Journal of Applied Pharmaceutical Research
Volume 11 Issue 5, Year of Publication 2023, Page 01 – 08
DOI: 10.18231/j.joapr.2023.11.5.1.8

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

POTENT ANTIBACTERIAL ACTIVITY OF TERMINALIA CHEBULA-BASED HERBAL SOAP FORMULATION AGAINST PROPIONIBACTERIUM ACNES AND ITS CYTOTOXIC EVALUATION ON HUMAN SKIN FIBROBLAST CELLS

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Article Information
Received: 14th June 2023
Revised: 29th November 2023
Accepted: 12th December 2023
Published: 31st December 2023

Keywords
Terminalia chebula, Herbal soap, Propinobacterium, Human Skin Fibroblast Cells

ABSTRACT

Background: Recently, many approaches have been conducted in natural skin healthcare, with herbal remedies gaining prominence due to their perceived efficacy and fewer side effects. Herbal soaps, in particular, are noted for their antimicrobial, anti-inflammatory, and antioxidant properties, making them appealing for acne-causing bacteria such as *Propionibacterium acnes*, *Terminalia chebula*, a medicinal plant with a rich history of traditional medicinal properties, including antibacterial effects. However, limited information exists on its activity against acne-causing bacteria and its cytotoxicity on human skin cells. **Methods:** Our study demonstrated the anti-propionibacterium activity using a novel *Terminalia chebula* herbal soap formulation (TC-HSF), and to determine its cytotoxicity on Human skin fibroblast cells, *Terminalia chebula* aqueous extract was used to make herbal soap, the antibacterial activity was performed against wild isolated propionibacterium acne strain using well-diffusion method, MIC and time-kill Kinetics, several concentrations were evaluated for cytotoxicity on Human skin fibroblast cell lines.

Results Our findings indicate a potent activity of TC-HSF against P.acne at 0.18mg\ml with lower CFU\ml in tested intervals and high cell viability 98%, IC_{50} Value was 50mg\ml. **Conclusion:** Our study highlights the promising antibacterial efficacy of TC-HSF against Propionibacterium acnes and its cytotoxic effects on human skin fibroblast cells. The research adds valuable insights into the potential of TC-HSF for acne management and underscores the importance of dosage considerations in the formulation of herbal soap products.

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INTRODUCTION

Acne vulgaris is a broad dermatological disorder that primarily affects the face and the skin of the human body in different regions, with the highest incidence occurring in individuals at the early stages of adolescence [1]. The pathogenesis of acne vulgaris is multifactorial and involves increased sebum production, hyperkeratinization, elevated skin inflammatory mediators, and colonization by Propionibacterium acnes [2,3]. *P. acnes*, a predominant bacterium found on the skin and the primary etiological agent of acne, has long been associated with acne vulgaris [4]. It produces various virulence factors and is recognized for its inflammatory and immunomodulatory properties [5], leading to acne vulgaris development. Additionally, *P. acnes* has been associated with many infections and clinical conditions, highlighting its significant pathogenic potential; *P. acnes* has acquired resistance to topical antibiotics such as macrolides, with resistance rates reported being more significant than 50% in many countries [6]. The conventional treatment approach for acne vulgaris typically involves using chemical agents such as retinoic acid, benzoyl peroxide, salicylic acid, and specific vitamins. Antibiotics such as Clindamycin and tetracyclines are also commonly prescribed [2]. However, these traditional treatment options may have adverse effects. Alternative therapies, including laser and light therapy, have shown promising results in improving scarring and skin texture. Nevertheless, these treatments can be costly and uncomfortable [7,8]. Natural products such as aloe vera and tea tree oil have emerged as potential alternatives for managing acne vulgaris [9]. Interest has grown in recent years in herbal remedies and a shift toward a more holistic and natural approach to healthcare [10]. Therefore, this evolving theory consistently investigates the potential of herbal-based products such as soaps, creams, and gels in treating acne vulgaris. One key advantage of herbal soaps is their antimicrobial properties, which may potentially aid in regulating bacterial proliferation, specifically in the case of *P. acnes,* and reduce inflammation associated with acne vulgaris [11][12]. Moreover, herbal soap exhibits anti-inflammatory and antioxidant effects, further contributing to its potential efficacy in managing acne vulgaris. *Terminalia chebula*, or "chebulic myrobalan," is an indigenous medicinal plant from Southeast Asia that belongs to the Combretaceae family. It has a long history of use in traditional Ayurvedic medicine ash. The dried fruit of *Terminalia chebula* is highly valued for its therapeutic properties. *Terminalia chebula* has multiple medicinal properties, including antibacterial, antifungal, anticarcinogenic, antidiabetic, antiproliferative, antiarthritic, anticaries, and multiprotective effects [13–16]. However, despite the widespread use of *Terminalia chebula*, there is limited information on its specific mechanisms of action against acne-causing bacteria and its cytotoxic effect on human skin cells. Further investigation is necessary to elucidate its interactions with the skin, the use of its extract in herbal soap formulation, and its potential benefits for various skin conditions using in vitro models. In addition, more research is required to determine the optimal concentration and formulation of *Terminalia chebula* in soaps to maximize its effectiveness while minimizing potential side effects. By conducting rigorous scientific studies, we can better understand the therapeutic potential of *Terminalia chebula* in herbal soap formulations for treating acne vulgaris.

