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Phytochemical Profiling and Antibacterial Activity of Physalis Peruviana Fruit Extract in a Carbomer-Based Topical Gel for Acne Vulgaris

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Abstract

Background: Acne vulgaris is a globally prevalent inflammatory skin condition affecting approximately 9.4% of the population, driven by factors such as microbial colonization by Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. While conventional treatments like antibiotics and retinoids are standard, they are increasingly hindered by antibiotic resistance and adverse skin reactions. This has sparked a growing interest in plant-based alternatives that offer both efficacy and a higher safety profile. Physalis peruviana (locally known as lobo-lobohan), a medicinal plant found in the Philippines, has long been recognized for its potential therapeutic properties but currently lacks robust scientific evidence in the local pharmaceutical context.

Purpose: This study aimed to investigate the phytochemical profile and antibacterial activity of Physalis peruviana fruit extracts and develop a stable, carbomer-based topical gel for acne management.

Methodology: An experimental research design was employed using ethanolic and aqueous extracts of Physalis peruviana fruits. Phytochemical screening was conducted via Thin Layer Chromatography (TLC) to identify key secondary metabolites, while phytochemical profiling was further detailed through FTIR-ATR spectroscopy, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity via DPPH assay. The antibacterial efficacy of the extracts against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis was evaluated using agar-well diffusion and broth microdilution assays to determine the Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC). Subsequently, carbomer-based gels were formulated at concentrations of 2%, 5%, and 10% and subjected to physicochemical testing for pH and homogeneity. All tests were performed in triplicate to ensure reproducibility and accuracy. Statistical analyses, including one-way ANOVA followed by Tukey's HSD post-hoc test, were performed at a significance level of $p < 0.05$.

Results: The extracts contained bioactive components, including alkaloids, phenols, tannins, flavonoids, and steroids, in both extracts. Quantitative analysis showed the ethanolic extract had significantly higher TPC ($p = 0.014$), while the aqueous extract had higher TFC ($p = 0.013$). The extracts demonstrated radical scavenging activity via the DPPH assay, indicating potential for reducing inflammation. Antibacterial assays revealed a significant difference across treatments ($p = 0.000$), though effectiveness was not directly proportional to concentration. Specifically, the 2% gel formulation demonstrated the highest Zone of Inhibition (ZOI) against S. aureus (14.54 mm) and C. acnes (18.55 mm), exhibiting activity comparable to or exceeding the positive control (clindamycin). In contrast, the 10% formulation showed the lowest ZOI across all tested strains, potentially due to restricted diffusion within the more viscous gel matrix. MIC and MBC values were significantly lower in ethanolic extracts ($p < 0.05$), indicating superior potency over aqueous extracts. Staphylococcus aureus and Staphylococcus epidermidis generally showed higher

sensitivity to the extracts compared to Cutibacterium acnes in broth microdilution assays. All gel formulations maintained a skin-compatible pH (5.0–6.2) and exhibited excellent homogeneity and stability. One-way ANOVA and Tukey's HSD post-hoc tests confirmed that the differences in antibacterial activity across concentrations were statistically significant ($p < 0.05$).

Conclusion: The results confirm that Physalis peruviana fruit extract possesses significant antibacterial properties against acne-associated pathogens. The successful formulation into a carbomer-based gel provides a scientifically validated, stable, and locally sourced phytopharmaceutical alternative for the management of acne vulgaris, particularly in the context of rising antibiotic resistance.

Keywords: Physalis peruviana, Acne Vulgaris, Antibacterial Activity, Phytochemical Profiling, Carbomer-Based Gel, MIC/MBC.

Chapter 1
THE PROBLEM AND ITS SETTING
Introduction

Dermatological diseases are among the most prevalent health problems in the world in every age group. Skin and subcutaneous diseases are also a significant component of the global burden of disease, and more and more these illnesses are seen as more than physical illness, with an impact on life quality and mental health (Yakupu et al., 2023; Meena et al., 2025). Of these dermatological diseases, acne vulgaris is one of the most common inflammatory skin diseases. Acne vulgaris is a skin disease that is not just a physical problem but also a disease with psychological and social implications, with reduced self-esteem and quality of life. Acne vulgaris is a common skin condition that impacts the lives of around 9.4% of the global population, particularly young adults and adolescents aged 10-24 years (Alexis et al., 2024; Vasam et al., 2023; Zhu et al., 2024; Guguluș et al., 2025). While acne is not a life-threatening condition, it was a significant dermatological and psychosocial burden, which had a significant impact on self-esteem, emotional distress, and quality of life, especially in adolescents (Birgul, 2021). The Philippine setting presented other misconceptions and cultural perceptions of acne that compounded the psychological and social effects of acne in Filipino youth (Villanueva, 2024).

Acne vulgaris has a multifactorial pathogenesis that includes hyperseborrhea, hyperkeratinization of the follicles, microbial colonization (mainly C. acnes),, and inflammatory responses. Aggravating inflammatory lesions were caused by secondary bacterial species, including Staphylococcus aureus and Staphylococcus epidermidis. Most traditional acne treatments involve topical and systemic medications such as retinoids, benzoyl peroxide, and antibiotics. After a longer period of treatment, however, adverse symptoms of the therapy were observed, along with a decrease in adherence and the development of antibiotic resistance (Otlewska et al., 2021; Abozeid et al., 2023; Niedzwiedzka et al., 2024).

To counteract the above limitations, recent studies highlighted the plant-based therapeutic effects, which also present the advantage of having less effect on the skin and exhibiting antibacterial properties (Cristani & Micale, 2024; Chellathurai et al., 2023). Physalis peruviana (cape gooseberry, locally known as "lobo-lobohan") is a member of the Solanaceae family that is widely used as an ethnomedicinal plant in the Philippines and has been found to possess bioactive properties. The phenolics, flavonoids, and withholds present in Physalis peruviana were revealed by the studies to be responsible for its antioxidant and antibacterial properties (Kasali et al., 2021; Gonzales et al., 2024; Verohanitra et al., 2025).

Although there is an increasing body of evidence for the therapeutic activity of Physalis peruviana, research has been mainly conducted with leaves, calyx or crude extracts, and only a few studies have detected the activity of P. peruviana in the fruit, especially in the context of acne management. Additionally, little has been done in the Philippines yet to explore the potential of the plant for use in the creation of a pharmaceutical product, particularly in the topical management of acne vulgaris. (Anwar et al., 2022; Kasali et al., 2021; Ngingo et al., 2024). Furthermore, it was reported that it had antibacterial activity, but few studies have specifically assessed its efficacy against the microorganism that causes acne, Cutibacterium acnes. In addition, the majority of investigations were limited to initial laboratory screening; very little work was done to advance to dosage-form development of a topical product.

In the Philippines, the tests used most frequently for the antibacterial activity of medicinal plants were the agar diffusion tests, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests being used rarely. The methodological limitation limited the clinical relevance and applicability of the existing findings. In addition, a significant amount of work is missing that combines phytochemical characterization, antibacterial testing, and formulation of topical gels containing carbomer, which are very suitable for the treatment of acne because they do not contain grease, are easy to apply, and have an excellent compatibility with the skin.

Physalis peruviana (cape gooseberry) has potential bioactive and medicinal properties, but it has not yet been extensively studied for its complete pharmaceutical use. To the best of our knowledge, it is for the first time that it has been studied in detail in order to perform a phytochemical analysis, assess its antibacterial activity, and formulate a topical gel with carbomer to combat acne vulgaris, creating a significant gap in existing literature.

To address these gaps in the literature regarding plant-based acne treatments, along with the production of topical herbal medications, the phytochemical profile and antibacterial activity of Physalis peruviana fruit extract were investigated, and a carbomer-based topical gel was formulated for the treatment of acne vulgaris. In particular, bioactive compounds in P. peruviana have previously been reported, some of which have been shown to have antibacterial activity, and most of which have been used for the leaves and calyx of the plant, but not the fruit (Kasali et al., 2021; Gonzales et al., 2024). Furthermore, literature on acne treatment has shown that conventional and natural antimicrobial agents have been shown to effectively target acne-causing microorganisms like Cutibacterium acnes, and that there is a need for additional quantitative antibacterial testing, like MIC and MBC, to evaluate efficacy.

The aim of this study was to isolate the most important bioactive components, assess antibacterial activity against acne-causing bacteria with quantitative assays, and formulate a stable and skin-compatible topical gel product. This research aims to create a scientific foundation for the formulation of a locally sourced, phytochemical-based alternative to manage acne in the Philippines by combining all three methods: phytochemical analysis, antibacterial testing, and pharmaceutical formulation.

Theoretical Framework/Conceptual Framework

The study is based on the theories on the medicinal plants having therapeutic potential, the mechanism of action of antimicrobial agents, and the efficacy of topical drug delivery systems. These theories lay the scientific background for the study of phytochemical analysis, antibacterial activity, and formulation of Physalis peruviana fruit extract into a topical gel for the management of acne vulgaris.

The study is based on the **Phytochemical Theory of Medicinal Plants** (Kasali, 2021), which states that plants have biologically active secondary metabolites like flavonoids, tannins, saponins, and withanolides that exhibit therapeutic activity. The bioactive compounds have antioxidant and antibacterial activity and

are useful for dermatological applications. This theory is in accordance with the phytochemical profiling carried out in this study and justifies the possible use of Physalis peruviana fruit extract as a natural treatment for acne vulgaris.

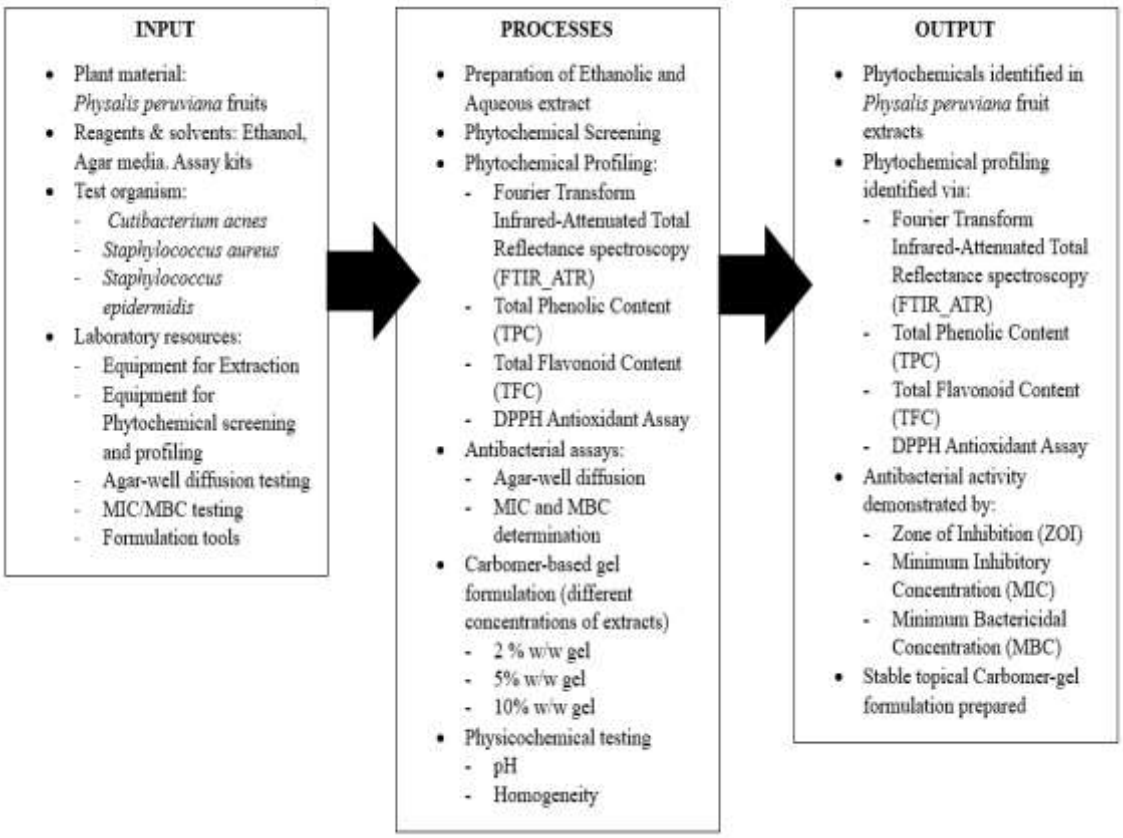
Furthermore, the study follows the **Germ Theory of Disease** (Carlsson & Råberg, 2024) that states that many infectious diseases are caused by microorganisms. Cutibacterium acnes, Staphylococcus epidermidis and Staphylococcus aureus play a key role in the initiation of inflammation and lesions of acne vulgaris. This theory is used for the study of antimicrobial activities of Physalis peruviana extract against these pathogens related to acne and the requirement for effective antimicrobial agents against them.

