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# Comprehensive evaluation of antiacne properties: HPLC analysis of crude extract from *Aloe buettneri* leaves against *Cutibacterium acnes*-induced acne

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**The hydroethanolic extract of *Aloe buettneri* was assessed for its anti-acne activity in a *Cutibacterium acnes*-induced acne model in rats as part of this study. Conventional therapies for acne often entail serious side effects. Total phenols, quantified using the Folin-Ciocalteu method, were found to be at a concentration of 16 ± 0.3 mg GAE/g, while total flavonoids, determined using the aluminum chloride method, were measured at 2.83 ± 0.5 mg QE/g. High-performance liquid chromatography (HPLC) techniques identified four polysaccharidic flavonoids in the extract: protocatechuic acid, caffeic acid, homo-orientin, and ferulic acid. In the acne-induced rat model, the disease group exhibited increased ear thickness, rectal temperature, and MDA level, along with a decrease in glutathione level (###p < 0.001). However, significant improvement in these parameters was observed after 12 days of topical treatment (***p < 0.001). The identified molecules in the *A. buettneri* extract, including protocatechuic acid, caffeic acid, homo-orientin, and ferulic acid, may contribute to the observed anti-acne properties.**

**Key words:** *Aloe buettneri*, hydroethanolic extract, anti-acne activity, chemical study.

## INTRODUCTION

Acne, a chronic inflammatory skin disorder, predominantly manifests during puberty, with prevalence estimates among adolescents ranging from 35 to over 90% (Wolkenstein et al., 2018). In Togo, a multicenter study conducted in Lome indicated that acne appeared on the face in 100% of cases, with the most common clinical form being papulopustular acne (66.7%) (Saka et al., 2018). This persistent inflammatory condition gives rise to emotional distress, psychosocial issues (Sachdeva et al., 2021) and psychiatric disorders, including depression, emotional isolation, and, in extreme cases, even suicidal thoughts (Misery, 2011).

The principal recognized pathophysiological pathways in acne lesion development involve excessive and altered sebum production, abnormal keratinization of the pilosebaceous follicle, microbial colonization, the inflammatory response, and oxidative stress (Suh and Kwon, 2015). Inflammatory acne, linked to a reduction in the diversity of *Cutibacterium acnes* phylotypes, is associated with the participation of *C. acnes* and

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*Staphylococcus epidermidis* in skin inflammation (Dréno et al., 2020). Consequently, both topical and systemic antibiotics are required to restore bacterial species' diversity and balance. Immunity has also begun to emerge as a factor involved in acne, paving the way for vaccine research (Contassot, 2018). Similarly, recent studies have disclosed that oxidative stress is a contributing factor in acne pathophysiology (Sahib et al., 2013; Anggraeni et al., 2017).

Various treatments, such as anti-seborrheic agents, anti-androgens, antimicrobials, anti-inflammatories, antioxidants, and keratolytics, are employed against acne (Araviiskaia and Dréno, 2016). However, these treatments may lead to drug resistance (Varghese, 2020), and some pose a risk of toxicity (Akhavan and Bershad, 2003). The primary adverse effects of topical acne treatments encompass erythema, desquamation, itching, burning, and increased skin sensitivity (Otlewska et al., 2020).

Hence, it is imperative to explore new therapeutic molecules capable of effectively treating acne. Plants, being longstanding sources of remedies for humans, emerge as excellent candidates for acne therapy research. Plant-based therapy provides a promising approach to address the various pathophysiological components involved in acne, employing a "multi-target" strategy. The documented efficacy of antioxidants derived from plant extracts in combating this pathology is well-established (Vora et al., 2018). There is a growing interest in developing alternative antioxidants for acne from natural sources, particularly plants.

The hydroalcoholic extract of *Aloe buettneri* is recognized for containing phenolic compounds with antioxidant and antimicrobial properties against *Cutibacterium acnes*, the microorganism responsible for acne (Kombate et al., 2022), as well as exhibiting anti-inflammatory activities (Metowogo et al., 2008). The objective of this study was to characterize the phenolic compounds in the *A. buettneri* extract and assess its anti-acne properties in Sprague Dawley rats.

## Experimental

### Phytochemical study

#### *Extraction*

Fresh leaves of *A. buettneri* were collected in Lomé (Togo) in September 2019. The reference specimens were authenticated and archived in the Herbarium of the Laboratory of Botany and Plant Ecology at the University of Lome (Togo) with the designation Togo 15668. The gathered samples were dried under controlled air conditions and subsequently pulverized into powder. A total of four hundred fifty grams (450 g) of powder were subjected to extraction through maceration in 4 l of a mixture of ethanol and distilled water (50:50), with regular manual agitation over a period of 72 h. Following this, the mixture underwent filtration through a hydrophilic cotton filter and then a filter paper (Whatman). The resulting filtrate was further evaporated to dryness under vacuum at 45ºC using a rotavapor R114. The resulting extract is a brownish paste that is soluble in water.

#### *Quantitative analysis of total phenols*

Total phenols were determined utilizing the Folin-Ciocalteu method as described by Maksimović et al. (2005). The extract (50 mg) was diluted in 5 mL of a methanol-water mixture (1:1) to achieve a final absorbance ranging from 0.5 to 1. A standard curve was prepared using gallic acid dissolved in the same solvent, with six points and concentrations ranging from 0 to 0.25 mg/mL. For the assay, 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times in a methanol-water mixture) was added to 0.25 mL of the diluted extract or the gallic acid standard solution. Subsequently, 2 mL of 1 M sodium carbonate ($Na_2CO_3$) was added. In the "blank" reaction, 0.25 mL of the solubilizing solvent was used instead of gallic acid or the extract under study. The obtained value was considered as the 0 mg/mL point on the standard curve.

The reaction mixture, corresponding to each solution on the gallic acid calibration curve or each sample to be assayed, was agitated and then left to stand for 15 min at room temperature. Absorbance was measured in triplicate for each sample at 765 nm using a cuvette-based molecular absorption spectrophotometer (Pierron). The average concentration of polyphenols present in the plant extract is expressed in milligrams of gallic acid equivalent per gram of extract using the equation of the gallic acid calibration curve.