Our goal is to examine the antibacterial activity of *Terminalia chebula* aqueous extract formulated herbal soap formulations against acne-causing *propionibacterium acne* and determine its cytotoxicity against human skin fibroblast cell lines.

MATERIALS AND METHODS

Plant materials

The aerial fruits were collected from trees in the surrounding areas of Sabha city, where these trees are abundant. The fruits were brought to the laboratory and Herbarium at the Botany Department, the College of Science Sebha University, identified and confirmed the plant type and genus, the plant was authenticated with reference No (COM\TC\123-12) . The collected fruits were washed with sterilized distilled water and dried at room temperature. The fruit peels were carefully removed using a scalpel, and the seeds were discarded. The peels were then ground into a powder using a household grinder. The powder was thoroughly checked to ensure purity before proceeding with further experiments.

Terminalia chebula aqueous extract preparation

100g of finely powdered *Terminalia chebula* was mixed with 1L of distilled water. The mixture was then refluxed for 30 minutes for 4 hours at 80°C[17]. The extract was filtered using Whatman no.3 and then filter-sterilized. The extract was stored at 4°C for further experiments.

Terminalia chebula Herbal Soap formulation (TC-HSF) Preparation

The method mentioned bysindsin [18] was performed with slight modifications. Three grams of sodium hydroxide (Fluka. USA)

were mixed with 15 ml of ddH_2O in a magnetic stirrer (200 rpm) and heated to 80 degrees Celsius. Three grams of sodium silicate (Fluka. USA) were added gradually. The mixture was left on the stirrer without heating. Subsequently, 15 ml of the desired *Terminalia chebula* extract concentrations were added while stirring continued. Fifteen milliliters of paraffin oil (Carlo erba. Italy) were added to the mixture. The mixture was left on the magnetic stirrer for 8 hours until the soap layer could be observed. The obtained soapy mixture was transferred to small molds and left to solidify. The formed *Terminalia chebula* herbal soap bars were stored at room temperature until use.

Propinobactreium acnes isolation

Following the instructions outlined by[19], thioglycolate broth (Shcarlab. Spain) and anaerobic basal agar (Shcarlab. Spain) were used in this study. A volunteer visiting the central dermatology clinic in Sebha, Libya, diagnosed with acne, was selected for sample collection. The volunteer agreed to take part in the study after reading explicit consent. The procedure involved swabbing soaked in 0.85% saline solution of the affected acne-prone face area. The swab was immediately transferred to the laboratory and placed in tubes containing thioglycolate broth. These tubes were subsequently incubated at 37°C for seven days using BD GasPak anaerobic jar system, with the addition of a candle to ensure anaerobic conditions. Following the observation of bacterial growth, the samples were cultured onto plates containing Anaerobic basal agar supplemented with Furoxone (400mg/ml), nalidixic acid (15 mg/L), and colistin sulfate (5 mg/L) (Bioanalyse. Turkey). The plates were then placed in the incubator for five days under the same anaerobic conditions described previously. Bacterial morphology and chemical characteristics and phenotypic conformation using the API Coryne system (BioMérieux, France) were subsequently used, and one isolate of *Propionibacterium acnes* with confirmed phenotypic characteristics was used in this study. Bacterial suspensions were prepared in 5 mL of 0.85% saline solution, and turbidity was compared to the McFarland standard of 0.5 to standardize cell concentrations.

