Original Article

In-Vitro Study of Antibacterial Activities of *Phyllanthus emblica* L. Leaves, *Punica granatum* L. Peels, and *Curcuma longa* L. Rhizomes Crude Extracts to *Propionibacterium acnes* Isolated from Acne Vulgaris Patients

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Background: Nowadays, the prevalence of antibiotic-resistant *Propionibacterium acnes* [P. acnes] has been increasing continuously due to easy access to use monotherapy antibiotics in Thailand. According to the recent study of antibiotic-resistant *P. acnes* in Thai population, the prevalence of these bacterial resistance to antibiotics has been highly emerging, particular to clindamycin and erythromycin. Numerous botanical extracts have been shown to have antimicrobial activities because of their phytochemical compounds such as tannins, phenols, flavonoids, etc.

Objective: To perform an in-vitro study of antimicrobial activities of some herbal crude extracts against clinical isolated *P. acnes*.

Materials and Methods: The present report was an experimental, cross-sectional study. Seventy-five *P. acnes* specimens from clinical isolations were collected. *Punica granatum* L. peels, *Phyllanthus emblica* L. leaves, and *Curcuma longa* L. rhizomes were extracted with 95% ethanol by maceration methods for 3 days and repeated the procedure twice. Then crude extracts were collected. *P. acnes* were tested with the herbal crude extracts by agar well diffusion assay to evaluate minimum inhibition zone [MIZ] and broth microdilution method to evaluate minimum inhibitory concentration [MIC]. Then, the authors produced the lotion products of each crude extract and their MIZ activity was tested.

Results: The means MIZ of *Phyllanthus emblica* L. at 5 mg/ml and 10 mg/ml were 14.99 and 20.90 mm, respectively. For *Punica granatum* L., the means MIZ at the concentration of 5 and 10 mg/ml were 4.07 and 9.95 mm. On the other hand, no inhibition zone was seen among *Curcuma longa* L. rhizomes crude extract. The means MIC of each crude extract from *Phyllanthus emblica* L., *Punica granatum* L., and *Curcuma longa* L. were 0.559, 1.003, and 3.804 mg/ml, respectively. Concerning the difference in the MIC activities of each extract, there was no statistically significant difference between *Phyllanthus emblica* L. and *Punica granatum* L. ($p = 0.092$). However, there was significant difference between *Phyllanthus emblica* L. with *Curcuma longa* L. ($p<0.001$) and *Punica granatum* L. with *Curcuma longa* L. ($p<0.001$) as well. The lotion products of each crude extract were turbid and had strong smell. The MIZ of the lotion of *Phyllanthus emblica* L., *Punica granatum* L. were 21.67 and 17.25 mm, respectively, without statistical significant difference. However, no inhibition zone was seen in the lotion of *Curcuma longa* L.

Conclusion: These three crude herbal extracts could inhibit the growth of clinical isolated *P. acnes*. The extract of *Phyllanthus emblica* L. leaves had the best antimicrobial activity following by *Punica granatum* L. peels and *Curcuma longa* L. rhizomes.

Keywords: *Propionibacterium acnes*, Antibacterial activities, *Punica granatum* L., *Phyllanthus emblica* L., *Curcuma longa* L., Acne vulgaris

J Med Assoc Thai 2018; 101 (9): 1169-76
Website: http://www.jmatonline.com

Acne vulgaris is one of the most common dermatologic disease. A recent review article stated that acne vulgaris affected more than 85% of adolescence worldwide[1]. It may not only impact on physical problems but also it can induce anxiety, low self-esteem, stress, depression and the worst is suicidal idea[2].

Pathogenesis of acne are multifactorial factors[3]. *Propionibacterium acnes* [*P. acnes*], gram positive rod non-motile anaerobic bacteria which located in pilosebaceous gland mostly on faces, upper chests and backs[4], is one of the main pathogenesis factors.

