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ANTIBACTERIAL POTENTIALS OF ALOE BARBADENSIS MILLER AND CITRUS LIMON PLANT EXTRACTS AGAINST SKIN INFECTING PROPIONIBACTERIUM ACNES: BOTH IN-VITRO AND IN-VIVO ATTEMPTS

Original Research

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Acknowledgement: The authors gratefully acknowledge the technical support provided by the Molecular Laboratory, Department of Zoology, University of Science and Technology Bannu.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Acne vulgaris is a chronic skin condition that significantly affects facial aesthetics and psychological well-being. It is primarily caused by *Propionibacterium acnes*, a Gram-positive anaerobic bacterium residing in the pilosebaceous units. The increasing resistance of *P. acnes* to conventional antibiotics has led to reduced treatment efficacy and rising healthcare costs. As a result, attention has shifted toward herbal therapies that offer safer, cost-effective, and environmentally friendly alternatives. Among the various botanicals, *Aloe vera* and *Citrus lemon* have shown promising antimicrobial properties and are widely used in traditional medicine.

Objective: This study aimed to evaluate the antibacterial effects of *Aloe vera* and *Citrus lemon* extracts against *P. acnes* using both in-vitro and in-vivo experimental models.

Methods: Leaves of *Aloe vera* and fruits of *Citrus lemon* were collected from District Bannu. Methanolic and aqueous extracts were prepared in concentrations of 40, 60, 80, and 100 µg/ml. In-vitro testing was performed using the agar well diffusion method with Clindamycin as the positive control. In-vivo analysis involved topical application of aqueous extracts on acne lesions across multiple body parts of participants over a seven-day period. Antibacterial effects were evaluated based on the zone of inhibition and percentage reduction in acne.

Results: *Aloe vera* extract showed inhibition zones of 12, 14, 20, and 23 mm at increasing concentrations, while *Citrus lemon* produced zones of 13, 16, 19, and 21 mm. Combination therapy yielded enhanced inhibition zones of 14, 17, 21, and 24 mm. In-vivo findings revealed a 61% reduction in acne with *Aloe vera*, 51% with *Citrus lemon*, and 67% when used in combination.

Conclusion: Both *Aloe vera* and *Citrus lemon* demonstrated substantial antibacterial activity against *P. acnes*, with combined use yielding superior results. These findings support further investigation into the phytochemical constituents for potential large-scale therapeutic use.

Keywords: *Aloe vera*, *Antibacterial*, *Citrus lemon*, *Phytotherapy*, *Propionibacterium acnes*, *Skin infections*, *Topical herbal treatment.*

INTRODUCTION

The human skin, accounting for approximately 16% of total body weight, serves as the largest and most exposed organ of the body. It plays a vital role as the first line of defense against a range of environmental threats, including biological, chemical, and physical pathogens (1). As a dynamic and complex organ, the skin not only acts as a physical barrier but also supports a diverse microbial ecosystem. Research indicates that around 50 million microorganisms may inhabit just 6.5 square centimeters (1 square inch) of skin, though this figure can vary significantly across the average total skin surface of 1.9 square meters (20 square feet). Notably, oily regions such as the face may host up to 78 million bacteria per square centimeter (or up to 500 million per square inch), highlighting the dense colonization of the skin microbiota (2,3). Despite the immense microbial load, these microorganisms do not merely coexist with the human host but actively contribute to skin health. They form a commensal microbial community that helps regulate one another, maintaining a balanced ecosystem. However, when this delicate equilibrium is disrupted, it can result in microbial overgrowth and subsequent skin infections (4). The skin microbiota also includes opportunistic organisms with the genetic potential to develop resistance to therapeutic agents. This underscores the importance of maintaining skin integrity and health through various strategies, including pharmacological treatment, hygiene, and non-pharmacological interventions such as phototherapy (5,6).

