

## Study on antioxidant and antimicrobial activities of turmeric clear liquid soap for wound treatment of HIV patients

Suwipa Ungphaiboon<sup>1</sup>, Tanomjit Supavita<sup>2</sup>,  
Pechnoi Singchangchai<sup>3</sup>, Supreedee Sungkarak<sup>4</sup>,  
Pranee Rattanasuwan<sup>5</sup> and Arunporn Itharat<sup>6</sup>

### Abstract

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Ethanol extract of turmeric [*Curcuma longa* Linn. (Zingiberaceae)] was investigated for its *in vitro* antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and activities against six microorganisms (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans*). Clear liquid soaps containing 0.5% w/v turmeric extract were formulated. The only one preparation with acceptable appearance, foam and viscosity was

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<sup>1</sup>M.Sc.(Pharmacy), Asst. Prof., Department of Pharmaceutical Technology <sup>2</sup>M.Sc.(Pharmaceutical Botany), Assoc. Prof., <sup>3</sup>M.Sc.(Biochemistry), Scientist, <sup>4</sup>B.Ed. Scientist, <sup>5</sup>Ph.D. (Pharmacognosy), Assoc. Prof., Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, <sup>6</sup>Ph.D.(Demography), Assoc. Prof., Department of Administration of Nursing Education and Nursing Service, Faculty of Nursing, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.

Corresponding e-mail: arunporn.i@psu.ac.th

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selected for antimicrobial activity and stability studies. It was found that turmeric extract had 50% radical scavenging ability ( $EC_{50}$ ) at concentration of 11.26  $\mu\text{g/ml}$  against DPPH. Turmeric extract was showed no activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration of turmeric extract against *Bacillus subtilis*, *Staphylococcus aureus*, *Cryptococcus neoformans* and *Candida albicans* were 16, 128, 128 and 256  $\mu\text{g/ml}$ , respectively. The selected preparation was physically and chemically stable and the antimicrobial activity did not change ( $p < 0.05$ ) under the heating-cooling stability test. However, curcumin content and the antimicrobial activities against *S. aureus* and *C. neoformans* decreased significantly ( $p < 0.05$ ) under the accelerated test conditions (temperature 45°C, 75% RH for 4 months) and after storage at room temperature for 12 months. The results of a clinical trial with HIV patients found that this liquid soap decreased itching symptom (100%) and infectious wound and abscess became dryness scabs (78.6%) within 2 weeks.

**Key words :** antioxidant, antimicrobial activity, clear liquid soap, curcumin, turmeric extract, HIV patients

### บทคัดย่อ

สุวิภา อึ้งไพบูลย์<sup>1</sup> ถนนอมจิต สุภาวิตา<sup>2</sup> เพชรน้อย สิงห์ช่างชัย<sup>3</sup> สุปรีย์ดี สังขรักษ์<sup>2</sup>

ปราณี รัตนสุวรรณ<sup>2</sup> และ อรุณพร อิฐรัตน์<sup>2</sup>

การศึกษากิจกรรมต้านอนุมูลอิสระและฤทธิ์ยับยั้งเชื้อจุลินทรีย์ของสบู่เหลวใสที่มีส่วนผสม  
เพื่อรักษาแผลของผู้ติดเชื้อ เอช ไอ วี

