A randomized, double-blind, controlled study on the safety and efficacy of 25% Tinospora rumphii (Makabuhay) cream versus 2% mupirocin cream on superficial pyodermas caused by Staphylococcus aureus

Grace Monica Ibaviosa, MD, DPDS[a], Johannes Dayrit, MD, FPDS[b], Ma. Teresita G. Gabriel, MD, FPDS[b], Gracia B. Teodosio, MD, FPDS[b], Cara Lynn Chia, MD[a]

ABSTRACT

BACKGROUND: Superficial pyoderma is an infection most commonly caused by Staphyloccoccus aureus. The drug of choice is 2% mupirocin cream. However, high cost and emerging drug resistance affect compliance and overall cure. Tinospora rumphii has demonstrated antibacterial activity in vivo rendering it a potential cost-effective alternative treatment.

OBJECTIVES: To determine the safety and efficacy of 25% T. rumphii cream versus 2% mupirocin cream in the treatment of superficial pyodermas caused by S. aureus.

METHODS: A randomized, double-blind, controlled study of 60 patients with superficial pyodermas caused by S aureus, aged 18-60, were given either 25% T. rumphii or 2% mupirocin cream for two weeks. Bactericidal activity, erythema, edema, induration and size of lesion were evaluated at baseline, days 3, 7, and 14. Participants Global Assessment (PGA) score and adverse events were noted. Statistical analysis was done using Mann-Whitney U and Pearson Chi square test. RESULTS: Fifty-one subjects (85%) completed the trial. There were no statistically significant differences between the two treatment groups for bactericial activity against Staphylococcus aureus (p=0.687) at day 14, for erythema (p=0.923, 0.5335, 0.3726, 0.6949), edema (p=0.0972, 0.5967, 0.2052, 0.2783), induration (p=0.0855, 0.3113, 0.281, 0.3161), and size of lesions (p=0.7262, 0.169, 0.15, 0.3988) at baseline, days 3, 7 and 14. There was no significant difference in PGA score (p=0.3086, 0.3483, 0.2234) at Days 3, 7 and 14 in both groups. No adverse events were noted.

CONCLUSION: Twenty five percent T. *rumphii* cream is equally safe and effective as 2% mupirocin cream for treatment of superficial pyodermas caused by S. aureus.

Key words: Tinospora rumphii, Mupirocin, Superficial pyoderma, Staphylococcus aureus

INTRODUCTION

Superficial pyoderma is a heterogeneous group of conditions that includes impetigo, folliculitis, furuncle, carbuncle ecthyma, erthyrasma, and suppurative paronychia with involvement of the epidermis, upper part of the dermis and the superficial part of the hair follicles and nails. Majority of these skin infections are caused by Gram-positive bacteria, most commonly *Staphylococcus aureus* and *Streptococcus pyogenes*.[1] At a global level, the burden of disease is large with an estimate upwards of the number of children affected at 162 million in low and low-middle income countries.[2] According to the Philippine Dermatological Society Health Information System (PDS HIS), there are a total of 5780 cases of superficial pyodermas seen at the Dermatology out patient department from the 11 institutions for the year 2016 alone.[3] There are various factors linked for its higher incidence in the lower socio-economic class and

Department of Dermatology, Research Institute of Tropical Medicine, Muntinlupa City
[a]Graduate Resident, [b]Consultant

Commercial funding: None
Conflict of interest: None

developing countries such as poverty, malnutrition, overcrowding in the household, poor hygiene and poor access to water and certain skin conditions such as secondary infections due to insect bites and scabies. [4, 5, 6]

Staphylococcus aureus is both a commensal bacterium and a human pathogen and is a major cause of numerous infections in both communities and health care facilities.[7,8] It is the leading cause of bacteremia, infective endocarditis, osteoarticular, skin and soft tissue, pleuropulmonary and device related infections.[7] The universal use of antibiotics has produced changes in the bacterial flora of man and established the development of increased resistance.[9] Infections due to *Staphylococcus aureus* present a significant health problem due to the emergence and spread of Methicillin Resistant *Staphylococcus aureus* (MRSA).[8,10,11]

Majority of these infections can be managed on an Out-patient basis with the first line treatment of superficial pyodermas being topical antibiotics.[5] Two percent mupirocin cream or ointment is recommended for mild to moderate cases of superficial pyoderma applied twice daily for 14 days.[5]

Mupirocin (pseudomonic acid A) is the major metabolite produced by *Pseudomonas fluorescens* under submerged fermentation.[11] It inhibits bacterial protein synthesis by binding competitively to isoleucyl - tRNA synthetase.[11] In vitro, mupirocin exhibits a high level of activity against gram-positive cocci such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, and other hemolytic streptococci, the pathogens most frequently encountered in primary and secondary skin infections.[11] In vitro, it has been shown to exhibit high activity against gram-positive cocci and is now widely used for treatment of skin infections caused by Staphyloccocus.[11] In a study by Eells et al, 88% of skin infections caused by Staphyloccocus aureus was eliminated with the treatment of mupirocin ointment.[12] Clinical usage of over more than 10 years has demonstrated the efficacy and safety of mupirocin for treating primary and secondary skin infections.[11,13] However, there are concerns over the development of drug resistance with the use of mupirocin.[10,14,15,16] Unrestricted use was associated with an increased mupirocin resistance among S aureus isolates from almost zero in the early 1990s to 28% in 1999. [10]

Tinospora rumphii, (syn. *Tinospora crispa*) a member of Menispermaceae family, is a vine growing in the Philippines in both rural and urban areas. It is a climbing, dioecious vine reaching a height of 4 to 10 m with stems up to 1 cm thick and somewhat fleshy, with scattered protuberances.[17] It is commonly used in traditional and indigenous medicine for topical ulcers, malaria, jaundice, rheumatism, urinary diseases, intermittent fevers, eye and liver ailment.[18,19,20] The effectiveness of extracts from stems of *Tinospora rumphii* has been previously demonstrated against parasites specifically scabies and *Pediculosis humanus capitis*.[20,21] In vitro studies done on the leaf and stem extract demonstrated that *Tinospora rumphii* has potential angiogenetic and anti-inflammatory effects.[22,23]

Phytochemical studies showed that various constituents have been isolated from the *Tinospora rumphii* plant. They belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides.[24,25,26,27] Three major groups of compounds; protoberberine alkaloids, terpenoids and polysaccharides are considered as putative active constituents of *T. rumphii*.[24] *T. rumphii* leaves and stems have also been reported to cure certain skin infections.