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How to cite this article: Deewongkij P, Taweechotipatr M, Kamanamool N, Udompataikul M. In-vitro study of antibacterial activities of *Phyllanthus emblica* L. Leaves, *Punica granatum* L. Peels, and *Curcuma longa* L. rhizomes crude extracts to *Propionibacterium acnes* isolated from acne vulgaris patients. J Med Assoc Thai 2018;101:1169-76.

P. acnes itself can cause both inflammatory acnes and non-inflammatory acnes via stimulation of pro-inflammatory cytokines, innate immunity, humoral immunity, and complement system[5]. Also, there was a study showing that acne patients had more *P. acnes* colonization than normal[6].

Topical antibiotics are one of the treatment modalities that are commonly used against *P. acnes* in acne vulgaris patients[7]. Resistance of *P. acnes* was reported to be associated with monotherapy, misuse, and overuse of topical antibiotics[8]. According to the review articles, the resistant situation in many countries had been rapidly increasing including Thailand[9]. From 2002 study, the prevalence of the *P. acnes* resistance to clindamycin and erythromycin were 4.62% and 6.15%[10]. Surprisingly, the *P. acnes* resistance prevalence of 2016 study had become much higher with 62.66% resistant to clindamycin and 64% resistant to erythromycin[11]. The objective of the present research was to find an alternative medicine of herbs against acne vulgaris.

From past to present, there have been numerous researches on using herbs to treat diseases. Reviewing the literature of herbs, the authors found *Phyllanthus emblica* L., *Punica granatum* L., and *Curcuma longa* L. have phytochemicals such as tannins, flavonoids, and alkaloids which have antibacterial and anti-inflammatory action[12-14]. Some studies showed the three herbs could inhibit commercial *P. acnes* obtained from laboratory, but none had done with the *P. acnes* clinically isolated from the patients[15,16]. Additionally, in Thailand, many herbs are available and inexpensive. Hence, by choosing *Phyllanthus emblica* L., *Punica granatum* L., and *Curcuma longa* L. to be tested with the *P. acnes* isolated from the acne patients was the topic of the present research.

Materials and Methods

Plant materials

The three plant materials used in the present study were shown in Table 1. The plant materials were collected in Bangkok, Thailand, in October 2017.

Preparation of plant extracts

One-hundred grams of each plant material were washed with clean water, then cut into small pieces using knife. Dried them at 50°C for 72 hours in a hot air oven and grounded to powder using a grinder. Recorded the weight of the dried powder, each dried powder was extracted with ethanol in 1:4 ratio (the dried powder (g): ethanol (ml)) for 48 hours[19]. After the maceration, spun the extractions at 25 revolutions per minute [RPM] at 25°C with a spinning machine. Filtered each of the plant's extraction and stored in 4°C fridge. Repeated the process of the maceration with the leftover herbal waste again. After macerating and filtering twice, then evaporated the filtrates to dryness with rotary evaporator and rotary vacuum evaporator. Three plants crude extracts were obtained.

Microorganism and media

Using 75 specimens of the *P. acnes* clinically isolated from 75 acne vulgaris patients and stored in brain heart infusion broth with 20% glycerol at -80°C deep freezer in the microbiology department of the Faculty of Medicine, Srinakharinwirot University, to maintain the viability of bacteria[11]. Brought out 75 specimens of *P. acnes* from the deep freezer and cultured with brain heart infusion agar added 10% horse serum.

Antibacterial test methods

The antibacterial susceptibility test was performed using 75 specimens of the isolated *P. acnes* from Thai acne patients according to previous study[11]. Brain Heart Infusion [BHI] agar (Becton Dickinson, USA) and Brain Heart Infusion [BHI] broth (Becton Dickinson, USA) supplemented with 10% horse serum (GIBCO Invitrogen, England) were chosen to culture the *P. acnes* and incubated under anaerobic condition at 37°C for 72 hours. The authors prepared the *P. acnes* in appropriate broth by following the McFarland standard.