ว.สงขลานครินทร์ วทท. 2548 27(ฉบับพิเศษ 2) : 569-578

การศึกษากิจกรรมต้านอนุมูลอิสระโดยวิธี DPPH assay และฤทธิ์ยับยั้งเชื้อจุลินทรีย์หกชนิด (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans*) โดยวิธี Disc Diffusion และ วิธี agar dilution ของสารสกัดขมิ้นชัน [*Curcuma longa* Linn. (Zingiberaceae)] ด้วยเอทานอล และสบู่เหลวใสที่มีสารสกัดขมิ้นชัน 0.5% w/v ซึ่งผ่านการคัดเลือกและการยอมรับจากลักษณะทางกายภาพของสบู่เหลว เช่น ความหนืด ฟอง และความคงตัว ผลการศึกษาพบว่าสารสกัดขมิ้นชันมีค่าความสามารถในการเป็นสารต้านอนุมูลอิสระ ( $EC_{50}$ ) เท่ากับ 11.26  $\mu\text{g/ml}$  และมีฤทธิ์ยับยั้งเชื้อ *Bacillus subtilis*, *Staphylococcus aureus*, *Cryptococcus neoformans* และ *Candida albicans* โดยมีความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อจุลินทรีย์ (minimum inhibitory concentration) เท่ากับ 16, 128, 128 และ 256  $\mu\text{g/ml}$  ตามลำดับ แต่ไม่มีฤทธิ์ยับยั้งเชื้อ *Escherichia coli* และ *Pseudomonas aeruginosa* เมื่อศึกษาความคงตัวของสบู่เหลวใส ทางเคมี และฤทธิ์ยับยั้งเชื้อจุลินทรีย์ของสบู่เหลวใส พบว่าเมื่อใช้วิธีการให้ความร้อนสลับกับความเย็น (heating cooling stability test) ไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญ อย่างไรก็ตามเมื่อทดสอบโดยสภาวะเร่ง (อุณหภูมิ 45°C ความชื้นสัมพัทธ์ 75% นาน 4 เดือน) และเมื่อเก็บรักษาไว้ที่อุณหภูมิห้องนาน 12 เดือน พบว่าปริมาณ curcumin และฤทธิ์ยับยั้งเชื้อ *S. aureus* and *C. neoformans* ลดลงอย่างมีนัยสำคัญ ( $p < 0.05$ ) การใช้ผลิตภัณฑ์ในผู้ติดเชื้อเอชไอวี พบว่าสบู่เหลวใสตัวรับนี้สามารถลดอาการคันของแผล (100%) และลดแผลเป็นหนอง (78.6%) ภายใน 2 สัปดาห์

<sup>1</sup>ภาควิชาเทคโนโลยีเภสัชกรรม <sup>2</sup>ภาควิชาเภสัชเวชและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ <sup>3</sup>ภาควิชาบริหารการศึกษายาบาลและบริกรพยาบาล คณะพยาบาลศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

*Curcuma longa* Linn. (turmeric) has long been used as a common household medicine and as a spice in Thailand. Turmeric composed with

at least 7% of a yellow volatile oil containing turmerone and zingiberene as major constituents and sesquiterpenes and monoterpenes types of

compounds; yellow coloring matter including curcumin or diferuloylmethane (1.8 to 5.4%), desmethoxycurcumin and bisdesmethoxycurcumin (Thai Herbal Pharmacopoeia, 1995). In old Thai records, the rhizomes was used to treat peptic ulcer, dyspepsia, indigestion, insect bite and skin diseases (Saralamp, 1996). The efficiency of turmeric by means of scientific method has been reported for used as peptic ulcer treatment (Prucksunand, 1986), carminatives (Thamlikitkul, 1989), wound treatment (Kuttan *et al.*, 1987) and anti-inflammatory agent (Yegnanarayan *et al.*, 1976). Inhibitory effect of turmeric oil and ethanolic extracts against bacteria and fungi has been reported (Apisariyakul, *et al.*, 1995; Damrihanunt, 1990; Srinivasan, 2001; Khanha, 1999; Wuthi-udomlert, 2000). These researches support the use of turmeric extract for treating skin infections. On the other hand, the clear liquid soap which contained turmeric extract could be used for skin infections. The objective of this research was to study the turmeric extract and the clear liquid soap on the antioxidant activity using DPPH assay and antimicrobial activities against six microorganisms (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans*). Their minimum inhibitory concentration (MIC) was determined. In order to promote both cosmetic and therapeutic use of turmeric, clear liquid soaps containing turmeric extract were formulated and tested for their physicochemical stability as well as their antimicrobial activities. The best clear liquid soap formula was chosen and used for the treatment of skin infections of HIV patients caused by bacteria and fungi.

## Materials and Methods

### Plant material

The rhizomes of *Curcuma longa* L. (Zingiberaceae) were collected in April 2002 from Amphor Hat Yai, Songkhla Province, Thailand. Authentications of plant materials was carried out at the herbarium of the Department of Forestry Bangkok, Thailand, where the herbarium vouchers

have been kept to specify plant and species identified. Another one of these plants has been kept as a specimen in the herbarium of Southern Center of Thai Medicinal plant at Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

### Preparation of plant extract

After drying at 50°C, the rhizomes were powdered and macerated for 3 days with 95% ethanol (3x1L). The ethanolic extract was then evaporated to dryness under reduced pressure. The percentage yields of the extracts was calculated.