In vitro studies done on the crude stem extract of *T rumphii* showed an impressive antibacterial effect. A study by Choudhary and colleagues, showed that the antimicrobial mechanism of alkaloids present in a very closely related species, *Tinospora cordifolia*, intercalates the cell wall and DNA of the microorganism. Terpenes cause bacterial membrane disruption and inhibit the release of autocoids and prostaglandins.[24, 25]

A preliminary study done by Lagda et al, revealed that lower concentrations of the *Tinospora rumphii* stem extract were found to be equally effective as the higher concentrations in inhibiting S aureus and S pyogenes organisms with a mean zone of inhibition of 10.00+8.66. [17] Phytochemical analysis of the *Tinospora rumphii* done at the Department of Science and Technology Industrial Technology Development Institute of the stem showed moderate amounts of triterpenes, abundant flavonoids, trace alkaloids, sterols, saponins and tannins.[17]

Previous studies with *Tinospora rumphii* cream has been shown to be safe for use in both animals and humans. A study by Sutantoyo et al, on patch testing of *Tinospora rumphii* cream on rabbits showed no irritation after 72 hours and the succeeding 7 days after the initial application and can be used safely in concentrations of 25%, 50%, 75% and 90%.28 The study by Galang et al, on patch testing of the *Tinospora rumphii* cream on human subjects showed

no irritation after 72 hours and the succeeding 2 weeks after the initial application and can be used safely in concentrations of 25%, 50% and 90%.[29]

All preliminary studies were approved by the Institutional Review Board and Institutional Animal Care and Use Committee and were conducted prior to the initiation of the study.

There is an abundance of literature on the phytochemical and *in vitro* antimicrobial activity of *Tinospora rumphii* against gram-positive bacteria Extensive literature review showed no published clinical trials on the safety and efficacy of *Tinospora rumphii* as an antimicrobial agent. This has encouraged the investigator to conduct a randomized, double-blind, controlled clinical trial on the safety and efficacy of 25% *Tinospora rumphii* (Makabuhay) cream versus 2% mupirocin cream on superficial pyodermas caused by *Staphylococcus aureus*.

This study aims to discover an effective and cheaper option to mupirocin in the treatment of pyodermas. If proven safe and effective on superficial pyodermas caused by *S aureus*, *Tinospora rumphii* cream may reduce morbidity and disease burden of bacterial infections and possibly be an alternative treatment to mupirocin resistant strains.

OBJECTIVES

The General Objective of this study was to determine the safety and efficacy of 25% *Tinospora rumphii* cream versus 2% mupirocin cream in the treatment of superficial pyodermas caused by *S aureus*.

The specific objectives were to compare the bactericidal activity based on the percentage of patients bacteriologically cured (negative for S. aureus) at day 14 of treatment, to compare the efficacy of 25% *Tinospora rumphii* cream and 2% mupirocin cream based on the clinical efficacy grading scale of erythema, edema, induration and size of lesions at baseline, days 3, 7 and 14 of treatment by having a 2 point scale improvement at the end of the treatment period. Another objective was to compare the Participants Global Assessment (PGA) score using a 7 point scale at days 3, 7, and 14 of treatment and to compare the occurrence and severity of adverse cutaneous reactions in the 25% *Tinospora rumphii* cream and 2% mupirocin treatment groups using a 4 point scale on days 3, 7 and 14 of treatment.

METHODOLOGY

Patients and Study design

A randomized, double-blind, controlled trial of 25% Tinosporum rumphii cream versus 2% mupirocin cream on patients with superficial pyoderma was conducted at the Dermatology out-patient department of a tertiary hospital for three months.

Subjects included in the study were males and females aged 18-60 years old, with clinical diagnosis of mild superficial bacterial skin infection defined as a lesion that measures less than 5 cm with absence of co-morbid diseases (diabetes mellitus, chronic liver or renal disease, vascular insufficiency, liver or renal disease) and constitutional signs and symptoms (fever, hypotension, tachycardia, hemorrhage, anesthesia), with lesions infected by Staphylococcus aureus as confirmed by Gram stain and culture of wound discharge and who were willing to participate in the study and sign a written informed consent.

Those with known hypersensitivity reactions to any ingredient of the test medications, have used topical antifungal, antibacterial or corticosteroid preparation within two weeks prior to consult and enrollment in the study, with co-morbid diseases such as hypertension, diabetes mellitus, asthma, with co-existing dermatitis such as atopic dermatitis and contact dermatitis and pregnant and lactating women were excluded from the study.

This study was approved by the institutional review board of the Research Institute for Tropical Medicine and was conducted at the Department of Dermatology of the same institution. Informed consent from the subjects included in the study was secured after being briefed on the study procedure.

Materials

Mature stems of *Tinospora rumphii* were obtained from the Sierra Madre mountain range at Infanta, Quezon and was identified by a certified taxonomist at the Research Center for Natural and Applied Health Sciences Herbarium.