Anti-propionibacterium activity

The antibacterial activity of the *Terminalia chebula* herbal soap formulation was determined using the Agar-well diffusion protocol [20]. In this study, suspensions of *Propionibacterium acnes* isolate were appropriately adjusted to a McFarland turbidity of 0.5. The suspension was sawbed onto Mueller Hinton agar plates (Oxoid, UK). The plates were equilibrated for 20 min. Subsequently, wells with a diameter of 12 mm were created in the agar with a cork borer, *Terminalia chebula* herbal soap formulation, dissolved in sterile distilled water to obtain concentrations of 50mg, 100mg, 150mg & 250mg, respectively. The solutions were pipetted into the wells of the plates (100ul) and then incubated in anaerobic conditions at 37 °C for 72 h. zone of inhibition diameter was measured. The experiment involved conducting three replicates for each treatment. Clindamycin 10ug disk (Bioanalyse. Turkey) was used as a positive control, and the soap formulation without the plant extract was used as a negative control.

MIC determination (Macrodilution Method)

Broth macro-dilution method was performed [21]with twofold dilutions of filter-sterilized TC-HSF in an aqueous solution. The final concentration range of TC-HSF was 0.09 to 250.00 mg\mL. 500 µL of the resulting mixture of each dilution was added into designated tubes containing 500 µL of Muller Hinton broth (Oxoid, UK). Subsequently, 50 µL of a bacterial suspension was added to these test tubes, with the suspension containing bacterial strains at a final concentration of 1×10^5(CFU\ml). The tubes were checked for bacterial growth inhibition after a 72-hour anaerobic incubation at 35 °C. MBC was determined by aseptically pipetting 10 µL of each tube used in the MIC to anaerobic basal agar plates, then incubated at 37°C under anaerobic conditions for up to 72 hours. Clindamycin 10ug was used as the experimental control in all cases.

Time-Kill Kinetics Assay

The study assessed the time-dependent antibacterial effectiveness of TC-HSF against *Propionibacterium acnes* using a modified [22]. MIC concentration of TC-HSF was used. Bacterial inoculums were adjusted at a final concentration of 1×10^5(CFU\ml) and then incubated at 37°C. At specific time intervals (0 to 80 h), 1.0 mL cultures were aseptically subsequently transferred to Anaerobic Basel agar plates and incubated at 37°C for 72 h at anaerobic conditions. A control experiment was performed without TC-HSF or the reference antibiotic Clindamycin. The colony-forming unit (CFU) counts of the bacteria were determined, and this procedure was conducted in triplicate (three separate experiments). A graphical representation was created, plotting the log CFU/mL against time, spanning approximately 80 hours.

Cytotoxic assay of TC-HSF on skin fibroblast cell lines

HSF: Human skin fibroblasts (Creative-bioarry, USA) were cultured in DMEM media (Sigma-Aldrich. USA) with 150 mg/mL streptomycin, 200 units/mL penicillin, and 10% heat-inactivated fetal bovine serum for 24 hours at a density of 1.5×10^7 cells per well. After initial incubation, the cells were treated with *Terminalia chebula* herbal soap at doses ranging from 0 to 100 mg\ml. Control vehicle DMSO was used. The cells were incubated for 24 hours after treatment. After removing the culture media, 300 µL of MTS reagent (Creative-bioarry, USA) was added to each well. The cells were cultured for 5 hours at 37°C in a controlled CO2 incubator [23]. Finally, absorbance at 492 nm of each well was measured using a microplate reader (Promega. USA). The proportion of cell viability compared to control cells was used to determine the IC50 (50% inhibitory concentration). The tests were done in triplicates.