Finally, this study is based on the **Theory of Transdermal Drug Delivery System,** which suggests the delivery of therapeutic agents via the skin for either local or systemic effects, with a major focus on the capacity of the active ingredients to pass through the skin barrier to the target site (Berkers et al., 2021). This indicates the need for formulation for the improvement of transdermal drug delivery. This theory gives justification to formulate a topical gel using carbomer as an extract carrier of Physalis peruviana fruit, which can penetrate, stabilize, and deliver the extract to the acne development site effectively.

Operational Framework

The operational structure shows the logical sequence for the research process in terms of its input variables and its research output. It shows the key components involved in examining the antibacterial properties of Physalis peruviana and its formulation feasibility. This framework explains how the research aims are met and the relationship between the various phases of the research.

Figure 1. Paradigm of the Study (IPO Model)



I present the IPO model in this figure, which depicts the entire framework of the study, the Input–Process–Output.

The study focused on the real-world and theoretical aspects, beginning at the input stage. The practical part was carried out by collecting the fruits of Physalis peruviana and preparing the extracts (aqueous and ethanolic) rich in bioactive phytochemicals. For the antibacterial activity of these extracts, the Cutibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis were used as bio-indicator organisms. Positive and negative controls were used to ensure the reliability of the results. A comprehensive literature review was also undertaken to lay the foundations of the study in terms of its literature basis and to explore the potential use of Physalis peruviana as a natural remedy for acne.

The **process stage** involved a series of experimental and analytical procedures. The crude extracts were subjected to screening tests for the existence of secondary metabolites such as flavonoids, saponins, tannins, and alkaloids. In addition, phytochemical characterization was performed using Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) to identify functional groups, while Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined using standard colorimetric methods. Furthermore, antioxidant activity was evaluated using the DPPH radical scavenging assay.

The extracts were then used in an antibacterial screening test using the agar well diffusion method to determine the diameter of the Zone of Inhibition (ZOI); the antibacterial effects were measured across different concentrations of the extracts.

The qualitative experiments are authenticated by the quantitative experiments, composed of the measurement of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Data obtained were triplicated and analyzed statistically using one-way ANOVA followed by Tukey's HSD test to compare the difference based on zone of inhibition; the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by comparative statistics to ensure accuracy and reproducibility. Finally, the different concentrations of extracts in the carbomer-based gel formulations were prepared and tested for physicochemical stability, such as pH and homogeneity, which confirmed the formulations' consistency and shelf life.

The **output** of this research was the development of a scientific formulation with the addition of Physalis peruviana fruit extract in the form of a gel as an anti-acne product that has been proven to have antibacterial activity against the bacteria that cause acne. The methodological innovation of the study was the inclusion of both the diffusion and confirmatory MIC and MBC assays, which was not often used in Philippine phytochemical studies. This contribution was an initial yet strong scientific foundation for the potential use of Physalis peruviana as a natural therapeutic agent in the context of antibiotic resistance and sustainable dermatologic interventions for acne vulgaris.

Statement of the Problem

This study seeks to investigate the antibacterial properties of Physalis peruviana fruit extracts to develop a carbomer-based gel formula as an alternative to existing topical gels for acne vulgaris management and treatment. This study seeks to answer the following questions:

1. What bioactive compounds are present in the ethanolic and aqueous fruit extracts of Physalis peruviana, as determined through qualitative and quantitative phytochemical analyses using appropriate analytical techniques?
2. What is the antibacterial activity of Physalis peruviana fruit extracts against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis using the agar-well diffusion assay?

3. What are the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against the identified microorganisms?
4. How is a carbomer-based topical gel containing Physalis peruviana fruit extract formulated?
5. What are the physicochemical properties of the formulated carbomer-based topical gels containing varying concentrations of Physalis peruviana extracts in terms of pH and homogeneity?
6. Is there a significant difference in antibacterial effectiveness between the formulated carbomer-based gel containing Physalis peruviana fruit extract and the positive control (clindamycin gel), as measured by the Zone of Inhibition (ZOI)?

Statement of Hypothesis

To determine the antibacterial effectiveness of the formulated carbomer-based topical gel containing Physalis peruviana fruit extract, this study is guided by the following null hypothesis:

H_0: There is no significant difference in antibacterial effectiveness between the carbomer-based topical gel containing Physalis peruviana fruit extract and the positive control (clindamycin gel) against acne-causing microorganisms (Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis), as measured by the Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC).

Assumptions of the Study

This study delved into the following assumptions:

1. The fruit of Physalis peruviana contains flavonoids, tannins, saponins, and phenolic compounds that contribute to its antibacterial activity.
2. The extraction processes, such as ethanolic and aqueous, are sufficient to obtain the components necessary for phytochemical evaluation.
3. The qualitative phytochemical techniques are appropriate for identifying secondary metabolites according to standard chemical reactions.
4. The in vitro antibacterial tests are enough to validate the efficacy of the extracts and gel formations.
5. The carbomer-based gel serves as an appropriate vehicle for topical delivery, allowing the incorporation of Physalis peruviana fruit extracts without affecting antibacterial activity or physicochemical properties.

Scope and Delimitation

This study aimed to investigate the phytochemical profile of Physalis peruviana fruit extract and its antimicrobial activity against cutaneous bacteria that cause acne, namely Cutibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus. It involves the formulation of a topical gel with the fruit extract and the determination of the pH and homogeneity of the formulation. The study only includes in vitro antibacterial testing and physicochemical evaluation and does not include clinical testing with human subjects.

The study is not deep in terms of delimitation, and it does not investigate the detailed mechanism of action of the bioactive compounds. The gel formulation is limited to topical application only, not other dosage forms like creams, lotions, or ointments. Only extracts obtained from the fruit with ethanol or water are taken into account, with other parts (such as leaves or calyx) and other solvents not being acceptable. Additionally, antimicrobial activity is limited to the microbial strains listed above and the extra screening of additional microbes is not provided. This study will take place in the school year of 2025-2026.

Significance of the Study

The research on Physalis peruviana fruit extract for its potential as a natural remedy for acne vulgaris is

important due to the increasing problem of antibiotic resistance and the restrictions of synthetic acne treatments. The formulation and evaluation of topical gels made from locally available Physalis peruviana fruits in the Philippines is significant to the development of evidence-based phytopharmaceuticals in the country. The study could be considered significant under the following aspects:

Patients. Experience the advantage of a safer, simpler, and environmentally friendly solution for acne vulgaris, hopefully minimizing the need for synthetic or antibiotic medications.

Dermatologists. May find the study helpful as a scientific foundation for the study of plant-based materials to consider in dermatologic care and expand treatments in acne management.

Pharmaceutical Scientist. To gain an edge in creating new topical formulations that may complement the trend of going green and natural in product development.

Farmers. The findings of this study can help farmers to promote the agricultural value and use of Physalis peruviana to be a medicinal plant for pharmaceutical and dermatological applications.

The researcher. May leverage the results to pursue areas of research that are very integrated with dermatology and medicine.

Future Researchers. Can use results as a basis for further investigation—moving to in vivo assays, formulation optimization, and clinical trials to investigate the wider pharmacological activities of Physalis peruviana.

This study's findings significantly add to the existing local scientific literature on medicinal plants, thereby encouraging the use of native natural resources for health and wellness innovation.

Definition of Terms

The following section presents the conceptual and operational definitions of key terms used in this study. Each term is defined to clarify its specific meaning within the context of Physalis peruviana-based antibacterial gel formulation and in vitro testing, ensuring a consistent understanding among readers. By providing clear, literature-supported definitions, this section helps prevent ambiguity in the description of procedures, variables, and results throughout the thesis.

Acne Vulgaris. A chronic inflammatory disorder of the pilosebaceous unit characterized by comedones, papules, pustules, and nodules, commonly associated with Cutibacterium acnes and secondary bacterial involvement. In this study, acne vulgaris refers to the dermatological condition targeted by the antibacterial activity of Physalis peruviana fruit extract formulated into a topical gel (Alexis et al., 2024). This is one of the variables of the study, where the Physalis peruviana (Cape gooseberry) was tested.

Antibacterial. Considered as the property of a substance to restrain or destroy the growth of bacteria. In this study, "antibacterial" indicates the activity of the Physalis peruviana fruit extract and the formulated carbomer-based gel in inhibiting the acne-forming microorganisms based on the zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determined (Balouiri et al., 2021; CLSI, 2023). This is one of the properties that was tested.

Antibacterial Testing. In this study, antibacterial testing refers to the evaluation of the antibacterial activity of ethanolic and aqueous extracts of Physalis peruviana fruit and the formulated carbomer-based topical gels against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis using agar well diffusion assay, broth microdilution method for MIC determination, and subculture plating for MBC determination (Kim et al., 2024; CLSI, 2023). In this study, this is the essential process done.

Carbomer-Based Topical Gel. It refers to a semi-solid formulation prepared using carbomer as the gelling agent, containing Physalis peruviana fruit extract, and evaluated based on its physical properties

(e.g., pH, homogeneity) and its effectiveness for acne management. (Chellathurai et al., 2023). This is the formulation being tested in this study.

Homogeneity A state in which components in a formulation are distributed evenly throughout without any lumps, coarser particles or phase separation. In this study, homogeneity is assessed by visual inspection and texture assessment of the physical consistency and uniformity of the topical gel with carbomer bases in this study (Chandrasekar et al., 2020; Chellathurai et al., 2023).

Phytochemical Profiling. It refers to the systematic identification and quantification of bioactive compounds (e.g., flavonoids, tannins, saponins, withanolides, anthocyanins, and antioxidants) present in Physalis peruviana fruit extracts using standard qualitative and quantitative analytical methods (Kasali et al., 2021). This is the process conducted by the researcher.

Physalis peruviana (Cape gooseberry). A fruit-bearing plant species belonging to the family Solanaceae, commonly known as the cape gooseberry. In this study, Physalis peruviana refers specifically to the fruit used as the source of bioactive compounds evaluated for antibacterial and anti-acne activity (Gonzales et al., 2024). In this study, this is the main component of the gel to be tested.

Total Phenolic Content. Denotes the quantity of phenolics in a plant extract in terms of milligrams of gallic acid equivalents per gram of extract (mg GAE/g). In this study, the qualitative determination of phenolics in the Physalis peruviana fruit extracts using TPC is related to the antioxidant and antibacterial properties of the plant. (Anwar et al., 2022; Añibarro-Ortega et al., 2025).

Total Flavonoid Content. A measure of the combined content of flavonoids in a plant extract expressed as milligrams Quercetin equivalents per gram of extract (mg QE/g). In this study, TFC was used to quantify the antioxidant and antibacterial activity of the fruit extracts of Physalis peruviana due to the presence of flavonoids (Añibarro-Ortega et al., 2025; Abd-ELmageed et al., 2024).

Zone of Inhibition (ZOI). It refers to the diameter, measured in millimeters, of the clear inhibitory area produced by ethanolic and aqueous Physalis peruviana fruit extracts and carbomer-based topical gel formulations against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis using the agar well diffusion method (Lozada et al., 2025).

Chapter 2
REVIEW OF RELATED LITERATURE

This chapter exhibits the related studies and literature significant for setting the direction of the study. A synthesis of a review of the related literature and studies, as well as the gaps bridged by the present study, is also included in this chapter.

Physalis peruviana

Physalis peruviana, commonly known as cape gooseberry, belongs to the Solanaceae family and is characterized by its distinctive papery calyx enclosing the fruit. Its morphology has been described in several studies, and it has been emphasized for its rich bioactive composition and medicinal importance (González et al., 2024; Güleşci et al., 2021). Its therapeutic properties, especially its antimicrobial effects, were confirmed by research conducted by both Kasali et al. (2021) and Añibarro-Ortega et al. (2025). Furthermore, Mohamed and Ali (2022) and Ngingo et al. (2024) reported that extracts from its calyx and leaves possess significant antibacterial properties, even against multidrug-resistant bacteria, which could make it valuable for skincare products, including for treating acne.