The agar well diffusion assay and the broth microdilution test were used for the antimicrobial activities of herb crude extracts. The agar well diffusion

Table 1. Plant materials used in the present study

Botanical name	Family	Local name	Part used	Ethnopharmacological uses
Curcuma longa L.	Zingiberaceae	ka-min-chan	Rhizomes	Antineoplastic, antiangiogenic, anti-apoptotic, cytotoxic, antithrombotic, immunomodulatory, wound healing, anti-stressor, anti- lithogenic and anti-diabetic actions[17]
Phyllanthus emblica L.	Phyllanthaceae	ma-kham-pom	Leaves	Asthma, sore throat, vomiting, hiccough, diarrhea, bleeding piles, gout and heart and bladder diseases[18]
Punica granatum L.	Lythraceae	tap-tim	Fruit peels	Diarrhea and in the treatment of inflammation and cancer[18]

assay was done by pouring the sterile culture media into each plate and left to dry. Then, the *P. acnes* (approximately 1.5×10^8 CFU/ml) was inoculated on the agar plates with three-dimensional swab method. Wells (4 mm in diameter) were made in media by using a of sterile cork borer. The crude extracts of each herb were diluted with 1% dimethyl sulfoxide [DMSO] to 5 mg/ml and 10 mg/ml. Then each herb with these two concentrations filled in each well (40 µl each). DMSO was used as a negative control. Thereafter, the agar plates were incubated at the condition as mentioned above. The diameters of inhibition zones around the well were measured in millimeters (mm) and were represented as no growth of *P. acnes*. This inhibition zone is described as the minimum inhibition zone [MIZ]. The agar well diffusion assay was done as to follow the previous research[20].

The minimum inhibitory concentration [MIC] was determined by broth microdilution method (NCCLS, 2017). A serial two-fold dilution of each crude extract were made in 96-well microtiter plates (Corning, USA) in a beginning concentration (5 mg/ml or 10 mg/ml) of the lowest concentration from the earlier result of the agar well diffusion assay that could inhibit the growth of *P. acnes*. The organism was adjusted to 0.5×10^6 CFU/ml and was combined in each well in equivalent volume of extract (50 µl). Then microtiter plates were incubated at the condition as described above. The method was performed in three separate experiments and the MIC was observed as the lowest concentration of extract that inhibited *P. acnes* growth. The MIC was reported in milligram per milliliter (mg/ml).

Preparing the formulas

The liquid formula of each herbal crude extracts was composed of glycerin, ethanol, sterile distilled water, and crude herbal extract. Then the agar well diffusion method was done by choosing the highest MIC from the earlier test. To exclude the antibacterial activity from ethanol, the negative control which had the same ingredients except the crude extract was formulated and compared. The agar well diffusion tests were performed in three separate experiments and the antibacterial activity was expressed as the MIZ.

Statistical analysis

Data were performed using the words statistics and data (Stata) version 14.0. Mean, Quantitative data was described by using mean, minimum, maximum, and standard deviation [SD]. Linear mixed-effects model and Wilcoxon rank-sum test were used to test the difference of quantitative data.

Ethics consideration

The present research was approved by the Srinakharinwirot University Ethical Committee. The certificate number is SWUEC/E-284/2560.

