A Double-blind, Randomized Controlled Trial of Ciplukan (*Physalis angulata Linn*) Extract on Skin Fibrosis, Inflammatory, Immunology, and Fibrosis Biomarkers in Scleroderma Patients

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ABSTRAK

Latar belakang: *skleroderma merupakan penyakit autoimun yang resisten terhadap pengobatan standar, penambahan ekstrak herba ciplukan (Physalis angulata Linn) diduga dapat memperbaiki fibrosis kulit skleroderma. Penelitian ini bertujuan mengkaji peran ekstrak herba Ciplukan sebagai terapi ajuvan untuk fibrosis kulit skleroderma yang mendapat terapi standar, berdasarkan MRSS, biomarker inflamasi, imunologi dan fibrosis serum.* ***Metode:*** *uji klinis acak tersamar ganda pada pasien skleroderma stabil yang berobat jalan di RSCM dan RSHS sejak November 2015−Maret 2017 yang memenuhi kriteria inklusi dan menerima terapi standar. Subjek secara random terbagi dua: kelompok uji yang mendapat ekstrak herba C iplukan 3x 250 mg/hari selama 12 minggu dan kelompok plasebo. Pemeriksaan MRSS, LED, P1NP, BAFF dan sCD40L dilakukan setiap 4 minggu hingga akhir penelitian.* ***Hasil:*** *lima puluh sembilan subjek menyelesaikan penelitian, 29 subjek kelompok uji dan 30 subjek kelompok plasebo, rerata usia 41 (SB 9) tahun, proporsi wanita : pria = 9 : 1. Ditemukan perbaikan fibrosis kulit bermakna pada kelompok uji dengan penurunan relatif MRSS sebesar 35,9% dibandingkan plasebo 6,3% dengan p < 0,001 dan penurunan relatif bermakna kadar P1NP sebesar 17,8% dibandingkan plasebo 0,7% dengan p = 0,002. Tidak ditemukan penurunan kadar LED, BAFF dan sCD40L pada kedua kelompok. Terdapat korelasi positif bermakna antara MRSS dengan kadar P1NP (r = 0,236, p = 0,036).* ***Kesimpulan:*** *pemberian ekstrak etanol herba ciplukan dosis 3 x 250 mg selama 12 minggu sebagai terapi ajuvan pada skleroderma dalam terapi standar, secara klinis dan statistik menunjukkan perbaikan kelainan fibrosis kulit berdasarkan MRSS dan biomarker fibrosis P1NP serum secara bermakna dibandingkan kontrol.*

Kata kunci: *Physalis angulata Linn, modified Rodnan's skin score (MRSS), erythrocyte sedimentation rate (ESR), procollagen type-1 N terminal proteinase (P1NP), skleroderma.*

ABSTRACT

Background: *scleroderma is an autoimmune disease characterized by organ fibrosis, resistant to standard treatment. It is suggested that he addition of Physalis angulata Linn. (Ciplukan) extract as adjuvant therapy can*

improve the scleroderma skin fibrosis. The aim at this study is to evaluate the effect of ciplukan extract as adjuvant on scleroderma skin fibrosis in standard therapy, based on modified Rodnan skin scale (MRSS), inflammatory biomarkers, immunology and serum fibrosis. ***Methods:*** *double-blind, randomized clinical trial was performed in scleroderma patients with stable disease at Cipto Mangunkusumo hospital and Hasan Sadikin hospital during November 2015− March 2017 who met the selection criteria and continued to receive standard therapy. The subjects were randomly allocated into two groups: the study group received the ciplukan extract 3 x 250 mg / day for 12 weeks and the placebo group. Examination of MRSS, ESR, P1NP, BAFF and sCD40L was performed every 4 weeks until the end of the study.* ***Results:*** *fifty-nine subjects completed the study. They consisted of 29 subjects of the treatment group and 30 of the placebo group, with an average age of 41 (SD 9) years, the proportion of women: male = 9 : 1. There was a significant improvement of skin fibrosis in the study group with a highly significant decrease in MRSS (35.9% VS 6.3%, p <0.001) and a relative decrease in P1NP levels (17.8% VS 0.7%, p = 0.002). No decrease in ESR, BAFF and sCD40L levels were observed in both groups. There was a weak but significant positive correlation between MRSS with P1NP levels (r=0.236, p=0.036).* ***Conclusion:*** *Ciplukan extract with dose 3 x 250 mg for 12 weeks as adjuvant on scleroderma standard therapy alleviates skin fibrosis significantly based on MRSS and P1NP levels.*