Acne vulgaris, a chronic inflammatory condition of the pilosebaceous unit, is one of the most prevalent dermatological conditions, particularly affecting approximately 80% of adolescents during puberty. Among the various microbial contributors, *Propionibacterium acnes* (now known as *Cutibacterium acnes*) is widely recognized as the principal causative agent (7). Acne can significantly impair facial aesthetics and psychosocial well-being, prompting individuals to seek medical treatment. Although several therapeutic modalities are available—ranging from topical agents and oral antibiotics to hormonal and retinoid therapies—the frequent use of antibiotics has led to a concerning rise in antibiotic-resistant strains of *P. acnes* (8,9). Moreover, these pharmacological treatments are often associated with adverse effects and substantial financial burden, particularly in lower-income populations. In light of these challenges, there has been renewed interest in natural remedies, especially medicinal plants, as safer and more cost-effective alternatives. Herbal medicine has been practiced for millennia and is increasingly valued for its antimicrobial potential, low toxicity, and accessibility. In recent years, various natural products have shown promise in managing infectious diseases while minimizing the risk of resistance development (10). However, data on the antibacterial efficacy of specific herbal agents, such as *Citrus lemon* and *Aloe vera*, against *P. acnes* remains limited, especially in both in-vitro and in-vivo contexts. Therefore, the present study aims to evaluate the antibacterial effects of *Citrus lemon* and *Aloe vera* against *Propionibacterium acnes* through comprehensive in-vitro and in-vivo investigations, with the objective of offering a potential natural alternative to conventional acne treatments while addressing concerns of antibiotic resistance and cost accessibility.

METHODS

The present study was designed as an experimental investigation to evaluate the antibacterial activity of *Aloe vera* leaves and *Citrus lemon* fruits against *Propionibacterium acnes*, using both in-vitro and in-vivo approaches. The plant materials were collected from the natural environment of District Bannu, Khyber Pakhtunkhwa, Pakistan. Specifically, mature leaves of *Aloe vera* and ripe fruits of *Citrus lemon* were harvested and transported to the Molecular Laboratory of the Department of Zoology at the University of Science and Technology, Bannu, for further processing. The collected plant materials were thoroughly rinsed with distilled water to remove surface contaminants. The outer peels of the *Aloe vera* leaves and *Citrus lemon* fruits were removed, and 20 grams of the inner crude portions of each plant were separately soaked in 50 ml of 70% methanol for the in-vitro assay. For the in-vivo application, another 20 grams of each sample were extracted in 50 ml of distilled water. These mixtures were agitated on a laboratory shaker to ensure uniform dissolution and then filtered through Whatman's filter paper. The resulting filtrates were air-dried under ambient conditions and stored for later use. To isolate the causative bacterial strains, clinical samples were collected using sterile cotton swabs from acne lesions, including blood and pus exudates, from individuals visiting healthcare centers in District Bannu. These samples were immediately placed into sterile tubes, diluted with normal saline in Eppendorf tubes, and streaked onto Bile Aesculin Agar and Nutrient Agar plates. The plates were incubated at 37°C for 24 hours to allow microbial growth. The isolated colonies were subjected to biochemical identification using standard protocols, including Catalase, Oxidase, and Triple Sugar Iron (TSI) tests. For microscopic confirmation, Gram staining was

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performed, and morphological characteristics were examined under a light microscope. Photomicrographs of the isolates were documented to confirm the presence of *P. acnes*.

The in-vitro antimicrobial activity of the plant extracts was assessed using the agar well diffusion method. Four different concentrations of the methanolic extracts were loaded into wells cut into the agar plates previously inoculated with bacterial cultures. Clindamycin, a commonly prescribed antibiotic for acne, was used as a positive control. All experimental procedures were performed in triplicate, and the zones of inhibition were measured using a scoring scale after 24 hours of incubation at 37°C to ensure reproducibility and accuracy of results. For the in-vivo assessment, aqueous extracts of both plants were prepared in concentrations of 40, 60, 80, and 100 µg/ml. Prior to clinical application, skin sensitivity was tested by applying the extracts on a small patch of skin for 15 minutes, following the safety protocol outlined by a study (11). Individuals showing no signs of allergic reactions were selected for the treatment phase. Informed verbal and written consent was obtained from all participants, and ethical clearance for the study was granted by the Institutional Review Board (IRB) of the University of Science and Technology Bannu. The participants were advised to apply the prepared formulations three times a day over a period of one week, and clinical improvement was monitored.

