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ORIGINAL ARTICLE

Trichilia catigua extracts with antibacterial activity for the treatment of acne vulgaris: topical formulation

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Abstract: The plants are possible sources of substances with antimicrobial activity, which have been extensively studied. The barks of *Trichilia catigua* are mainly used as stimulants, antioxidant, analgesic, vasodilator, antimicrobial, anti-inflammatory and anti-depressant. In the folk medicine their extracts are used as tonic for the treatment of fatigue, stress, impotence and memory deficits. The barks extract has a high content of phenolic compounds, including flavonoids and tannins. This study aimed to evaluate the *in vitro* antimicrobial activity against major microorganisms present on skin with acne of fifteen crude extracts obtained from *T. catigua* bark. We further aimed to use selected extracts to develop a cosmetic formulation for the treatment of acne vulgaris. The tests performed included disk diffusion in agar, determination of minimum inhibitory concentration (MIC), time-kill and survival curve analyses, and scanning electron microscopy analyses. The bacterial strains tested were *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus*, *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Cutibacterium acnes*, and *Bacillus cereus*. Potent antimicrobial activity was exhibited by the TCE01 (water), TCE02 (methanol), and TCE12 (water: methanol: ethanol: 1:1:1, v/v) extracts. However, when included in cosmetic formulations, they showed different activities. All formulations showed non-Newtonian behavior and pseudoplastic flow, in addition to thixotropy. Formulations containing TCE02 and TCE12 at 1.0% (w/w) of extracts exhibited antimicrobial activity in topical formulations for acne treatment. **Keywords:** Antimicrobial, Cosmetic, *Trichilia catigua*, Catuaba, Acne vulgaris.

INTRODUCTION

Trichilia catigua (A. Juss.), commonly known as catuaba or catiguá, is a tree that can reach 10 meters tall [1]. It has been used in common medicine as an aphrodisiac, and for treatment of sexual impotence, fatigue, memory deficit [2], insomnia, and depression [3,4]. It is reported to be effective without collateral effects or any toxicity toward humans [5,6]. According to Pizzolatti and collaborators [7], a flavonoid mixture including cinchonain isolated from *T. catigua* bark was shown to have antimicrobial properties. Furthermore, *T. catigua* crude extracts have been reported to exhibit antiviral activity [8].

To provide scientific validation of secondary metabolite for their use as therapeutic agents, *in vitro* and *in vivo* studies are required [9]. The results of these assays aid in identification of the biological mechanisms of action that are used as the basis for treatment in clinical practice [10]. Thus, products titled "naturals" have gained great attention in various industry segments [11].

Careful selection of solvents for preparation of extracts is critical, as this process plays a primary role in isolation of bioactive components. Large differences in efficacy can result from small variations in solvents used for extraction. Thus, a simplex centroid mixture design consisting of four solvents was used for extraction of *T. catigua* barks.

Acne vulgaris is a multifactorial inflammatory disease of the pilosebaceous follicles of the skin, affects approximately 85% of teenagers, and can persist into adulthood [12]. *Cutibacterium acnes*, related to acne pathogenesis, is a Gram-positive anaerobe that normally inhabits the skin [13]. This is an opportunistic pathogen implicated in progression of inflammatory acne vulgaris [14]. This bacterium is involved in production of proinflammatory cytokines, including interleukin (IL)-1B, IL-8, IL-12, and tumor necrosis factor alpha, resulting in inflammatory acne disease [15,16]. Gram-negative folliculitis is typically characterized by multiple papules and pustules in the middle of the face. It is caused by various bacterial strains and may be the result of prolonged antibacterial treatments in patients with acne [17].

The first line treatment for mild acne is topical therapy, administered as a monotherapy [18]. Antibiotics are commonly used to treat acne in combination with topical therapy. However, use of these agents for long-term treatment can contribute to development of bacterial resistance [19]. Other common treatment uses Accutane® called Roaccutane® outside the United States. This is a retinoid compound able to promote remission of acne, but its use involves an extremely dangerous teratogenicity with high absolute risk [20].

Topical herbal treatments are effective for treatment of acne skin due to their characteristics. Tannins, as an example, have natural astringent properties and can be used topically to treat acne [21]. Lonni et al. [22] indicated that *T. catigua* extract (TCE) had high total polyphenol content and antioxidant activity. Furthermore, Lonni et al. [23] indicated that W/O/W multiple emulsion is an effective platform for the delivery of TCE.

Emulsions contain two immiscible liquid phases dispersed into each other as fine droplets. Emulsions are extensively used as drug vehicles for treatment of skin diseases, and generally produce a pleasing sensation when applied to the skin [24]. Raw materials obtained from plant extraction, formulation composition, and temperature can affect viscosity, which can affect delivery of extracts to the application site. To demonstrate development of an appropriate system to deliver extracts of *T. catigua*, mechanical and rheological properties should be evaluated [25].

Therefore, the aim of the present study was to determine the antimicrobial activity against major microorganisms present on skin with acne of 15 extracts, and develop and characterize cosmetic formulations containing TCE for acne vulgaris treatment.

MATERIAL AND METHODS

Plant material

Barks of *T. catigua* were collected in Caetité, Bahia, Brazil (2011). The voucher specimen was identified by Dr. Cássia Mônica Sakuragui, and was deposited at the Herbarium of Universidade Estadual de Maringá (HUEM#306253), Maringá, PR, Brazil. The collection of the plant material was registered with IBAMA-SISBIO under No. 11995-6, May 13, 2016, authentication code 48926652 under the responsibility of J.C.P. Mello. Access to the botanical material was registered by the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado- SisGen* under No. A8B4204.