Keywords: *Physalis angulata Linn, modified Rodnan's skin score (MRSS), erythrocyte sedimentation rate (ESR), procollagen type-1 N terminal proteinase (P1NP), scleroderma.*

INTRODUCTION

Scleroderma is an autoimmune connective tissue disease involving fibrosis of the skin, which is characterized by the excessive accumulation of extracellular matrix (ECM) proteins, vascular injuries, and immune abnormalities. In early stages of scleroderma, activated fibroblasts in the affected areas produce high amounts of collagen.[1] Numerous studies have demonstrated the crucial role of several fibrogenic cytokines released from immunocytes in initiating the sequence of events leading to fibrosis in the pathogenesis of scleroderma.[1,2] Moreover, vascular injury and apoptosis, extracellular matrix overproduction, oxidative stress (reactive oxygen species) also played role.[1,2,3-5] The standard therapy for scleroderma is modifying disease anti-rheumatic drugs (DMARD).[6] However, there are some problems such as high risk of drugs resistance; drug side effects, and price of the drug.[2,6] Some immunosuppresant agents are not covered by the national health insurance. Therefore, some patients discontinue the treatment, resulting in disease progresses, higher morbidities, and mortality. Some new medicines are still under research and not accessible. Others are too expensive for most of Indonesian communities.

Ciplukan herb (Physalis angulata Linn.) is known widely as a medicinal plant. It grows in Indonesia and has been known for its efficacy to stimulate the activity of lymphocyte, and modulate the immune system in human.[7-8] Ciplukan herb is also known for its antiinflamatory, antiproliferative, anti-angiogenesis, and anti-cancer activities because it contains phenol, secosteroid (physalin, withanguline, and others), and saponin. It has three major pathways in immunomodulatory. The first pathway is phenol components in Ciplukan herb, such as flavonoid, tannin, phenylpropane, and other simple phenol compounds which show antioxidant and immunomodulating effects that control the autoimmune activity.[8-10] The second and third pathways are due to secosteroid and saponin compounds which have anti-inflammatory and antiproliferative effects that is thought to inhibit the fibrosis process in scleroderma.[10-12] Ciplukan herb is proved to be safe in human. No side effects were reported in long-term use.[8-9]

There are no published clinical trials regarding the effects of ciplukan herb on scleroderma, in terms of skin fibrosis changes based on MRSS and the level of biomarkers serum in scleroderma. The aim of this study to evaluate the effect of the addition ciplukan extract as adjuvant to scleroderma standard therapy in suppressing inflammatory, immunological, and fibrosis processes to accelerate clinical improvement of skin fibrosis based on MRSS in

scleroderma patients (primary outcome).[13-14] We evaluated disease activity based on erythrocyte sedimentation rate for inflammatory biomarker; the levels of B-cell activation factor (BAFF) and soluble CD40 ligand (sCD40L) serum for immunological biomarkers; and the level of procollagen Type I N-Terminal Propeptide (P1NP) serum for biomarker of fibrotic process (secondary outcome).[15-22]

METHODS

This was a randomized, double blind, placebo controlled trial. Subjects were recruited consecutively from January 2016 to March 2017. This study has been approved by the Ethical Research Comission of Faculty of Medicine Universitas Indonesia and Research unit of Cipto Mangunkusumo Hospital (25/UN2.F1/ETIK/2016).

Subjects

Inclusion criteria were limited and diffused type of scleroderma patients in rheumatology outpatient clinic in Cipto Mangunkusumo Hospital (RSCM) or Hasan Sadikin Hospital (RSHS) Bandung, aged 15−60 years old, received standard therapy with stable dose in the last 3 months. Exclusion criteria were not willing to participate in the study, impaired liver function or cirrhosis, impaired renal function, overlap syndrome, active tuberculosis, heart disease, allergy to Ciplukan herb or history of hypersensitivity to certain drugs, hypotension and hypoglycemia.

Subjects with consent for clinical trials were randomly assigned into 2 groups: intervention group (ciplukan herb 250 mg TID) or control group (placebo containing amylum 250 mg TID). Subjects were observed for 12 weeks. Allocation was done using block permuted randomization technique with block size combination 4. The result was put in sealed envelopes. The researchers, responsible physicians, and study subjects did not know the allocation group until the end of the study (double-blind).