RESULTS

Biochemical Tests and Gram Stain Analysis:

Following initial biochemical tests including Catalase, Oxidase, and Triple Sugar Iron (TSI), the isolates were confirmed as *Propionibacterium acnes* through microscopic examination using Gram staining. The bacteria appeared as Gram-positive rods under the microscope, and the photomicrograph confirmed the identification of the targeted microbial species.

In-Vitro Assessment of Antimicrobial Activity and Determination of the Zone of Inhibition:

The antimicrobial potential of *Aloe vera*, *Citrus lemon*, and their combined extracts was assessed against *P. acnes* using the agar well diffusion method. Four concentrations—40, 60, 80, and 100 µg/ml—were tested for each extract, and Clindamycin was used as the positive control. The zone of inhibition for *Aloe vera* extract increased with concentration, measuring 12 mm, 14 mm, 20 mm, and 23 mm respectively, compared to Clindamycin, which produced zones of 16 mm, 20 mm, 24 mm, and 27 mm. The *Citrus lemon* extract exhibited inhibition zones of 13 mm, 16 mm, 19 mm, and 21 mm at the same concentrations. When combined, *Aloe vera* and *Citrus lemon* extracts demonstrated a stronger inhibitory effect than when used individually. At concentrations of 40, 60, 80, and 100 µg/ml, the combined extract produced inhibition zones of 14 mm, 17 mm, 21 mm, and 24 mm respectively. These values approached the antibacterial efficacy of Clindamycin, especially at higher concentrations.

In-vivo Analysis of the Extracts:

In-vivo evaluation was carried out by testing the aqueous extracts on different body regions of acne patients after ensuring no allergic reactions occurred. The extracts were applied topically three times a day for one week. The *Aloe vera* extract achieved a 75% reduction of acne on the face, 80% on the forehead, 66% on the neck, 55% on the shoulders, 44% on the upper arm, and 59% on the back shoulder, with an overall treatment effectiveness of 61%.

Similarly, the *Citrus lemon* extract showed comparatively lower effectiveness, with inhibition percentages of 56% on the face, 60% on the forehead, 66% on the neck, 44% on the shoulders, 33% on the upper arm, and 54% on the back shoulder, resulting in an overall inhibition of 51%. The combined use of *Aloe vera* and *Citrus lemon* yielded the highest in-vivo effectiveness, achieving 75% acne inhibition on the face, 80% on the forehead, 66% on the neck, 55% on the shoulder, 44% on the upper arm, and 59% on the back shoulder, with a cumulative inhibition rate of 67%.

Table 1: Shows the antibacterial effect of *Aloe vera* against *P. acnes*.

Concentrations	Zone of inhibition	
	Clindamycin	*Aloe vera* Plant Extract
40 ug/ml	16 mm	12 mm
60 ug/ml	20 mm	14 mm
80 ug/ml	24 mm	20 mm
100 ug/ml	27 mm	23 mm

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Table 2: Shows the antibacterial effect of Lemon against *P. acnes*.

Concentrations	Zone of inhibition	
	Clindamycin	***Citrus lemon* Plant Extract**
40 ug/ml	16 mm	13 mm
60 ug/ml	20 mm	16 mm
80 ug/ml	24 mm	19 mm
100 ug/ml	27 mm	21 mm

Table 3: Represents the combined antibacterial effect of *Aloe vera* and Lemon plant against *P. acnes*.

Concentrations	Zone of inhibition	
	Clindamycin	***Aloe vera* and *citrus lemon* plant Extract**
40 ug/ml	16 mm	14 mm
60 ug/ml	20 mm	17 mm
80ug/ml	24 mm	21 mm
100 ug/ml	27 mm	24 mm

Table 4: Represents the in-vivo antibacterial effect of *Aloe vera* against acnes on different body parts of the patients sampled in this research work.

Body Parts	No of acnes	Treated Treated	Inhibition (%)
Face	16	12	75
Forehead	05	04	80
Neck	06	04	66
Shoulder	18	10	55
Upper arm	09	04	44
Back side shoulder	22	13	59
Total	76	47	61 %

Table 5: Represents the in-vivo antibacterial effect of *Citrus lemon* against acnes on different parts of the body.