Other studies have confirmed its pharmacological benefits. Zhang et al. (2022) discovered new withanolides with antibacterial activity, and Cristani and Micale (2024) highlighted the importance of

bioactive compounds from plants for the treatment of acne. The ecological and pharmaceutical significance of cape gooseberry in sustainable medicine was also mentioned by Verohanitra and colleagues (2025). All these studies show that Physalis peruviana is a rich phytochemical and therapeutically useful plant.

Dela Cruz and Santos (2022) reported the antimicrobial and antioxidant activities of Physalis species in the Philippines, while Balangcod and Balangcod (2021) reported the ethnopharmacological uses of Physalis species in skin treatment in Benguet and Mountain Province. Lozada et al. (2025) also reported the topical safety of the oils extracted from such plants for their respective uses. The results indicate a scientific and cultural significance of Physalis peruviana and its potential use in dermatological applications.

To summarize, Physalis peruviana is a scientifically and culturally proven plant. The strong candidates for the formulation of anti-acne products in the present study are its phytochemical richness and therapeutic properties.

Figure 2.

The whole Plant and Fruits of the Cape gooseberry (Physalis peruviana).

Source: Premier Seeds Direct. (n.d.). cape gooseberry (Physalis peruviana). PremierSeedsDirect.com. Retrieved January 19, 2026, from https://premierseedsdirect.com

Phytochemical Profiling

Physalis peruviana is widely known as an abundant source of secondary metabolites, which are involved in its therapeutic properties. Añibarro-Ortega et al. (2025) reported that its fruits contain flavonoids, tannins, saponins, and withanolides, which exhibit antimicrobial and antioxidant properties. Zhang et al. (2022) also discovered new antibacterial compounds known as novel withanolides (Peruranolides A-D), highlighting its therapeutic applications. Together, these discoveries created a profile of Physalis peruviana as a cornucopia of phytochemicals for its possible biomedical applications.

These phytochemicals have been found to be beneficial in acne treatment. Flavonoids have high antioxidant and antibacterial properties; tannins interfere with the cell walls of bacteria, and saponins increase the permeability of the cell membranes (Cristani & Micale 2024). Verohanitra et al. (2025) also highlighted the significance of cape gooseberries in the fields of green chemistry and pharmaceutical science.

Dela Cruz and Santos (2022) were able to confirm the presence of flavonoids and tannins in Physalis in the Philippine context, and Lozada et al. (2025) demonstrated the safe use of plant oils in topically applied formulations. Its traditional usage was further elaborated by Balangcod and Balangcod (2021), who emphasized its importance in skin care applications.

Phytochemical studies have been boosted with the advent of modern analytical techniques. Bioactive molecule identification can be conducted using Fourier Transform Infrared (FTIR-ATR) spectroscopy, whereas total phenolic content (TPC) and total flavonoid content (TFC) analyses can be used to quantify bioactive molecules (Kamau et al., 2020; Añibarro-Ortega et al., 2025). Furthermore, DPPH is a commonly employed antioxidant activity assay (Anwar et al., 2022).

In conclusion, phytochemical profiling gives a scientific foundation for comprehending the therapeutic potential of Physalis peruviana and connects its bioactive constituents with antibacterial properties pertinent to the management of acne.

Antibacterial Activity

Acne vulgaris is a multifactorial inflammatory condition that involves microbial colonization, mainly by Cutibacterium acnes, Staphylococcus epidermidis and Staphylococcus aureus (Cristani & Micale, 2024; Guguluș et al., 2025; González et al., 2024; Zhu et al., 2024). Although conventional treatments are effective, they have some negative side effects, low adherence rates and high levels of antibiotic resistance (Otlewska et al., 2021). It is clear that better antimicrobial susceptibility testing is needed to track trends and patterns in antimicrobial resistance (ASST) (CLSI, 2024).

Antibiotics have been found to have drawbacks and are under scrutiny in recent studies for alternatives that are less risky. The plant-based treatments have attracted attention because of their antibacterial and antioxidant properties. Physalis peruviana has been shown to have promising antibacterial properties against acne-causing bacteria (Dela Cruz & Santos, 2022).

Moreover, Lozada et al. (2025) verified the safe and effective use of plant-based formulations in topical medications, and Balangcod and Balangcod (2021) highlighted the ongoing use of medicinal plants in traditional dermatological management practices. The results indicate that there is a need for further investigation of natural alternatives to anti-acne drugs based on plants.

Concluding, the literature highlights the disadvantages of traditional treatments and the possibility of using P. peruviana as a natural antibacterial. Considering antibacterial activity as a crucial consideration in this study, phytochemical profiling is integrated with antibacterial evaluation to gain a comprehensive understanding of the therapeutic potential.

Carbomer-Based Topical gel

The carbomer-based topical gel is widely used in pharmaceutical and dermatological formulations for its excellent gelling, stabilizing, and drug delivery qualities. Carbomers are high molecular weight cross-linked polyacrylic acid polymers that make clear, stable gels when neutralized. Non-greasy, easy to apply, high spreadability, and favorable acceptability by the patient are characteristic features of these gels, making them particularly suitable for use in topical administration (Chellathurai et al., 2023; Mazova et al., 2023).

Carbomer gels are an effective local treatment in dermatology, especially in the treatment of acne vulgaris. They are able to carry active ingredients directly into the dermis to the site of action, the pilosebaceous unit, which increases the efficacy of treatment and decreases systemic absorption and possible side effects, respectively (Chellathurai et al., 2023; Cristani & Micale, 2024). Carbomer gels are a targeted delivery system, the ideal carrier for the anti-acne active ingredient, whether synthetic or natural from plants.

In addition, carbomer gels have also been recommended for treatment of bacterial skin infections because they can be used as antimicrobial agents for pathogens like Cutibacterium acnes and Staphylococcus aureus (Tiwari et al., 2025). These gels are also employed in the treatment of bacterial skin diseases because they allow delivery of the antibacterial compounds while keeping the comfort of the skin and its hydration (Mazova et al., 2023). Their roles in wound healing and skin repair have also been noted as they create a moist environment and release of active substances that are crucial for skin repair and regeneration (Anwar et al., 2022).

The pharmaceutical benefits of the carbomer-based gels have been highlighted in some studies. These include drug stability, controlled drug release, appropriate dermal penetration and associated desirable physicochemical properties like appropriate drug viscosity and pH (Tiwari et al., 2025; Chellathurai et al., 2023; Mazova et al., 2023). Moreover, they are non-greasy and cooling agents that can improve patient compliance and satisfaction (Anwar et al., 2022).

In the field of dermatology, carbomer-based topical gels are an effective and versatile delivery system in general. They are useful in improving the drug's performance and patient acceptability and can be used as a drug carrier for a specific therapy such as acne vulgaris, where Physalis peruviana fruit extract was used in the current study.

Topical Gel Formulation

The topical gels allow for local application of the active ingredients, reducing systemic uptake and improving compliance. Chellathurai et al. (2023), Mazova et al. (2023), and Tiwari et al. (2025) have shown that carbomer gels have good spreadability, stability, and improved dermal absorption properties. They have these properties that make them good carriers to use for dermatological application. In addition, Anwar et al. (2022) have found that extracts of Physalis peruviana (goldenberry) maintained their antibacterial activity in gel formulations, suggesting their potential application in topical formulations. In the same way, Mohamed and Ali (2022) showed the antimicrobial activities of the extracts of Physalis calyx in topical formulations. Overall, these results support the use of carbomer gels as an effective delivery system for plant-based therapies.

New studies focused on gels' effectiveness rather than regular ointments. Chellathurai et al. (2023) reported that herbal gels were well accepted by the patients, and Mazova et al. (2023) ensured their physicochemical stability. Tiwari et al. (2025) reported better adherence and controlled release of active constituents, proving their usability and effectiveness. Goldenberry extracts were found to improve the stability of the product in gels by Anwar et al. (2022). All these findings together were evidence that topical gels were effective and patient friendly delivery systems.

The benefits of topical gels over traditional ointments have been highlighted by recent research. Chellathurai et al. (2023) stated that herbal gels are easily accepted by the patients because of their non-greasy nature, and Mazova et al. (2023) reported good physicochemical stability of herbal gels. Tiwari et al. (2025) also found that the formulation exhibited better adherence and sustained release, which resulted in better therapeutic efficacy. Moreover, Anwar et al. (2022) reported that the use of goldenberry extracts in gel formulation enhanced the stability of the gel. All these studies have shown that topical gels are an effective and patient-friendly drug delivery system.

Topical formulations are also highlighted for their relevance in the Philippine context. Lozada et al. (2025) showed that the biodiversity oils are able to be used effectively in cosmetic gels, and this shows that they are culturally acceptable. A few studies (Dela Cruz & Santos, 2022; Kuper, 2018) have shown that Physalis species have antimicrobial and antioxidant properties, which makes them recommended for

dermatological purposes. Additionally, Balangcod and Balangcod (2021) highlighted the incorporation of traditional herbal medicine into contemporary topical delivery mechanisms, reinforcing the topical gel's role in acne treatment.

Based on these results, the production of Physalis peruviana-based gels using carbomer is deemed to be justifiable. Formulations that have been shown to be effective, stable, and culturally acceptable are promising alternatives for acne treatment. In addition, the integration of phytochemical profiling, antibacterial evaluation, and gel formulation offers a holistic and evidence-based strategy for acne management, making the formulation of a topical gel an important aspect of the present study.

Synthesis of the Reviewed Literature

The literature reviewed sheds light on the rising interest of the use of plant sources as alternatives in dermatological applications, especially in acne vulgaris. Physalis peruviana has consistently been shown to be a phytochemically rich medicinal plant with beneficial bioactive compounds like flavonoids, tannins, saponins, and withanolides, which are known to possess antibacterial, antioxidant, and anti-inflammatory properties (Añibarro-Ortega et al., 2025; Zhang et al., 2022; Cristani & Micale, 2024). Their activities are important to overcome the most important components of acne pathogenesis, such as microbial colonization, inflammation, and oxidative stress. These bioactive compounds are further authenticated by various advanced analytical techniques including: FTIR-ATR, total phenol content (TPC), total flavonoid content (TFC) and DPPH assays (Kamau et al., 2020; Leite et al., 2022; Anwar et al., 2022).

Furthermore, a number of studies have proven the antibacterial activity of Physalis peruviana, such as its activity against antibiotic-resistant microorganisms (Mohamed & Ali, 2022; Ngingo et al., 2024; Dela Cruz & Santos, 2022). Most studies, however, have investigated the leaves and calyx while a few studies investigated the fruit extract with limited studies against acne associated bacteria like Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. Furthermore, the majority of studies are limited to qualitative screening methods (agar diffusion assays), and quantitative methods, like minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), have only limited applications in clinical practice (CLSI, 2024).

Carbomer based topical gels have been well documented for their stability, ability to increase dermal penetration, controlled release and high patient acceptability, making them effective and patient friendly drug delivery systems (Chellathurai et al., 2023; Mazova et al., 2023; Tiwari et al., 2025). Phenolic extracts of plants like Physalis peruviana have also been demonstrated to effectively be added in the gel formulation without losing their antibacterial activity (Anwar et al., 2022). Medicinal plants in the Philippines continue to be culturally significant and still being used for their medicinal properties; there are studies that support their use in topical formulations (Balangcod & Balangcod, 2021; Lozada et al., 2025).

In general, literature has confirmed the therapeutic potential of Physalis peruviana and the efficacy of topical gels with carbomer in dermatological applications. It also points to the fact that there are some gaps in the scope and integration of the studies already performed, especially when it comes to the use of said studies for acne-specific applications and full pharmaceutical development.

Gap/s Bridged by the Present Study

Although much literature has been published about Physalis peruviana, research for its use in pharmaceutical development in the Philippines is still limited, especially for the topical treatment of acne vulgaris. Although its antioxidant, anticancer, and metabolic potential have been well investigated, its

dermatological potential and formulation into effective topical preparations have not been sufficiently explored. Based on the review of related literature and studies above, the following gaps were identified:

1. Acne-associated microorganisms such as Cutibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus have limited studies related to the phytochemical composition and antimicrobial activity of Physalis peruviana fruit extracts.
2. Existing local studies are mostly qualitative (agar diffusion assays), and quantitative (MIC and MBC) assays are very few and little used to determine clinical significance.
3. Comparative studies to compare the efficacy of plant-based formulations with commercially available products for anti-acne effects are limited.

