. and MARTIN, W.J. ed. 1982. "Diagnostic Microbiology." 6th Edition. (The C.V. Mosby Company : St. Louis, Missouri, USA.)

LENNETTE, E.H., BALOW, A., HAUSLER, W.J. Jr. and TRUANT, J.P. ed. 1980. "Manual of Clinical Microbiology." 3rd Edition. (American Society for Microbiology : Washington, D.C.)

TANTIVATANA, Payom. 1983. "Spices." p. 27. (Chulalongkorn University Press : Bangkok.)

WASUWAT, Sasithorn, WANNISSORN, Puttarin, CHAMCHAANG, Wilaiporn, SUNTORNTANASAT, Taweesak and CHOTIPONG, Arubol. 1986. The study of inhibition of bacterial and fungal growth of extracted materials from Alpinia conchigera Griff. TISTR : Bangkok (Report no. 2, Research Project no. 21/2.)

WILKINSON, D.S. EREGERT, S. and MAGNUSS, B. 1970. Terminology of contact dermatitis. Acta. Derm. Venereol. 50 : 92

APPENDIX

TABLE 1 THE CONSTITUENTS ANALYSIS OF

ALPINIA CONCHIGERA (% IN GRAM OF DRIED WEIGHT)

Description	Per cent of 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 TISTR

TABLE 2. PRODUCTS OF ALPINIA CONCHIGERA EXTRACTION

70% ethanolic extract	9%
80% ethanolic 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)	S. aureus			P. aeruginosa		
	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)	Trichophyton mentagrophytes	Microsporum gypseum	E. floccosum	S. aureus	P. aeruginosa
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	T. mentagrophytes	M. gypseum	E. floccosum	S. aureus	P. aeruginosa
-Conchigera oil 0.001 g 0.003 g	1.5 2.3	0.5 1.4	2.3 3.2	0.7 0.7	0 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	1.9	1.9	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	
	S. aureus	P. aeruginosa
1 Control	1.66×10^{11}	1.01×10^{11}
2 1% oil cream	2.9×10^{5}	2.0×10^{8}
3 3% oil cream	1.4×10^{5}	1.81×10^{6}
4 Cream 70% ext.	<100	<100

S. aureus initial number = 4.17×10^{7} cells

P aeruginosa initial number = 2.10×10^{7} cells

TABLE 7. CLINICAL REPORTS OF PATIENTS TREATED WITH ANTIBACTERIAL DRUGS

No.	Diagnosis	Sex	Age (year)	Duration (day)	Position	Size in diameter (mm)	Drugs	Position No. of Abscess
1	Foliculitis	Female	27	180	Right hip	0.3 (60 points)	A	1
					Left hip	0.3 (40 points)	G	2
2	Pyoderma	Female	29	7	Right ankle (3 areas)	5 (each area)	A	3, 4, 5
3	Pyoderma	Female	74	21	No. 1 at right hand	5	A	6
					No. 2 at right hand	11	G	7
4	Infected eczema	Female	30	30	Right foot	20	A	8
5	Infected eczema	Female	15	30	Big toe	5	A	9
					Foot	10	G	10
6	Infected eczema	Female	28	7	Left back of foot	40	A	11
7	Pyoderma	Female	20	20	Foot	5	A	12
					Arm	20	G	13
8	Pyoderma	Female	31	7	Foot	10	A	14
9	Infected eczema	Female	57	7	Toe	10	A	15
10	Foliculitis	Female	30	2	Head	2 (20 points)	A	16
11	Pyoderma	Male	19	7	Leg 1	3	A	17
					Leg 2	8	G	18

A = Conchigera cream "A"

G = Gentamycin cream

TABLE 8. RESULTS OF THE TREATMENT BY ANTIBACTERIAL DRUGS

Position number	Drugs		Results				
			First day	Third day		Seventh day	
	A	G	Cultured*	Cure (%)	Cultured	Cure (%)	Cultured
1	/		1	-	-	25	1
2		/	1	-	-	50	1
3,4,5	/		1	50	-	100	-
6	/		1	50	-	100	-
7		/	1	50	-	100	-
8	/		1	25	1	25	-
9	/		1	-	-	50	1
10		/	1,3	-	-	100	-
11	/		1	25	1,4	25	1,4
12	/		1,4	25	1,4	25	-
13		/	4	50	4	100	-
14	/		1,4	50	1,4	100	-
15	/		1	increase	1	-	-
16	/		5	100	-		
17	/		4	50	2,1	50	1,2
18		/	4	50	2,1	50	1,2

1 = Staph. aureus

2 = Strep. pyogenes

3 = Staph. epidermidis

4 = Beta hemolytic strep. gr.

5 = Micrococcus spp.

- = Not done

A = Conchigera cream "A"

G = Gentamycin cream

TABLE 9. CLINICAL REPORTS OF PATIENTS TREATED WITH ANTIFUNGAL DRUGS

Number	Sex	Age	Duration (day)	Position	Rash size (cm)	Rash number	Drugs
1	Female	27	150	(Inguinal) Groin Right leg	5x8	1	B
				(Inguinal) Groin Left leg	5x8	2	B
				(Umbilicus) Navel	5x5	3	T
				Back	5x5	4	T
2	Male	19	10	Left arm	5x6	5	B
				Left leg	3.5x5	6	T
3	Female	20	21	(Inguinal) Groin Left leg	5x12	7	B
				(Inguinal) Groin Right leg	5x8	8	T
4	Male	19	30	(Inguinal) Groin Left leg	5x5	9	B
				(Inguinal) Groin Right leg	3x3	10	T
5	Male	19	14	Knee	5x5	11	B
6	Male	17	30	Hand	5x9	12	B
7	Male	19	150	Left ankle	4x4	13	B
				Right foot	5x5	14	B
				Left hand	5x5	15	T
8	Male	15	4	Waist	4x20	16	B
				Arm	5x5	17	B
				Leg	5x5	18	T

B = Conchigera cream "B"

T = 1% Tolnaftate cream

TABLE 10. RESULTS OF THE TREATMENT BY ANTIFUNGAL DRUGS

Rash number	Drugs		Cultured Date	Results					
				7th day		14th day		21th day	
	B	T		Cure (%)	Cultured	Cure (%)	Cultured	Cure (%)	Cultured
1	√		+	-	-	100	N		
2	√		+	-	-	100	N		
3		√	+	-	-	100	N		
4		√	+	-	-	100	N		
5	√		1	25	-	50	1	50	1
6		√	1	50	N	100	N	100	N
7	√		1	100	N	100	N	100	-
8		√	1	100	N	100	N	100	-
9	√		1	-	-	100	+	100	N
10		√	1	-	-	100	+	100	N
11	√		2	50	2	50	2	75	2
12	√		+	25	1	100	N		
13		√	+	25	1	50	-	50	1
14	√		1	25	-	100	-	100	N
15		√	+	25	-	100	-	100	N
16	√		2	-	-	50	+	100	N
17	√		2	-	-	50	+	50	2
18		√	2	-	-	50	+	100	N

B = 3% Conchigera cream

T = 1% Tolnaftate cream

+ = Positive reaction for KOH only

- = Not done

1 = Positive reaction for Trichophyton rubrum

2 = Positive reaction for T. mentagrophytes

N = Negative reaction