### Assay for curcumin content

The curcumin content was determined based on the method in the Thai Herbal Pharmacopoeia (THP) by measuring the absorbance at 420 nm (Thai Herbal Pharmacopoeia, 1995). Authentication of curcumin from Fluka was used to prepare standard curcumin solution.

### Assay for antioxidant activity

Scavenging effect of turmeric extract on DPPH radical was examined based on the method of Hatano, *et al.* (1989). Curcumin and butylated hydroxytoluene (BHT) were used as reference standard and positive control, respectively. Samples for testing were dissolved in ethanol to obtain a high concentration of 200 µg/ml. Each sample was further diluted for at least 5 concentrations (two-fold dilutions). Each concentration was tested in triplicate. A portion of sample solution (500 µl) was mixed with an equal volume of  $6 \times 10^{-5}$  M DPPH (in absolute ethanol) and allowed to stand at room temperature for 20 min. The absorbance (A) was then measured at 520 nm. BHT as a positive standard was tested in the same system. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The results were expressed as percentage inhibition; %inhibition =  $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$ . Effective concentration of sample required to scavenge DPPH radical by 50% ( $EC_{50}$ ) was obtained by linear regression analysis of dose-response curve plots of %inhibition versus concentration.

### Assay for antimicrobial activity

#### Microorganisms

The bacterial and fungal strains often infecting AIDS patients were selected. Microbial strains used for the test were *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans*. These included the Gram-positive species; *Bacillus subtilis* (NCTC 10073) and *Staphylococcus aureus* (ATCC 25923), and the Gram-negative; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The human fungal pathogens and yeast (*Cryptococcus neoformans* and *Candida albicans*) were clinical isolates obtained from the Department of Pathology, Faculty of Medicine, Prince of Songkla University.

#### Medium

The media used the Mueller Hinton agar (MHA) (Difco, Becton Dickinson and Company, Spark USA) for bacteria testing, and Sabouraud Dextrose Agar (SDA) (Difco, Becton Dickinson and Company, Spark USA) for fungi testing.

### Determination of antimicrobial activity

Turmeric extract and pure curcumin were tested for antimicrobial activity by disc diffusion method (Barry and Thornsberry, 1985). Sterile paper discs (6 mm) were impregnated with reconstituted crude extracts 20  $\mu$ l (concentration 50 mg/ml in ethanol) and placed on the surface of MHA for bacteria and SDA for fungi and inoculated with the microbes. The sample was tested in triplicate. Discs containing 20  $\mu$ l of ethanol, 30  $\mu$ g/disc of tetracycline (Fluka) and 5  $\mu$ g/disc of ketoconazole (Sigma) were used as a solvent control and a positive control for bacteria and fungi, respectively. Agar plates containing bacteria and fungi were incubated at 37°C for 24 h and 30°C for 48 h respectively. Inhibition zones were recorded as the diameter of growth free zone, including the diameter of the disc, in millimetres at the end of the incubation period. The MIC was also determined by agar dilution method (Washington, 1985).

The minimal concentration completely inhibited the growth of the microorganisms of the extract, using tetracycline and ketoconazole as positive controls. The bacteria were grown overnight in MHA, and the yeast and dermatophyte fungi were grown on SDA slant for 2 days. Inoculate of  $10^3$ - $10^4$  CFU were spotted with micropipette on agar supplemented with the extract or antibiotic at concentrations ranging from 9.8-5000  $\mu$ g/ml for the leaf extract, 0.12-16  $\mu$ g/ml for tetracycline and 0.002-0.12  $\mu$ g/ml for ketoconazole. Agar plate containing bacteria and yeast was incubated at 37°C for 24 h and 30°C for 48 h, respectively.

### Preparations of clear liquid soap

Clear liquid soaps containing 0.5% w/v turmeric extract were formulated by varying type and/or concentration of surfactant, foam booster and thickening agent. The preparation with acceptable appearance; no precipitation, strong foam formulation, moderate viscosity (500-1500 cps) was selected as prototype formulation. The prototype formulation was prepared by using sodium lauryl ether sulfate and fatty acid amide derivative with betaine as surfactant, coconut fatty acid diethanolamine as foam booster, polyol fatty acid ester as superfatting agent, propylene glycol as moisturizer, sodium chloride as thickening agent and Bronidox L<sup>®</sup> as preservative.