This research is designed to evaluate Physalis peruviana using phytochemical profiling, quantitative antibacterial activity, and a comparative study with commercial anti-acne products to validate its use as an alternative medicine for the treatment of acne vulgaris based on the identified gaps.

Chapter 3
RESEARCH METHODOLOGY

This chapter presents the methodology employed in the study, including the research design, materials, procedures for extraction, phytochemical analysis, antibacterial testing, formulation of the carbomer-based topical gel, and data analysis. It outlines the systematic approaches used to ensure the validity and reliability of the results.

Research Design

The experimental research design was used for this study to assess the phytochemical profile, antibacterial activity, and formulation of topical gel with Physalis peruviana fruit extract for acne vulgaris treatment. The method of experimentation enabled the systematic manipulation and control of the variables involved to establish the efficiency of the formulated product. The most scientific method for determining cause and effect relationships between independent and dependent variables is experimental research design that involves manipulating the independent variables in an experiment with controlled variables (Takona et al, 2024). A hypothesis is systematically and deliberately tested in a controlled environment; the results will be assessed in a credible and reliable way. Similarly, this design is likely to be essential in medical and microbiological studies for the evaluation of treatment effectiveness and the comparison of different treatments to control medicines (Balouiri et al., 2021). This method is appropriate for the present study since the efficacy of the bacteria inhibition and formulation can be assessed.

In particular, a laboratory-based experimental design with both control and intervention groups was used. The intervention group consisted of topical gels containing different concentrations (2%, 5% and 10%) of extract from the Physalis peruviana fruit, and the control group comprised a commercial anti-acne gel (clindamycin gel). The elements of the study were selected as independent variables type of extract (aqueous and ethanolic) and concentration level of the formulated gels. The dependent variables were the zone of inhibition (ZOI), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and the physicochemical property of the formulations such as pH value and homogeneity. Standard microbiological methods were used to assess and compare their anti-bacterial activity of both the groups. The study was carried out in three major stages: (1) phytochemical profiling of the fruit extracts by qualitative and quantitative analysis; (2) antibacterial evaluation through agar-well diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC); and (3)

formulation and evaluation of the topical gel based on carbomer which included determination of physicochemical parameters like pH and homogeneity.

The data collected from the experiments was analyzed in a suitable statistical manner to find differences between the control and intervention groups. The design provided thorough and systematic research on the therapeutic effects of Physalis peruviana fruit extract as an acne management tool.

Preparation

1. Collection, Preparation, and Authentication

The researchers applied the purposive sampling technique in obtaining fresh fruits of Physalis peruviana in cultivated plants in Cauayan City, Isabela Province, during March-April 2025. This technique was used to ensure selection of the physiologically mature, undamaged, and morphologically representative fruits necessary for optimal bioactive compounds.

All the collected plant materials used in the study were sent to the Department of Agriculture – Regional Crop Protection Center (DA-RCPC), Ilagan City, Isabela, for taxonomic identification and authentication of the plant material.

2. Preparation of Plant Sample

Collected fruits of Physalis peruviana were washed extensively with distilled water to wash out the impurities in the fruits and then air-dried at room temperature. They were then cut into smaller pieces and oven-dried at 40-45 °C to get to a constant weight. Dried samples were ground in a mechanical grinder to get coarse powder and then stored in an airtight container for extraction.

Procedures

1. Extraction of Plant Sample

Two extracts, ethanolic extract and aqueous extract, were prepared at the University of Perpetual Help System – Cauayan Campus, Isabela, Cauayan City, Isabela.

1.1. Ethanolic Extract via Maceration

The maceration method was used to extract the bioactive compounds from the fruit material of Physalis peruviana by adding about 50 g of powdered fruit material in 95% ethanol and stirring occasionally for 48-72 hours to get maximum extraction. This was then filtered on Whatman filter paper. The crude ethanolic extract was obtained by concentrating the filtrate with a rotary evaporator under reduced pressure and stored in a clean labeled container under refrigerated conditions for further use.

1.2. Aqueous Extract via Maceration

Another portion of 50 g of powdered sample was soaked in distilled water and subjected to maceration for 24–48 hours. The mixture was filtered and the filtrate concentrated by water bath evaporation at a controlled temperature. The aqueous extract thus obtained was placed in sterilized containers and kept in a refrigerator for further analysis. The prepared extracts were all properly labeled and stored at 4°C until they were used for phytochemical analysis and antibacterial testing to ensure the preservation of bioactive components.

2. Phytochemical Analysis

a. Qualitative Phytochemical Analysis

Standard qualitative phytochemical tests were carried out on the ethanolic extract and aqueous extract of Physalis peruviana fruits to determine the presence of the major bioactive compounds present in the fruits. Thin Layer Chromatography (TLC) was used to carry out phytochemical screening to identify flavonoids, tannins, phenols, steroids, and alkaloids.

The analysis was conducted at Saint Mary's University, Bayombong, Nueva Vizcaya, where the laboratory is equipped with appropriate chromatographic materials and reagents for both qualitative and quantitative phytochemical analyses.

Test for Alkaloids

- The TLC plate was prepared, and a baseline will be drawn approximately 1–2 cm from the bottom.
- The ethanolic and aqueous extract solutions were reconstituted in methanol.
- Small aliquots of each extract were spotted onto the TLC plate using capillary tubes.
- The plate was developed in a pre-saturated chamber containing chloroform, methanol, and ammonia (8:2:0.1).
- The plate was removed once the solvent front reached about 80% of the plate height and was air-dried.
- The developed plate will be sprayed with Dragendorff's reagent.
- The plate was observed for the appearance of orange to reddish-brown spots, which indicated the presence of alkaloids.

Test for Phenols

- The TLC plate was prepared and marked with a baseline 1–2 cm from the bottom.
- The ethanolic and aqueous extracts were dissolved in methanol.
- The samples were applied onto the plate.
- The plate was developed in toluene: ethyl acetate: formic acid (5:4:1).
- The plate was removed and air-dried.
- The plate was sprayed with the Ferric chloride test.
- The appearance of blue, green, or violet spots indicated the presence of phenolic compounds.

Test for Tannins

- The TLC plate was prepared, and a baseline was drawn 1–2 cm from the bottom.
- The ethanolic and aqueous extracts were reconstituted in methanol.
- The samples were spotted onto the plate using capillary tubes.
- The plate was developed in ethyl acetate: methanol: water (40:5:5).
- The plate was removed, dried, and sprayed with the Ferric chloride test.
- The plate was observed for blue-black or green spots, which indicated the presence of tannins.

Test for Flavonoids

- The TLC plate was prepared and marked with a baseline 1–2 cm from the bottom.
- The ethanolic and aqueous extracts were dissolved in methanol.
- The samples were applied onto the plate using capillary tubes.
- The plate was developed in ethyl acetate: formic acid: acetic acid: water (100:11:11:26).
- The plate was removed, dried, and observed under UV light at 366 nm.
- The plate was sprayed with Aluminum chloride spray reagent.
- The plate was observed for yellow or fluorescent spots, indicating the presence of flavonoids.

Test for Steroids

- The TLC plate was prepared, and a baseline was drawn 1–2 cm from the bottom.
- The ethanolic and aqueous extracts were reconstituted in methanol.
- The samples were spotted onto the plate.

- The plate was developed in hexane: ethyl acetate (8:2).
- The plate was removed, dried, and sprayed with Liebermann–Burchard reagent.
- The plate was gently heated if necessary.
- The development of blue-green coloration indicated the presence of steroids.

Test for Triterpenoids

- The TLC plate was prepared and marked with a baseline 1–2 cm from the bottom.
- The ethanolic and aqueous extracts were dissolved in methanol.
- The samples were applied onto the plate using capillary tubes.
- The plate was developed in toluene: ethyl acetate (7:3).
- The plate was removed, dried, and sprayed with Liebermann– Burchard reagent.
- The plate was observed for reddish-violet or purple spots, indicating the presence of triterpenoids.

Data recording and interpretation

- All developed TLC plates were observed under ultraviolet (UV) light (254 nm and 366 nm), and the presence of visible or fluorescent spots was recorded. The plates were further examined after spraying with appropriate detecting reagents, and the resulting colors and spot characteristics were documented. The distance traveled by each spot, and the solvent front was measured, and the corresponding Rf values were calculated.
- The intensity of each spot was rated qualitatively as absent (-), weak (+), moderate (++), or strong (+++).

b. Quantitative Phytochemical Analysis

Quantitative determination of bioactive compounds was carried out using appropriate analytical techniques. Instrumental analyses, including Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and the DPPH antioxidant assay, were performed at the Research Center for the Natural and Applied Sciences, University of Santo Tomas, Sampaloc, Manila. The facility is equipped with advanced instrumentation and supported by technical expertise in natural product analysis.

Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR_ATR)

Fourier Transform Infrared (FTIR) Spectroscopy is applied to determine the functional groups existing in plant extracts owing to their unique ability to absorb infrared rays. FTIR analysis is to be conducted by means of the SHIMADZU IR Prestige-21 Spectrophotometer with Attenuated Total Reflectance (ATR) attachment.

Analysis Procedure

1. The dried sample was placed directly onto the zinc selenide (ZnSe) single-reflection attenuated total reflectance (ATR) crystal plate.
2. The infrared spectrum was obtained using a SHIMADZU IR Prestige-21 Fourier Transform Infrared (FTIR) spectrophotometer equipped with a Pike Miracle ATR accessory.
3. Spectral acquisition was performed in the wavenumber range of 4000–400 cm^{-1}, with a resolution of 4 cm^{-1}.
4. The measurements were conducted using the ATR mode, allowing direct analysis of the sample without additional preparation
5. The obtained spectra were recorded and analyzed for the identification of functional groups present in the sample.

Data Interpretation

The FTIR spectra were analyzed for characteristic absorption peaks corresponding to functional groups such as hydroxyl, carbonyl, aromatic, and aliphatic groups. Peak values (cm^{-1}) were compared with standard reference libraries and published literature. Comparative analysis between ethanolic and aqueous extracts were performed based on differences in peak intensity and functional group distribution.

Table A

Standard FTIR Reference Ranges

Wavenumber (cm^{-1})	**Functional Group**	**Meaning**
3200–3600	O–H stretch	Alcohols / Phenols
2850–3000	C–H stretch	Alkanes
3100–3000	=C–H	Alkenes
1600–1650	C=O stretch	Carbonyl (ketones, aldehydes, esters)
1650–1750	C=C	Alkenes / Aromatics
1000–1300	C–O stretch	Alcohols, ethers, and esters
650–900	C–H bending	Aromatic compounds

(Khakhalary, S., & Narzari, S., 2025)

Total Phenolic Content

The total phenolic content of the ethanolic and aqueous fruit extracts of Physalis peruviana was determined using the Folin–Ciocalteu colorimetric method. Increasing concentrations of Gallic acid was used as a standard. This assay was selected due to its wide acceptance and reliability in estimating total phenolic compounds in plant extracts.

Assay Procedure

1. An aliquot of 12.5 µL of the diluted sample or standard solution was mixed with 50 µL of water and 12.5 µL of Folin-Ciocalteau reagent.
2. After 5 minutes, 125 µL of 7% Na2CO3 solution was added
3. The mixtures were allowed to stand at room temperature for 90 minutes.
4. The absorbance was measured at 750 nm wavelength using a microplate reader
5. The total phenolic content of the sample was measured against the Gallic acid standard calibration.

Data Recording and Interpretation

A calibration curve was constructed using the absorbance values of the gallic acid standards. The total phenolic content (TPC) of the samples was determined from the calibration curve. Results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract). All measurements were performed in triplicate, and results will be expressed as mean ± standard deviation.

Higher absorbance values were indicative of higher phenolic content. The phenolic content of the ethanolic and aqueous extracts was compared based on their calculated mg GAE/g values.

Statistical Treatment

Statistical analysis was performed using an independent t-test with a level of significance of $p < 0.05$ to determine whether there is a significant difference between the two extracts.

Total Flavonoid Content

The total phenolic content of the ethanolic and aqueous fruit extracts of Physalis peruviana was determined using the aluminum chloride colorimetric method.