The preparation of the extract was done by the Chemicals and Energy Division of the Department of Science and Technology Industrial Technology Development Institute in Bicutan, Taguig City, Metro Manila, Philippines. Since the 25% *T. rumphii* concentration was the lowest concentration that showed antimicrobial activity, this was the concentration used in the in vivo study.

A reputable, licensed pharmacist practicing for 25 years in the Philippines formulated a cream containing 25% *T. rumphii* extract. Cultures of the cream were performed to ensure the absence of contaminants. To assess the safety of the test product, patch testing was done on both animal and human subjects which showed no irritation after 72 hours to 2 weeks post application hence, the investigators proceeded with the clinical trial evaluating the safety and efficacy of 25% *T. rumphii* cream on subjects with superficial pyodermas caused by S. aureus.

Sample Size Estimation

Values were based on a study comparing 2% mupirocin ointment with 2.5% *Moringa oleifera* leaf extract (MOLE) ointment in the treatment of superficial pyoderma by Adasa et al. The mean change in lesion size from baseline to Day 14 in the mupirocin treatment group was 1.85 + 0.59 inches.31 Using this data as an estimate of the effect of a standard treatment and given that a clinically important difference of 0.5 between groups is considered acceptable, a sample size of 60 subjects, 30 in each treatment arm, is sufficient. This was calculated using a power of 90% and 95% confidence level using STATA 10.

Randomization, treatment allocation and blinding

Subjects were recruited at the Dermatology out-patient department of a tertiary government hospital. Attending resident physicians identified all prospective subjects via interview and physical examination and referred them to the principal investigator for initial examination and screening to determine their eligibility to participate in the study.

Patients who were clinically diagnosed with superficial pyoderma were screened for *S. aureus* infection. Specimen for Gram stain and culture were taken from the wound discharge by the principal investigator using the swab technique. Dry lesions were moistened with sterile normal saline solution prior to swabbing. Two specimen samples were taken from each prospective participant. Both samples were obtained by rotating a sterile cotton swab over 1 cm of open wound for 5 seconds without touching the edges of the wound. The first swab was placed in an empty sterile test tube for culturing while the second swab was used to make three rows of smear on a sterile slide for Gram staining. Both specimen samples were brought immediately to the Microbiology laboratory and was tested within two hours from the time of collection. All results were read by a licensed medical technologist within 48 hours. Treatment was started right after gram stain and culture has been done depending on the arm of treatment in which the patient was allocated. Patients were informed of the results (whether negative or positive for S. aureus) by phone.

Patients who fulfilled the inclusion criteria including a clinical diagnosis of superficial pyoderma were asked to sign the informed consent form. Once the informed consent was signed, measurements and photos of the lesions were taken.

Participants in the study were assigned randomly by a third person, someone who is not directly involved in the study, to one of the two groups using a computer-generated table of random numbers (http://stattrek.com/statistics/random-number-generator.aspx). On the other hand, a pharmacist was tasked to alphabet code each ointment and that code was known only to that person. The third person was again assigned to number the test ointments (that have been coded by the pharmacist) from 1 to 10 according to the computer generated allocation sequence. Both participants and principal investigator were blinded to the treatment allocations. The test products were placed in identical white colored tubes.

Pertinent history and clinical examination was gathered by the principal investigator.

Photographs of the lesions were taken under standardized settings on the initial visit, taking note of the changes in characteristics such as color, edema and size.

Study intervention

Subjects were given a white tube containing the medication (either 25% Tinospora rumphii cream or 2% mupirocin cream but the name was not indicated in the label). Subjects were instructed to apply the medications, 25% Tinosporum rumphii cream or 2% mupirocin cream, twice daily for 2 weeks with a sterile cotton applicator provided by the investigator and left on until it is rinsed off with soap and water upon taking a bath the next morning. Subjects were asked not to apply anything else other than the cream provided. They were asked to observe any sign of redness, itchiness, burning sensation, peeling, rashes, blisters or any new lesion that developed upon applying the cream or shortly after.

Patients were asked to return to the Dermatology out-patient department of a tertiary hospital for follow-up on days 3, 7 and 14 to assess the outcome measures. During the follow-up, the primary investigator took photographs of the lesions and assessed the outcome parameters with the aid of a checklist.