#### *Total flavonoids contents determination*

The method utilized was as described by Kosalec et al. (2004). The extract was dissolved in water and prepared at the same concentration used for the total phenol determination. A standard range was prepared with six concentrations of quercetin (1 to 100 µg/mL) using distilled water as the solubilizing solvent. For the assay, 1.2 mL of methanol was added to 0.400 mL of the diluted extract or quercetin standard solution. Subsequently, 10% aluminum chloride (0.080 mL) was added, followed by 1M sodium acetate (0.080 mL), and the solution was made up to 4 mL with distilled water. In the blank reaction, 0.400 mL of the solubilizing solvent (distilled water) was used instead of quercetin. The obtained value was considered as the 0 mg/mL point on the standard curve.

The reaction mixture, corresponding to each quercetin standard solution in the calibration curve or each sample to be assayed, was mixed and then allowed to stand for

30 min at room temperature. Absorbance was measured in triplicate for each of the samples at 415 nm using a cuvette-based molecular absorption spectrophotometer (Pierron). The average concentration of flavonoids present in the plant extract is expressed in milligrams equivalent quercetin per gram of extract using the calibration curve equation.

## Chromatographic analysis

### *Thin-layer chromatography (TLC)*

The crude hydroalcoholic extract underwent analysis using Thin-Layer Chromatography (TLC). Silica plates (aluminum-backed) from Merck served as the stationary phase. Various mobile phases were employed throughout the study, depending on the extract(s) under investigation. These mobile phases included Ethyl acetate/Methanol/Water (100/17/13); Ethyl acetate/Acetic acid/Formic acid/Water (100/11/11/26); and Propanol/ Water (70/30), specifically utilized for the analysis of highly polar fractions rich in polysaccharide compounds.

Different reagents were employed as visualizing agents. The NEU reagent (NP/PEG) facilitated the characterization of phenolic compounds under UV (ultraviolet) light at 365 nm. In the presence of phenolic acids, flavonols, and flavones, these compounds manifested as blue, yellow-orange, or green fluorescence (Rastija et al., 2004). Vanillin-sulfuric acid was also used to highlight polysaccharides.

## High-performance liquid chromatography (HPLC)

### *Method 1: Primary test*

The analyses were conducted using a Varian ProStar system equipped with a Varian Star diode array detector (DAD) for UV/VIS spectrum recording and a Varian Star 230 binary pump. Injections were manually performed using a 10 µL loop and a 100 µL syringe. The column utilized was a Nucleodur EC 250/4.6 100-10 C 18 EC, 5 µm, from Macherey Nagel, equipped with an 8/4 pre-column filled with the same stationary phase. For the analysis, the hydroethanolic extract was dissolved in water at respective concentrations of 10 mg/mL and 5 mg/mL, and then analyzed at a flow rate of 1 mL/min following an acetonitrile/water gradient with 0.1% trifluoroacetic acid, as detailed in Table 1. Detection was performed at 205, 280, and 324 nm by comparing their absorption spectra and retention times with those of the major peaks.

### *Method 2: Optimization of analytical conditions*

The system utilized was a Varian 920 LC equipped with a Diode Array Detector (DAD), a column oven, and an auto-injector. The column employed was a Nucleodur EC 250/4.6 100-10 C 18 ec, 5µm, from Macherey Nagel, with an 8/4 pre-column filled with the same stationary phase. For the analysis, the extract was dissolved in water at respective concentrations of 10 mg/mL and 5 mg/mL, and then analyzed by injecting at 20 µg/mL at a flow rate of 1 ml/min at 30°C. The mobile phase used was an acetonitrile/water gradient with 0.1% trifluoroacetic acid, as detailed in Table 2.

Detection was performed at 205 and 280 nm by comparing their absorption spectra and retention times with those of the major peaks.

## *In vivo* assay

### *Animals*

Sprague Dawley rats weighing between 145 to 170 g, sourced from the animal house of the Department of Animal Physiology at the University of Lomé, were employed in this study. These animals were housed in a well-lit room with a 12-h light/12-h dark cycle, maintained at a temperature of 24ºC, and provided with free access to food and water. All experiments involving rats were conducted with the approval of the ethics committee of the Department of Animal Physiology at the University of Lomé. This ethics committee is a branch of the broader ethics committee for the control and supervision of animal experiments and the use of blood, with reference number 006/2021/BC-BPA/FDS-UL.

## *Cutibacterium acnes*-induced acne model in the Sprague Dawley rat

### Preparation of the gel

The natural gel served as an excipient for the preparation of various formulations. Specifically, 2.5 and 5% gels containing hydroalcoholic extracts of *A. buettneri* were prepared. Following the induction of inflammatory acne lesions, the different gel formulations were topically applied. A daily application of 200 µL of gel was administered to the lesions until the ear was fully covered in the various batches of rats (Arias et al., 2004).

### Bacteria preparation

*C. acnes* ATCC 6919 was obtained from “Laboratoires Humeau” (La Chapelle-sur-Erdre, France). *C. acnes* in a 72-h full growth phase was utilized. The bacterium was cultured on Cooked Blood Agar (Oxoid) and incubated at 37ºC for 72 h. A *C. acnes* suspension containing 8 × 10^7 colony-forming units (CFU) per 20 µL was prepared in phosphate-buffered saline (PBS) using a densitometer.

**Table 1.** Gradient of mobile phases in method 1.

| Mobile phase | A: $H_2O$, 0.1% Trifluroacetic acid<br>B: Acetonitrile | | | | |
|---|---|---|---|---|---|
| Gradient | Time (min) | 0 | 30 | 70 | 75 |
| | %A | 88 | 74 | 30 | 88 |
| | %B | 12 | 26 | 70 | 12 |

**Table 2.** Gradient of mobile phases - Method 2.

| | | |
|---|---|---|
| Time (min) | 0 | 60 |
| A: $H_2O$, 0.1% TFA | 98 | 40 |
| B: ACN | 0 | 60 |

## Acne induced by *C. acnes*

Male Sprague Dawley rats weighing between 150 and 170g were employed for the experiments. During the acne induction phase, *C. acnes* were cultured in phosphate-buffered saline (PBS) with a pH of approximately 7.2 (Sigma-Aldrich, USA). In this phase, a *C. acnes* suspension containing 8 × 10^7 colony-forming units (CFU) per 20 µL was intradermally injected into the left ear of the rats to induce inflammation in different groups. The negative control group received PBS instead of the bacterial culture (Kumar et al., 2019). This induction phase lasted 48 h, after which inflammatory lesions, in the form of edema, appeared on the animals' ears at the site of inoculation.