Data Collection and Analysis

Collected data included demographic data (age, sex, weight, height, blood pressure), clinical data (duration of illness, history of illness and medication), skin fibrosis examination based on MRSS, and laboratory examination: ESR, the levels of P1NP, BAFF and sCD40L serum, complete blood count, transaminase enzyme, creatinine, blood glucose, sodium, potassium and urinalysis. All data were collected every 4 weeks during the visit. We also assessed subjects' compliance and adverse event during treatment.

Normally distributed data was presented in mean (SD); otherwise, presented in median (interquartile range). A 95% confidence interval (95% CI) was calculated around scleroderma-prevalence. Comparison between groups was done using Mann-Whitney U test. Data were analyzed using SPSS program. Drop out subjects were removed from the analysis (per protocol) to see the main output. Efficacy of the drugs was represented using the result of intention to treat (ITT) and number needed to harm (NNH). The main outcome of clinical assessment, improvement of skin fibrosis based on MRSS was analyzed using unpaired t-test, to assess the different effects between ciplukan herb group and placebo group. For the secondary outcome, numeric variables such as the level of ESR, BAFF, sCD40L, P1NP, and the relative changes of those variables after 12 weeks of treatment were analyzed using Wilcoxon test.

This study was conducted based on the principles of the "Guideline for Good Clinical Practice" of the ICH Tripartite Guideline (ICH-GCP). This study has been registered in the registry of clinical trial data at www.clinicaltrials.gov with ClinicalTrials.gov ID: NCT03141125 identification number to ensure the public scientific knowledge of this research.

RESULTS

Baseline Characteristics

There were 61 subjects randomly allocated into 2 groups. After 12 weeks of observation, one subject dropped out in ciplukan group due to loss to follow-up. In placebo group, one subject dropped out due to severe hypoglycemia. (**Figure 1**)

From 59 subjects, 92.1% were females with mean age of 41 (9) years old. Duration of scleroderma was 12 months to 88 months

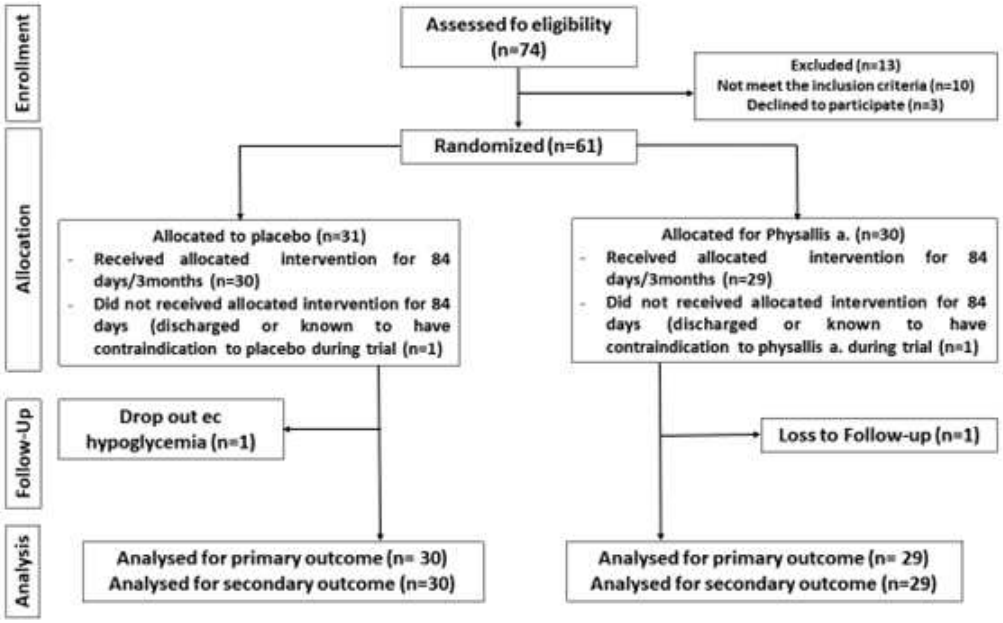

Figure 1. Flow of subjects

with the median of 34 months. Subjects were categorized into 35 subjects (59%) diffuse type scleroderma and 24 subjects (41%) limited type scleroderma. Diffuse type scleroderma were higher in intervention group (65.5%) than in placebo group (53.3%), the other subjects were categorized into limited-type scleroderma. (**Table 1**)

The most common encountered ACR EULAR diagnostic criteria 2013 clinical symptoms were finger fibrosis (100%), and Raynaud's phenomenon (100%), followed by scars in 36 (61%) subjects, finger ulcers in 32 (54%) subjects, salt and pepper appearance in 32 (54%) subjects, finger edema in 54 (91.5%) subjects, and telangiectasia in 17 (28.8%) subjects.