Preparation of T. catigua extract

Air-dried stem bark (10% w/w) was powdered and extracted using the following solvents: water, methanol, acetone, and ethanol by turbo-extraction (Ika T25), using the compositions specified in the simplex centroid mixture design (Table 1), resulting in 15 crude extracts (TCE). The TCE were filtered and concentrated using a rotary evaporator *in vacuum*, and then lyophilized according to Lonni et al. [22].

Evaluation of TCE antimicrobial activity

Bacterial strains

The following species of bacteria were used: *Staphylococcus aureus* ATCC 29213, Methicillin-Resistant *S. aureus* (MRSA) N315 and BEC 9393, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* 1E4248, *Bacillus cereus* ATCC 14579, and *Cutibacterium acnes* ATCC 6919 and ATCC 11827. These bacterial strains were obtained from the Laboratory of Basic and Applied Bacteriology, Department of Microbiology, State University of Londrina, Paraná, Brazil

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Disk agar diffusion test

TCE scattered in propylene glycol (Synth®) at concentrations of 1.0, 0.5, 0.1, and 0.05 g/mL were applied to paper disks. A control disk containing only propylene glycol (solvent control) was also prepared.

The disks containing TCE were placed on plates previously seeded with bacterial cultures. The plates were incubated at 37 ºC for 16-18 h. For evaluation of antibiotic bacterial sensitivity, the diameter of inhibition zone was measured. This procedure was performed according to the Clinical and Laboratory Standard Institute [26].

Minimum Inhibitory Concentration (MIC) for aerobic and anaerobic bacteria (on anaerobically jar - method for *C. acnes*)

MIC of aerobic bacteria was determined using the broth microdilution technique, according to CLSI [26], with some alterations. Bacterial samples were seeded on plates containing Mueller-Hinton (MH) agar (Difco®) at 37 ºC for 24 h, and diluted in saline (0.9 % NaCl) to reach a concentration of 1.5 x 10^8 CFU/mL, which corresponds to 0.5 on the McFarland scale. On microdilution plates containing 50 µL of MHB complemented with TCE at concentrations ranging from 0.5% to 10%, 50 µL of bacterial preparations were added, and the suspensions were incubated at 37 °C for 24 h. Bacterial growth was evaluated using a spectrophotometer with detection at 600 nm. The lowest concentration of extracts that inhibited visible bacterial growth was defined as MIC.

Anaerobic bacteria were evaluated using the microdilution method according to CLSI [26], with some alterations. Trypic soy broth (TSB) (Difco®) was used for this test, and reinforced clostridial medium (RCM) containing yeast extract (13 g/L), peptone (10 g/L), glucose (5 g/L), soluble starch (1 g/L), sodium chloride

(5 g/L), sodium acetate (3 g/L), and cysteine hydrochloride (0.5 g/L) (Oxoid Microbiology Product) was used for bacterial activation. The plates were incubated under anaerobic conditions using Gas-Pak (BD, Sparks, MD, USA) at 37 °C for 72 h.

Time-Kill Curve

Staphylococcus aureus ATCC 29213 was grown in MH agar medium at 37 °C for 24 h. A new inoculum was prepared by diluting the bacteria in fresh MH broth to obtain 10^6 cells/mL. TCE were added to the desired concentration. Solvents were added to MH broth as controls. The aliquots were removed, diluted, and plated on MH agar and incubated for different times (1, 2, 5, 7, 10, and 24 h), and colony forming units (CFU) were counted. The results were compared with cultures grown under the same conditions, but without TCE (control).

Scanning Electron Microscopy (SEM)

TCE02 (methanol) was evaluated at MIC for the optimal incubation time as determined by the time-kill curve method. The procedure was performed according to Oliveira and others [27]. *S. aureus* ATCC 25923 was prepared in MH broth and the cell density was adjusted to 10^8 CFU/mL. One milliliter of the cell suspension was distributed to two tubes. TCE02 at MIC was added to the first tube and solvent was added to the second tube (control). The cultures were shaken at 150 rpm at 37 ºC for 3 h. Ten microliters of each culture and of a fixative solution (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2) were added onto poly-L-lysine-coated glass slides. After 30 min, 500 µL of fixative solution was added to each slide, and slides were incubated for 20 h. Post-fixation was then performed in 1% OsO_4 for 1 h. Post-fixed cells were dehydrated using an ethanol gradient (Sigma-Aldrich) (70, 80, 90, and 100% ethanol), dried using CO_2 (BALTEC CPD 030 Critical Point Dryer), coated with gold (BALTEC SDC 050 Sputter Coater), and visualized using a scanning electron microscope (FEI Quanta 200). All reagents were obtained from Electron Microscopy Sciences.

Development of Formulations

Formulations (O/W emulsions) were prepared by the phase-inversion emulsification method in two steps, using hydroxyethylcellulose, emulsifying wax NF (cetylstearyl alcohol and polysorbate 60), caprylic/capric triglycerides, propylene glycol, cyclomethicone, distilled water, and 1.0% TCE (w/w). Hydroxyethylcellulose was dispersed in the water phase. Emulsifying wax NF and Caprylic/capric triglycerides were dispersed in the oil phase. The oil and aqueous phases were heated separately to 75 ± 2 °C. The oil phase was slowly added to the aqueous phase with stirring at 450 rpm (RW20 Digital Mixer, IKA, Wilmington, NC) until they reached 40 °C. Cyclomethicone was added to the formulation. TCE (01, 02, and 12) were separately dispersed in propylene glycol, then added to the emulsion at 25 °C. The compositions of four formulations of emulsions (O/W) were as follows: BF – formulation without TCE, F01 – formulation with 1.0% (w/w) TCE01, F02 – formulation with 1.0% (w/w) TCE02, and F12 – formulation with 1.0% (w/w) TCE12 (Table 2).