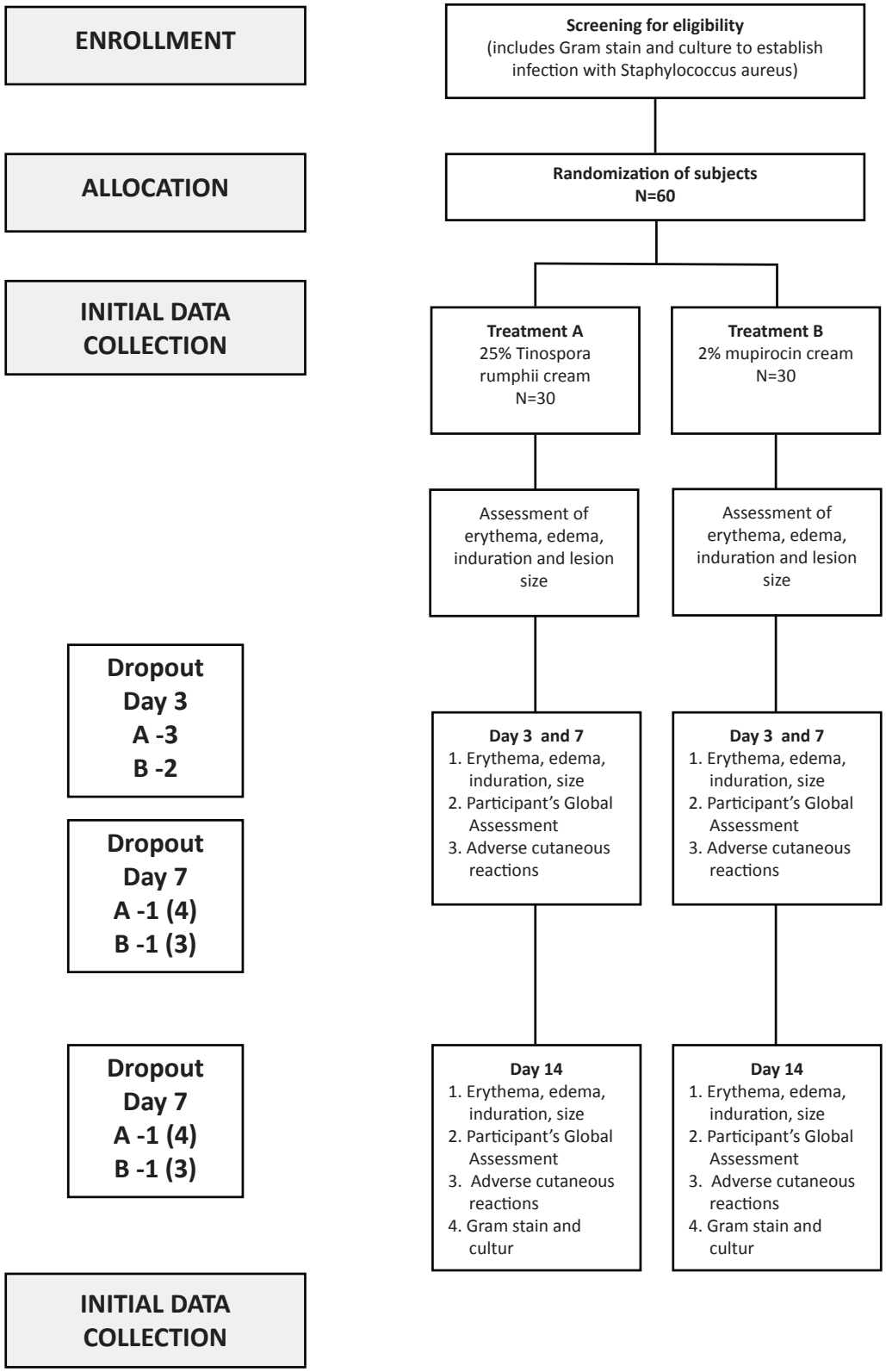

Figure 1. Flowchart of Study Procedure

OUTCOME MEASURES

Outcome measures were assessed at baseline, Day 3, 7 and Day 14 of treatment by the principal investigator.

The presence of *S. aureus* at baseline was confirmed by Gram stain and culture of wound discharge and was one of the inclusion criteria for enrollment into the study. The presence/absence of *S. aureus* was again determined at the end of the treatment period (Day 14) by Gram stain and culture of wound discharge to check for successful elimination of S. aureus infection due to the assigned ointment. Bactericidal activity of 25% *Tinospora rumphii* cream versus 2% mupirocin cream was expressed as the percentage of patients bacteriologically cured (negative for *S. aureus*) at the end of the treatment period (Day 14).

Clinical efficacy of the test products were assessed at baseline, days 3, 7 and 14 of treatment in terms of the following parameters: erythema, edema, induration and size of the lesions. The size of the lesion were obtained by measuring the widest diameter of the lesion in centimeters. Erythema, edema and induration on the other hand, were assessed using the following 5-point scale in the Guidelines for acute bacterial skin infections and developing drugs for treatment recommended by the Food and Drug Administration:

Table 2. Clinical Efficacy Grading (5-point scale)

SCORE	DESCRIPTION
0	Absent
1	Mild
2	Moderate
3	Severe
4	Very Severe

The Participant's Global Assessment Score was determined on days 3, 7 and 14 of treatment using the 7-point scale shown below.

Table 3. Participant's Global Assessment (7-point scale)

SCORE	DESCRIPTION
0	Complete clearance
1	Almost cleared; very significant clearance of disease with only traces of disease remaining; approximately 90% improvement
2	Marked response; significant improvement with some disease remaining; approximately 75% improvement
3	Moderate response; intermediate improvement between slight and marked response; approximately 50% improvement
4	Slight response with only some improvement but significant disease remains; approximately 25% improvement
5	Condition did not change
6	Condition worsened

Adverse cutaneous reactions (if any) were noted on days 3, 7 and 14 of treatment using the following 4-point scale by the Standards of International Drug and Test Products:

Table 4. Evaluation of Adverse Effects (4-point scale)

SCORE	DESCRIPTION	CHARACTERISTICS
0	None	
1	Mild	Erythema, dryness, pruritus
2	Moderate	Burning, desquamation
3	Severe	Vesicle, bullae, erosions

Participants were instructed to follow-up after another two weeks for evaluation of the maintenance of clinical response and possible delayed adverse reactions. If at the end of the study period, subjects are not satisfied with the results and wish to be treated with the standard medication (mupirocin 2% cream), the subject will be provided this treatment for free. Treatment for any adverse reaction will also be given free of charge.

DATA ANALYSIS/STATISTICAL CONSIDERATION

The Pearson chi-square test was used to compare the proportion of patients bacteriologically cured (determined by Gram stain and culture) between the two treatment groups at Day 14. Mann Whitney U test was used to compare the effect of 25% Tinospora rumphii cream with 2% Mupirocin cream in terms of clinical efficacy score for the different clinical responses (e.g. edema, erythema, induration, size of lesions) across the two treatment groups at baseline and at different periods of the intervention (Day 7 and Day 14). Friedman test was used for intra-group comparison. A 5% level of significance was used in making decisions for the hypotheses to be tested. All computations were made using STATA version 13.5.