Results

One hundred grams of *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels, and *Curcuma longa* L. rhizomes could be extracted and yielded 18.58 g, 24.76 g, and 29.79 g, respectively. The ethanol crude extract of *Phyllanthus emblica* L. was capable of inhibiting the growth of all isolated *P. acnes* at the concentration of 5 and 10 mg/ml via agar well diffusion method. *Punica granatum* L. was also capable of inhibiting the growth of some isolated *P. acnes* at the concentration of 5 and 10 mg/ml via agar well diffusion method. On the other hand, *Curcuma longa* L. was unable to inhibit the growth of isolated *P. acnes* at any concentration of 5 and 10 mg/ml. The MIZs of each herbal crude extracts at 5, 10 mg/ml concentration to 75 specimens isolated *P. acnes* (mean ± SD) were shown in Table 2. Means of the MIZ of extraction of *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels and *Curcuma longa* L. rhizomes at concentration of 5 mg/ml and 10 mg/ml were 14.99±3.77 mm, 20.90±3.45 mm; 4.07±4.17 mm, 9.95±3.65 mm; 0±0 mm, and 0±0 mm, respectively (Table 3). Furthermore, means of the MIZ of *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels and *Curcuma longa* L. rhizomes to resistant *P. acnes* at 5 mg/ml and 10 mg/ml were 14.87±4.60 mm, 21.17±3.94 mm; 4.45±4.63 mm, 10.06±4.03 mm; 0±0 mm, and 0±0 mm, respectively. Means of the MIZ of *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels and *Curcuma longa* L. rhizomes to susceptible *P. acnes* at 5 mg/ml and 10 mg/ml were 15.15±2.15 mm, 20.52±2.63 mm; 3.54±3.42 mm, 9.78±3.11 mm; 0±0 mm, and 0±0 mm, correspondingly (Table 3). These implied that both *Phyllanthus emblica* L. and *Punica granatum* L. had polar compounds as their active ingredients. All three of herbal crude extracts were subsequently subjected to determination of the MIC values.

Determination of means of the MIC values of the ethanol extract of *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels and *Curcuma longa* L. rhizomes, to all clinical isolated *P. acnes*, were 0.559±0.685 mg/ml, 1.003±0.954 mg/ml, and 3.804±2.901 mg/ml, respectively (mean ± SD). Means of the MIC of *Phyllanthus emblica* L., *Punica*

Table 2. Antimicrobial activities of each plant extract in inhibition zone (mm) and MIC (mg/ml)