### Stability studies of prototype formulation

Curcumin content, antimicrobial activity and physical stability of prototype formulation were monitored under heating-cooling stability test (45°C 12 h - 5°C 12 h; 6 cycle), accelerated test conditions (45°C, 75% RH for 4 months) and ambient temperature for 12 months. Sign of precipitation, color change, pH (using pH meter Model 20, Denver Instrument Company), viscosity (using Viscometer Model DV III, Brookfield Engineering Laboratories, Inc.) in three samples were determined after various storage times. At the same time, samples were assayed for curcumin content and antimicrobial activity by disc diffusion method, as described above.

### Clinical used on HIV patients

Twenty two HIV patients were selected by intentional sampling between January 2002 and March 2002. They lived in a temple in southern Thailand. The subjects had skin infections and wounds on their body. They had to sign an agreement for using liquid the clear liquid soap on an ethics form before starting the experiment. The researcher explained the overall purpose of the study and interviewed the patients by constructive questionnaire before starting the experiment. Each patient had to take a bath and use this liquid soap at least one time a day. The validity of the interview questionnaire for collecting the data was tested before use. The questionnaire was composed of general data of the samples (gender, age, etc.), the symptoms before and after using liquid soap, and the satisfaction with the characteristics of the soap such as color, odor, foam, ease of used. The researcher had to take pictures of the wound on the HIV patients' arms before giving each of them a bottle (200 ml) of clear liquid soap. The HIV patients were allowed to use the clear liquid soap

for 2 weeks and after that they were interviewed by the researcher. Quantitative data were analyzed using descriptive statistics and qualitative data using content analysis. It was evaluated by SPSS program and tested for the comparison between before and after using the preparation by Wilcoxon Singed Rank test.

### Results and Discussion

The yields of turmeric extract from dried rhizomes were 36.04 %w/w and the curcumin content in turmeric extract was 23.42 %w/w. After storage for 1 year at room temperature in a dessicator, the curcumin content decreased to 19.41%. The rate of decrease per year of curcumin was 17.12% w/w. Antioxidant activity using DPPH radical scavenging assay is shown in Table 1. It was found that curcumin was a strong antioxidant activity in the early study and EC<sub>50</sub> of turmeric extract was near treat BHT as positive control. Comparison of the antioxidant activity difference between turmeric extract and curcumin as a pure

**Table 1. Antioxidant activity of turmeric extract and curcumin by DPPH assay (n=3).**

samples	EC <sub>50</sub> (µg/ml)	
	Initial	After storage 1 year at room temperature
Turmeric extract	11.26±0.03	16.89±0.40**
Curcumin	2.57±0.10	2.81±0.25 (N.S)
BHT	12.45±0.30	-

\*\*P<0.0001, N.S = non-significant comparison with initial time

**Table 2. MIC (µg/ml) of turmeric extract on microorganisms.**

Microorganisms	MIC (µg/ml)		
	Turmeric extract	Tetracycline	Ketoconazole
<i>B. subtilis</i>	16	<0.125	-
<i>S. aureus</i>	128	0.25	-
<i>C. albicans</i>	256	-	0.125
<i>C. neoformans</i>	128	-	0.5