Initial assessment of modified Rodnan's Skin Score (MRSS) showed higher score in ciplukan group (median 19, range 8−34) compared to placebo group (median 15, range 6−36). This is caused by the diffuse type of scleroderma subjects in ciplukan group were higher than in placebo group with the ratio of 19:16. This is consistent with the extent of skin fibrosis predilection in diffuse type than in the limited type of scleroderma.

The result of laboratory tests at the baseline of the study were as followed: the average of ESR was 38 (22) mm/hour; P1NP level was 56.8 (31.8) ng/mL; BAFF level was 1,258 (5,473) pg/mL; and serum sCD40L level was 6,505 (3,415) pg/mL.

Response to Ciplukan Herb

Changes in skin abnormalities in the study subjects marked by a decrease in MRSS were shown in **Figures 2** and **3**.

MRSS decreased 35.9% in the ciplukan group, while it only decreased 6.3% in placebo group after 12 weeks. It means that Ciplukan herb can improve skin fibrosis clinically (**Figure 4**).

Further decrease of serum P1NP levels were also found in ciplukan herb group (17.8%) compared to the placebo group (0.7%) after 12 weeks (**Figure 5**). The response was better than the standard therapy alone.

There were no significant changes of the ESR value, serum BAFF, and sCD40L level in both groups. However we found that in placebo group, those values tend to increase compared to the baseline, but it was not statistically significant.

Bivariate analysis of skin fibrosis based on MRSS showed a weak correlation with serum P1NP level ($r = 0.236$; $p = 0.036$).

DISCUSSION

The previous study at Laboratorium Penelitian dan Pengujian Terpadu Universitas

Table 1. Baseline characteristics

Characteristic	*Physalis angulata* Group (n = 29)	Control Group (n =30)
Mean age, years (SD)	41 (9)	41 (9)
Gender, n (%)		
- Male	0 (0.0)	2 (9.5)
- Female	29 (100)	29 (90.5)
Type of Scleroderma, n (%)		
- Limited type	10 (34.5)	14 (46.7)
- Difuse type	19 (65.5)	16 (53.3)
Onset of the disease (months), median (IQR)	34 (12–88)	31 (13–72)
BMI (kg/m^2), mean (SD)	20.7 (3.7)	20.1 (2.5)
Standard Therapy, n (%)		
- metothrexate	28 (96.6)	28 (93.3)
- sulphasalazine	1 (3.4)	1 (3.3)
- mycophenolat mofetil	1 (3.4)	1 (3.3)
- colchicine	2 (6.8)	2 (6.6)
- nifedipin	12 (41.4)	14 (46.7)
- diltiazem	6 (20.7)	5 (16.7)
- amlodipine	11 (37.9)	10 (33.3)
- methylprednisolon: < 10 mg/day	15 (51.7)	16 (53.3)
- aspirin	24 (82.7)	22 (73.3)
- clopidogrel	3 (10.3)	4 (13.3)
- cilostazol	2 (6.8)	1 (3.3)
- folic acid	28 (96.6)	28 (93.3)
MRSS, median (IQR)	19 (8–34)	15 (6–36)
ESR (mm/hour), mean (SD)	35 (19)	40 (25)
BAFF (pg/mL), mean (SD)	1,132 (435)	1,380 (621)
P1NP (ng/mL), mean (SD)	50.5 (24.5)	62.9 (37)
sCD40L (pg/mL), mean (SD)	6,870 (4,125)	6,151 (2,576)

SD= Standard deviation; ESR= Erythrocyte sedimentation rate; MRSS= Modified Rodnan's Skin Score; BAFF= β cell activating factor; P1NP= Procollagen type-1 N terminal proteinase; sCD40L= soluble cluster of differentiation 40 Ligand.

Gadjah Mada (LPPT UGM) Yogyakarta, Ciplukan herb contained unspecific compounds, such as: Betasitosterols, tannins, phenols, saponins, flavonoids, and alkaloids.