Characterization of formulations

Organoleptic and morphological analysis

Appearance, odor, and color characteristics were analyzed in triplicate by evaluation of phase separation, color change, precipitation, and turbidity. The samples were classified in terms of color as normal, without alteration, slightly modified, modified, or heavily modified [28].

Each sample was evaluated for appearance, creaming, and coalescence after 24 h, and after the centrifugation test. Five grams of sample were centrifuged (Centrifuge Baby I 206 BL, Fanem) at 2,800 rpm for 30 min at room temperature.

Determination of pH

Formulation pH values were determined using a pH meter (Hanna) calibrated with buffer solutions (pH 4.0 and 7.0) at room temperature (25 ± 5 °C) (n=3).

Texture profile analysis of O/W emulsions

Texture profile analysis was performed using a TA-XTplus Texture Analyzer (Stable Micro Systems, Surrey) in TPA mode. After cautiously transferring formulations to small vials, the samples were compressed twice (2 mm/s) at a defined depth (15 mm) by an analytical probe (10 mm diameter), with a 15 s delay between the end of the first and the beginning of the second pass. Three replicates of each formulation were analyzed at temperatures of 25 °C and 37 °C. Force-time and force-distance graphs were used to calculate hardness, compressibility, elasticity, and cohesiveness parameters [25].

Continuous shear rheometry

Flow analysis of formulations was performed at 25 °C and 37 °C ± 0.1 °C using a controlled stress rheometer (MARSII, Haake Thermo Fisher Scientific) with parallel steel cone-plate geometry (35 mm, separated by a fixed distance of 0.052 mm). O/W emulsion samples with and without TCE were carefully applied to the lower plate to ensure that formulation shear was minimized, allowing at least 1 min of equilibration prior to analysis. The downward and upward flow curves were calculated over shear rates ranging from 0 to 2000/s, which increased over a period of 150 s, and were maintained at the upper limit for 10 s, then decreased over a period of 150 s. Modeling of upward flow curves was performed using the Oswald-deWaele equation (Power Law) (Eq.(1)). All analyses were performed at least in triplicate.

$$\tau = \kappa . \gamma^n \qquad (1)$$

Where τ is shear stress (Pa), γ is the rate of shear (s^{-1}), κ is the consistency index [$(Pa.s)^n$], and n is the flow behavior index (dimensionless). RheoWin 4.10.0000 (Haake) software was used to calculate the hysteresis area for each formulation.

Antimicrobial activity of formulations

Survival curve

Cultures of *S. aureus* ATCC 29213 were grown on MH agar for 24 h at 37 °C. A new inoculum was prepared by diluting the bacteria in fresh MH broth to 10^8 cells/mL. Ten microliters of diluted bacteria were added to 990 µg of each formulation containing TCE or 990 µg BF. The mixtures were homogenized, diluted, and plated on nutrient agar (Himedia). Different incubation times (1, 2, 5, 7, 10, and 24 h) were used for counting of colony forming units (CFU).

Statistical analysis

All experimental measurements were carried out in triplicate, and were expressed as an average of three analyses ± standard deviation. The effects of TCE concentration and TCE presence on pH and antimicrobial activity of formulations, and texture profile, and rheometry analyses were statistically compared using one-way analysis of variance (ANOVA). $p<0.05$ was considered statistically significant.

Table 1- Solvent proportions in the crude extracts of *trichilia catigua* barks.

Crude extract (TCE)	Extracts (solvent proportions, v/v)
01	*w (1)*
02	*m (1)*
03	*a (1)*
04	*e (1)*
05	*w/m (1/2:1/2)*
06	*w/a (1/2:1/2:1/2)*
07	*w/e (1/2:1/2)*
08	*m/a (1/2:1/2)*
09	*m/e (1/2:1/2)*
10	*a/e (1/2:1/2)*
11	*w/m/a (1/3:1/3:1/3)*
12	*w/m/e (1/3:1/3:1/3)*
13	*w/a/e (1/3:1/3:1/3)*
14	*m/a/e (1/4:1/4:1/4:1/4)*
15	*w/m/a/e (1/4:1/4:1/4:1/4)*

w = water; *m* = methanol; *a* = acetone; *e* = ethanol.

Table 2. Composition of four formulations of emulsions (O/W) without TCE (BF) and with 1.0% TCE (F01, F02, and F12).

	Formulations			
Composition (%; w/w)	BF	F01	F02	F12
Distilled water	Qsp 100.0	Qsp 100.0	Qsp 100.0	Qsp 100.0
Hydroxyethylcellulose	0.5	0.5	0.5	0.5
Emulsifying wax NF*	2.0	2.0	2.0	2.0
Caprylic/caprictriglycerides	3.0	3.0	3.0	3.0
TCE01	-	1.0	-	-
TCE02	-	-	1.0	-
TCE12	-	-	-	1.0
Propyilene glycol	5.0	5.0	5.0	5.0
Cyclomethicone	0.5	0.5	0.5	0.5

RESULTS AND DISCUSSION

Brazilian biodiversity is the largest in the world. However, studies on chemical and biological potential of all species are scarce [29]. Increased attention has focused on products obtained from plants for use in multiple industry segments [11]. As such, several types of plants, such as *T. catigua,* are being studied to evaluate potential biological activities.

