Phytochemical analysis, antioxidant and antibacterial activity of *Pereskia bleo* flowers

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Pereskia bleo, belongs to the Cactaceae family has been traditionally used for treating various diseases. This study aimed to determine the phytochemical analysis, antibacterial activity, antioxidant activity, total phenolic content, and total flavonoid content of Pereskia bleo flowers. Phytochemical screening revealed the presence of glycoside, alkaloid, flavonoid, saponin, tannin, steroid, and terpenoid. The antioxidant activity test indicated that the crude extract of Pereskia bleo flowers had an average IC50 value of 6 ± 0.4359, confirming positive results in the phenolic content test. The total phenolic content of the Pereskia bleo flower crude extract was calculated as 75.295 mg/g at a concentration of 15.059 µg/ml and absorbance of 0.165. In contrast, the calculated total flavonoid content was 7.385 mg/g at a concentration of 9.477 µg/ml, suggesting the present of flavonoid compounds in the flowers of Pereskia bleo. The antibacterial activity of this sample was also tested using the disc-diffusion method against Gram-negative (Salmonella sp., Pseudomonas sp., E. coli) and Gram-positive (Staphylococcus aureus, Bacillus sp., Staphylococcus epidermidis) bacterial strains. The collected data showed that both 100 µg/ml and 500 µg/ml sample concentrations exhibited potent activity against Salmonella sp. and Pseudomonas sp.

Keywords: Phytochemical; antibacterial; antioxidant; *Pereskia bleo*; flower

1.0 Introduction

Pereskia bleo belongs to the genus of cacti, commonly known as the 'Needle Seven Blade' in English. It is classified within the cacti genus due to its spiny shrub with large leaves and a thin stem, which typically reaches heights ranging from 2 to 8 meters. Pereskia bleo has been extensively utilized in traditional medicine for cancer-related treatments (Asmaa et al., 2014). According to Stevenson and Mauseth (2004), *Pereskia bleo* is characterized as a non-succulent, treelike cactus with large leaves. Additionally, Pereskia bleo exhibits monomorphic fibrous wood development, unlike the typical wood formed by vessels within a matrix of fibers. Intriguingly, *Pereskia bleo* is unique in that it develops shoots and roots under both watering systems. This species is regarded as a valuable nutritional supplement due to its protein, fiber, iron, calcium, and mineral content. Moreover, the leaves of Pereskia bleo are traditionally employed for treating various ailments such as hypertension, cancer, diabetes, rheumatism, and inflammation (Lucéia et al., 2016).

The genus to which *Pereskia bleo* belongs is Pereskia, and it was named after Nicolas-Claude Fabri de Peiresc, a 16th-century French botanist. The *Pereskia* genus is considered to be less advanced and comprises 17 tropical species divided into two subgroups. Originating from the region between Brazil and Mexico, Pereskia is cultivated in numerous tropical and subtropical countries, including Malaysia, India, and Singapore (Lucéia et al.,

2016). Locally, *Pereskia bleo* and *Pereskia grandifolia* have been traditionally utilized as natural remedies for cancer-related ailments, rheumatism, diabetes, high blood pressure, inflammation, and ulcers.

Previous studies have demonstrated the presence of phytochemical constituents such as saponins, flavonoids, tannins, and polyphenolic bioactive compounds extracted from Pereskia species (Wahab et al., 2009). Sharif et al. (2013) reported the presence of alkaloids, bioactive compounds, in both *Pereskia bleo* (Kunth) and *Pereskia grandifolia* (Haw). Four alkaloids were isolated from *Pereskia bleo* (Kunth), namely 3,4-dimethyl-β-phenethylamine, mescaline, 3-methoxytyramine, and tyramine. Additionally, *Pereskia grandifolia* (Haw) was found to contain three alkaloids: mescaline, 3-methoxytyramine, tyramine, and p-methoxy-β-hydroxy-β-phenethylamine.

Several studies have confirmed the presence of antioxidant agents in *Pereskia* extracts. According to Sharif et al. (2015), many foods and herbs possess antioxidant properties, and excessive consumption of antioxidants has been inversely associated with the development of certain pathologies, such as cardiovascular disease. Antioxidant-rich foods have also been identified as beneficial in preventing degenerative diseases. Their findings indicate that extracts derived from *Pereskia bleo* contain high levels of antioxidant compounds such as α-tocopherol, β-sitosterol, and erythritol, as well as non-polar antioxidant compounds such as unsaturated fatty acids.