Phenolic compounds like flavonoid, tannin, phenilpropan, and other phenols have immunomodulatory activity with complement

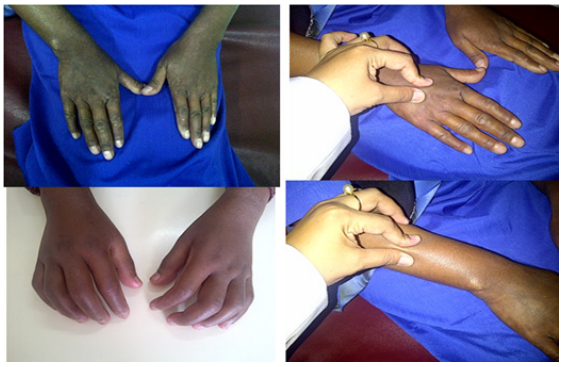

Figure 2. Skin of scleroderma patients before study.

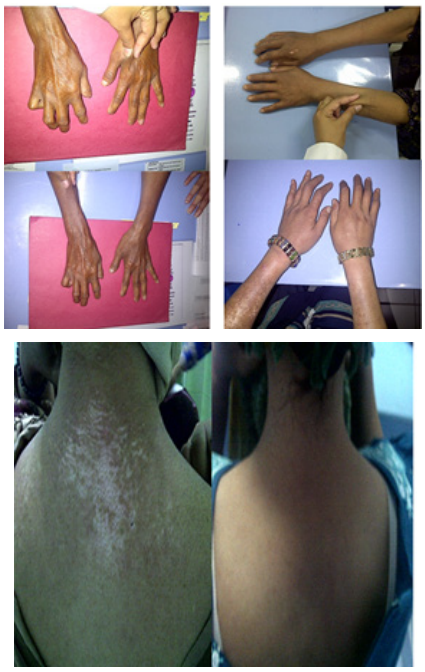

Figure 3. The skin of a scleroderma patient after completion of clinical trials (archive after open label) in the Ciplukan group.

system or intracellullar biochemistry reaction. Ethanol extract is capable of extracting more phenols, flavonoids, and tannins than methanol and hexsane do. This study used 50% ethanol extract, which contains the highest total phenols compared to other solvents. The saponins component in herbal plants is known to have a potentiating effect on chemotherapy drugs such as methotrexate.[25]

Several studies have shown that ethanol extract from ciplukan herb has a strong antioxidant effect due to the flavonoid content. Phenol derivatives of ciplukan herb have an important role as natural theurapeutic substances that can inhibit oxidative stress in chronic

inflammatory diseases such as scleroderma.[26-27] Inflammatory processes and oxidative stress have been shown to play a role in the pathogenesis of scleroderma. The antioxidant ability of phenols can also protect lymphocytes from the adverse effects of reactive oxygen molecules. The component is soluble in a polar solvent, inducing leukocytes proliferation, increased lymphocytes proliferation is influenced by several factors such as the phenols content attached to proteins forming a protein complex that binds to the hydrogen ions. Sitosterol in ciplukan herb, has a strong anti-inflammatory effect and may contribute to the clinical improvement of scleroderma patients.[11-12]

The exact role of proinflammatory markers in ciplukan herb such as thromboxane B2 (TXB2) and leukotriene B4 (LTB4) remains to be elucidated. TXB2 has the ability to increase platelet aggregation and LTB4 has a chemotaxis effect on neutrophils, causing direct damage to the cells.[12] Other proinflammatory markers that need further investigation are Interleukin-6 and C-reactive protein (CRP), which increase in the inflammatory phase. Assessment of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activities may also be considered for follow-up studies, because both of them are involved in the inflammatory process. Inhibition of this COX enzyme causes a decrease in arachidonic acid levels, by alteration of arachidonic acid into eicosanoid, prostaglandin and thromboxane in the inflammatory pathway.[11,12]

There are several novelties and strengths in this study. First, to the best of our knowledge, this is the first clinical trial in the world that investigated the effects of ciplukan herb extract as adjuvant therapy, i.e. adding traditional herb to the standard therapy on scleroderma patients.