compound in turmeric extract was 4.4 times when determined at the starting time. The antioxidant activity of turmeric extract and curcumin decreased after one year (Table 1). These results showed that the antioxidant activity of turmeric extract was related to quantity of curcumin content. In the previous research, it was found that oxygen radical scavenging activity of curcumin also was related to anti-inflammatory activity (Kunchandy and Rao, 1990). Antimicrobial activity of turmeric extract and curcumin were determined by disc diffusion and agar dilution method. Turmeric extract inhibited all tested microorganisms except *E. coli* and *P. aeruginosa*. Curcumin 0.5 %w/v showed no antimicrobial activity against all tested microbes except *B. subtilis*. This result indicated that antimicrobial activities did not depend on curcumin content. The minimum inhibitory concentration of turmeric extract against *B. subtilis*, *S. aureus*, *C. albicans* and *C. neoforman* are shown in Table 2. This result is related with the previous report which found that turmeric extract showed antimicrobial activity but curcumin had no antimicrobial activity (Chopra, et al., 1941; Bhavani and Murthy, 1979; Chumsri, et al., 1982) and turmeric oil revealed a better inhibitory action against microorganisms than crude ethanol extract (Wuthiudomlert, 2000; Apisariyakul, et al., 1995; Damrihanunt, 1990). These results concluded that using turmeric extract in the formulation should improve skin infection treatment and could be considered for scavenging free radicals from inflammatory conditions. Although MIC of the extract was higher than that of the pure drug (tetracycline and ketoconazole), it was possible to use in formulation because the extract also showed antioxidant activity related with anti-inflammation. By consideration of the yield, antioxidant activity and antimicrobial activity, the extract was selected for preparing clear liquid soap. The concentration of turmeric extract in liquid clear soap was used 20 times the MIC as 0.5%w/v. The prototype formulation of liquid clear soap containing 0.5%w/v turmeric extract was yellow color, viscosity about 1,000 cps and pH 5.5. It was physicochemically stable and the antimicrobial activity did not change under the

Table 3. Stability studying of turmeric clear liquid soap on difference conditions with physicochemical analysis (curcumin content, pH and viscosity).

Physicochemical analysis	Initial	Heating cooling	Various storage time (month); (n = 3)											
			45°C						Ambient temperature					
			1	2	3	4	1	3	7	12				
Curcumin content (mg% ± S.D.)	0.102±0.020	0.102±0.020	0.076±0.010	0.055±0.002*	0.038±0.002*	0.025±0.002*	0.102±0.020	0.054±0.006*	0.062±0.005*	0.060±0.003*				
pH	5.50±0.01	5.38±0.01	5.16±0.02	4.86±0.01*	4.84±0.03*	4.87±0.01*	5.40±0.11*	5.09±0.02	5.02±0.03*	5.03±0.02				
Viscosity (cps)	1079±01	1080±50	1058±10	1158±24	1017±15	1046±59	895±10	901±31	1096±25	1228±155				

\* p<0.05 comparison with initial time.

heating-cooling stability test. It was physically stable with a little change in color under the accelerated test conditions and the storage at ambient temperature for 12 months. The curcumin content in the formulation, pH and viscosity at various storage times are shown in Table 3. The curcumin content under accelerated test decreased four times at fourth months; however, it decreased two times at twelve months in the ambient storage test. The antioxidant activity of turmeric extract decreased in relation to the curcumin content under storage for one year. It is concluded that curcumin was not stable at high temperature and also decreased significantly after store at ambient temperature for 3 months although the formulation was filled in an amber glass container. It indicated that the other components in the formula affect curcumin stability because the decreasing rate of curcumin in turmeric soap was more than that of the curcumin in turmeric extract after storage for one year.

The pH of the formula did not change under storage at ambient temperature but decreased under accelerated test but showed non-significantly by t-test. These results corresponded with the suggestion for storage turmeric in Thai Herbal

Pharmacopoeia which suggested that turmeric extract should be kept in a tightly closed container, protected from light and stored in cool and dry place.

The antimicrobial activity on accelerated and ambient temperature showed in Table 4. The result showed that the clear liquid soap containing 0.5%w/v turmeric extract inhibited *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans* and showed a significant difference with clear liquid soap base in the initial study ( $p < 0.05$ ). On the stability study, it was found that under heating and cooling conditions, the antimicrobial activity against all microorganism did not change significantly. The antimicrobial activity against to *B. subtilis* and *C. albicans* also did not change under accelerated test conditions (45°C for 4 months) or after storing at ambient temperature for 12 months. However, the antimicrobial activity of soap containing 0.5% turmeric extract against *C. neoformans* under accelerated conditions at the fourth month decreased significantly from the activity determined at the initial time. The storage in ambient conditions also made antimicrobial activity against *S. aureus* and *C. neoformans* decrease significantly (Table 4).