Body Parts	No of acnes	Treated	Inhibition (%)
Face	17	09	56
Forehead	05	03	60
Neck	06	04	66
Shoulder	18	08	44
Upper arm	09	03	33
Back side shoulder	22	12	54
Total	76	39	51 %

Table 6: Represents the combined in-vivo antibacterial effect of *Aloe vera* and *Citrus lemon* against acnes on different parts of the body.

Body Parts	No of acnes	Treated	Inhibition (%)
Face	16	13	75
Forehead	05	05	80
Neck	06	05	66
Shoulder	18	11	55
Upper arm	09	04	44
Back side shoulder	22	13	59
Total	76	51	67 %

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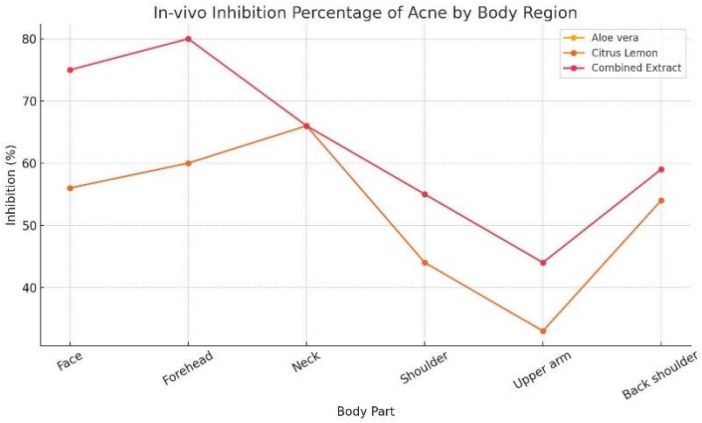

Figure 1 In-vivo Inhibition Percentage of Acne by Body Region

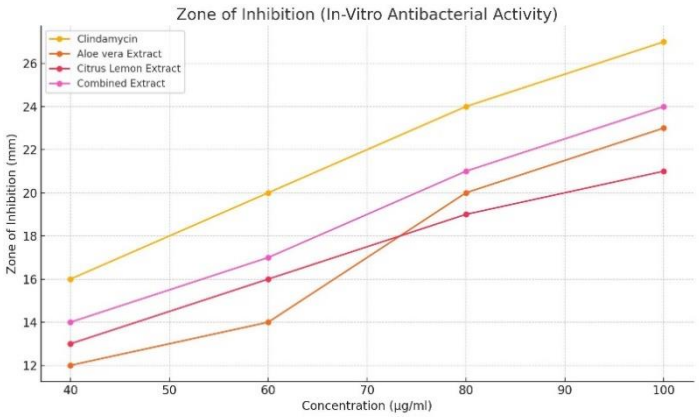

Figure 2 Zone of Inhibition (in-vivo Antibacterial Activity)

DISCUSSION

The findings of this study contribute to the growing body of evidence supporting the use of herbal remedies in managing skin infections, particularly acne vulgaris caused by *Propionibacterium acnes*. As a Gram-positive anaerobic bacterium, *P. acnes* has long been recognized as a key etiological agent in the development of inflammatory acne lesions. The increasing reliance on antibiotic therapies has led to a concerning rise in antimicrobial resistance, rendering many conventional treatments less effective and highlighting the urgent need for alternative approaches (12). Furthermore, the financial burden of continuous antibiotic use is particularly challenging for populations with limited healthcare access, making cost-effective herbal alternatives an appealing strategy. The study demonstrated that both *Aloe vera* and *Citrus lemon* extracts exhibited notable antibacterial activity against *P. acnes* in vitro, with inhibition zones increasing proportionally with concentration. At the highest tested concentration of 100 µg/ml, *Aloe vera* achieved a 23 mm inhibition zone, while *Citrus lemon* achieved 21 mm, confirming their significant antimicrobial properties (13). These findings are consistent with prior studies that reported similar inhibitory effects of plant-based compounds on acne-causing bacteria. The combination of both extracts yielded even greater inhibition, suggesting a synergistic interaction that enhances antibacterial efficacy beyond what either extract could achieve individually. This combinatory effect indicates the therapeutic potential of multi-herb formulations for enhanced outcomes (14-16).