Previous studies showed the presence of various chemical compounds in *Trichilia catigua* including cinchonains [7], anthocyanin glycosides, tannins (both hydrolysable and condensed), saponins [30], ciclolignans, alkaloids, flavonoids, and sesquiterpenes [31]. The antimicrobial activities of these compounds have been described [32, 33], and suggest potential activity of *T. catigua* against microorganisms. Antioxidant activities of substances (epicatechin, procyanidins B2 and C1, catiguanins A and B, cinchonains Ia, Ib, Ic, and Id) isolated from *T. catigua* bark were evaluated using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay, and demonstrated radical scavenging activity and reducing power of these compounds [34, 35].

The 15 TCE presented in this study were evaluated in a previous study for total polyphenol content (TPC), antioxidant activity, and identification of classes of compounds by optimization of the extraction procedure High TPC was found in TCE02 and TCE12 (48.31 ± 0.55 %, and 46.57 ± 0.74 %, respectively), and the presence of cinchonains, gallic acid derivatives, natural polyphenols, flavonoids, catechins, and epicatechins was verified by comparative evaluation of HPLC–DAD spectra of the chromatographic peaks [22].

Regarding disk agar diffusion test, TCE05, TCE06, TCE07, and TCE12 showed the largest inhibition zones for *S. aureus* (13, 12, 15, and 13 mm, respectively), *S. epidermidis* (14, 15, 16, and 15 mm, respectively), and *Methicillin Resistant S. aureus* (4, 14, 14, and 15 mm, respectively). No inhibition was observed for other bacterial strains.

MIC was determined for TCE01, TCE02, TCE05, TCE06, TCE07, and TCE12 against *S. aureus* ATCC 29213. The resultant MIC values were 500 µg/mL for TCE02, TCE05, TCE06, and TCE12, and 1000 µg/mL for TCE01 and TCE07.

For *C. acnes*, the lowest concentration of extract added to the microdilution plates was 31.25 µg/mL of TCE01, TCE07, and TCE12, and this concentration visibly inhibited growth of *C. acnes.* Therefore, MIC for these extracts was defined as less than 31.25 µg/mL.

Pizzolatti and collaborators [7] evaluated the antimicrobial activity of two epimeric flavalignan cinchonains against various species of bacteria, including *Staphylococcus aureus*. These compounds were contained in the ethanolic extract of stem bark of *T. catigua.* The results of this study showed that the ethanolic extract was able to inhibit growth of *B. cereus* and *S. aureus*. The MIC against these bacteria was comparable to our results for TCE02, TCE05, TCE06, and TCE12.

Antibacterial activity of various extracts against *S. aureus* (including MRSA) and *S. epidermidis* was verified in our study. Some compounds cannot diffuse in agar due to poor solubility, so microdilution in broth is important for confirmation of antibacterial activity. TCE01 and TCE02 exerted antibacterial activity only in the microdilution in broth assay. The concentrations were slightly high, but the antibacterial effects of these extracts could be improved in the future via further processing.

Time-kill curves were generated for TCE01, TCE02, TCE05, TCE06, TCE07, and TCE12 extracts against *S. aureus* ATCC 29213. Results are summarized in Figure 1.

TCE01 (1,000 µg/mL) eliminated all bacteria within 7 h, and the number of CFU was 2,160-fold lower following 5 h of incubation compared to that of the control (without extract). For TCE02, the number of CFU was reduced 10,000-fold after 10 h of incubation compared to that of the control. For TCE03, the number of CFU was reduced 158-fold after 7 h of incubation compared to that of the control, and the number of CFU was zero at the final time point. For TCE04, the number of CFU was 611-fold lower after 10 h of incubation compared to that of the control, and the number of CFU was zero after 24 h. For TCE05, of the number of CFU was reduced 15,220-fold after 10h of incubation compared to that of the control. TCE06 and TCE07 reduced the number of CFU 1,000,000-fold after 10 h of incubation compared to that of the control. For TCE12, the number of CFU was reduced 17-fold after 5 h of incubation.

Within 24 h TCE01, TCE03, TCE04, TCE06, and TCE12 eliminated all bacteria, and TCE01 was the most effective. SEM images of *Staphylococcus aureus* ATCC 29213 treated with TCE02 (2000 µg/mL) at 3 h are shown in Figure 2. Figure 2A shows untreated *S. aureus* ATCC 29213 (control) at 12,500X magnification prepared in standard MH broth. This sample was characterized by a large number of smooth, spherical cells, with unaltered average size. Figure 2B is an image of untreated *S. aureus* ATCC 29213 (control) at high magnification (25,000X), which shows unmodified surface of *S. aureus* 29213. Figure 2C is an image of *S. aureus* ATCC 29213 treated with TCE02 at 12,500X magnification, and shows cell surface alterations, including protrusions and cell debris. Moreover, the number of cells was reduced and an amorphous mass was observed. Figure 2D is an image of *S. aureus* ATCC 29213 treated with TCE02 at high magnification (25,000X), and shows that morphology of *S. aureus* 29213 changed. Furthermore, large amounts of organic matter, likely from cell death, were observed (Figure 2C and 2D).

Evaluation by SEM showed reduced bacterial populations, cellular morphological alterations, amorphous masses, and cell debris within a few hours of treatment with TCE02 extract. These results suggested that *T. catigua* extract inhibited the growth of *S. aureus* ATCC 29213 and also exerted high antibacterial activity.