A sensitivity analysis was conducted to assess whether results were consistent if lost to followup/ drop-out patients assumed to be observed until the end of the study. Imputation, using Stata 13.5 option for ordinal and continuous variable, with one imputation, was used for those who did not complete the 3 follow-up sessions. Imputation was done separately by randomy assigned treatment group. Same statistical analysis used in the primary analysis were followed.

RESULTS

Demographic profile

Sixty patients with Staphylococcus aureus infection were randomly assigned to either the Mupirocin or Tinospora rumphii treatment group at baseline. The ages ranged from 18 to 60 years old, the mean age of these patients being 31.48 years with a standard deviation of 11.30 years. 60% (36 out of 60) of the patients were males but the ratio of males to females per treatment groups was similar as presented in Figures 2 and 3.

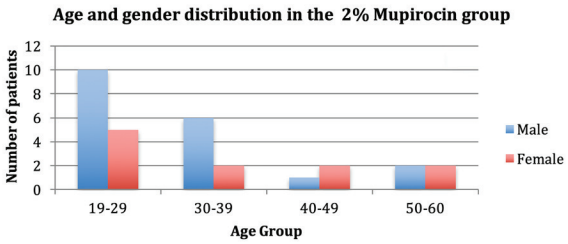

Figure 2. Age and gender distribution of patients in the 2% Mupirocin group

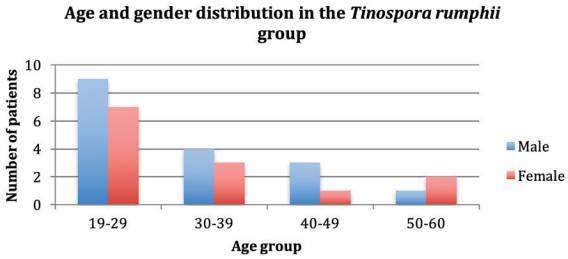

Figure 3. Age and gender distribution of patients in the 25% *Tinospora rumphii* group

Bactericidal activity

At Day 14, 68% of patients in the 25% *Tinospora rumphii* group and 76.92% in the 2% mupirocin group were bacteriologically cured at the end of the study period. Chi squared test showed that the proportion of patients with bactericidal activity did not have any significant difference between the 2 treatment groups with a p value of 0.687. Figure 4 shows the patients who were bacteriologically cured in both treatment groups at the end of the study period.

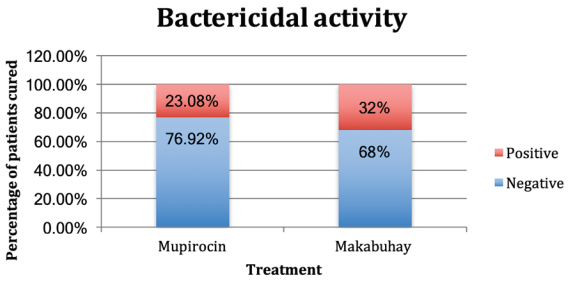

Figure 4. Percentage of patients bacteriologically cured at day 14 in the 25% *Tinospora rumphii* and 2% mupirocin group

Effect on Lesions

Erythema

There was a reduction in the severity of erythema in both treatment groups. The mean severity score for erythema in the *T rumphii* group was 2.8 ± 0.48 and 2.73 ± 0.64 in the mupirocin group at baseline; 2.25 ± 0.85 in the *T. rumphii* group and 2.1 ± 0.85 in the mupirocin group on day 3; 1.19 ± 0.61 in the *T. rumphii* group and 1.07 ± 0.56 in the mupirocin group on day 7; 0.36 ± 0.47 in the 25% *T. rumphii* group and 0.3 ± 0.45 in the 2% mupirocin group on day 14 as presented in Figure 5. Results of the Mann Whitney U test showed that the median severity score for erythema did not vary significantly between the two treatment groups at baseline (p=0.923), day 3 (p=0.5335), day 7 (p=0.3726), and day 14 (p=0.6949).

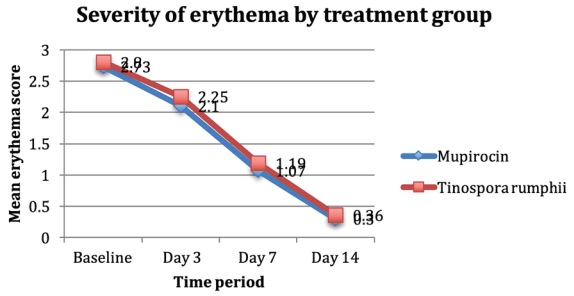

Figure 5. Severity of erythema in the 25% Tinospora rumphii group and 2% mupirocin group over time

Edema

There was a reduction in the severity of the edema in both *T. rumphii* and mupirocin trearment groups. The mean severity score for edema in the *T. rumphii* group was 2.76 ± 0.62 and 2.57 ± 0.50 in the mupirocin group at baseline; 2 ± 0.85 in the T. rumphii group and 1.85 ± 0.78 in the Mupirocin group on Day 3; 1.15 ± 0.69 in the Tinospora rumphii group and 0.85 ± 0.73 in the Mupirocin group on Day 7; 0.28 ± 0.43 in the Tinospora rumphii group and 0.15 ± 0.35 in the Mupirocin group on Day 14 as presented by Figure 6. Results of the Mann-Whitney test showed that the median severity score for edema did not vary significantly between the 2 trearment groups at baseline (p=0.0972), Day 3 (p=0.5967), Day 7 (p=0.2052) and Day 14 (p=0.2783).