P. acnes number	Crude extract of *Phyllanthus emblica* L. leaves (mean)			Crude extract of *Punica granatum* L. peels (mean)			Crude extract of *Curcuma longa* L. rhizomes (mean)		
	MIZ (mm)		MIC (mg/ml)	MIZ (mm)		MIC (mg/ml)	MIZ (mm)		MIC (mg/ml)
	5 mg/ml	10 mg/ml		5 mg/ml	10 mg/ml		5 mg/ml	10 mg/ml	
1	18.50	19.50	0.625	11.35	13.60	1.250	-	-	0.625
2	12.50	21.75	0.078	8.25	13.25	1.250	-	-	0.313
3	17.00	21.00	0.313	10.00	12.75	0.625	-	-	1.250
4	15.75	21.75	0.313	8.75	11.75	1.250	-	-	0.625
5	16.00	21.00	0.313	9.00	10.00	1.250	-	-	0.078
6	23.00	30.00	0.313	11.00	14.50	1.250	-	-	1.250
7	16.00	20.25	0.313	8.25	11.50	1.250	-	-	2.500
8	14.50	20.50	0.313	9.25	12.75	0.625	-	-	1.250
9	20.50	24.00	0.313	11.25	14.00	0.625	-	-	2.500
10	20.50	25.75	0.313	10.25	13.00	0.625	-	-	1.250
11	19.00	28.00	0.625	11.00	13.75	0.625	-	-	1.250
12	22.25	29.25	0.625	8.75	13.25	0.625	-	-	1.250
13	23.50	30.00	0.625	11.50	16.00	1.250	-	-	2.500
14	16.25	21.75	0.313	8.00	12.75	0.625	-	-	2.500
15	15.00	22.75	0.313	8.75	11.50	0.625	-	-	1.563
16	16.50	22.50	0.313	10.50	12.50	0.625	-	-	1.250
17	17.50	24.50	5.000	7.75	12.50	7.500	-	-	5.000
18	0.00	18.50	0.625	-	-	0.313	-	-	5.000
19	15.75	19.25	0.313	4.00	10.50	0.078	-	-	0.078
20	15.00	19.00	0.469	5.00	10.50	0.156	-	-	3.750
21	12.50	15.50	0.625	-	9.50	0.469	-	-	3.750
22	6.00	14.00	0.156	-	4.50	0.625	-	-	3.750
23	14.25	19.25	0.117	-	12.00	0.313	-	-	5.000
24	12.00	15.25	0.156	-	9.50	0.039	-	-	0.703
25	9.00	13.50	0.469	-	3.50	0.313	-	-	10.000
26	14.75	19.00	0.098	-	-	0.039	-	-	0.117
27	14.50	21.50	0.078	-	11.50	0.078	-	-	0.156
28	12.50	18.00	0.078	-	5.00	0.156	-	-	1.875
29	7.00	16.00	0.059	-	9.50	0.313	-	-	7.500
30	10.25	14.75	2.500	-	-	3.750	-	-	10.000
31	6.00	14.75	0.117	-	-	0.117	-	-	3.750
32	17.50	22.00	0.078	8.00	12.00	0.313	-	-	0.078
33	11.50	20.75	2.500	-	9.25	2.500	-	-	10.000
34	15.00	21.00	0.313	-	10.50	0.625	-	-	5.000
35	15.25	23.25	0.313	-	10.75	1.250	-	-	5.000
36	13.50	23.50	0.625	-	10.25	1.250	-	-	10.000
37	15.75	23.50	1.250	3.75	11.00	1.250	-	-	5.000
38	14.25	22.00	0.313	-	9.50	1.250	-	-	5.000
39	15.50	22.50	0.313	-	11.75	0.938	-	-	5.000
40	17.00	22.00	0.313	-	11.00	1.250	-	-	1.875
41	15.75	21.00	0.625	7.75	10.75	1.250	-	-	5.000
42	12.50	22.25	0.313	-	8.00	0.625	-	-	5.000
43	15.25	21.00	0.313	3.50	10.50	0.938	-	-	5.000
44	21.75	24.00	0.469	-	12.00	0.625	-	-	5.000
45	16.50	22.50	0.625	4.50	11.00	1.250	-	-	5.000
46	17.75	20.00	0.625	9.00	12.00	1.250	-	-	10.000
47	15.25	21.75	0.625	4.25	11.25	1.250	-	-	5.000
48	13.25	18.00	0.157	3.25	10.00	0.938	-	-	2.500
49	17.50	21.50	0.313	7.75	11.25	0.313	-	-	10.000
50	16.00	22.25	1.250	-	-	1.250	-	-	10.000
51	18.50	22.25	0.157	9.00	12.75	0.625	-	-	2.500
52	13.75	19.00	0.469	-	4.50	1.250	-	-	5.000
53	16.75	20.50	0.235	4.25	10.00	0.625	-	-	2.500
54	13.50	23.50	0.625	4.25	9.25	1.250	-	-	10.000
55	15.00	20.50	0.313	4.25	9.00	1.250	-	-	5.000
56	17.25	20.50	0.313	-	9.00	0.938	-	-	5.000
57	9.75	12.00	0.313	9.00	11.25	0.938	-	-	10.000
58	16.75	23.75	0.313	4.25	11.75	0.625	-	-	5.000
59	13.00	20.00	0.625	4.00	8.75	1.250	-	-	5.000
60	15.75	21.50	0.313	8.25	11.00	0.625	-	-	3.750
61	15.50	22.50	0.313	-	11.50	0.625	-	-	1.250
62	14.75	20.50	0.469	9.75	12.75	1.250	-	-	1.875
63	12.75	18.25	1.250	-	10.75	1.250	-	-	5.000
64	13.50	18.00	1.250	-	10.25	1.250	-	-	5.000
65	12.50	15.75	1.250	-	-	1.250	-	-	5.000
66	14.50	17.50	0.313	-	8.00	1.250	-	-	0.625
67	16.50	23.00	0.625	-	11.25	1.250	-	-	1.250
68	16.00	21.75	0.625	-	9.50	1.875	-	-	2.500
69	14.00	20.00	0.625	-	10.00	1.250	-	-	1.250
70	10.25	17.00	1.250	4.50	9.00	1.250	-	-	1.250
71	17.50	22.00	0.625	-	13.00	1.875	-	-	3.750
72	18.00	24.00	0.157	3.50	10.75	0.625	-	-	0.938
73	16.00	22.75	0.313	8.75	10.50	0.625	-	-	3.750
74	16.75	23.00	0.625	3.50	13.00	0.625	-	-	3.750
75	15.00	20.50	0.313	3.75	10.25	0.625	-	-	2.500