For the treatments, six groups of six rats were formed and received the following treatments by topical application an additional 12 days after oedema induction:

Group 1: negative control (injection of PBS), animals were treated with 0.9% sodium chloride
Group 2: *C. acnes* induced group (disease control), animals were treated with distilled water
Group 3: *C. acnes* induced group; animals received natural gel as a vehicle.
Group 4: *C. acnes* induced group, animals received 5% Cutacnyl gel, used as a reference substance.
Group 5 and 6: *C. acnes* induced group, the rats received 2.5 and 5% *A. buettneri* gels respectively.

During this treatment phase, ear thickness and rectal temperature were measured daily until the end of the study.

On the last day, following the final topical application, the rats underwent a fasting period. After 12 h of fasting, the rats were anesthetized with ether, and blood was collected from the retroorbital sinus into tubes containing EDTA. The blood was collected in dry tubes, centrifuged at 2500 rpm for 15 min, and the serum was then collected for the determination of oxidative stress markers. The organs (thymus and spleen) were subsequently ground, and their homogenates were utilized for the determination of malondialdehyde (MDA) and glutathione to assess the antioxidant status of these vital organs.

## Determination of oxidative stress markers

### Determination of MDA

The determination of malondialdehyde (MDA) was carried out in the thymus, spleen, and serum using a colorimetric method (Gérard-Monnier et al., 1998). Thymus and spleen samples in each batch were individually ground in tris-HCl at a pH of 7.4 at a rate of 2 g in 10mL, and homogenates were obtained. A 1-methyl-2-phenylindole solution was prepared at 10.3 mM in acetonitrile. This solution was activated by adding a 32 µM $FeCl_3$ solution (prepared in methanol) to the phenylindole solution in the proportions of 25 and 75%, respectively (to obtain 100 mL of activated phenylindole, 25 mL of $FeCl_3$ was added to 75 mL of the phenylindole solution). A calibration range of 1,1,3,3 tetraethoxypropane (MDA standard) was prepared (0; 1.25; 2.5; 5; 10, and 20nM).

To 1.3 mL of the activated phenylindole solution, 500 µL of standard MDA or homogenate and 300 µL of concentrated HCl (37%) were added, respectively. The mixture was homogenized and incubated at 45ºC for 1 h. After cooling, the contents of each tube were centrifuged at 4000 rpm for 10 min, and the absorbance was read at 586 nm. The absorbance of each homogenate relative to the standard MDA was used to determine the concentration of MDA (nM) in each organ. Three tests were performed for each homogenate.

### Glutathione assay

The colorimetric method as described by Kadébé et al. (2016) was employed for this assay. Standard concentrations of reduced glutathione (GSH) ranging from 100 to 600 µg/mL were prepared. To 50 µL of homogenate or GSH, 150 µL of Tris (0.2 M; pH = 8.2) and 10 µL of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (0.01 M) were added. The mixture was then adjusted to a total volume of 1 mL with 790 µL of absolute methanol. All tubes were sealed, homogenized, and incubated at room temperature for half an hour. Subsequently, all tubes were centrifuged at 3000 rpm for 15 min, and the absorbance was read at 412 nm after five minutes of incubation.

## Statistical analysis

Statistical analysis was conducted using GraphPad.

**Table 3.** Relative contents of total phenols and total flavonoids in the hydroalcoholic extract of *A. buettneri.*

| Total phenols (mg GAE/g dry extract) | Total flavonoids (mg QE/g dry extract) |
|---|---|
| 16 ± 0.4 | 2.83 ± 0.5 |

GAE = Gallic acid equivalent, QE = Quercetin equivalent.

Prism 9 software. The results were expressed as Mean ± standard error of the mean. Statistical differences among treatments were tested with One-way ANOVA, followed by Tukey's post hoc test for multiple comparison tests at a significant level of $p \leq 0.05$

## RESULTS

### Yield of Extraction of plant material

The amount of crude extract obtained from 450 g of powdered material macerated in a 70% hydro-ethanolic solution is 109 g, representing a yield of 24.25%.

### Total phenols and total flavonoids contents

The contents of total phenols and total flavonoids, determined by the Folin-Ciocalteu and aluminum chloride methods, respectively, are recorded in Table 3. The results obtained demonstrate richness in polyphenols in the hydroalcoholic extract (16 ± 0.4 mg GAE/g dry extract). Among these polyphenols, a high presence of flavonoids (2.83 ± 0.5 mg QE/g dry extract) has been shown through the aluminum chloride assay. These results confirm those obtained from the preliminary qualitative analyses.

### TLC profile

TLC profiles of hydroalcoholic extract are presented in Figure 1.

### HPLC analysis chromatograms obtained with methods 1 and 2

The hydroalcoholic extract was analyzed by HPLC using Method 1 and the optimization method (Method 2). A comparison of their absorption spectra and retention times with those of the major peaks in the extract allowed us to identify four compounds (Table 4).

### Effect of the extract on the ear thickness and acne lesions

The ear thickness of the rats was measured every day until the 14th day of the study. A significant increase in ear thickness ($p < 0.001$) was observed in the induced group compared to the negative control group. After 12 days of treatment with *A. buettneri* extracts, ear thickness significantly decreased in all treated groups, approaching the normal level (Figure 2). On the 8th day, both 5% cutacnyl and 5% *A. buettneri* completely healed the inflammatory lesions, while the 2.5% *A. buettneri* extracts completely healed the lesions on the 12th day. Figure 3 depicts the various lesions after 12 days of treatment.