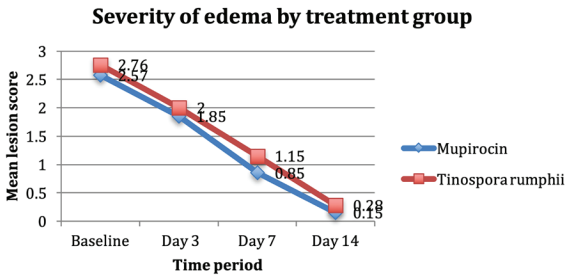

Figure 6. Severity of edema in the 25% *Tinospora rumphii* group and 2% mupirocin group over time

Induration

There was a reduction in the severity of induration in both treatment groups. The mean severity score for induration in the Tinospora rumphii group was 2.93 ± 0.37 and 2.77 ± 0.56 in the Mupirocin group at baseline; 2.18 ± 0.82 in the Tinospora rumphii group and 1.92 ± 0.85 in the Mupirocin group on Day 3; 1.35 ± 0.70 in the Tinospora rumphii group and 1.1 ± 0.61 in the Mupirocin group on Day 7; 0.36 ± 0.47 in the Tinospora rumphii group and 0.23 ± 0.41 in the Mupirocin group on Day 14 as presented by Figure 7. Mann Whitney test showed that the median severity score for induration did not vary significantly between the 2 treatment groups at baseline (p=0.0855), Day 3 (p=0.3113), Day 7 (p=0.281) and Day 14 (p=0.3161).

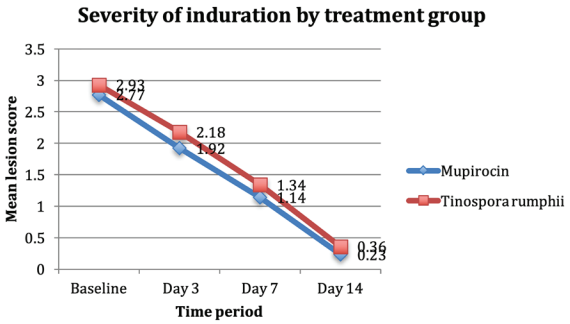

Figure 7. Severity of induration in the 25% *Tinospora rumphii* group and 2% mupirocin group over time

Lesion size

There was a decrease in the lesion size in both treatment groups. The mean lesion size in the Tinospora rumphii group was 2.7 ± 0.40 and 2.62 ± 0.41 in the Mupirocin group at baseline; 2.06 ± 0.71 in the Tinospora rumphii group and 1.91 ± 0.63 in the Mupirocin group on Day 3; 1.05 ± 0.49 in the Tinospora rumphii group and 0.9 ± 0.47 in the Mupirocin group on Day 7; 0.24 ± 0.31 in the Tinospora rumphii group and 0.17 ± 0.30 in the Mupirocin group on Day 14 as presented by Figure 8. Mann Whitney test showed that the median lesion size did not vary significantly between the 2 treatment groups at baseline (p=0.7262), Day 3 (p=0.169), Day 7 (p=0.15) and Day 14 (p=0.3988).

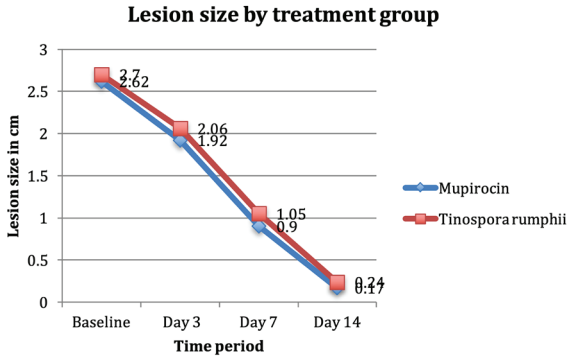

Figure 8. Lesion size in the 25% *Tinospora rumphii* group and 2% mupirocin group over time

Participants Global Assessment

There was a decrease in the Participant's Global Assessment score in both treatment groups. The mean PGA score in the Tinospora rumphii group was 3.33 ± 1.20 and 3.07 ± 1.04 in the Mupirocin group on Day 3; 1.96 ± 0.87 in the Tinospora rumphii group and 1.78 ± 0.89 in the Mupirocin group on Day 7 and 0.68 ± 0.62 in the Tinospora rumphii group and 0.46 ± 0.50 in the Mupirocin group on Day 14 as presented by Figure 9. Mann Whitney test showed that the PGA score did not vary significantly between the 2 treatment groups at baseline (p=0.3086), Day 3 (p=0.169), Day 7 (p=0.3483) and Day 14 (p=0.2234).

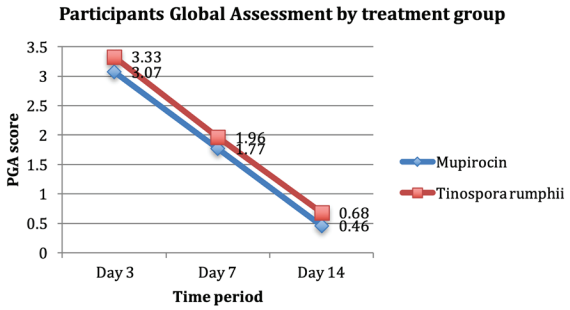

Figure 9. Participants Global Assessment in the 25% *Tinospora rumphii* group and 2% mupirocin group on Days 3, 7, and 14.

Adverse Events

No adverse events were reported in both the 25% *Tinospora rumphii* group and the 2% Mupirocin group during the entire duration of the study.

Drop out/Withdrawals

There were a total of 9 patients who left the trial. 4 patients were from the Mupirocin group and 5 patients from the Tinospora rumphii group. Three subjects from the Tinsopora rumphii group and 2 subjects from the Mupirocin group did not follow up on day 3. 1 subject for each group did not return at day 7. 1 subject for each group did not appear on the final day of the study. The 9 subjects were contacted via phone call. However, eight subjects claimed improvement of the lesions hence did not seek to return for follow up for the rest of the study period and 1 subject cited travel as the reason for not finishing the clinical trial but claimed improvement of the lesion. Participants who withdrew from the study were not included in the statistical analysis of the results.