Assay Procedure

- Equal volumes (100µL) of the diluted sample and 2% AlCl3 methanol solution were added in wells of a 96-well plate.
- An equal volume (100 µL) of 2% aluminum chloride ($AlCl_3$) in methanol will be added to each well.
- A blank was prepared containing all reagents except the sample solution.
- After 1 hour at room temperature, the absorbance was measured at 420nm using a microplate reader.

Data Recording and Interpretation

The total flavonoid content (TFC) of the samples was determined from the Quercetin standard calibration curve, where absorbance was plotted against known standard concentrations (ppm). All measurements were performed in triplicate, and results were expressed as mean ± standard deviation. Higher absorbance values were indicated higher flavonoid content. The flavonoid content of the ethanolic and aqueous extracts will be compared based on their calculated mg QE/g values.

Statistical Treatment

Statistical analysis was performed using an independent t-test with a level of significance of $p < 0.05$ to determine whether there is a significant difference between the two extracts.

DPPH Antioxidant Assay

The antioxidant activity of the ethanolic and aqueous fruit extracts of Physalis peruviana was evaluated using the DPPH Antioxidant assay. Ascorbic acid was used as the standard reference compound. This method was selected due to its sensitivity, reproducibility, and widespread application in evaluating the radical scavenging potential of plant-derived bioactive compounds.

Assay Procedure

1. 20 µL of each extract was prepared in dimethyl sulfoxide (DMSO) and transferred into a microplate.
2. Subsequently, 40 µL of 0.96 mM DPPH solution prepared in ethanol was added to each well.
3. The plate was kept in the dark for 15 minutes, after which the absorbance of the solution was measured at 540 nm in a plate reader.
4. DMSO served as a blank, and Ascorbic Acid (4mg/mL) served as the standard.
5. Samples were tested at a single concentration of 4mg/mL to determine the antioxidant activity.

Calculation of Antioxidant Activity

The data was reported as percent DPPH scavenging effect using the following equation:

$$DPPH\ scavenging\ effect\ (\%)\ or\ Percent\ inhibition = \frac{Absorbance\ of\ DPPH - Absorbance\ of\ sample}{Absorbance\ of\ DPPH} x100$$

Data Recording and Interpretation

Each sample was analyzed in triplicate. The DPPH control and Ascorbic acid standard and Ethanolic extract and Aqueous extract samples showed specific absorbance values that researchers measured. The team used standard formulas to calculate DPPH radical scavenging activity, which they expressed as percent DPPH radical scavenging activity. The mean value together with standard deviation (SD) results were obtained through calculation of all duplicate test results.

Statistical Treatment

Statistical analysis was performed using an independent t-test with a level of significance of $p < 0.05$ to determine whether there is a significant difference between the two extracts.

3. Antibacterial Activity Testing

The Bioscience Research Laboratories at De La Salle Medical and Health Sciences Institute located in Dasmariñas, Cavite conducted all antibacterial tests which measured Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against Staphylococcus aureus and Staphylococcus epidermidis and Cutibacterium acnes. The laboratory has suitable equipment that enables microbiological testing and antimicrobial assessment to be conducted in controlled environments.

3.1 Test Microorganisms

The study used three bacterial strains that are known to cause acne as test organisms. The test organisms for this study included Cutibacterium acnes (ATCC 6919), Staphylococcus aureus (ATCC 25923), and Staphylococcus epidermidis (ATCC 12228). The research identifies these microorganisms as primary factors that lead to the development of acne and its associated skin infections (Chen et al. 2022; Huang et al. 2023).

The researchers obtained bacterial isolates from an accredited microbiological culture collection at the Bioscience Research Laboratories of De La Salle Medical and Health Sciences Institute. The researchers followed standard microbiological procedures to maintain and subculture each strain according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2021).

The researchers assessed the antibacterial properties of Physalis peruviana fruit extracts against acne-related bacteria. The laboratory maintained pure cultures of test organisms under suitable conditions before testing to confirm both their viability and the testing results.

3.2 Agar Well Diffusion Method

The researchers evaluated the antibacterial properties of the extracts through testing, which used the agar-well diffusion method. The researchers inoculated sterile Mueller-Hinton agar plates with standardized bacterial suspensions. The researchers made aseptic wells on the agar surface and added measured volumes of the extracts into each well. The researchers incubated the plates under suitable environmental conditions for a period of 18 to 24 hours. The researchers measured the zones of inhibition, which appeared after incubation, in millimeters. The antibacterial activity of the extracts increased with the size of the zones, which resulted from the extract testing.

Table B.
Agar Well Diffusion Assay Setup

Species	**Control 1: Clindamycin gel (Positive Control**	**Control 2: Gel Base (Negative Control**	**Test Samples: Physalis peruviana**
Staphylococcus aureus	**Control 1A: Clindamycin gel** vs Staphylococcus aureus	**Control 2A:** Gel base vs Staphylococcus aureus	**Test 1A:** Ethanolic extract (100 µL) vs Staphylococcus aureus **Test 1B:** Aqueous extract (100 µL) vs Staphylococcus aureus

			Test 1C: 2% Gel formulation vs Staphylococcus aureus **Test 1D:** 5% Gel formulation vs Staphylococcus aureus **Test 1E:** 10% Gel formulation vs Staphylococcus aureus
Staphylococcus epidermidis	**Control 1B:** Clindamycin gel vs Staphylococcus epidermidis	**Control 2B:** Gel base vs Staphylococcus epidermidis	**Test 2A:** Ethanolic extract (100 µL) vs Staphylococcus epidermidis **Test 2B:** Aqueous extract (100 µL) vs Staphylococcus epidermidis **Test 2C:** 2% Gel formulation vs Staphylococcus epidermidis **Test 2D:** 5% Gel formulation vs Staphylococcus epidermidis **Test 2E:** 10% Gel formulation vs Staphylococcus epidermidis
Cutibacterium acnes	**Control 1C:** Clindamycin gel vs Cutibacterium acnes	**Control 2C:** Gel base vs Cutibacterium acnes	**Test 3A:** Ethanolic extract (100 µL) vs Cutibacterium acnes **Test 3B:** Aqueous extract (100 µL) vs Cutibacterium acnes **Test 3C:** 2% Gel formulation vs Cutibacterium acnes **Test 3D:** 5% Gel formulation vs Cutibacterium acnes

			Test 3E: 10% Gel formulation vs Cutibacterium acnes

5.3 Minimum Inhibitory Concentration

The study used a sterile 96-well microplate to determine the Minimum Inhibitory Concentration (MIC) of Physalis peruviana fruit aqueous and ethanolic extracts through the broth microdilution method. The experimenter distributed 100 µL of sterile Mueller–Hinton broth to each well of the microplate. The test extract was introduced into the first well at 100 µL and two-fold serial dilution was performed to create concentrations that ranged between different mg/mL levels across the remaining wells.

The researchers created a standardized bacterial inoculum by adjusting test organisms to 0.5 McFarland standard turbidity which corresponds to (~1 × 10^8 CFU/mL), and then conducting further dilution. The researchers added 100 µL of the bacterial suspension to each well to reach a total volume of 200 µL.

The positive control wells contained bacterial inoculum, which did not receive any extract treatment, whereas negative control wells contained only broth solution. The experiment included a solvent control of DMSO and a standard antibiotic, clindamycin.

The microplate underwent incubation at 37°C for a period between 18 and 24 hours. The researchers used turbidity measurements to evaluate bacterial growth after the incubation period. The MIC represents the minimal extract concentration that resulted in total bacterial growth inhibition.

5.4 Minimum Bactericidal Concentration

The MBC was performed after the Minimum Inhibitory Concentration (MIC). Aliquots of 10–20 µL from wells exhibiting no visible growth were inoculated onto Mueller–Hinton agar plates aseptically.

The plates were incubated at 37°C for 18–24 hours. After incubation, the plates were examined for bacterial colony formation. The MBC was defined as the lowest concentration of the extract that resulted in no visible colony growth, indicating complete bactericidal activity.

All assays were performed in triplicate to ensure accuracy, reproducibility, and reliability.

6. Formulation of carbomer-based topical gel containing Physalis peruviana fruit extract

Carbomer-based gel was prepared as the base component. Ethanolic extract and Aqueous extract of Physalis peruviana were added in 2%, 5%, and 10% (w/w) concentrations. A homogenized solution, subsequent cooling, followed by storage in sterilized containers, was done. One copy of the formulation was kept, labelled, and stored at ambient conditions until evaluation.

Formulation of carbomer-based gels containing the plant extract was done at the University of Perpetual Help System Cauayan – Isabela Campus, located in Cauayan City, Isabela.

6.1 Procedure

Carbomer 940 was dispersed slowly into approximately two-thirds of the required distilled water (room temperature, ~25°C) with gentle stirring to avoid clumping. Hydration was allowed for 60-90 minutes until a smooth, lump-free gel base formed.

1. The weighed ethanolic extract was dissolved in propylene glycol (with minimal water/ethanol if needed for solubility) to achieve the target concentration (2%, 5%, or 10% w/w relative to final gel
2. weight).
3. The extract-propylene glycol solution was gradually incorporated into the hydrated carbomer gel under continuous stirring until it was homogeneous.

4. Methylparaben was dissolved in a small volume of propylene glycol or water, then added to the gel mixture with stirring.
5. The pH monitoring process was continued until the neutralization process reached its target pH range of 5.0-6.0 which scientists consider optimal for skin compatibility and the gel's viscosity.
6. The remaining distilled water was added q.s. to 100 g, mixed thoroughly to remove air bubbles (standing or gentle vacuum degassing), and transferred to sterile, airtight containers.
7. Formulations were labeled as "2% P. peruviana Gel," "5% P. peruviana Gel," and "10% P. peruviana Gel." They were stored at room temperature (25±2°C) protected from light until evaluation. The plain gel base was prepared similarly (no extract) for the negative control All preparations were performed in triplicate under aseptic conditions. The method creates non-greasy gels that spread easily while providing better skin absorption for acne treatment.

7. Physicochemical Evaluation

The physicochemical assessment of prepared gels determined the quality, stability, and suitability for topical application. Furthermore, the evaluations would generate data required for the determination of prepared carbomer-based gel (different concentration extracts) characteristics, consistencies, uniformities, and stabilities. Moreover, the assessment shall take place at the University of Perpetual Help System Laguna-Isabela campus located in Cauayan City, Isabela.

Physicochemical properties of formulated carbomer-based topical gels containing different concentrations of Physalis peruviana extracts in terms of.

7.1 pH

A clean beaker was taken, and 1 gram of the gel sample (carbomer-based gel) was weighed, after which it was dispersed in 10 ml of distilled water and then stirred thoroughly for uniform dispersion. The pH of the specified gels was measured after calibration of the digital pH meter using the standard buffer at pH 4.0 and 7.0. By immersing the electrode in the sample suspensions, the pH value was recorded once stabilization had occurred. The measurement was repeated three times for each sample formulation, and mean pH ± SD was calculated and recorded.

7.2 Homogeneity

A small amount of the carbomer gel was taken and applied evenly onto a clean glass slide using a spatula. The gel smear was then observed from the starting point to the end of spreading. It was examined for the presence of lumps, coarse particles, grittiness, phase separation, or any non-uniform texture. The gel was considered homogeneous if it exhibited a smooth and uniform appearance without visible aggregates or phase separation and showed consistent texture throughout the formulation. The samples were then rated using a three-point scale: Excellent (smooth, uniform in color, and no lumps or separation), Good (mostly smooth with very minimal irregularities); and Poor (with visible lumps, uneven color, or separation),

Data Gathering Procedures

The experimental procedures, including the preparation of plant samples, extraction, and formulation of carbomer-based topical gels of Physalis peruviana fruit extracts, were conducted at the Pharmacy Simulation laboratory of the University of Perpetual Help System Cauayan from February to March 2026. Before the conduct of the study, the researcher secured an official letter of request and coordinated with the designated laboratory personnel for the use of the facilities and equipment necessary for the study.

Qualitative phytochemical analysis through Thin Layer Chromatography (TLC) was conducted at Saint Mary's University from February to March 2026. Instrumental phytochemical analyses, including Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH antioxidant assay, were performed at the Research Center for the Natural and Applied Sciences of the University of Santo Tomas during the same period. Prior coordination with the respective laboratories was carried out to confirm the availability of testing services, laboratory schedules, and required fees before sample submission and analysis.