Survival curves for each formulation are shown in Figure 3. The bacteria survived in BF for 5 h. F02 and F12 reduced the number of CFU/mL more than BF after 2 h of contact. However, F01 did not reduce microbial growth.

TCE01 showed high antibacterial activity within a few hours of incubation, and all bacterial populations were killed within 7 h. However, this antibacterial activity was not observed in the F01 formulation. The extraction solvent for TCE01 was water, which not provided total extraction of actives with antimicrobial activity. F01 showed high thixotropic behavior, which is appropriate for skin applications, but not adequate for *in vitro* microbial assays. As such, TCE01 was not suitable for this formulation. It is possible that pH, solvents, composition of formulation, solubility, viscosity, micelles, and other factors may have interfered with compounds from this extract.

In contrast, TCE02 and TCE12 in formulations F02 and F12, respectively, showed high efficiency in controlling bacterial populations, considerably reducing the number of bacteria within 2 h of incubation. This high antimicrobial activity was related to high concentrations of polyphenols extracted from *T. catigua* and preparation of a suitable delivery system, which allowed activity at the target site. These results suggested that methanol (TCE02) and ternary solvent mixtures (water: methanol:

ethanol; TCE12) were extracted compounds with antimicrobial activity that was maintained when included in formulations. These results demonstrated that extraction solvents and extracted compounds may interact differently with formulation components, and may affect antimicrobial tests following formulation. Thus, antimicrobial activity of 1.0 % of TCE in formulations was observed after 24 h of incubation and suggested potential application of these extracts in formulations for treatment of acne.

These extracts did not show antibacterial activity against Gram-negative bacteria. However, many skin infections are caused by Gram-positive bacteria such as *S. aureus,* including MRSA, in hospitals and in the community, suggesting potential of these formulations for topical application.

TCE01, TCE02, and TCE12 showed the best antibacterial activity and had the greatest potential for use in cosmetic or pharmaceutical formulations. Thus, formulations were developed containing 1.0 % (w/w) of each of these extracts.

Morphological analysis showed that BF appeared milky and F01, F02, and F12 showed the characteristic color of *T. catigua* with a milky appearance. After the centrifugation test at room temperature (25 ± 2 ºC), no formulations showed creaming, coalescence, or phase separation. The pH of the samples were as follows: BF (6.66 ± 0.02), F02 (5.43 ± 0.07), F01 (5.13 ± 0.04), and F12 (4.65 ± 0.01). Formulations containing TCE differed significantly from BF ($p<0.05$).

Microbial tests showed that the formulations were not contaminated [36]. Compressional flow was evaluated by measurement of elasticity, hardness, compressibility, and cohesiveness properties. Texture profile analysis results of BF, F01, F02 and F12 are summarized in Table 3.

In general, similar results for mechanical properties were observed. No statistically significant difference were observed for hardness ($p < 0.05$) of formulations containing TCE compared to BF at 25 °C.

Increased temperature (25 °C to 37 °C) promoted a decrease in all mechanical properties in the F01 formulation. F02 showed a decrease in hardness and elasticity, and F12 a decrease in compressibility and cohesiveness, with increased temperature. In addition, all formulations containing TCE showed decreased compressibility compared to BF at 37 °C.

Suitable flow properties are important to local actions of products, and can provide relevant information regarding optimization of formulation composition and stability [24]. Flow properties of formulations BF, F01, F02, and F12 at 25 °C and 37 °C are shown in Figure 4.

Pseudoplastic flow was observed, independent of temperature, for all formulations. A power law model was used to fit and compare the additive effects of each extract and temperature, allowing for determination of the consistency index (κ) and flow behavior index (n) from the up-curve of each rheogram (Table 4).

Increased temperature resulted in decreased κ values for BF, F02, and F12, and a slight increase in κ value for F01. The n value decreased in F01, and increased in BF, F02, and F12, in response to increased temperature.

All formulations showed positive values for hysteresis area at 25 °C (Table 4). BF hysteresis area at 37 °C was negative, while hysteresis area for formulations containing TCE was positive.

Rheological parameters such as apparent viscosity, shear stress, shear rate, and consistency were measured to evaluate changes in flow behavior of topical formulations depending on factors such as formulation composition and temperature. This evaluation

demonstrated formulation homogeneity and suitability for extract delivery.

The formulations (O/W emulsions prepared using a two-step emulsification method) were simple and reproducible. Furthermore, they were homogeneous when mixed with TCE. In O/W emulsions, water is the external phase, which is indicative of higher water content, resulting in lower cost of formulation and tendency to feel less greasy [37]. Absence of creaming, coalescence, or phase separation was verified in all formulations, and may have been due to the presence of an emulsifier, which decreased interfacial tension between the liquids and stabilized the dispersed phase against coalescence [38].

Determination of compressional flow properties (elasticity, hardness, compressibility and cohesiveness) provides fundamental information regarding mechanical performance [39]. The mechanical properties of the formulations with and without TCE were evaluated to system performance at room temperature and physiological temperature. Hardness and compressibility demonstrate the force required to remove the sample from the packaging material and to apply to the target site [25]. Therefore, low values of these properties are suitable for topical formulations. Low elasticity values were observed for BF, F01, F02, and F12, indicating a shorter time to stretch and remodel formulation structure. Higher cohesiveness values are associated with greater restructuring of formulations [25]. In general, higher cohesiveness values were observed at 25 °C, demonstrating that this temperature is suitable for the formulations evaluated in this study.