### Effect of the extract on rectal temperature

The rectal temperature of the rats increased in the *C. acnes*-induced acne groups compared to the negative control group ($p < 0.0001$). After topical treatment with extracts of 2.5 and 5% *A. buettneri* gels for 12 days, the temperature significantly decreased towards normal ($p < 0.001$) (Figure 4)

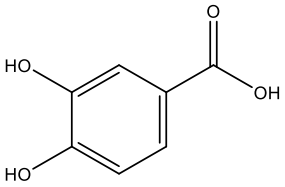
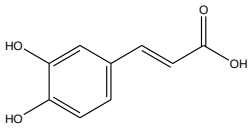
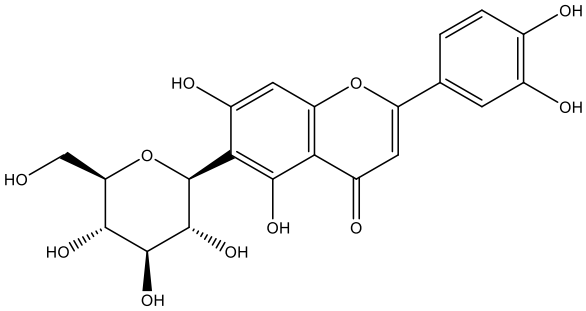
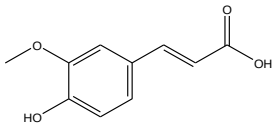
### Effect of extracts on glutathione levels in the spleen, thymus, and serum

The summary of the effect of extracts on the glutathione levels in the spleen, thymus, and the serum is presented in Figures 5 to 7, respectively. These figures show that the glutathione levels decreased in the induced group compared to the normal control ($p < 0.001$). In the spleen homogenates, the 5% Cutacnyl gel and the different extracts significantly increased the glutathione levels ($p < 0.001$) (Figure 5). Similarly, in the thymus homogenates, the 5% Cutacnyl gel and the different extracts also significantly increased the glutathione levels ($p < 0.001$) (Figure 6). In the serum, *A. buettneri* 5% significantly decreased the glutathione levels ($p < 0.01$), while the 5% Cutacnyl gel and 2.5% *A. buettneri* gel did not significantly affect this level ($p > 0.05$) (Figure 7).

### Effect of the extract on the MDA levels in the serum, spleen, and thymus

Figures 8 to 10 show the levels of MDA in the serum, spleen homogenates, and thymus homogenates, respectively, which significantly increased in the induced group compared to the normal control ($p < 0.01$). Different

**Table 4.** Compounds identified in the *Aloe buettneri* hydroalcoholic extract.

| N° | Name of compound | Chemical structure | Raw formula | Retention time (min) |
|---|---|---|---|---|
| 1 | Protocatechuic acid |  | $C_7H_6O_4$ | 13.92 |
| 2 | Caffeic acid |  | $C_9H_8O_4$ | 20.87 |
| 3 | Homo-orientin |  | $C_{21}H_{20}O_{11}$ | 23.42 |
| 4 | Ferulic acid |  | $C_{10}H_{10}O_4$ | 27.26 |

concentrations and the Cutacnyl gel used as a reference drug did not affect serum MDA levels ($p > 0.05$; treated groups compared to the normal control). Figures 9 and 10 show that only the groups treated with 2.5% *A. buettneri* gel and 5% *A. buettneri* gel significantly decreased the MDA levels in spleen and thymus homogenates ($p < 0.01$).

## DISCUSSION

The anti-acne activities of *A. buettneri* extract formulations were assessed in vivo using a *C. acnes*-induced acne model in Sprague Dawley rats. In this model, *C. acnes* suspension was injected into the dorsal side of the rats' ears to induce local and chronic inflammation. The primary difference between the development of acne lesions in humans and the development of cutaneous lesions in inflamed rat ears is that these animal models essentially bypass the gradual formation of comedones and develop changes partially equivalent to the stage of comedone rupture. On the other hand, C. acnes (bacteria isolated from acne lesions) induce local inflammation with the formation of papules and pustules in the form of edema (Hall et al:, 2018). The increase in ear thickness confirmed the induction of inflammatory acne in rats. Similar results of induction were obtained by different investigations (Mingsan et al., 2016; Li et al., 2017; Han et al., 2018; Kumar et al., 2019).

The different extract formulations significantly reduced ear thickness during 12 days of treatment. Similarly, the increase in body temperature in infected groups confirmed the pathological state of the animals. The decrease in rectal temperature to the normal value following treatment with hydroethanolic extract also confirms the efficacy.

Hyperkeratosis, the abnormal proliferation of keratinocytes in the epidermis, is an etiological factor in acne. These different formulations would have keratolytic activities (Ruan et al., 2020) and would limit comedo formation. Reactive oxygen species (ROS) are released by neutrophils in inflammatory tissue. These free radicals attack DNA and/or membrane lipids, causing damage to the skin, including normal tissues (Aruoma, 1998; Nakai and Tsuruta, 2021).