The sensitivity analysis done showed similar results with the primary analysis.

DISCUSSION

In this study, the safety and efficacy of 25% *Tinospora rumphii* cream was compared with the standard 2% mupirocin cream in treating superficial pyodermas caused by *Staphylococcus aureus* using the following: 1) bactericidal activity 2) clinical efficiency grading, 3) Participants Global Assessment (PGA) score and 4) adverse events.

Results showed that 68% of patients treated with 25% *Tinospora rumphii* cream were bacteriologically cured (negative gram stain and negative culture for S. aureus) while 76.92% were bacteriologically cured in the mupirocin treatment group at the end of the study period. Statistical analysis showed that the median cure rate in both treatment groups did not have any significant difference. This suggests that the bactericidal activity of 25% *Tinospora rumphii* cream against S. aureus is comparable with 2% mupirocin cream.

The effect of 25% *Tinospora rumphii* cream on superficial pyoderma lesions as determined by erythema, edema, and induration showed that the grading of the lesions in both groups were moderate to severe at baseline and constantly decreased to less than mild at the end of the study period. Statistical analysis showed that the median severity scores for erythema, edema, and induation did not have any significant difference between both treatment groups.

Lesion size was also observed to constantly decrease from baseline to the end of the study period. Statistical analysis showed that there was no significant difference between the two treatment groups.

The preliminary study done by Lagda and colleagues, on the *in vitro* evaluation of *Tinospora rumphii boerlage* (Makabuhay) stem extract as an antimicrobial agent showed that it possessed activity against *S. aureus*. Different concentrations of makabuhay extracts were used. The 25% extract yielded a mean zone of inhibition (MZI) of 10.00±8.66, 50% yielded an MZI of 14.00±1.73 , 75% yielded a MZI of 14.00±1.73 while 100% yielded a MZI of 14.67±0.58 for S. aureus. A mean zone of inhibition more than or equal to 10 mm was considered to have positive antimicrobial activity. Since the 25% *T. rumphii* concentration was the lowest concentration that showed antimicrobial activity, this was the concentration used in the clinical trial.

These findings support published reports on the antimicrobial components of *Tinospora spp*. done by Choudhary et al, Tavera and Hamid et al.[32,33] Our findings provide supplementary information that T. rumphii stem extract exerts antimicrobial activity against bacteria.[7] Choudhary and colleagues suggested that the antimicrobial mechanism of alkaloids present in *Tinospora cordifolia*, intercalates the cell wall and DNA of the microorganism. Terpenes cause bacterial membrane disruption and inhibits the release of autocoids and prostaglandins.[18]

The Participant's Global Assessment (PGA) scores given by the participants also decreased from slight response (approximately 25% improvement) to moderate response (approximately 50% improvement) at Day 3 to almost cleared (more than 90% improvement) at day 14. The scores did not have any significant difference between the 2 treatment groups during the entire study period.

There were no reported adverse events from both the *T. rumphii* group and the 2% mupirocin group. These findings validate the preliminary studies done by Sutantoyo and colleagues on the safety of different concentrations of *T. rumphii* cream on rabbit skin irritation. A study by Galang and colleagues demonstrated the safety of *T. rumphii* in 25%, 50% and 90% cream on normal human skin. There were no adverse events noted 72 hours and 2 weeks after application of the *T. rumphii* cream in all concentrations. Another study by Mamaril et al, showed that T. rumphii compounded into pediculocidal shampoo showed no adverse events. [17]

CONCLUSION

25% Tinospora rumphii cream is equally effective and safe as 2% mupirocin cream in the treatment of superficial pyodermas caused by S. aureus. The combination of bactericidal cure, resolution of lesions, absence of adverse reactions and its low cost makes 25% Tinospora rumphii cream a cost effective alternative for the treatment of superficial pyodermas.

RECOMMENDATIONS

Culture count was not used to measure bactericidal activity. It merely was reported as positive or negative and cases of pyoderma due to organisms other than S. aureus were not included in this study. Researchers should conduct a study on the safety and efficacy of 25% *Tinospora rumphii* cream on superficial pyodermas caused by other skin pathogens such as *S. pyogenes* or Methicillin Resistant *Staphylococcus aureus* and to conduct a study on the safety and efficacy of 25% *Tinospora rumphii* cream on younger age groups.

REFERENCE

1. Kalshetti VT, Bhate VM, Haswani N, Bothikar ST. Staphylococcus aureus: a major causative agent of community - acquired pyoderma. Int.J.Curr. Microbiol.App.Sci (2014) 3(9) 94-97

2. Bowen AC, Mahe A, Hay RJ, Andrews RM, Steer AC, Tong ST, et al. The global epidemiology of impetigo: a systematic review of the population prevalence of impetigo and pyoderma. PLoS One 2015;10(8):e0136789.

3. Philippine Dermatological Society Health Information System. Internet. Available from http://192.168.2.140/pdshis/index.php

4. Gandhi S, Ojha SK, Ranjan KP, and Neelima. Clinical and Bacteriological Aspects of Pyoderma. N Am J Med Sci. 2012; 4(10): 492–495.

5. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. The Canadian Journal of Infectious Diseases & Medical Microbiology. 2008;19(2):173-184.