Addition of TCE to BF promoted small flow curve changes (Figure 4). Since the base composition was fixed, each behavior change was attributed to the different solvents used in the extraction process of *T. catigua*, which were water (TCE 01), methanol (TCE 02), and water, methanol, and ethanol (TCE 12).

The flow rate values (n) obtained from the power-law model (Table 4) showed results below one, confirming that all formulations demonstrated non-Newtonian behavior and pseudoplastic flow [40, 41]. Therefore, viscosity decreased with strain rate, confirming O/W emulsion behaviors described in previous studies [38].

O/W emulsions containing TCE exhibited thixotropic behavior, as confirmed by positive hysteresis area values. This characteristic is advantageous to delivery systems because the material become more fluid upon application. After removal of shear stress, the thixotropic system returns to its initial structure with lower viscosity in a short time, which may favor stability of the formulation.

W/O/W multiple emulsions containing different vegetable oils were previously evaluated for delivery of *T. catigua* extracts [23]. Good retention of TCE in the skin was observed, indicating that this formulation was suitable to carry TCE. Moreover, this formulation can be used for many topical treatments for infectious and skin diseases. Topical formulations are cost-effective, easy to use, and comfortable for patients.

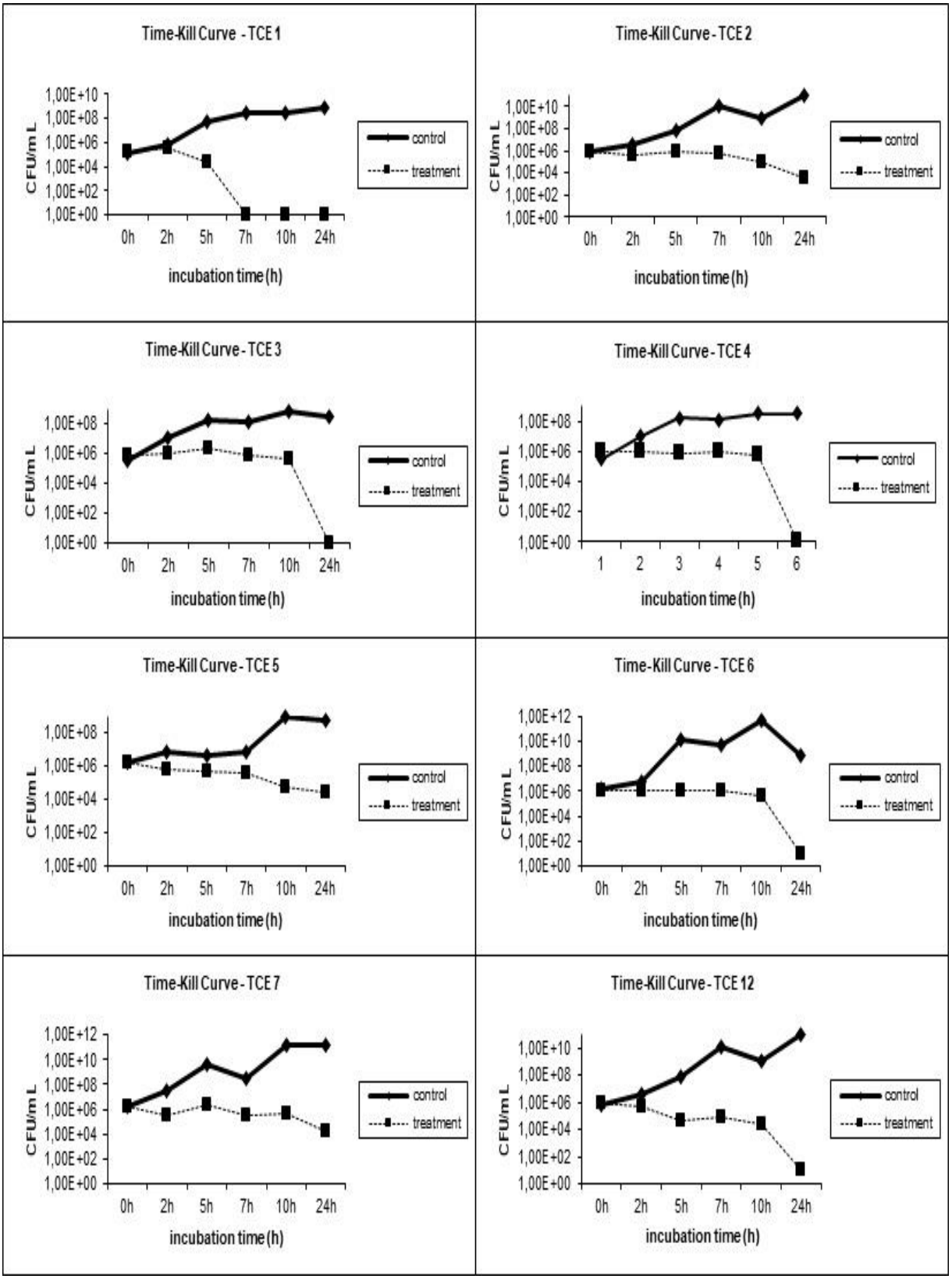

Figure 1. Time-kill curve of *S. aureus* 29213 versus TCE01, TCE02, TCE03, TCE04, TCE05, TCE06, TCE07, and TCE12 extracts. Treatment: aliquots containing medium of culture, TCE 20% (w/v) and bacteria inoculum. Control: aliquots containing medium of culture and bacteria inoculum.