The antibacterial activity testing of the ethanolic and aqueous fruit extracts, including agar well diffusion assay, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC), was conducted at the Bioscience Research Laboratories of De La Salle Medical and Health Sciences Institute from March to April 2026. The researcher coordinated with the laboratory personnel regarding the availability of microbiological testing, submission of samples, and scheduling of laboratory procedures. All microbiological analyses were performed using standard laboratory protocols under controlled conditions.

Upon receiving the results, the researcher proceeded with the statistical analysis of the gathered data.

Statistical Treatment of Data

All experimental data were statistically analyzed to evaluate the Phytochemical profiling and antibacterial activity of the formulated carbomer-based gels. Each test will be performed in triplicate, and the results were expressed as mean ± standard deviation (SD) to reflect variability within replicates.

Statistical analysis was performed on Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and the DPPH antioxidant assay using an independent-samples t-test to determine whether a significant difference exists between the antioxidant activities of the ethanolic and aqueous extracts.

The mean zones of inhibition (ZOI) obtained from the agar-well diffusion assay were compared across treatments (aqueous extract, ethanolic extract, 2%, 5%, and 10% carbomer-based gel formulations, and positive and negative controls). Data were subjected to one-way Analysis of Variance (ANOVA) to determine whether significant differences exist among the treatments. For MIC and MBC results, values were tabulated descriptively and compared between extract types and concentrations. Where numerical comparison is applicable, one-way ANOVA may be used to detect statistical differences in antibacterial potency. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to identify which specific groups differ from each other.

Quantitative parameters such as pH from the physicochemical testing are analyzed descriptively using mean and standard deviation. Qualitative parameters like color, appearance, homogeneity, and stability were visually inspected and tabulated. For homogeneity, it was examined by observing the samples in good lighting conditions and noting down their texture and nature. Each formulation was observed for lumps, coarse particles, different color distribution, or phase separation using a three-point scale such as Excellent (no lump, no phase separation and uniformity in color), Good (mostly uniform and absence of lump with no phase separation) and poor (presence of lump, no phase separation, or difference in color).

The rule of thumb to consider the results statistically significant was 95% confidence level, $p<0.05$.The data were be presented in a manner such that it is easy to compare the data extracted and the carbomer-based gel formulations, and it was also be compared with the literature to interpret and draw conclusions.

Ethical Consideration

This study was purely on the basis of plant-based material, with in vitro experiments on the standard lab strains of Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis, without any

human or animal subjects. Hence, formal approval on the basis of human clinical ethics committees is not mandatory, but the approach complies with overall human values.

The handling of the cultures was done in accordance with biosafety level 2 standards since the test organisms can cause human pathogens. The handling of the organisms was done in an accredited microbiological laboratory with controlled entry, and proper personal protective equipment was utilized. In some procedures where aerosol and splash generation are likely (vortexing of cultures, making plate cultures, opening culture tubes), handling of organisms is done in an accredited class II biosafety cabinet. The standard biosafety practices applicable at all times include the proper use of labels of cultures, the prevention of the consumption of eatables; the immediate disinfection of spills with approved biocide agents, and the disinfection/decontamination of all cultures and plates, as well as contaminated disposables, through autoclaving prior to disposal. Similarly, the disinfection, sterilization, and disposal of sharps through the use of biohazard sharps containers applicable in the area could also be observed.

To ensure scientific integrity and the validity and reliability of data, all the assay procedures strictly followed a validated method for antimicrobial susceptibility testing and plant extract screening assays. All data was also documented appropriately and honestly, irrespective of the outcome, in compliance with current recommendations for ethical antimicrobial research and biosafety risk management.

Chapter 4
PRESENTATION, ANALYSIS, AND INTERPRETATION OF DATA

This chapter presents the results, analysis, and interpretation of phytochemical, antioxidant, antibacterial, and physicochemical properties of Physalis peruviana fruit extracts. The results consist of FTIR analysis, total phenolic content (TPC), total flavonoid content (TFC), and DPPH antioxidant activity. Significant differences between the extracts and treatment groups were determined using statistical tests like the independent samples t-test and one-way ANOVA. Also, the presence of antibacterial activity was evaluated based on zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All results are presented and discussed in relation to the objectives of the study.

Phytochemical Analysis

a. Qualitative Phytochemical Analysis

The phytochemical screening of Physalis peruviana extracts showed that the extracts contained various bioactive compounds, both in ethanolic and aqueous extracts.

Table 1. Phytochemical Screening of Physalis peruviana Fruit Extract.

Plant Constituent	Reagent / Test Performed	Expected Result (TLC Visualization)	Ethanolic Extract (ETOH)	Aqueous Extract (AQ)	Remarks
Alkaloids	Dragendorff's Reagent	Orange to reddish-brown spots	+	+	Present
Phenols	Ferric Chloride Test	Blue, green, or violet spots	+	+	Present
Tannins	Ferric Chloride Test	Blue-black or green spots	+	+	Present

Flavonoids	Aluminum Chloride + UV	Yellow or fluorescent spots	+	+	Present
Steroids	Liebermann–Burchard	Blue-green coloration	+	+	Present
Triterpenoids	Liebermann–Burchard	Reddish-violet or purple spots	+	+	Present

Legend: (+) Present; (-) Absent

Table 1 represents the phytochemical screening of extract of fruit of Physalis peruviana using TLC. Here are the reagents that was used, the results that is expected and the observed results for each solvent used for the extraction. The result of this test shows presence of Alkaloids when the spot appears orangish or reddish brown when sprayed using Dragendorff's reagent. Presence of phenolic compounds and tannins was tested with the use of Ferric chloride reagent whereby yellow to green or violet spots was observed for phenolic compounds whereas blue black or green spots for tannins. Presence of flavonoid is determined when yellow fluorescent spot is observed when viewed under UV light at 366nm when Aluminium chloride spray is used. While using Liebermann-Burchard reagent presence of steroides appeared blue-green while triterpenoids appear reddish-violet or purple color respectively.

It shows that triterpenes, sterols, phenols, anthraquinones, anthrones, tannins, flavonoids, steroids, and alkaloids are present in both extracts; this proves that these compounds are stable and can be extracted regardless of the polar solvent that has been used.

The presence of phenols, flavonoids, tannins, and alkaloids is interesting to this research. These compounds are known to have a significant amount of anti-microbial activity and anti-inflammatory properties. This shows that the phytochemical presence within the extract of Physalis peruviana fruit could contribute to its antibacterial activity against the strains that have been tested (Kasali et al., 2021) hence making it suitable as the active component for the topical gel that could be used for the treatment of acne.

b. Quantitative Phytochemical Analysis

The present section includes phytochemical analysis and biological activity, using total phenolic content (TPC), total flavonoid content (TFC), FTIR analysis, and DPPH assay applied to extracts of P. Peruviana fruit. All the analyses above were carried out in order to detect the phytochemical compounds present in the extract and evaluate their ability to present the observed biological effects.

FTIR Analysis

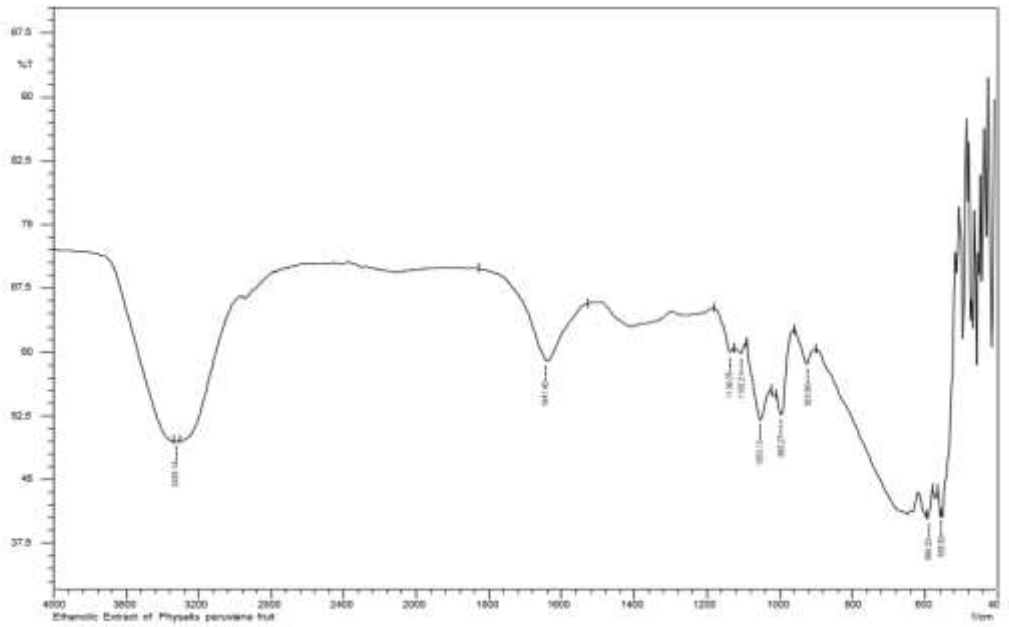


Figure 3. FTIR spectrum of ethanolic extract of Physalis peruviana fruit.

Figure 3 Shows FTIR spectrum of the ethanolic extract of Physalis peruviana fruit. There are absorption bands around 3200–3400 cm 1 showing the presence of O-H groups, which is commonly characteristic of phenols and flavonoids. There are distinct peaks around 1600–1650 cm 1 due to stretching vibrations of the carbonyl C=O groups, suggesting presence of ketone, aldehyde or carboxylic acid functional group. Absorption bands around 1000–1100 cm 1 identified corresponding to C-O stretching of alcohol, ether or ester functional group were also observed. Presence of these bioactive functional groups indicate that the ethanolic extract contains active compounds that contribute to its biological activity (Kozhantayeva et al., 2024).

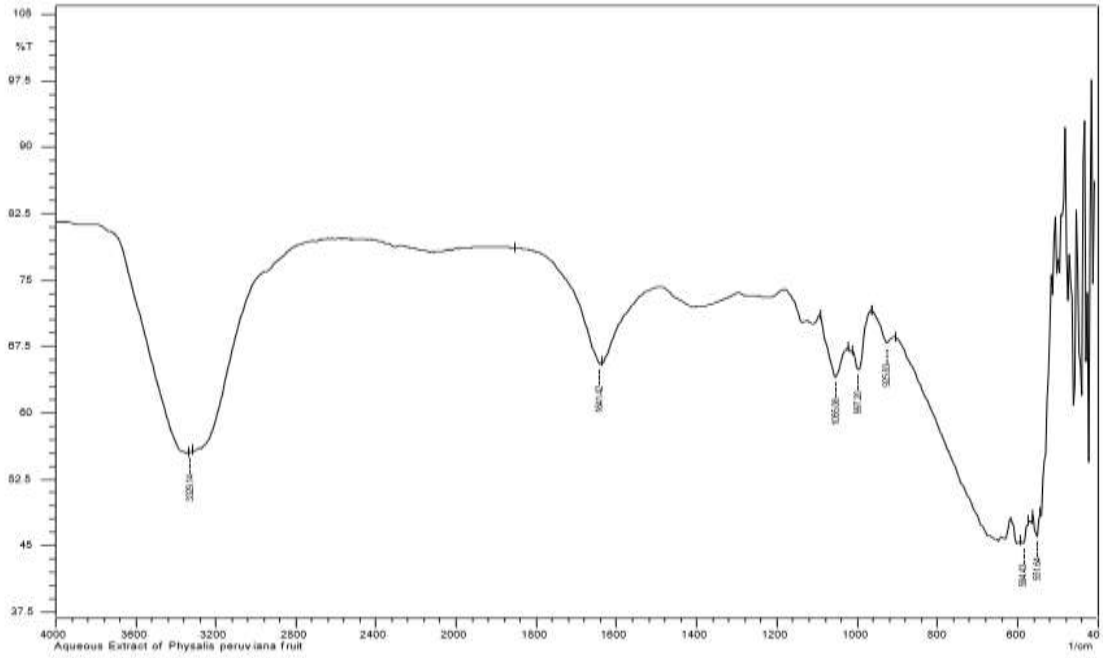


Figure 4. FTIR spectrum of aqueous extract of Physalis peruviana fruit

Figure 4 shows the FTIR spectrum of the aqueous extract of Physalis peruviana fruit. The spectrum also displayed a broad peak between 3200 and 3400 cm^{-1}, which corresponds to the O-H vibration of the phenolic and flavonoid compounds. The peak at around 1600-1650 cm^{-1} corresponds to the stretching vibration of C=O the group in the compounds. Other absorption peaks in the range of 1000-1100 cm^{-1} are due to C-O stretching vibrations for alcohols, ethers, or esters. Compared to the ethanolic extract, fewer peaks with lower intensity were observed in the spectrum of the aqueous extract, suggesting it has a relatively lower range of phytochemical compounds than the ethanolic extract. This is in accordance with previous studies that have shown lower content of phenolics and flavonoids in aqueous extracts compared to alcoholic extracts due to the difference in solvent polarity and extraction rate (Kaczorov et al., 2021).