Statistical analysis

Using Minitab 20 (Minitab, LLC.) Three replicates were done for all experiments. The data is presented as mean ± SD. We used ANOVA and simple t-test to find significant value differences. The difference was considered significant at 5% ($p < 0.05$).

RESULT

Anti-propionibacterium activity of *Terminalia chebula* herbal soap formulation

The results given in Table (1) show an analysis of the anti-propionibacterium activity exhibited by the *Terminalia chebula* herbal soap formulation (TC-HSF) against *Propionibacterium* isolates; the data represents the antibacterial efficacy and demonstrates a significant ($p<0.05$) correlation with the concentration of the TC-HSF. Specifically, as the concentration of the TC-HSF increased, the zone of inhibition also exhibited a proportional increase.

At a concentration of 10mg, the zone of inhibition was 12mm; this increased to 18mm at 100mg, 21mm at 150mg, and reached its peak at 25mm for the 250mg concentration. The results also show significant differences between the treatments compared to the negative (Soap formulation without extract) and positive control (Clindamycin 10ug) at 0.05.

Table 1 demonstrates the effect (inhibition zone) of tested concertation of TC-HSF against *Propionibacterium* isolate

Mean Diameter of inhibition zone (MM)						
	TC-HSF \ Concentration (mg)				Controls	
Propinobactreium isolates	1	100	150	250	Clindamycin 10ug	Soap formulation without extract
	12	18	21	25	23	0
p-value (< 0.05)						

Table 2 indicates MIC and MBC values of TC-HSF and the positive control Clindamycin

Tested compound	Concentration (Mean)	
TC-HSF	MIC (mg/mL)	MBC (mg/mL)
	0.18	0.36
Clindamycin (Control)	0.04	0.24
p-value (< 0.05)		

The mean MIC and MBC values of Clindamycin and TC-HSF against an isolate of *Propinobacterium* are shown in Table (2). The mean MIC value TC-HSF was 0.9 mg/mL. The mean MIC value for Clindamycin was 0.04 mg/mL, which was lower than TC-HSF. The MBC values represent the lowest concentration required to kill the bacteria, with TC-HSF requiring 0.36 mg/mL and Clindamycin requiring 0.24 mg/mL. These results highlight the significant ($p<0.05$) susceptibility of *Propionibacterium* isolate to different concentrations of the tested compound.

The Time-Kill Kinetics graph figure (1) represents the bactericidal activity of TC-HSF at 0.18mg/ml, Clindamycin 10ug, and a control (bacteria culture only) over approximately 80 hours. Clindamycin and TC-HSF at 0.18mg/ml exhibited significant bactericidal activity, as evidenced by their consistent Log10(CFU/ml) values around 5 Log10(CFU/ml) across the timeline. However, TC-HSF at 0.18mg/ml demonstrated potent

bactericidal effects, with a clear linear escalation in bacterial reduction from an initial Log10 (CFU/ml) value of around 5, *propionibacterium acnes* culture only served as a control shows Log10(CFU/ml) nearly 17.5 by the 70-hour point. Figure (2) shows the mean Log10 (CFU\ml) reduction as TC-HSF at 0.18mg/ml, and Clindamycin 10ug exhibited a significant reduction in the total tested interval compared to the control.

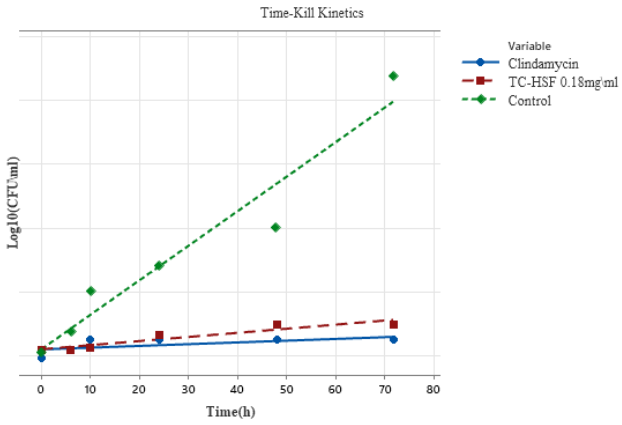

Figure 1 shows the plot of time-kill kinetics of the treatment effect on Log10(CFU\ml) of *propionibacterium acnes* isolate