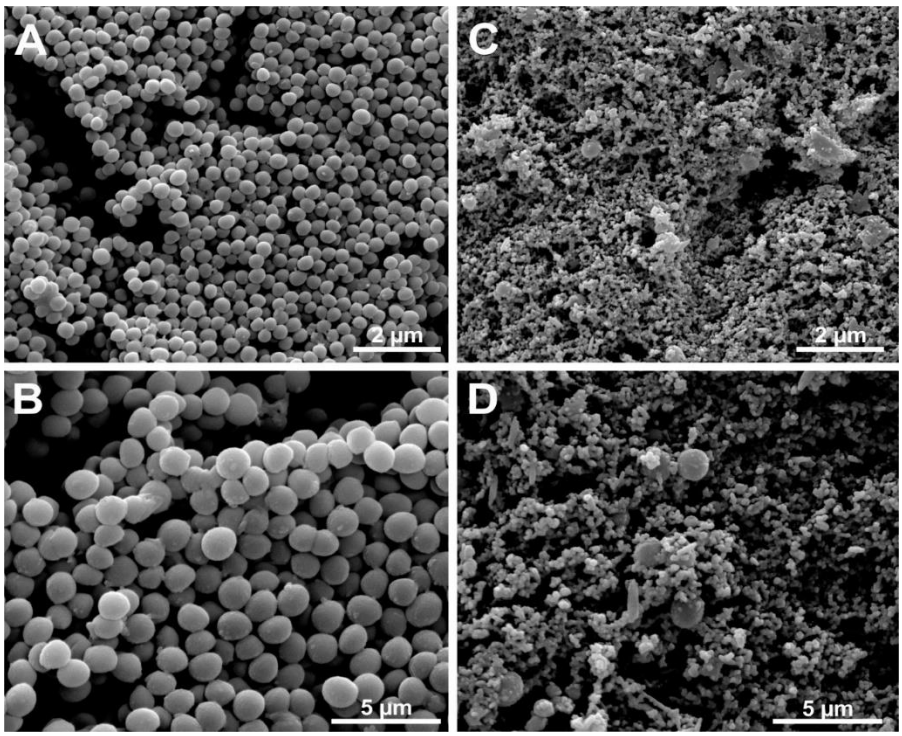

Figure 2. Scanning electron microscopy of strains *Staphylococcus aureus* ATCC 29213 treated with TCE02 (2,000 µg/mL) at 3 h. A: untreated *S. aureus* ATCC 29213 (control) at 12,500 x magnification; B: untreated *S. aureus* ATCC 29213 (control) at 25,000 x magnification; C: *S. aureus* ATCC 29213 treated with TCE02 extract at 12,500 x magnification; D: *S. aureus* ATCC 29213 treated with TCE02 extract at 25,000 x magnification.

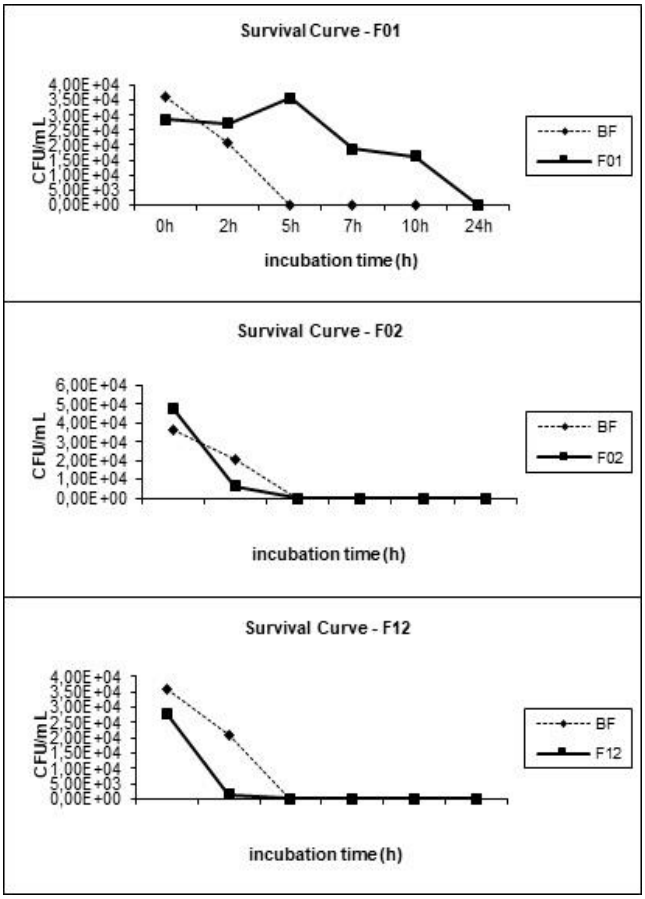

Figure 3. Survival Curves of *S. aureus* 29213 versus BF, F01, F02, and F12, respectively.

Table 3. Mechanical properties (elasticity, hardness, compressibility and cohesiveness) of BF, F01, F02 and F12, at 25 °C and 37 °C.