MIC = minimum inhibitory concentration; MIZ = minimum inhibitory zone; - = no zone inhibition; - = no inhibitory activity at the highest cut off concentration at 10 mg/ml

Table 3. Antimicrobial activities of each plant extract in inhibition zone (mm)

Crude extracts	*P. acnes*	MIZ at 5 mg/ml (mean ± SD)	MIZ at 10 mg/ml (mean ± SD)
Phyllanthus emblica L. leaves	Overall *P. acnes* (n = 75)	14.99±3.77	20.90±3.45
	Resistant *P. acnes* (n = 44)	14.87±4.60	21.17±3.94
	Susceptible *P. acnes* (n = 31)	15.15±2.15	20.52±2.63
Punica granatum L. peels	Overall *P. acnes* (n = 75)	4.07±4.17	9.95±3.65
	Resistant *P. acnes* (n = 44)	4.45±4.63	10.06±4.03
	Susceptible *P. acnes* (n = 31)	3.54±3.42	9.78±3.11
Curcuma longa L. rhizomes	Overall *P. acnes* (n = 75)	-	-
	Resistant *P. acnes* (n = 44)	-	-
	Susceptible *P. acnes* (n = 31)	-	-

MIZ = minimum inhibitory zone; - = no zone inhibition; - = no inhibitory activity at the highest cut off concentration at 10 mg/ml

Well diameter 4 mm

granatum L., and *Curcuma longa* L. to resistant *P. acnes* were 0.560±0.850 mg/ml, 0.970±1.211 mg/ml, and 3.394±2.863 mg/ml, respectively. Means of the MIC of *Phyllanthus emblica* L., *Punica granatum* L. and *Curcuma longa* L. to resistant *P. acnes* were 0.560±0.850 mg/ml, 0.970±1.211 mg/ml, and 3.394±2.863 mg/ml, subsequently. Additionally, means of the MIC of *Phyllanthus emblica* L., *Punica granatum* L., and *Curcuma longa* L. to susceptible *P. acnes* were 0.557±0.348 mg/ml, 1.048±0.375 mg/ml, and 4.385±2.901 mg/ml, correspondingly (Table 4). Table 2 showed the MIC values to 75 specimens of isolated *P. acnes* (mean ± SD). The difference of antibacterial activity of each plant extraction using the MIC values were shown in Table 4, among the extraction of *Phyllanthus emblica* L. leaves and *Punica granatum* L. peels were no statistically significant all three groups; overall *P. acnes*, resistant *P. acnes*, and susceptible *P. acnes* (p = 0.092, 0.214, and 0.240, correspondingly, by using Linear mixed-effects model). There was statistically significant in all three groups of *P. acnes* between the extraction of *Phyllanthus emblica* L. leaves and *Curcuma longa* L. rhizomes (p<0.001 all). There was also statistically significant among the extraction of *Punica granatum* L. peels and *Curcuma longa* L. rhizomes in all three groups (p<0.001 all).

After collecting all the MIC data, generating the lotion type products for each of plant extraction was done. *Phyllanthus emblica* L. leaves product was green, turbid, and strong smell solution. It had pH 3 with no sedimentation and after applied the solution to skin it penetrated within 10 seconds. It also did not leave the stain after applying. *Punica granatum* L. peels product was pale yellow, turbid and no smell solution. It had pH 5 with no sedimentation and after applied the solution to skin it penetrated within 10 seconds. It also leaved no stain on the skin. *Curcuma longa* L. rhizomes product was brick colored, turbid, and strong smell solution. It had pH 3 with sedimentation and after applied the solution to skin it penetrated within 5 seconds. It leaved the stain after applying.