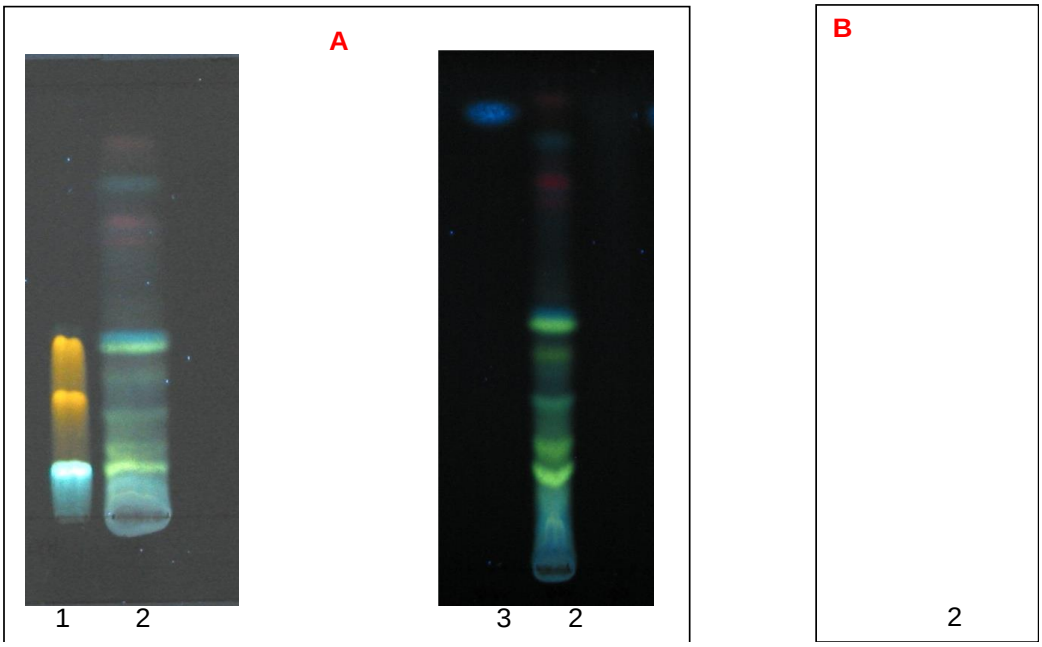



**Figure 1.** TLC profiles of the hydroalcoholic extract of *A. buettneri* using different solvents. Stationary phase: Silica gel plate (Merck); mobile phases: A, ethyl acetate/methanol/water (100/17/13); B, ethyl acetate/acetic acid/formic acid/water (100/11/11/26): spots:1:mixture of reference compounds (Hyperoside, chlorogenic acid, rutin); 2:Hydroalcoholic extract of *A. buettneri*;3:Anthrone;Visualization: NP/PEG, observation under UV light at 365 nm.

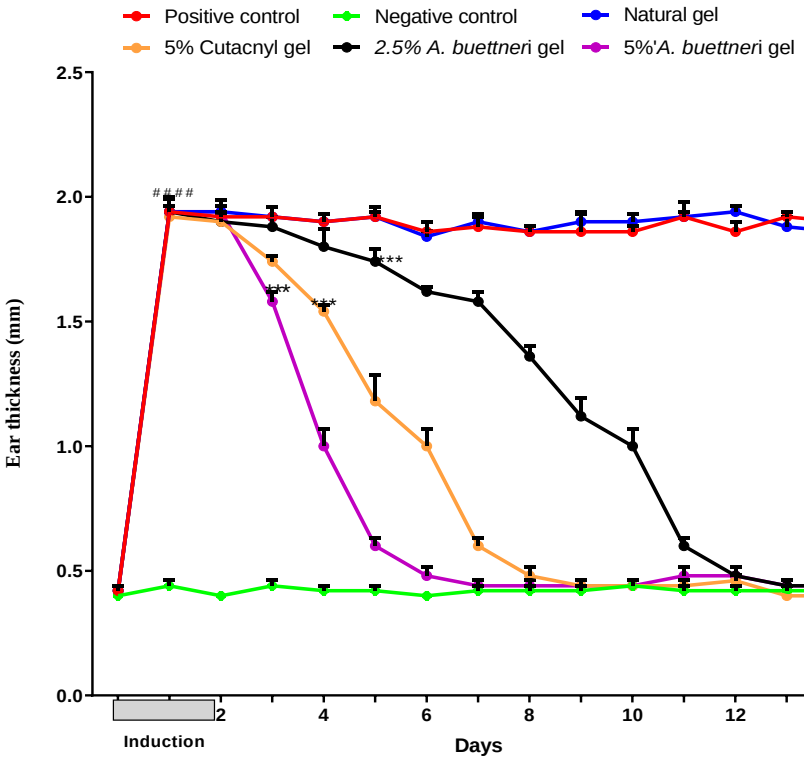



**Figure 2.** Effect of the extract on ear thickness. Each value is expressed as Mean ± SEM, n = 6. ####p<0.01 (positive control compared to negative control). ***p<0.001 (treated compared to positive control). ANOVA followed by Tukey's test.


**Figure 3.** Effect of the extracts on acne lesions in rats A = Negative control; B = disease control; C = Natural gel; D = 5% Cutacnyl gel; E = 2.5% *A. buettneri gel*; F = 5% *A. buettneri gel*.

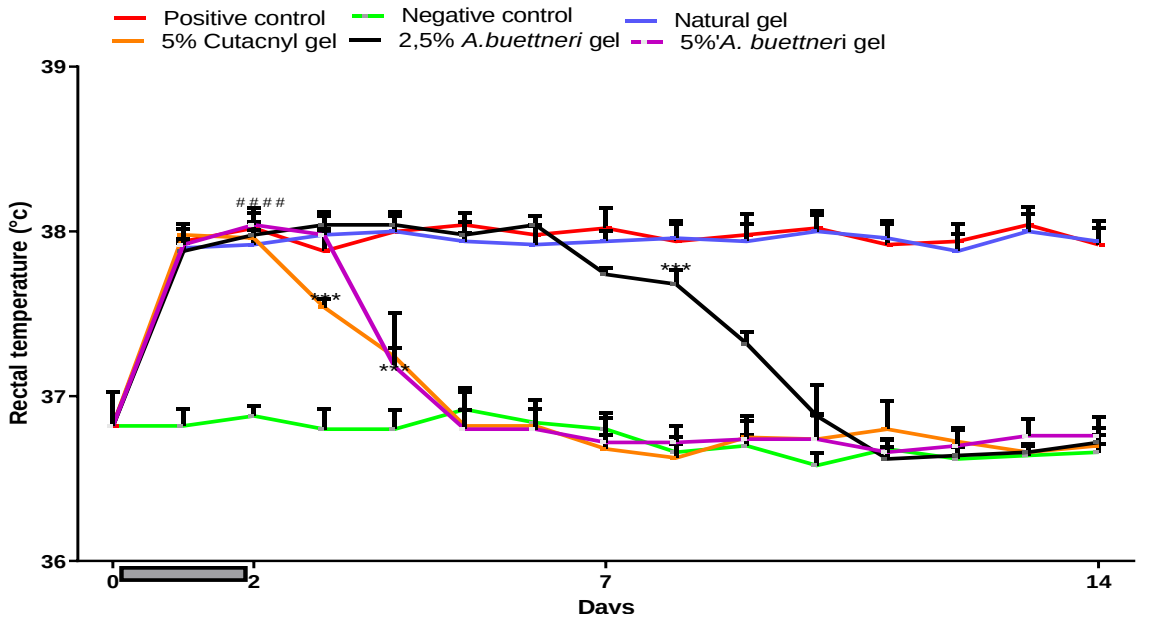



**Figure 4.** Effect of *A. buettneri* extract on the rectal temperature of rats. Results are presented as mean ± SEM, n=6. ####p<0.0001 (Positive control compared to negative control); ***p<0.001 (positive controls compared to treated). Rats treated with different extracts significantly reduced rectal temperature.