Formulation	Temperature (°C)	Mechanical (compressional) properties			
		Hardness (N)	Compressibility (N.mm)	Elasticity (mm)	Cohesiveness (Dimensionless)
BF	25	0.055 ± 0.003	0.138 ± 0.003	0.982 ± 0.019	1.479 ± 0.064
	37	0.055 ± 0.001	0.142 ± 0.004	0.998 ± 0.048	1.001 ± 0.036
F01	25	0.051 ± 0.003	0.097 ± 0.005	1.063 ± 0.017	1.123 ± 0.061
	37	0.051 ± 0.002	0.088 ± 0.003	0.971 ± 0.014	1.048 ± 0.022
F02	25	0.052 ± 0.002	0.085 ± 0.004	0.986 ± 0.024	1.029 ± 0.060
	37	0.046 ± 0.003	0.090 ± 0.004	0.970 ± 0.034	1.107 ± 0.025
F12	25	0.049 ± 0.002	0.104 ± 0.002	0.917 ± 0.043	1.007 ± 0.020
	37	0.058 ± 0.003	0.092 ± 0.003	1.041 ± 0.045	0.990 ± 0.037

Data represents mean ± SD (n = 3)

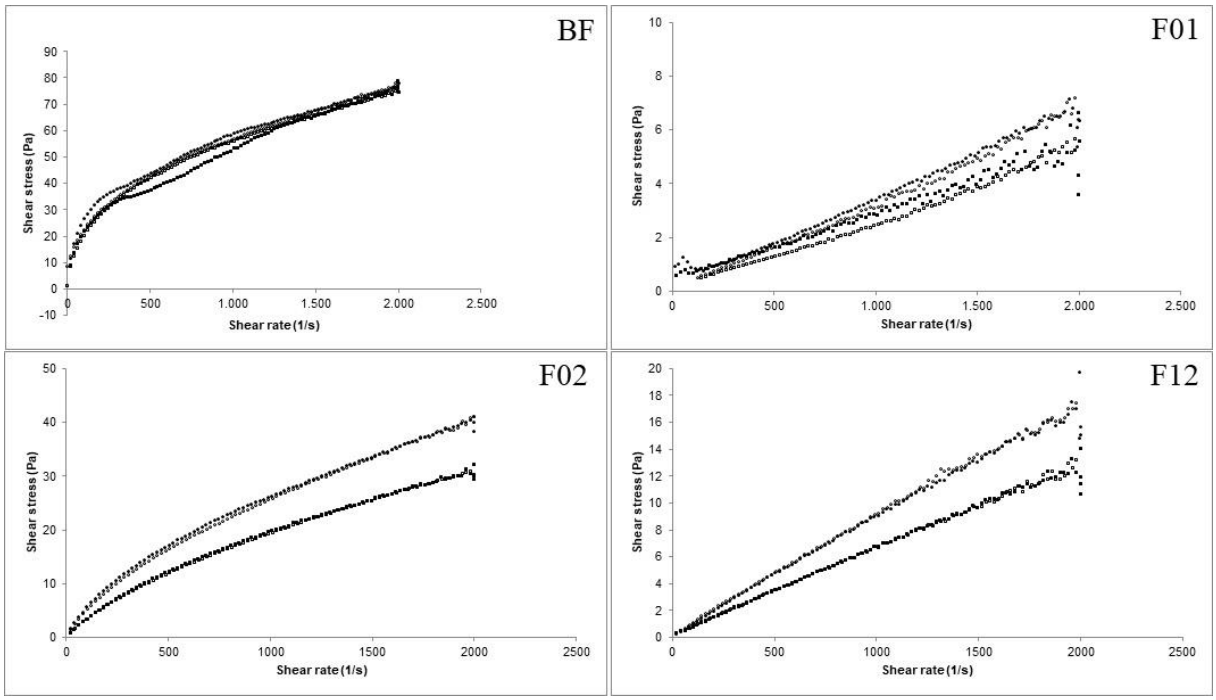

Figure 4. Flow rheograms of BF (A), F01 (B), F02 (C) and F12 (D), at 25 °C (●) and 37 °C (■). Closed symbol represents up-curve and open symbol represents down curve. In all cases, the coefficient of variation of replicate analysis was less than 10%.

Table 4. Power law parameters and hysteresis area of formulations in absence and containing TCE.

Formulation	Consistency Index (κ) (Pa.s)		Flow Behavior Index (n) (Dimensionless)		Hysteresis area[a]	
	25 ºC	37 ºC	25 ºC	37 ºC	25 ºC	37 ºC
BF	4.340 ± 0.667	2.218 ± 0.087	0.378 ± 0.021	0.464 ±0.004	4.154 ± 1.561	-3.259 ± 0.970
F01	0.006 ± 0.002	0.009 ± 0.004	0.938 ± 0.037	0.846 ± 0.059	435.420 ± 126.722	642.60 ± 142.267
F02	0.317 ± 0.060	0.199 ± 0.018	0.625 ± 0.005	0.672 ± 0.010	1.081 ± 0.244	0.236 ± 0.617
F12	0.016 ± 0.002	0.012 ± 0.001	0.920 ± 0.014	0.934 ± 0.037	9.611 ± 2.973	22.800 ± 18.364

Data represents mean ± SD (n = 3). [a] Thixotropy (+) and Rheopexy (-).

CONCLUSION

The results of our *in vitro* experiments demonstrated that *Trichilia catigua* extracts exhibited significant antibacterial activity. Design mixtures of different solvents were used for extraction optimization. Potent antimicrobial activity was observed in TCE01, TCE02 and TCE12. Scanning electron microscopy confirmed bacterial structural alterations in response to these extracts. Moreover, formulations containing 1.0 % (w/w) TCE02 and TCE12 exerted antibacterial activity, suggesting potential use of these extracts in anti-acne formulations. Non-Newtonian behavior and pseudoplastic flow were verified in all formulations. The O/W emulsions containing TCE provided a thixotropic system that was favorable for formulation retention at the application target. In this study, the use of cosmetic technology and microbiological techniques indicated that extracts obtained from *T. catigua* barks included in formulations at 1.0 % (w/w) have potential activity as antimicrobial agents and may be effective for treatment of acne. Further studies in humans are necessary to confirm these findings.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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