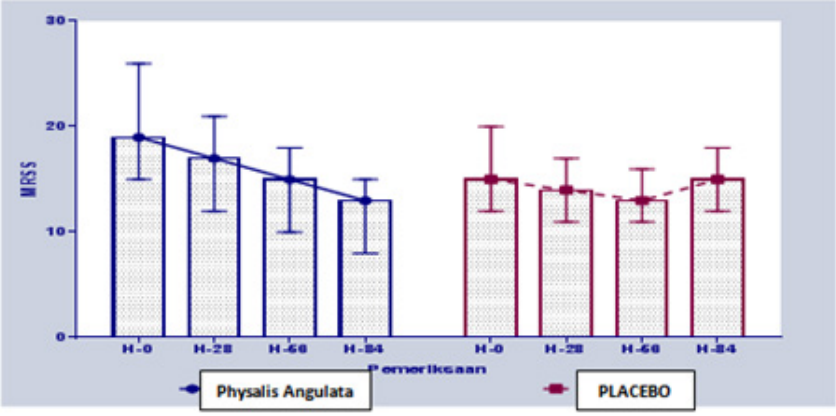

Figure 4. Difference of MRSS changes from *Physalis angulata* and placebo group.

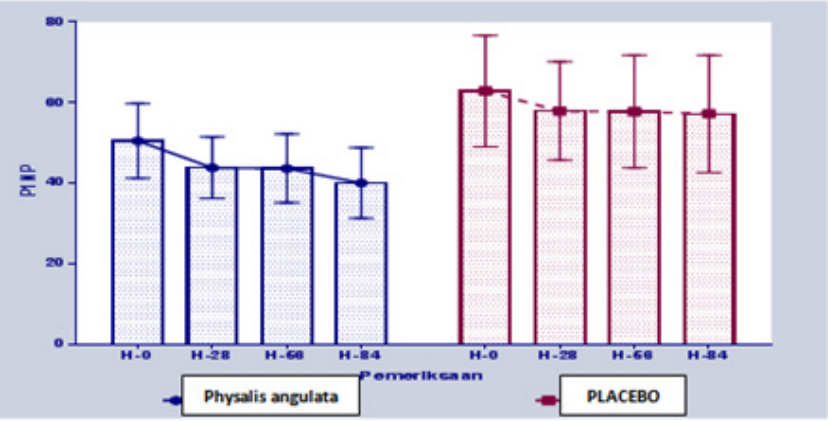

Figure 5. Difference of P1NP changes from *Physalis angulata* and Placebo group.

Second, ethanol extract has the highest content of phenols, flavonoids, tannins compared to methanol extract and hexane extract.[8,9] Third, in this ciplukan extract contains saponins substances that are not found in ciplukan plants abroad.[16] Fourth, clinical and laboratory data support antifibrotic effects of ethanol extract of ciplukan herb for scleroderma. The addition of ciplukan herb has been proven to accelerate clinical improvement based on decreased MRSS values in assessing skin fibrosis. In general, standard scleroderma treatment needs longer time to reduce MRSS values from the baseline, up to 2 years.[13,14] In this study, the reduction of MRSS to 6 points lower than the baseline was achieved within 3 months. We also found a significant decrease in serum P1NP level clinically and statistically as the evidence of fibrosis process of improvement.

Eventhough the correlation was weak between MRSS and P1NP levels, our finding supports the theory that P1NP has a role in skin fibrosis process of scleroderma patients who show an excessive synthesis of collagen and release its metabolite products to the circulation. We found no correlation between MRSS and the result of ESR value, serum BAFF and CD40L levels in both groups. ESR, as the biomarker for inflammation used in this study, failed to indicate the presence of inflammation process in scleroderma due to less sensitive. Further study using other inflammatory markers are needed to prove the anti-inflamation effects of Ciplukan herb. There were no significant changes of serum BAFF and sCD40L levels.

The outcome of this study was analyzed per protocol. Although there were 2 subjects excluded from the study, the number of our study subjects (29 and 30 for the treatment and the control group, respectively) still exceeded the minimum sample size from the calculated sample size, i.e. at least 27 subjects for each group. The number of samples in this study has met the criteria, thus the result of the research outcomes analysis is valid, with good research power (80%).

The ciplukan extract is suggested to have synergic effects as antifibrotic on skin scleroderma, by its antioxidant, antiinflammatory, antiproliferative effects, and saponins content in ciplukan herb may also have a similar effect to methotrexate action, with the end result of clinical improvement of scleroderma skin fibrosis.[10-12,25-27]

CONCLUSION

Our results suggested that administration of 3x250 mg per day dose of ciplukan herb extract as adjuvant therapy in scleroderma standard therapy for 12 weeks, was statistically significant and clinically important in alleviating skin fibrosis based on assessment of MRSS and P1NP levels.

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