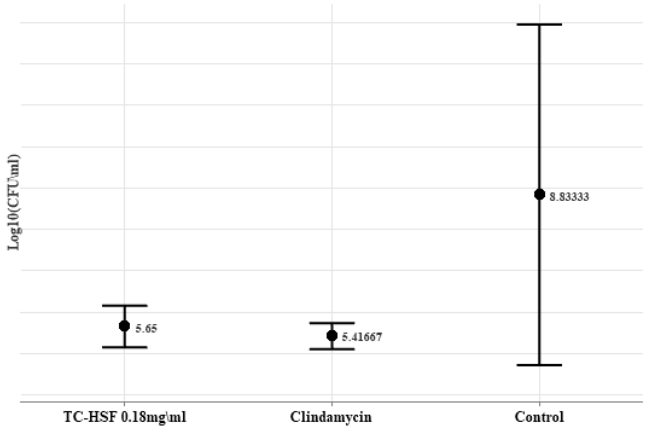

Figure 2 shows the total mean log10 (CFU\ml) reduction of treatment compared to the control.

The Cytotoxic assay of TC-HSF on skin fibroblast cell lines was evaluated across different concentrations, as illustrated in Figure (3). Skin fibroblast cell lines without any treatment served as the control and showed approximately 100% cell viability with no IC_{50}. For the concentrations of 0.78 to 12.50 mg/ml of TC-HSF, the cell viability remained close to 100%, indicating insignificant cytotoxic effects. However, a slight decrease in cell viability was observed at 25 mg/ml, with viability dropping further to below 60% at 50 mg/ml. The IC_{50} value was recorded at a 50 mg/ml concentration, where cell viability was below 50%.

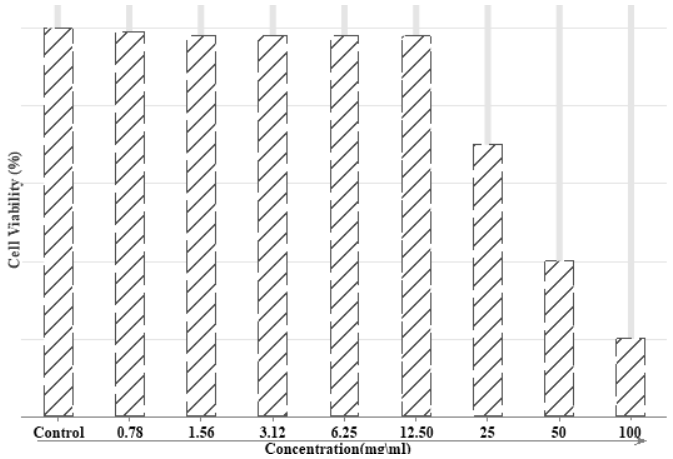

Figure 3 shows Skin fibroblast Cell viability % treated with different concentrations of TC-HSF

DISCUSSION

Alternative treatments of acne-causing bacteria using natural product soap are common since many medicinal plants have skin-health-promoting benefits. The current study aims to examine the potential in vitro effect of *T. chebula* aqueous extract soap formation against the growth of *propionibacterium acnes* and to determine its skin fibroblast cell cytotoxicity. Our results present a potent anti-*propionibacterium* activity of the *Terminalia chebula* herbal soap formulation (TC-HSF). The increasing zone of inhibition with higher concentrations of TC-HSF underlines its potential as an effective antibacterial agent. The observed positive correlation between TC-HSF concentration and the zone of inhibition signifies a dose-dependent antibacterial efficacy against *Propionibacterium* isolate.

Comparative analysis with Clindamycin, a well-known antibiotic, as reported[24], revealed that at a concentration of 250mg, TC-HSF surpassed Clindamycin in terms of zone of inhibition. This finding is noteworthy, highlighting the promising antimicrobial potency of TC-HSF, particularly at higher concentrations. However, it's essential to acknowledge the superior zone of inhibition exhibited by Clindamycin at concentrations below 250mg. Such comparisons are vital for assessing the efficacy of herbal formulations against conventional antibiotics.