Controlling the production of free radicals is essential for normal physiological cell functioning (Cho et al.;2023). The increased level of MDA in induced groups showed an elevated level of lipid peroxidation in acne for this model. This also confirms and supports the findings of other authors regarding the role of oxidative stress in *C. acnes*-induced acne models (Kurutas et al., 2005; Kumar et al., 2019). Studies have reported that antioxidant enzymes such as superoxide dismutase and myeloperoxidase activities were reduced in acne patients (Gollnick et al., 2003). The level of MDA is higher in the induced group compared to the negative control group. Various studies have reported that the superoxide radical can damage surrounding healthy epidermal cells.

The crude extract would have increased antioxidant enzyme activities. This hydroethanolic extract increased the glutathione level and allowed a decrease in the MDA level in the plasma of the treated groups compared to the infected groups. When batches treated with the extract were compared to the induced and untreated batch, the glutathione level increased, while the MDA level decreased significantly in thymus and spleen homogenates; two key organs involved in the immune system. The choice of these two organs is justified by the fact that immunity is an integral component of the pathophysiological factors of acne (Contassot, 2018). Protecting these two organs from oxidative stress damage would help limit inflammation and, consequently,

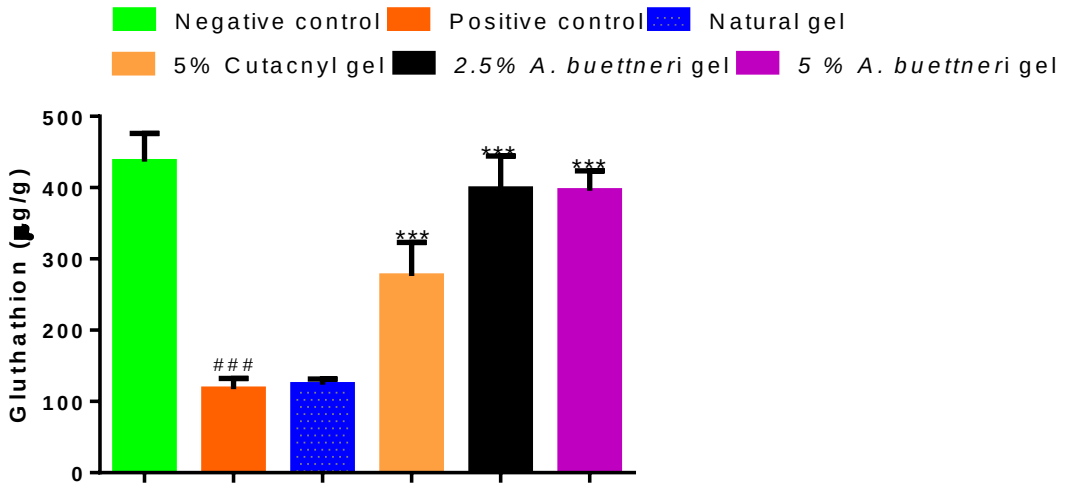



**Figure 5.** Effect of extracts on glutathione levels in the spleen.Each value is expressed as Mean ± SEM, n = 6. ### p<0.001: positive control compared to negative control. ***p<0.001: cutacnyl gel 5%, *A. buettneri* 2.5%, *A. buettneri* 5% compared to positive control."

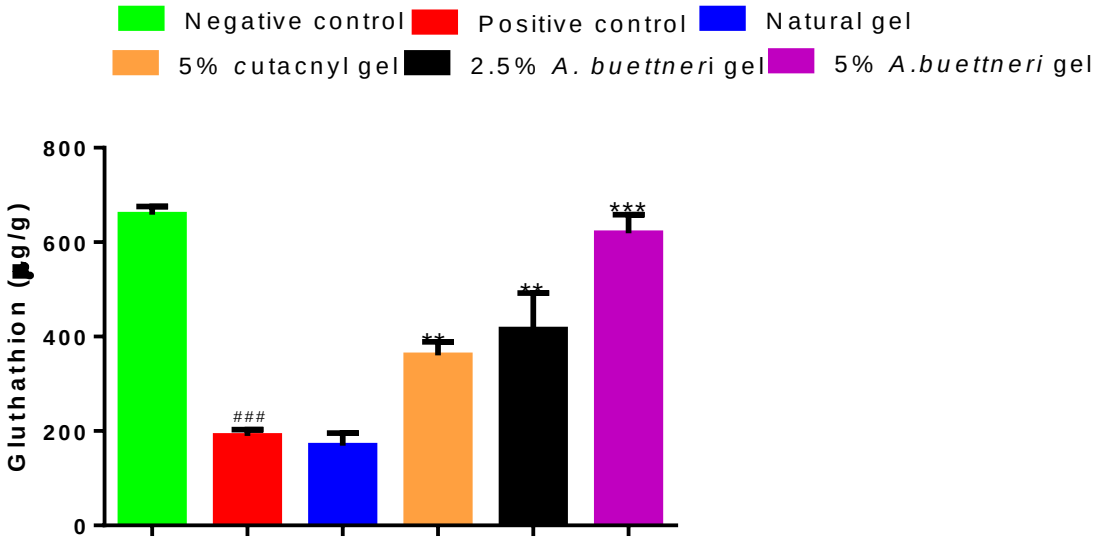



**Figure 6.** Effect of *A. buettneri* formulations on glutathione levels in the thymus. Each value is expressed as Mean ± SEM, n = 6. ### p<0.001: positive control compared to negative control. ***p<0.001: 5% Cutacnyl gel, 2.5%, *A. buettneri* gel, 5% *A. buettneri* gel compared to positive control.

potentially fight lesions in this in vivo model.