In vivo testing further validated the antimicrobial effects of both extracts when applied topically to affected individuals. Improvement in acne reduction across various body regions was observed, with the combination therapy once again outperforming individual applications (17). Importantly, no adverse immunological responses were recorded during the patch testing phase, reinforcing the safety profile of these herbal preparations for short-term topical use (18,19). One of the strengths of this study lies in its dual approach—employing both in-vitro and in-vivo methodologies—which allowed for a more comprehensive evaluation of the antibacterial effects under controlled as well as real-world conditions. The use of multiple concentrations further added to the robustness of the data, providing a dose-dependent profile of efficacy. The study also contributed to a relatively underexplored research gap, as there is limited published data focusing on the antimicrobial properties of *Aloe vera* and *Citrus lemon* specifically against *P. acnes*.

Nevertheless, the study has several limitations that warrant consideration. The sample size for in-vivo testing was not specified in terms of participant diversity or statistical power, which limits the generalizability of the findings. Additionally, the study did not quantify or isolate the active phytochemicals responsible for the observed antimicrobial effects, which would be critical for standardization and clinical translation. The lack of advanced statistical analysis, such as significance testing (e.g., p-values, confidence intervals), also limits the interpretability and comparability of the results with other peer-reviewed studies. Furthermore, no long-term follow-up was conducted to assess the sustainability of the therapeutic effect or the potential for delayed hypersensitivity reactions. Future studies should aim to isolate and characterize the bioactive compounds in these plant extracts to better understand their mechanisms of action against *P. acnes*. Larger, randomized controlled trials with placebo groups and standardized application protocols would provide more definitive evidence of efficacy and safety. Additionally, exploring the potential of these extracts in combination with low-dose antibiotics could offer a means of reducing antibiotic usage while preserving therapeutic effectiveness (20). Overall, the study underscores the

promising role of plant-based therapies in managing antibiotic-resistant dermatological conditions. The observed enhancement in antibacterial activity through combination therapy highlights a direction for future research focused on developing synergistic, affordable, and sustainable treatments for acne and other microbial skin infections.

CONCLUSION

This study concluded that *Aloe vera* and *Citrus lemon* possess significant antibacterial properties against *Propionibacterium acnes*, the bacterium responsible for acne. Their use individually showed promising results, while the combination therapy demonstrated enhanced effectiveness, offering a potential natural alternative to conventional antibiotic treatments. These findings support the integration of herbal remedies into dermatological care, particularly in addressing the growing concern of antibiotic resistance and treatment accessibility. The results emphasize the practical value of exploring plant-based therapies for managing acne and encourage further research into the development of safe, effective, and affordable topical formulations derived from medicinal plants.

AUTHOR CONTRIBUTION

Author	Contribution
Sumera Afzal	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Laiq Zaman*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Shawana Nawaz	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Shehzad Zareen*	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Zaib Un Nisa	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Saira Jabeen	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Batab Khan	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Sumbal Nisar	Writing - Review & Editing, Assistance with Data Curation
Muhammad Wali	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Kaur S, Bains K. Aloe Barbadensis Miller (Aloe Vera). Int J Vitam Nutr Res. 2024;94(3-4):308-21.
2. Catalano A, Ceramella J, Iacopetta D, Marra M, Conforti F, Lupi FR, et al. Aloe vera-An Extensive Review Focused on Recent Studies. Foods. 2024;13(13).
3. Liang J, Cui L, Li J, Guan S, Zhang K, Li J. Aloe vera: A Medicinal Plant Used in Skin Wound Healing. Tissue Eng Part B Rev. 2021;27(5):455-74.
4. Zhang R, Hou XJ, Zhang Y, Wang CD, Zhao F, Zhao DP, et al. [Analysis on formulation regularity and characteristics of acne-relieving Chinese medicinal health products and Chinese patent medicines]. Zhongguo Zhong Yao Za Zhi. 2021;46(13):3234-9.
5. Jones VA, Patel PM, Wilson C, Wang H, Ashack KA. Complementary and alternative medicine treatments for common skin diseases: A systematic review and meta-analysis. JAAD Int. 2021;2:76-93.
6. Chellathurai BJ, Anburose R, Alyami MH, Sellappan M, Bayan MF, Chandrasekaran B, et al. Development of a Polyherbal Topical Gel for the Treatment of Acne. Gels. 2023;9(2).