The mean Minimum Inhibitory Concentration (MIC) values for TC-HSF and Clindamycin reaffirmed their varying antibacterial effectiveness. *Propionibacterium* isolate displayed a higher MIC for TC-HSF than Clindamycin, suggesting higher concentrations

of TC-HSF to inhibit bacterial growth effectively. As mentioned,[25], these MIC values are still suitable for indicating antibacterial activity.

As illustrated through Time-Kill Kinetics obtained results, the bactericidal activity revealed a potent bactericidal effect of TC-HSF at MIC 0.18mg/ml over 80 hours. In contrast, Clindamycin exhibited significant bactericidal activity within the same timeframe. This outcome points out the potential of TC-HSF as a bactericidal agent against *Propionibacterium* isolates, underscoring its therapeutic relevance in specific intervals. Moreover, our result showed a significant decrease of TC-HSF in log10(CFU\ml) total mean compared to the control.

At lower concentrations, the assessment of TC-HSF's cytotoxicity on skin fibroblast cell lines indicated minimal cytotoxic effects in the concentration range from 0.78mg\ml to 25mg\ml on human fibroblast cells. However, cytotoxicity IC_{50} was observed at higher concentrations, particularly at 50 mg/ml and 100 mg/ml. Our findings regarding the cytotoxicity were significantly similar t[15,26,27], which test the toxicity of several extracts of *T. chebula* despite the differences as we test *T. chebula* in a soap formulation and address a novel application of TC-HSF on human fibroblast cells.

Our study did not definitively identify the specific phytochemicals in TC-HSF. However, many reports indicate that *T. chebula* contains many bioactive components responsible for antimicrobial activity, such as tannins that have demonstrated the ability to inhibit the growth of various bacteria through multiple mechanisms. Although many reports[28,29] had tested the antimicrobial activity of *T. chebula* extracts, our findings were similar to many reports [30][31]that have mentioned its safety and cellular effect at different concentrations against several cell lines, such as keratinocyte cells. Our study was the first to add aqueous extract in a soap formulation and test it against acne-causing bacteria such as *propionibacterium acne* isolate, isolated from a patient suffering from acne vulgaris. Our findings provide valuable novel insights into the antibacterial efficacy of TC-HSF and advance our understanding of combating *Propionibacterium* infections. Additionally, we determine lower MIC and intervals to inhibit *Propionibacterium* growth. Notably, the MIC of TC-HSF was within the safe concentration that did not affect skin fibroblast cell lines.

Further exploration is required to determine a safe and effective therapeutic concentration of TC-HSF. Our study was only conducted on a wild strain of *propionibacterium* isolated from a patient, and more testing of TC-HSF on other dermatological microbial pathogens is needed. Moreover, several chemical characteristics of the newly formed soap formulations were not performed, as we aim to continue our study on such herbal soap formulations. Additional investigations are needed to understand the mechanism and pinpoint the specific bioactive compounds responsible for the observed growth inhibition, especially when incorporated into skin care products such as soap and lotions.

CONCLUSION

Alternative treatments utilizing natural products have long been explored for their potential against acne-causing bacteria. The presented study was an implied into the antibacterial capabilities of *Terminalia chebula* aqueous extract soap formulation (TC-HSF) against Propionibacterium acnes. The outcomes highlight the promising antibacterial efficacy of TC-HSF, particularly at higher concentrations, such efficacy was particularly emphasized through the dose-dependent zone of inhibition, as well as the Time-Kill Kinetics, which showcased TC-HSF's strong bactericidal activity. Cytotoxicity assessments further emphasized the safety of TC-HSF on human fibroblast cells at specific concentrations, laying foundational grounds for its therapeutic application. Further research and clinical studies are warranted to validate these findings and translate them into practical applications into pharmaceutical industry of herbal soap production.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All authors contributed to the study's conception and design by Shamsi Saad Shamsi and Mukhtar Almukashir. Material preparation and lab data result collection were performed by Enas Ali, Heba Zidan, Shamsi Saad Shamsi. Salahaldin Alfurjany performed analysis. Shamsi Saad Shamsi wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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