Choosing each of the *P. acnes* from both resistant *P. acnes* group and susceptible *P. acnes* group based on the highest MIC values from earlier experiment. The MIZ values were shown in Table 5. There was no statistically significance between *Phyllanthus emblica* L. leaves product and the negative control (p = 0.053,

Table 4. Antimicrobial activities of each plant extract in MIC (mg/ml)

P. acnes	Crude extracts	MIC (mean ± SD)	*p*-value*
Overall *P. acnes* (n = 75)	*Phyllanthus emblica* L. leaves	0.559±0.685	Reference
	Punica granatum L. peels	1.003±0.954	0.092
	Curcuma longa L. rhizomes	3.804±2.901	0.001
Resistant *P. acnes* (n = 44)	*Phyllanthus emblica* L. leaves	0.560±0.850	Reference
	Punica granatum L. peels	0.970±1.211	0.214
	Curcuma longa L. rhizomes	3.394±2.863	<0.001
Susceptible *P. acnes* (n = 31)	*Phyllanthus emblica* L. leaves	0.557±0.348	Reference
	Punica granatum L. peels	1.048±0.375	0.240
	Curcuma longa L. rhizomes	4.385±2.901	<0.001

MIC = minimum inhibitory concentration

* Result from Linear mixed-effects model

Table 5. Antimicrobial activities of each plant extract in lotion form in inhibition zone (mm)

Types of crude extract in lotion form	*P. acnes*	MIZ	*p*-value**
Phyllanthus emblica L. leaves	Resistant *P. acnes*	25.33	0.053
	Susceptible *P. acnes*	18.00	
Negative control*	Overall *P. acnes*	-	
Punica granatum L. peels	Resistant *P. acnes*	22.00	0.053
	Susceptible *P. acnes*	12.50	
Negative control*	Overall *P. acnes*	-	
Curcuma longa L. rhizomes	Resistant *P. acnes*	-	-
	Susceptible *P. acnes*	-	
Negative control*	Overall *P. acnes*	-	

MIZ = minimum inhibitory zone (mm); - = no zone inhibition; - = no inhibitory activity at the highest cut off concentration at 10 times of MIC

Well diameter 4 mm, * Negative control: lotion form without crude extracts, ** Result from Wilcoxon rank-sum test

Table 6. Comparing the antibacterial activity of plant extractions' lotion form

Types of crude extract in lotion form	*P. acnes*	MIZ (mm)	*p*-value*
Phyllanthus emblica L. leaves	Resistant *P. acnes*	25.33	0.439
	Susceptible *P. acnes*	18.00	
Punica granatum L. peels	Resistant *P. acnes*	22.00	
	Susceptible *P. acnes*	12.50	

MIZ = minimum inhibitory zone

Well diameter 4 mm, * Result from Wilcoxon rank-sum test

by using Wilcoxon Rank-Sum test) as well as *Punica granatum* L. peels product and the negative control (p = 0.053). Although, *Curcuma longa* L. rhizomes product was not capable of inhibiting the growth of *P. acnes* due to the aforementioned reason. On top of that, comparing between *Phyllanthus emblica* L. leaves product and *Punica granatum* L. peels product, there was no statistically significance of the antibacterial activity (p = 0.439, Table 6).

Discussion

For the qualitative method of antimicrobial activity of the crude extracts (MIZ), *Phyllanthus emblica* L. and *Punica granatum* L. could inhibit *P. acnes* growth. On the other hand, *Curcuma longa* L. was no zone of inhibition assuming that the active ingredient of *Curcuma longa* L. was non-polar but the components of brain heart infusion agar were polar. The brain heart infusion agar is composed of dextrose, disodium hydrogen phosphate, sodium chloride, deionized water, agar, Brain Heart Infusion from (solids), peptic digest of animal tissue, and pancreatic digest of casein. *Curcuma longa* L. rhizome has curcumin as a main phytochemical that has an antimicrobial effect. Curcumin is a lipophilic substance whereas brain heart infusion agar is composed of polar substances as mentioned; therefore, curcumin could not diffuse through the agar[17,21]. *Phyllanthus emblica* L. leaf has tannins (chebulinic acid, chebulagic acid) which are hydrophilic substances[22]. *Punica granatum* L. peel has hydrolysable tannins as the major components: punicalin, pedunculagin, and punicalagin which are hydrophilic substances[23]. With experimental results on agar well diffusion assay, tannins have antibacterial activity[24].