7. Pal S, Raj M, Singh M, Saurav K, Paliwal C, Saha S, et al. The Effect of Aloe vera on Skin and Its Commensals: Contribution of Acemannan in Curing Acne Caused by Propionibacterium acnes. Microorganisms. 2024;12(10).
8. Kaminaka C, Yamamoto Y, Sakata M, Hamamoto C, Misawa E, Nabeshima K, et al. Effects of low-dose Aloe sterol supplementation on skin moisture, collagen score and objective or subjective symptoms: 12-week, double-blind, randomized controlled trial. J Dermatol. 2020;47(9):998-1006.
9. Zhong H, Li X, Zhang W, Shen X, Lu Y, Li H. Efficacy of a New Non-drug Acne Therapy: Aloe Vera Gel Combined With Ultrasound and Soft Mask for the Treatment of Mild to Severe Facial Acne. Front Med (Lausanne). 2021;8:662640.
10. Ansong JA, Asante E, Johnson R, Boakye-Gyasi ME, Kuntworbe N, Owusu FWA, et al. Formulation and Evaluation of Herbal-Based Antiacne Gel Preparations. Biomed Res Int. 2023;2023:7838299.
11. Chauhan, A., & Chauhan, S. B. (2022). Formulation and Evaluation of Polyherbal Emulgel for Treatment of Acne. *Current Research in Pharmaceutical Sciences*, 132-137.
12. Pozos-Nonato S, Domínguez-Delgado CL, Campos-Santander KA, Benavides AA, Pacheco-Ortin SM, Higuera-Piedrahita RI, et al. Novel Nanotechnological Strategies for Skin Anti-aging. Curr Pharm Biotechnol. 2023;24(11):1397-419.
13. Sadgrove NJ, Simmonds MSJ. Pharmacodynamics of Aloe vera and acemannan in therapeutic applications for skin, digestion, and immunomodulation. Phytother Res. 2021;35(12):6572-84.
14. Michalak M. Plant Extracts as Skin Care and Therapeutic Agents. Int J Mol Sci. 2023;24(20).
15. Mansoor K, Aburjai T, Al-Mamoori F, Schmidt M. Plants with cosmetic uses. Phytother Res. 2023;37(12):5755-68.
16. Manouchehri A, Abbaszadeh S, Ahmadi M, Nejad FK, Bahmani M, Dastyar N. Polycystic ovaries and herbal remedies: A systematic review. JBRA Assist Reprod. 2023;27(1):85-91.
17. Zeng LH, Rana S, Hussain L, Asif M, Mehmood MH, Imran I, et al. Polycystic Ovary Syndrome: A Disorder of Reproductive Age, Its Pathogenesis, and a Discussion on the Emerging Role of Herbal Remedies. Front Pharmacol. 2022;13:874914.
18. Gaur PK, Mishra R, Kaushik R, Verma KK, Kumar N, Lata K. Polyherbal Antiacne Gel: In Vitro Antibacterial Activity and Efficacy Evaluation Against Cutibacterium acnes. Assay Drug Dev Technol. 2024;22(7):373-86.
19. Hosny KM, Rizg WY, Alfayez E, Elgebaly SS, Alamoudi AJ, Felimban RI, et al. Preparation and optimization of aloe ferox gel loaded with Finasteride-Oregano oil nanocubosomes for treatment of alopecia. Drug Deliv. 2022;29(1):284-93.
20. Zago LR, Prado K, Benedito VL, Pereira MM. The use of babosa (Aloe vera) in treating burns: a literature review. Braz J Biol. 2021;83:e249209.