Total Phenolic Content (TPC)

Table 2. Total Phenolic Content (TPC) of Physalis peruviana Fruit Extracts

Extract Type	Mean	SD	t-value	p-value	Interpretation
Ethanolic Extract	0.385	0.00404			
Aqueous Extract	0.372	0.00351	4.21	0.014	Significant

Table 2 presents the total phenolic content (TPC) of the ethanolic and aqueous extracts of Physalis peruviana fruit. As the results indicate, the ethanolic extract had a higher mean value (M = 0.385, SD = 0.00404) than the aqueous one (M = 0.372, SD = 0.00351) which demonstrates that the ethanol was more efficient than the aqueous in extracting the phenolic compounds. The independent samples t-test indicated a statistically significant difference between the two extracts (t = 4.21, p = 0.014), and this may imply that the solvent type has a significant effect on the phenolic's solubility. This observation contributes to the concept mentioned in the research that organic extracts, especially ethanol, increase the solubility and recovery of phenolic compounds because they are intermediate in polarity.

The same findings were found in literature, with previous research revealing that the ethanolic extraction is more capable of extracting phenolic content than the aqueous one due to the higher capacity of ethanol to dissolve the bioactive compounds (Tourabi et al., 2023).

Total Flavonoid Content (TFC)

Table 3. Total Flavonoid Content (TFC) of Ethanolic and Aqueous Extracts of Physalis Peruviana

Extract Type	Mean	SD	t-value	p-value	Interpretation
Ethanolic Extract	0.0497	0.00100	8.61	0.013	Significant
Aqueous Extract	0.186	0.0275			

Table 3 presents the total flavonoid content (TFC) of the ethanolic and aqueous extracts of Physalis peruviana. Results reveal that the aqueous extract (M = 0.186, SD = 0.0275) contained significantly higher flavonoid content than the ethanolic extract (M = 0.0497, SD = 0.00100). Then, statistical analysis with an independent sample t-test showed statistically significant difference between extracts, (t = -8.61, p = 0.013), and thus the nature of the extract has a significant effect on flavonoid extraction.

This result implies that water is more effective to extract flavonoid compounds in Physalis peruviana which could be explained by the polarity of flavonoids that can be better soluble in aqueous system. The same result has been observed in other previous research studies, in which aqueous extracts had a better flavonoid production than organic solvents because greater polar phytochemicals could be extracted (Chaves et al., 2020).

DPPH Antioxidant Activity

Table 4. DPPH Antioxidant Activity of Ethanolic and Aqueous Extracts of Physalis Peruviana

Extract Type	Mean	SD	t-value	p-value	Interpretation
Ethanolic Extract	0.417	0.00764			
Aqueous Extract	0.421	0.0142	0.48	0.664	Not Significant

Table 4 presents the DPPH antioxidant activity of the ethanolic and aqueous extracts of Physalis peruviana. The aqueous extract (M = 0.421, SD = 0.0142) showed slightly higher antioxidant activity compared to the ethanolic extract (M = 0.417, SD = 0.00764). However, statistical analysis using an independent sample t-test revealed that the difference between the two extracts was not statistically significant (t = -0.48, p = 0.664), indicating that both extracts exhibit comparable antioxidant activity.

The absence of a significant difference may be attributed to the presence of similar antioxidant compounds in both extracts, such as phenolics and flavonoids, which contribute collectively to free radical scavenging activity. Even though extraction solvents may affect the quantity of phytochemicals extracted, the antioxidant effect is not entirely concentration dependent but instead the interaction and synergy of the various compounds. This further justifies the fact that both ethanolic and aqueous extracts had comparable antioxidant properties despite the differences observed in TPC and TFC. Similar findings have been reported in previous studies, where variations in solvent polarity did not necessarily result in significant differences in DPPH radical scavenging activity (Baliyan et al., 2022). This implies that the antioxidant activity cannot be determined only based on phenolic or flavonoid content.

Table 5. Comparison of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH Antioxidant Activity of Ethanolic and Aqueous Physalis peruviana Extracts

Type		Mean	SD	t-test	p-value	Interpretation
TPC	Ethanolic	98.37	1.14	4.202	0.014*	Significant
	Aqueous	94.72	0.98			
TFC	Ethanolic	10.39	0.07	8.605	0.013*	Significant
	Aqueous	19.33	1.80			
DPPH activity	Ethanolic	15.13	2.86	4.68	0.664	Not Significant
	Aqueous	16.00	1.54			
*Significant @ 0.05						

Table 5 shows the comparison of the total phenol content (TPC), total flavonoids content (TFC), and the DPPH antioxidant activity of the ethanolic extract and aqueous extract of Physalis peruvina. The findings showed different amounts of phytochemical and antioxidant activity, and significant differences as well as non-significant ones were found between both types of extracts.

For total phenolic content (TPC), statistically significant difference was found between the ethanolic and aqueous extracts (t = 4.202, p = 0.014). The ethanolic extract had a higher mean value (98.37) than did the aqueous extract (94.72), thus showing that ethanol was more efficient to extract phenolic compounds. This

suggests that phenolic compounds are more soluble in ethanol, resulting in higher extraction efficiency. (Tourabi et al., 2023).

Similarly, for total flavonoid content (TFC), a huge difference was also evident (t = -8.605, p = 0.013). In this instance, the mean value of the aqueous extract (19.33) was higher than the ethanolic extract (10.39) hence water was more efficient in extracting flavonoid compounds. This difference implies that various phytochemicals are soluble to different extents in different solvents used in extraction (Chaves et al., 2020).

In contrast, there was no statistically significant difference between DPPH antioxidant activity of the ethanol and aqueous extracts (t = -4.68, p = 0.664). Though the aqueous extract (Mean = 16.00) showed a slightly better antioxidant activity than the ethanolic extract (Mean = 15.13) the difference was not significant. This means that these two extracts have similar antioxidant potentials even though they vary in phytochemical content. The finding indicates that that several bioactive compounds might act in a synergistic manner in antioxidant effects leading to similar overall activities (Baliyan et al., 2022).

Antibacterial Activity

Zone of Inhibition (ZOI)

Table 6. Zone of Inhibition (ZOI) of Physalis peruviana Extracts Against Selected Bacterial Strains

	Type	Mean	SD	F-value	p-value	Interpretation
S.aureus	Ethanolic	14.36	0.45	23.100	0.000*	Significant
	Aqueous	13.73	1.07			
	T1 (10%)	12.63	0.64			
	T2 (5%)	13.32	1.45			
	T3 (2%)	14.54	0.53			
	Positive control	14.14	0.77			
	Negative control	8.00	0.00			
S.epi	Ethanolic	14.21	0.38	17.250	0.000*	Significant
	Aqueous	14.11	0.71			
	T1 (10%)	12.99	1.95			
	T2 (5%)	14.16	0.62			
	T3 (2%)	14.13	0.55			
	Positive control	14.32	1.00			
	Negative control	8.14	0.39			
C,anes	Ethanolic (1)	18.86	0.42	193.626	0.000	Significant
	Aqueous (2)	18.42	0.52			
	T1 (10%)	17.18	0.20			
	T2 (5%)	18.33	0.60			
	T3 (2%)	18.55	0.52			
	Positive control (6)	14.14	0.77			

	Negative control (7)	8.00	0. 00			
*Significant @ 0.05						

Table 6 presents the zone of inhibition (ZOI) of Physalis peruviana extracts of S. aureus, S. epidermidis, and C.anes. These results showed that there was a statistically significant difference among the treatment groups since the results of the one-way ANOVA were statistically significant ($p < 0.05$), and therefore, the level of antibacterial activity differs with the type of extract and concentration used. The post hoc analysis also showed that the differences were significant mostly between the negative control and all the treatment groups.

In Staphylococcus aureus, ethanolic extract (Mean = 14.36 mm), had a slightly higher antibacterial activity compared to the aqueous extract (Mean = 13.73 mm). The positive control had a 2% extract with a comparable antibacterial activity (Mean = 14.54 mm) or slightly better than the positive control, suggesting a high potential antibacterial activity. The negative control was used to confirm the absence of antibacterial activity (Mean = 8.00 mm), the most poorly inhibited. The post hoc results confirmed all of the observed differences in the negative control, which supports the observed antibacterial effects of the extracts. Moreover, most treatment groups have a relatively low value for the standard deviation, which ensures uniformity and reliability of the results and demonstrates little variation in the response to the antibacterial. The results did not demonstrate a direct dose-dependent relationship among the gel formulations, as the 2% formulation exhibited the largest mean zone of inhibition. This finding may be attributed to differences in gel viscosity, diffusion capacity through the agar medium, or variability in formulation characteristics affecting antimicrobial diffusion.

Likewise, the ethanolic (Mean = 14.21 mm) and aqueous extracts (Mean = 14.11 mm) had similar antibacterial effects in the case of Staphylococcus epidermidis. The 5% (Mean = 14.16 mm) and 2% (Mean = 14.13 mm) concentrations exhibited a relatively higher inhibition as compared to the 10% (Mean = 12.99 mm) indicating that antibacterial efficacy was not directly proportional to increasing extract concentration. This suggests that higher concentrations may have affected the diffusion of the active compounds in the agar medium, resulting in lower apparent inhibition zones. ANOVA results (F 23.100 in the case of S. aureus and F 17.250 in the case of S. epidermidis, $p = 0.000$) indicated that the difference between treatments was significant. Post hoc also indicated that the negative control significantly varied among all the treatment groups. Furthermore, the similar mean values of the two extracts indicate that both ethanolic and aqueous extracts are effective for extracting antibacterial compounds against Staphylococcus epidermidis. However, the observed variation among gel concentrations indicates that antibacterial activity in the formulation may be influenced not only by extract concentration but also by formulation factors such as viscosity and diffusion capacity within the agar medium.

There was a significant difference among treatment groups with the F value and p value for Cutibacterium acnes, 193.626 and 0.000, respectively; the antibacterial activity varied significantly depending on the type of extracts, formulation concentration, and controls. The ethanolic extract (Mean = 18.86 mm) exhibited the highest antibacterial activity, followed by the aqueous extract (Mean = 18.42 mm) indicating both methods of extraction used are able to extract active compounds that possess antibacterial effects against Cutibacterium acnes. Among the formulated gel concentrations, 2% (Mean = 18.55 mm) showed the highest zone of inhibition, followed by 5% (Mean = 18.33 mm) and 10% (Mean = 17.18 mm) showed the lowest antibacterial activity showing that antibacterial efficacy of formulation formulation against

Cutibacterium acnes is not directly proportional with increasing concentration. Post hoc analysis further showed significant differences between several pairs of treatments; there were significant differences between the negative control against all extract treatments and certain concentrations of the formulation against the positive control.