The crude extract inhibited lipid peroxidation induced by *C. acnes* in serum, thymus, and spleen homogenates by significantly inhibiting the formation of MDA and stimulating glutathione production. This extract could restore the balance between the pro-oxidant and antioxidant system. Preliminary work has shown that *A. buettneri* extract has antioxidant, bactericidal, and bacteriostatic activities against acne-causing bacteria (Kombate et al., 2022).

These various activities would be beneficial for acne patients. The hydroethanolic extract of *A. buettneri* could potentially be used as an alternative treatment for acne. Excessive sebum production contributes to acne pathogenesis, and suppressing sebum production reduces the incidence and severity of acne (Akhtar et al., 2010; Ruan et al., 2020).

This suggests that the extract of *A. buettneri* may have lipogenesis-inhibiting properties and could limit sebum secretion. Literature has shown that polyphenolic compounds are known to have antimicrobial, antioxidant, and anti-inflammatory activities (Bouarab et al., 2019).

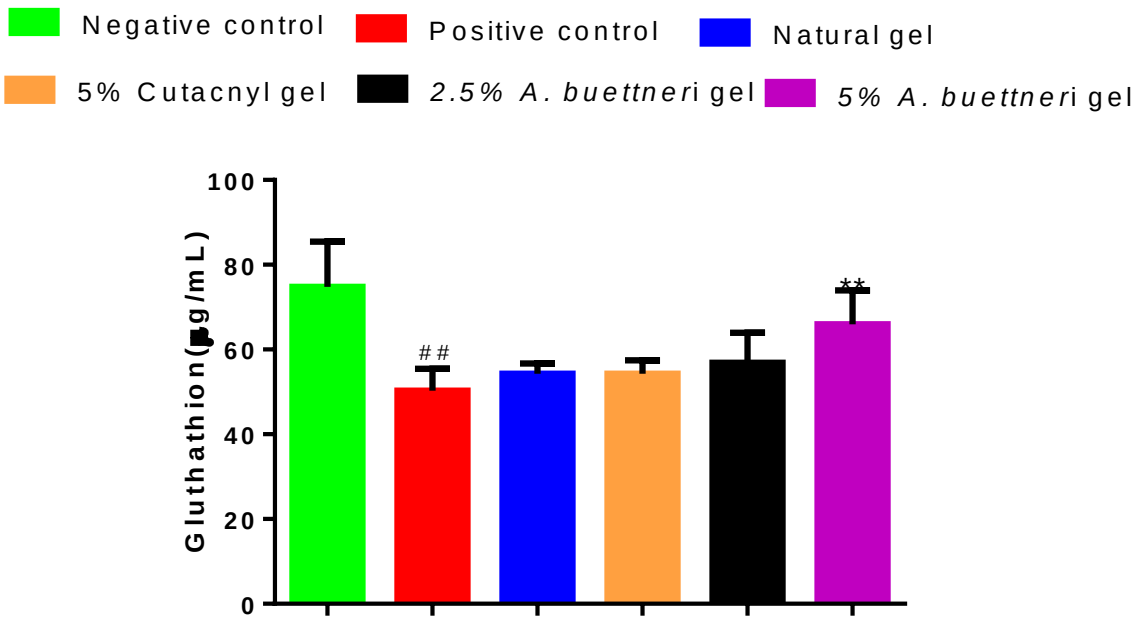



**Figure 7.** Effect of extracts on glutathione levels in the serum. Each value is expressed as Mean ± SEM, n = 6. ## p<0.01: positive control compared to negative control. **p<0.01: 5% *A. buettneri* gel compared to positive control

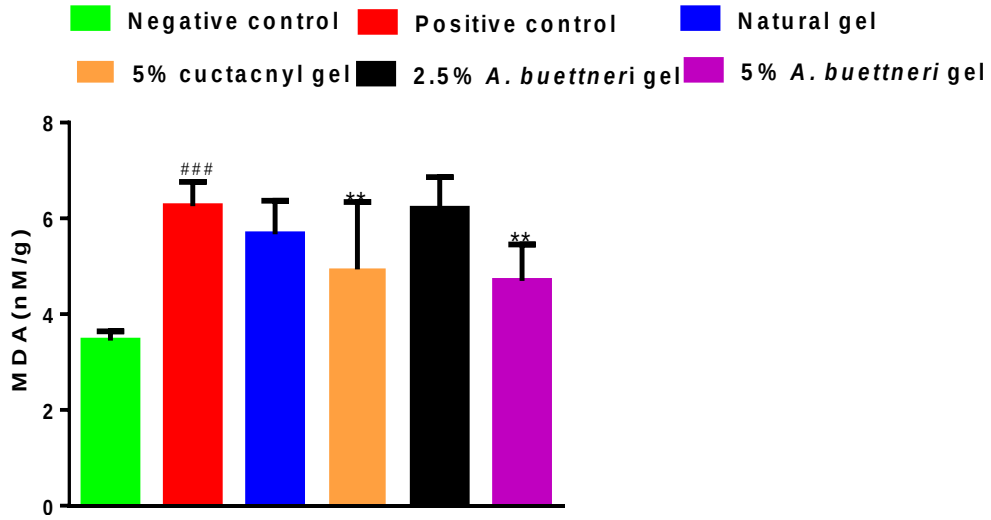



**Figure 8.** Effect of extracts on MDA levels in the spleen. Each value is expressed as Mean ± SEM, n = 6. ### p <0.001: positive control compared to negative control. ** p <0.01: cutacnyl 5%, *A. buettneri* 5% compared to positive control."

Therefore, a phytochemical screening of the extract and its fractions was conducted. The Folin-Cioacalteu method, TLC, and the HPLC approach were used complementarily to identify the phenolic compounds of *A. buettneri* extract. Highly polar polysaccharide compounds were identified and could be involved in the extract's activity. Four polysaccharidic flavonoid molecules were identified by HPLC. These include protocatechuic acid, caffeic acid, homo-orientin, and ferulic acid. Caffeic acid is known for its antioxidant activities.