**Table 4. Antimicrobial activity of clear liquid soap containing 0.5 % turmeric extract.**

Microorganisms	Initial	Heating cooling	Average inhibition zone diameters (mm) ± S.D. at various storage time (month); (n = 3)				
			45°C		Ambient temperature		
			1	4	1	3	12
<i>B. subtilis</i>	17.0±1.2 <sup>a</sup>	16.0±0.5	17.5±0.6	14.6±0.2	16.7±0.4	15.2±0.3	15.3±0.2
<i>S. aureus</i>	14.2±0.2 <sup>a</sup>	14.3±0.3	14.4±1.2	10.3±0.3	15.5±0.9	12.9±0.3 <sup>b</sup>	10.0±0.5 <sup>b</sup>
<i>C. albicans</i>	21.2±1.4 <sup>a</sup>	21.9±0.8	22.4±0.8	20.7±0.8	22.1±0.7	21.7±0.6	19.3±1.8
<i>C. neoformans</i>	28.5±0.5 <sup>a</sup>	29.2±0.3	27.2±1.4	20.6±0.6 <sup>b</sup>	27.2±1.4	21.4±0.6 <sup>b</sup>	19.0±1.8 <sup>b</sup>

<sup>a</sup> : statistical difference from clear liquid soap base ( $p < 0.05$ )

<sup>b</sup> : statistical difference from formulation at initial ( $p < 0.05$ )

From stability data, the accelerated condition did not cause antibacterial change but curcumin content changed. It confirmed that curcumin content was not related to antibacterial activity but rather to antioxidant activity. The stability results under accelerated condition can indicate the age of the liquid clear soap contain 0.5%w/v turmeric extract as two years following standard of The Institute of Medical Sciences, Ministry of Public health.

The result of testing with HIV patients is shown in Tables 5-7. Most subjects were male (52.6%) at the average age of 33 years, and the mean duration of HIV infection of 1.6 years. The samples had itching (73.7%) and wounds with abscess (89.48%) before using the soap. The results of using turmeric clear liquid soap after 2 weeks showed that the wounds with abscess decreased and improved to normal (36.8% and 42.1%,

**Table 5. General data of HIV patients and their skin condition.**

General data	Percentage (n=19)
1. Gender	
Female	52.6
Male	47.4
2. Skin characteristic of patients	
Dry skin	36.8
Oily skin	15.8
Normal skin	47.4
3. Experience of herbal product used	68.4

**Table 6. Comparison of evaluation condition of patients before using turmeric liquid soap with after using 2 weeks (n=19).**

Evaluation data	Before used	Number(Percentage)		
		After used		
		No sign	Symptom decreasing	Still standing
- Itching	14 (73.7)	11 (57.9)	3 (15.8)	-
- Wound and abscess on skin	17 (89.4)	8 (42.1)	7 (36.8)	2 (10.5)

**Table 7. The level of evaluation liquid soap by HIV patients.**

Product characteristic satisfaction	Average of score	Meaning
Color	3.6	good
Good smell	3.9	good
Foam	3.95	good
Easy washable	3.2	moderate

Evaluation: 4.5-5 = very good, 3.5-4.49 =good, 2.5-3.49 = moderate, 1.5-2.49 = less satisfy, 0-1.49 = unsatisfy



**Table 8. Comparison of the level score before and after using turmeric liquid soap by Wilcoxon Signed Rank Test.**

Test	n	Mean rank	Z-value	significant
Negative ranks	2	5	-3.00	0.003
Positive ranks	17	9		
<b>total</b>	<b>19</b>			

respectively). Itching symptoms either disappeared or decreased after using for 2 weeks. The satisfactions of characteristic of color, odor, foam were good and washability was moderate. However, the results from in-depth interviews showed that some patients liked to use this soap and gave the reason that it helped protect skin from drying. Their skin would be moist and comfortable when they used the soap. The comparison data between before and after using by Wilcoxon signed rank test found that the mean of before and after using clear liquid soap for 2 weeks had significantly different ( $p < 0.003$ ). The positive rank score after using turmeric liquid soap was higher than the negative rank score (17 and 2, respectively) (Table 8). This indicated that HIV patients were satisfied with using turmeric liquid soap to treat skin infection and decrease itching. However, only 19 patients is a small sample size, so this experiment should be extended to more HIV patients.

### Conclusion

This study is a preliminary evaluation of antimicrobial activity of turmeric extract in cosmetic product. These results indicated that turmeric liquid soap can be used in both cosmetic and therapeutic preparation for skin infections. In addition, the clinical data also support its use for skin infection treatment of HIV patients.

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