For most cases the conclusions point to the fact that the nature and quantity of extract used greatly influenced the antibacterial properties of Physalis peruviana. Generally, there was no consistent concentration-dependent antibacterial response shown because a higher concentration did not lead to a higher zone of inhibition in several cases; some lower concentrations did give higher antibacterial activity compared to higher concentrations in some cases, thus suggesting there is a non-linear relationship between the concentration and activity. In addition, ethanolic extract showed generally higher activity than aqueous extract, indicating that ethanol is better than aqueous extract for extracting active compounds with antibacterial properties, although antibacterial activity seems dependent not only on the concentration but also on the solvent used and the formulation of the product.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 7. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Physalis peruviana Extracts

Type		Mean	SD	F-test	p-value	Interpretation
C. acnes	Ethanolic (1)	0.24	0.15	64.78	0.000*	Significant
	Aqueous (2)	0.41	0.20			
	2% (3)	0.34	0.15			
	5% (4)	0.41	0.13			
	10% (5)	0.45	0.07			
	Positive control (6)	0.07	0.11			
	Negative control (7)	1.25	0.04			
S. aureus	Ethanolic	0.41	0.21	44.039	0.000*	Significant
	Aqueous	0.53	0.19			
	2%	0.25	0.23			
	5%	0.53	0.21			
	10%	0.57	0.16			
	Positive control	0.06	0.05			
	Negative control	1.28	0.08			
S. epi	Ethanolic	0.16	0.13	72.781	0.000*	Significant
	Aqueous	0.45	0.23			
	2%	0.34	0.14			
	5%	0.41	0.13			
	10%	0.39	0.04			
	Positive control	0.08	0.14			

	Negative control	1.32	0.04			
*Significant @ 0.05						

Table 7 is a summary of the MIC and MBC values of the Physalis peruviana extracts against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. MIC and MBC values were displayed together, in order to assess both the inhibitory and the bactericidal efficacy of the treatments. Results revealed a significant variation of these values among treatment groups, with the result of ANOVA test having $p < 0.05$ which implies that the antibacterial effect varies according to the type and concentration of extract. Post hoc analysis shows pairwise comparison among treatments; the most obvious pair is between negative control and treatments.

For Cutibacterium acnes, the difference was highly significant ($F = 64.78$, $p = 0.000$), which implies that antibacterial activity significantly varied among treatments. The ethanolic extract showed lower MIC/MBC values (Mean = 0.24) compared to the aqueous extract, indicating stronger antibacterial and bactericidal activities. The positive control (Mean = 0.07) exhibited the highest antibacterial activity, while the negative control (Mean = 1.25) showed no inhibitory effect. Post hoc results revealed significant differences between the ethanolic and aqueous extracts, between extract concentrations and the positive control, and between all treatment groups and the negative control. This analysis suggests that the effectiveness of the antibacterial activity depends on the concentration and type of solvent used with stronger antibacterial activity exhibited by ethanolic extracts due to better extraction of the active phytochemicals (Rubayiza & Mukisa, 2025).

For Staphylococcus aureus, statistically significant difference ($F = 44.039$, $p = 0.000$) was also found, showing variability of antibacterial activity between treatments. The ethanolic extract (Mean = 0.41) exhibited stronger antibacterial activity than the aqueous extract (Mean = 0.53), as indicated by lower MIC/MBC values. The antibacterial effect was highest on the positive control (Mean = -0.06) with the negative control (Mean = 1.28) having no antibacterial activity. Post hoc analysis confirmed that the negative control significantly differed from all other treatment groups, while significant differences were also observed between selected extract concentrations and the controls. These findings justify the idea that the antimicrobial properties of plant extracts rely on their concentration and mode of extraction (Sun et al., 2025).

For Staphylococcus epidermidis, a highly significant difference was observed ($F = 72.781$, $p = 0.000$), indicating that the treatments differ significantly in their antibacterial effects. The ethanolic extract exhibited lower MIC/MBC values than the aqueous extract (Mean = 0.16), indicating stronger antibacterial activity. The efficacy of the positive control (Mean = 0.08) was maximal whereas the negative control (Mean = 1.32) did not have an antibacterial effect. Post hoc findings likewise showed significant differences between the negative control and all treatment groups, as well as between selected extract concentrations and the positive control. These results also ensure that ethanol is a superior solvent to extract antimicrobial compounds thus enhancing the antibacterial effectiveness of the extract Kozhantayeva et al., 2024).

Physicochemical Properties of the Formulated Gel

This section presents the physicochemical properties of the formulated carbomer-based gel containing Physalis peruviana extracts, specifically pH and homogeneity. These parameters were considered to assess the stability, consistency and applicability of the formulations to the topical application.

pH Determination

Table 8. pH of Physalis peruviana Extracts and Gel Formulations

Formulation	pH (Undiluted) Mean ± SD	pH (Diluted) Mean ± SD
Topical Gel 2%	6.1 ± 0.10	6.2 ± 0.10
Topical Gel 5%	5.8 ± 0.10	6.1 ± 0.10
Topical Gel 10%	5.0 ± 0.10	5.9 ± 0.10

Table 8 presents the pH values of the formulated gel samples expressed as mean ± standard deviation. The formulation gels exhibited pH values ranging from 5.0 ± 0.10 to 6.2 ± 0.10 for undiluted samples and 5.9 ± 0.10 to 6.83 ± 0.06 for diluted samples.

A decreasing trend in pH was observed with increasing extract concentration, with the 10% gel formulation exhibiting the lowest pH (5.0 ± 0.10 undiluted; 5.9 ± 0.10 diluted). This indicates that as the concentration of extract increases, the pH decreases, or in other words it increases the acidity, perhaps owing to presence of acid phytoconstituents such as phenolic compounds in the formulation. However, 2% and 5% gel formulations of extract showed a neutral range of pH.

Low standard deviation values for all the formulations signify the consistency of the pH value and thus the stability and uniformity of the gel formulations. It depicts a good control on formulation procedures and reproducibility of results. However, it is to be also mentioned that the different formulations did not differ among themselves with regards to concentration of extract and all of them showed dermatologically compatible pH range (necessary for maintaining barrier of skin)

Moreover, increase of pH was observed on dilution with water, reflecting the decreasing acidity. This also increases the skin compatibility since skin accepts the least acidic formulations well. However, all the values for the gel formulations remained well within the normal pH range of the skin, which is between 4.5 to 6.5 and thus it is a valid product for topical application and will not irritate the skin. An appropriate pH range is important in the formulation because of the skin barrier functions and in maintenance of microbial homeostasis (Prvnescu et al., 2025).

Homogeneity Test

Table 9. Physicochemical and Qualitative Evaluation of Physalis peruviana Gel Formulations

Formulation	Color	Appearance	Homogeneity	Stability
Topical Gel 2%	Colorless to slightly pale yellow	Clear, smooth gel	Excellent	Stable (no phase separation)
Topical Gel 5%	Light yellow	Clear to slightly translucent, smooth	Excellent	Stable (no phase separation)
Topical Gel 10%	Yellow to golden yellow	Translucent, smooth gel	Excellent	Stable (no phase separation)

Table 9 presents the homogeneity test, which showed that all the prepared samples were found to be excellent, meaning that all formulations had a smooth texture, homogenous color, and no lumps or phase separation. This indicates that the mixing was successful in attaining the uniform distribution of ingredients all over the gel matrix.

Good physical stability of the formulations is also exhibited by the lack of apparent anomalies and is valuable when considering uniform application and delivery of active compounds. The formulations in the topical preparations should be homogeneous because they help to enhance the quality of the products, their effectiveness, and their acceptability by the users (Zhao et al., 2024).

Chapter 5
SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

This chapter presents the summary of findings, conclusions, and recommendations of the study results. It identifies the important findings of the phytochemical, antioxidant, antibacterial, and physicochemical studies of Physalis peruviana fruit extracts and how these could be applied in topical formulations. This study seeks to answer the following questions:

1. What bioactive compounds are present in the ethanolic and aqueous fruit extracts of Physalis peruviana, as determined through qualitative and quantitative phytochemical analyses using appropriate analytical techniques?
2. What is the antibacterial activity of Physalis peruviana fruit extracts against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis using the agar-well diffusion assay?
3. What are the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against the identified microorganisms?
4. How is a carbomer-based topical gel containing Physalis peruviana fruit extract formulated?
5. What are the physicochemical properties of the formulated carbomer-based topical gels containing varying concentrations of Physalis peruviana extracts in terms of pH and homogeneity?
6. Is there a significant difference in antibacterial effectiveness between the formulated carbomer-based gel containing Physalis peruviana fruit extract and the positive control (clindamycin gel), as measured by the Zone of Inhibition (ZOI)?

Summary of Findings

This study evaluated the phytochemical content, antioxidant, antimicrobial and physicochemical properties of Physalis peruviana fruit extracts, which were measured in ethanol and aqueous solvents.

1. Phytochemical screening showed that the two extracts contained significant bioactive compounds, specifically alkaloids, phenols, tannins, flavonoids, steroids, and triterpenoids. FTIR analysis also validated these findings and indicated the presence of functional groups that are indicative of phenolic and flavonoid compounds. In terms of TPC and TFC, there were notable differences and the ethanolic extract had a statistically higher phenolic content ($p=0.014$) and aqueous extract a statistically higher flavonoid content ($p=0.013$), thus showing that the solvent used impacts the extraction of phytochemicals. As the DPPH assay indicated, there was no statistically significant difference in antioxidant activity between the two extracts ($p=0.665$).
2. There was a significant difference between the zone of inhibition (ZOI) between the treatment groups ($p=0.000$) between Cutibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis, demonstrating that different levels of antibacterial activity relate to type of extract and concentration of the extract. There was not a proportional relationship between concentration and antibacterial activity; the 2% formulations often showed a larger zone of inhibition compared to the 10% formulation, perhaps because the lower concentrations allow for better diffusion into the agar.

3. MIC and MBC values showed statistically significant differences across treatments ($p = 0.000$), with lower MIC/MBC values in ethanolic extracts, which showed increased antibacterial/bactericidal effects. The post hoc analysis also indicated the existence of a significant difference, especially between the negative control group and the treatment groups.
4. The formulated gel was incorporated by Physalis peruviana fruit extract into a carbomer-based vehicle to create a preparation suitable for topical use. The Formulations were developed at varying concentrations (2%, 5%, and 10 %), with the 2% concentration identified as particularly effective.
5. Physicochemical studies showed that all the formulated gels had a pH value that was within the acceptable range of 5.0 to 6.2 and thus could be used topically. The formulations also exhibited an outstanding homogeneity, which is smooth and uniform.
6. There were significant differences between treatment groups and the negative control. The 2% gel formulation showed the highest Zone of Inhibition against S. aureus (14.54 mm) and C. acnes (18.55 mm), exhibiting activity comparable to or higher than the positive control (Clindamycin). The results also indicate the influence of extract concentration, though the effect was not proportional to the concentration possibly because of formulation aspects like viscosity and diffusion rate.

The overall results conclude that Physalis peruviana extracts possess significant phytochemical and antibacterial potential and are adaptable for topical application.

Conclusions

Based on the findings of the study, the following conclusions are drawn:

1. Both the aqueous and ethanolic extracts from Physalis peruviana fruit were found to have some interesting phytochemical constituents such as the presence of flavonoids, tannins, alkaloid and phenols which is a good indication of its biological activities.
2. The extracts were found to have antioxidant properties; but there was no significant difference between treatment by DPPH assay so the extracts require more confirmation with another antioxidant method.
3. The aqueous and ethanolic extracts have antibacterial activities against Cutibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis as there was zones of inhibition formed in the agar disk diffusion test and the activity differs in concentrations of both extracts.
4. Quantitative assays such as MIC and MBC also show the extract to have an effect as antibacterial on bacteria that also correlated with the results from the diffusion test.
5. There were significant differences shown between treatment, according to the one-way ANOVA and Turkey HSD that suggest that the antibacterial activity of extract varies depending on its concentration.
7. The carbomer gel formulated was physically and chemically acceptable, appropriate in terms of the parameters measured such as the pH and homogeneity of the gel.

Recommendations

Based on the findings of the study, the following recommendations are proposed:

1. Subsequent research is urged to use the aforementioned techniques (HPLC, GC-MS) in order to characterize and quantify the responsible bioactive compounds for the antibacterial activity of Physalis peruviana. In addition, other antioxidant assays (ABTS, FRAP) should be used in future research, in the case where significant results are not found with the DPPH assay, for a wider evaluation of these properties.

2. A suggestion is made to perform further antimicrobial studies, using standardized quantitative assays (MIC, MBC) and test against more relevant micro-organisms to skin infections or related to acne, and isolation and identification of the responsible compounds.
3. Further optimization of topical gel formulation of carbomer. For improving physical stability, skin permeability and controlled delivery. In vivo or clinical studies in humans to establish safety and efficiency of Physalis peruviana topical preparations in acne treatment.
4. Alternative and/or complementary treatment for acne vulgaris using plant-derived topical preparations such as Physalis peruviana gel by physicians, pharmacists and other healthcare professionals in the area of skin treatments

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