Previous studies have indicated that protocatechuic acid possesses various beneficial pharmacological properties in the context of acne treatment (Jalali et al., 2020; Liang et al., 2022). Ferulic acid is a phenolic compound used and combined with other ingredients for topical applications (Cavalcanti et al., 2021). Other studies have demonstrated the relative effectiveness of ascorbic acid in capturing reactive species, particularly

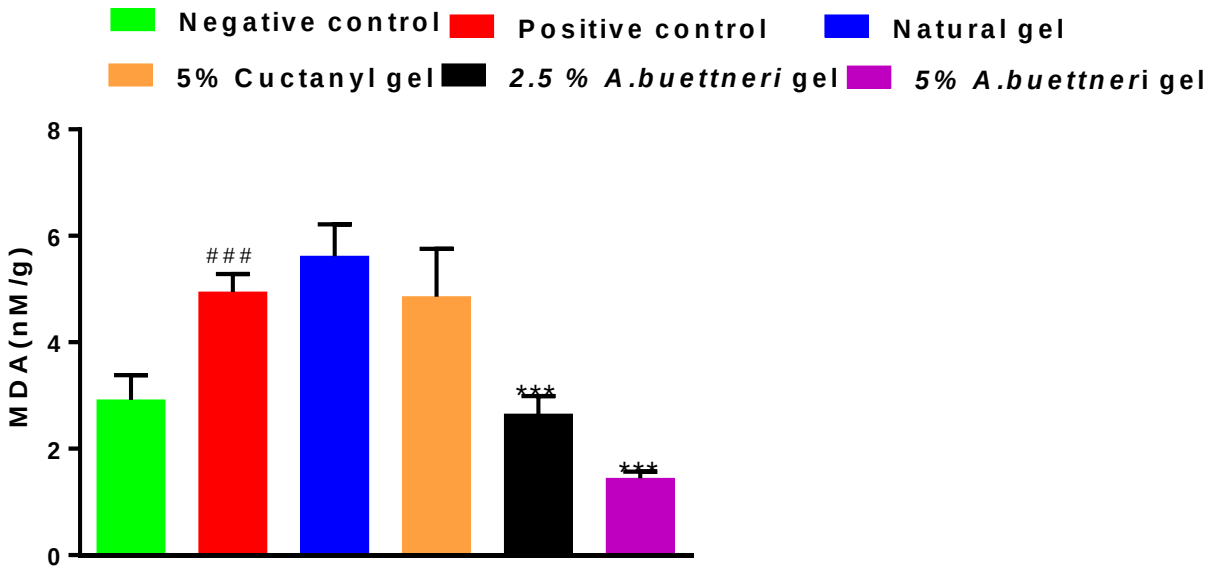



**Figure 9.** Effect of extracts on MDA levels in the thymus. Each value is expressed as Mean ± SEM, n = 6. ## p <0.01: positive control compared to negative control. *** p <0.001: Cutacnyl 5%, *A. buettneri* 5% compared to positive control.

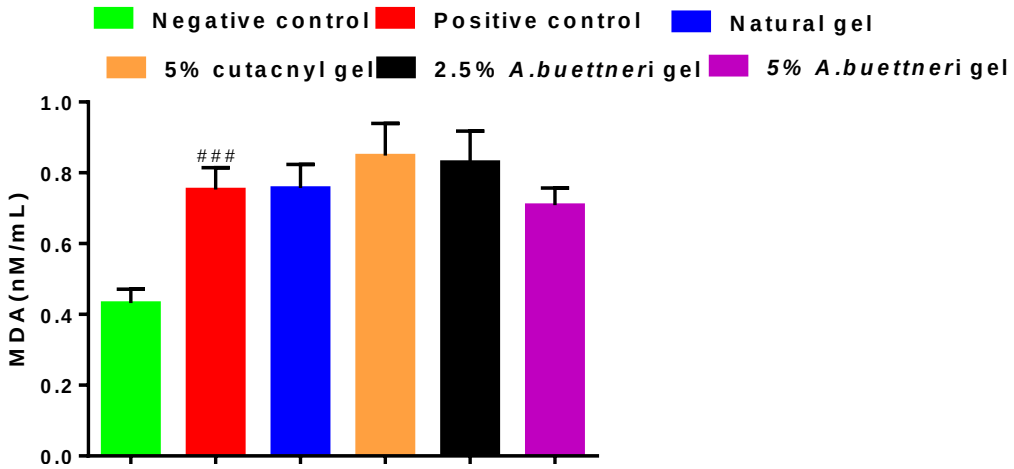



**Figure 10.** Effect of extracts on MDA levels in serum. Each value is expressed as Mean ± SEM, n = 6. ### p <0.001: positive control compared to negative control.

O2•− and HOCl/OCl−, which are relevant in the signaling and defense processes of organisms (Spagnol et al., 2019). Some authors demonstrated the presence of mucilages, polysaccharides, and oligosaccharides in extracts of several Aloe species (Vázquez et al., 1996; Gallagher and Gray, 2003, Beppu et al., 2004). Another study has shown that polysaccharides isolated from A. barbadensis extract possess various pharmacological activities. Thus, the polysaccharide and polyphenolic compounds identified in the hydroalcoholic extract of *A. buettneri* leaves could be the basis for the extract's anti-acne effects. The hydroethanolic extract of *A. buettneri* contains biomolecules with antioxidant and anti-acne properties, and it is important to investigate its fractions for therapeutic purposes in an acne model. Further work will attempt to demonstrate this.

## Conclusion

The formulations derived from *A. buettneri* extract exhibit anti-acne properties in a rat model. The phytochemical composition of the extract has been analyzed, revealing the presence of phenols and flavonoids through quantitative assessments. Additionally, TLC and HPLC analyses of the extract confirmed the presence of

polyphenols, including flavonoids, phenolic acids, and polysaccharides. The predominant components consist of polyphenols and polar saccharide-like substances. In total, with HPLC, four polysaccharidic flavonoid molecules are identified: Protocatechuic acid, caffeic acid, homo-orientin, and ferulic acid. These research findings provide validation for the traditional use of this plant resource in treating acne within the Togolese population. *A. buettneri* stands as a promising botanical resource for the cosmetics industry. Further research is needed to investigate the specific mechanisms by which these identified molecules act on the pathophysiological components of acne.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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