For the quantitative evaluation of antimicrobial activity of the crude extracts (MIC), *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels, and *Curcuma longa* L. rhizomes could inhibit clinical isolated *P. acnes* growth. *Phyllanthus emblica* L. had the highest potency of antimicrobial effect and followed by *Punica granatum* L. The lowest potency was *Curcuma longa* L. There was no statistical significant difference between *Phyllanthus emblica* L. and *Punica granatum* L.. However, there were statistical significant difference between *Phyllanthus emblica* L. and *Curcuma longa* L. as well as *Punica granatum* L. and *Curcuma longa* L.

The clinical isolated *P. acnes* used in the present research were isolated from 75 Thai acne patients from the previous study. The main advantage of this isolated *P. acnes* is it better represents the current population than the commercial *P. acnes*. Moreover, the antibacterial activities of these three herbs have never been investigated with clinical isolated *P. acnes*. The MIC results of *Phyllanthus emblica* L., *Punica granatum* L., and *Curcuma longa* L. from the previous studies were 2.500 mg/ml, 0.156 mg/ml, and 0.024 mg/ml, respectively[15,25]. The results were opposite to the present study. This was apparently due to population-based *P. acnes*. Apart from this, these three herbs can eventually be used as an alternative treatment for today's acne patients.

Producing the lotion products was to confirm the antimicrobial effect of each crude extract that could inhibit *P. acnes* growth, not the component of ethanol. The MIZ results showed that *Phyllanthus emblica* L.'s and *Punica granatum* L.'s lotion could inhibit *P. acnes* growth compared to negative control, but there was no zone of inhibition around turmeric's lotion. The cause of inability to inhibit the bacteria was as mentioned earlier. The quality of the crude extract lotions revealed the unpleasant texture and smell, because the crude extract was not the pure active ingredient of herb.

Conclusion

Among the three samples tested, the crude extract of *Phyllanthus emblica* L. leaves had the best antimicrobial effect in term of bacteriostatic activity against the clinical isolated *P. acnes* followed by *Punica granatum* L. peels and *Curcuma longa* L. rhizomes.

The active ingredient of *Curcuma longa* L. rhizomes crude extract had lipophilic property, whereas *Phyllanthus emblica* L. leaves and *Punica granatum* L. peels crude extracts had hydrophilic properties. Until now, there have been no studies that used these three herbs to test their antimicrobial effects on the population-based *P. acnes*. *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels, and *Curcuma longa* L. rhizomes could be an alternative treatment for acne that will suit current population. For further study, it

is recommended to analyze the active ingredients of these three herbs and to develop the usable products. In the future clinical phase study should be done.

What is already known on this topic?

Phyllanthus emblica L. leaves, *Punica granatum* L. peels, and *Curcuma longa* L. rhizomes are known to contain many phytochemicals which have antibacterial activity. These three herb crude extracts could inhibit the growth of standard *P. acnes*.

What this study adds?

Crude extracts of *Phyllanthus emblica* L. leaves, *Punica granatum* L. peels, and *Curcuma longa* L. rhizomes could inhibit the growth of *P. acnes* isolated from Thai acne vulgaris patients. These three herbs maybe an alternative medicine for acne vulgaris that suit the current population.

Acknowledgement

The authors would like to thank the Faculty of Medicine, Srinakharinwirot University for providing the grant for the present study, and all the staffs of the Microbiology Department, Srinakharinwirot University for helping in this study.

Potential conflicts of interest

The authors declare no conflict of interest.

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