6. Templer J, Maximo SB. Bacterial Skin and Soft Tissue Infections. Curr Opin Infect Dis. 2009; 45(3): 9-16, 26

7. Tong, SYC, Davis, J, Eichenberger, E., Holland, T, Fowler VG. Staphylococcus Aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin Microbio Rev 2015: 603–661

8. Chen, C.J, Huang, Y.C, New epidemiology of Staphylococcus aureus infection in Asia. Clinical Microbiology and Infection 2014: 20(17): 605-2

9. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. Microbio Mol Biol Rev 2010; 74(3): 417–433

10. Hetem, D.J., Bonten, M.J. Clinical relevance of mupirocin resistance in Staphylococcus aureus. J Hosp Infect. 2013; 85(4): 249-56

11. Gisby J, Bryant J. Efficacy of a New Cream Formulation of Mupirocin: Comparison with Oral and Topical Agents in Experimental Skin Infections. Antimicrobial Agents and Chemotherapy 2000; 44(2): 255-260.

12. Eells L.D., Mertz P.M., Piovanetti Y., Pekoe G.M., Eaglstein W.H. Topical antibiotic treatment for impetigo with mupirocin. Arch Dermatol 1986; 122: 1273-6.

13. Breneman D.L. Use of mupirocin ointment in the treatment of secondarily infected dermatoses. J AM Acad Dermatol 1990; 22: 886-92.

14. Upton, A., Lang, S., Heffernan, H. Mupirocin and Staphylococcus aureus: a recent paradigm of emerging antibiotic resistance. Journal of Antimicrobial Chemotherapy. 2003;51:613-617

15. A.R. Caffrey, B.J. Quilliam, K.L. LaPlante, Risk factors associated with mupirocin resistance in meticillin-resistant Staphylococcus aureus, J Hosp Infect 2010; 76(3): 206-210

16. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin Resistance in Staphylococcus aureus Causing Recurrent Skin and Soft Tissue Infections in Children. Antimicrob Agents and Chemother. 2011;55(5):2431-2433

17. Lagda D, Sutantoyo CJ, Galang MC. In vitro evaluation of Makabuhay stem extract as an Antimicrobial Agent against Staphylococcus aureus Steptococcus pyogenes and Pseudomonas aeruginosa. 2015, Unpublished manuscript

18. Ahmad W, Jantan I, Bukhari SNA. Tinospora crispa (L.) Hook. f. & Thomson: A Review of Its Ethnobotanical, Phytochemical, and Pharmacological Aspects. Front Pharmacol. 2016;7:59

19. Tan RS, Bajo. LM. Modulation of Tinospora rumphii and zinc salt on DNA damage in quinoline-induced genotoxicity and hepatotoxicity in male albino mice. Advances in Toxicology. 2014

20. Castillo, A, Osi, MO, Ramos, JD, De Francia JL, Dujunco MU, Quilala, PF. Efficacy and safety of Tinospora cordifolia lotion in Sarcoptes scabiei var hominis-infected pediatric patients: A single blind, randomized controlled trial. J Pharmacol Pharmacother. 2013; 4(1):39-46

21. Dela Torre, LT, Ponsaran G, Ponsaran, MG, De Guzman, KD, De Guzman, LD, Manalo, AM, et al. Safety, efficacy, and physicochemical characterization of Tinospora crispa ointment: a community-based formulation against Pediculus humanus capitis. The Korean Journal of Parasitology. 2017; 55(4): 409-416

22. Hipol, R.L., Cariaga, M.F.N.M, and Hipol, R.M. Antiinflammatory activities of the aqueous extract of the stem of Tinospora crispa (Family Menispermaceae). Journal of Nature Sudies. 2012; 11(1&2): 88-95

23. Galia, MLD., Galia, J.D. Angiogenetic activity of Tinospora rumphii Boerl (Makabuhay) Leaf and Stem Extracts. International Research Journal of Biological Sciences. 2016; 5(1): 54-59

24. Choudhary N, Siddiqui MB, Azmat S, Khatoon S. Tinospora cordifolia: Ethnobotany, Phytopharmacology and Phytochemistry Aspects. IJPSR, 2013; 4(3): 891-899.

25. Choudhary, MI, Ismail M, Ali, Z, Shaari K, Lajis NH, Atta-ur-Rhaman. Alkaloidal Constituents of Tinospora crispa. Nat Prod Commun. 2010;5(11):1747-50

26. Mohammed, AI. Antimicrobial activity of Tinospora crispa root extracts. International Journal of Research in Ayurveda and Pharmacy. May-June 2012 3(3):417-419

27. Ragasa, C, Cruz, MC. Antimicrobial Diterpenes from Tineaspora rumphii. Journal of Research Science, Computing and Engineering, 2005;2(1)

28. Sutantoyo CJ, Lagda D, , Dayrit JF, Gabriel, MT. An Evaluation of Rabbit Skin Irritation from Makabuhay Cream. 2016. Unpublished manuscript

29. Galang MC, Chia, CLN, , Gabriel, MT, Dayrit, JF, Teodosio, GB. Use of skin patch testing in determining safely of Tinospora rumphii 25%, 50%, 90% cream on normal skin. 2016. Unpublished manuscript

30. Panis L, Cejar R, Gabriel MT, Dayrit JF, A randomized, double-blind, controlled study on the safety and efficacy of 10% bee propolis ointment versus 2% superficial pyodermas caused by Staphylococcus aureus. 2014. Unpublished manuscript

31. Adasa, G, A Randomized, Double-blind, Controlled Study on the Safety and Efficacy of 2.5% Moringa oleifera Lam (Moringaceae) Leaf Extract Ointment versus 2% Mupirocin Ointment on Superficial Pyodermas caused by Staphylococcus aureus . Unpublished manuscript

32. Yusoff, M, Hamid, H, Houghton, P. Anticholinesterase inhibitory activity of quaternary alkaloids from Tinospora crispa. Molecules 2014; 19:1201-1211