The extracts of *Pereskia bleo* were examined against bacteria *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Streptococcus pyogenes* (SP), and *Escherichia coli* (EC) by Johari and Khong (2019). The assessment involved qualitative and quantitative analysis, determining the presence or absence of bacteria through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The methanolic extract exhibited strong activity against Gram-negative bacteria SA and SP, with MIC values of 225µg/mL, while exhibiting the same potency against Gram-positive bacteria PA and EC at a MIC value of 450µg/mL. Similarly, the chloroform crude extract demonstrated robust activity against SA, SP, and EC at a MIC value of 225µg/mL, and against PA at a MIC value of 450µg/mL. Subsequent confirmation of MBC values was obtained through the absence of bacterial growth on nutrient agar streaked from the lowest clear MIC values. Both methanolic and chloroform extracts of *Pereskia bleo* exhibited significant inhibition of bacteria SA, SP, PA, and EC.

Despite indications of *Pereskia bleo's* potential as antibacterial and antioxidant agents, there remains a gap in comprehensive studies exploring the chemical composition and biological properties of these flowers. Therefore, this study is specifically designed to identify the phytochemical compounds present in *Pereskia bleo* flowers, assess their antioxidant capabilities, and evaluate their efficacy against various bacterial strains. Through this research endeavor, valuable insights into the potential medicinal and therapeutic attributes of *Pereskia bleo* flowers are anticipated, with the overarching objective of advancing the development of natural products for healthcare and pharmaceutical applications.

2.0 Materials and methods

2.1 Materials

All the chemicals and solvents used during this study were methanol, sulfuric acid, hydrochloric acid, sodium hydroxide, ferric chloride, acetic anhydride, iodine, potassium iodide, chloroform, copper sulphate, mercuric chloride, ammonia, dichloromethane, aluminium chloride, potassium acetate, sodium carbonate, acetone, ethanol 90%, nutrient agar, 0.5 McFarland standard, and muller Hilton brooth.

2.2. Sample extraction

The flowers of *Pereskia bleo* were cut into small pieces and left to dry inside the laboratory oven. Once fully dried (119.4 g), they were ground into a fine powder (96.4g) using a blender before proceeding to the extraction process. The dried, ground flowers of Pereskia bleo (119.4 g) were soaked in methanol for 24 hours at room temperature. Subsequently, the solvent-containing extract sample underwent filtration at room temperature. The extraction with the solvent was repeated three times with methanol to obtain more extracts. The filtrate from each extraction was combined, and the solvent was evaporated under vacuum at 30℃ using a rotary evaporator. The resulting extract was then freeze-dried for two days to ensure no methanol remained, yielding crude extracts (43.7 g). These crude extracts were then subjected to isolation through thin-layer chromatography (TLC), phytochemical tests, and evaluation of their antibacterial and antioxidant activities.

2.3 Phytochemical Screening

The crude extracts obtained from *Pereskia bleo* flowers underwent phytochemical tests to identify the presence of glycosides, alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids using standard methods. A positive response was indicated by a change in color or the formation of a precipitate.

2.3.1Test for Glycoside Compounds

The extract was dissolved in 1 ml of distilled water, and a few drops of aqueous sodium hydroxide solution were added. The formation of a yellow-colored solution indicated the presence of glycosides. This color change is a characteristic reaction associated with the presence of glycosides, providing initial evidence of their existence within the extract.

2.3.2 Test for Alkaloid Compounds

The Wagner’s reagent (2 ml) was added to the extract. This reagent consists of a solution of iodine in potassium iodide and is commonly used as a qualitative test for the detection of alkaloids in plant extracts. Upon the addition of Wagner's reagent to the extract, the formation of a reddish-brown precipitate was observed. This color change is indicative of a positive reaction and suggests the presence of alkaloids in the extract (Firdouse and Alam, 2011).

2.3.3 Test for Flavonoid Compounds

Flavonoids were tested using the alkaline reagent method. Initially, the extract was dissolved in a solution of diluted sodium hydroxide (NaOH). Subsequently, diluted hydrochloric acid (HCl) was cautiously added to the solution. The appearance of a colorless solution following the addition of HCl served as an indicator of the presence of flavonoids in the extract (Tiwari *et al.*, 2011). Flavonoids are a diverse group of secondary metabolites commonly found in plants, known for their antioxidant and health-promoting properties. The utilization of the alkaline reagent method enables the rapid and qualitative assessment of flavonoid content in the extract.

2.3.4 Test for Saponin Compounds

The foam test was employed for saponin compounds. Initially, 1ml of the extract was mixed with 5 ml of distilled water in a test tube. The resulting mixture was vigorously shaken to facilitate thorough mixing of the extract with the water. Subsequently, the test tube was allowed to stand undisturbed for a period of 30 minutes. During this resting period, the formation of foam on the surface of the solution was observed. The presence of foam indicated the presence of saponins in the extract (Firdouse and Alam, 2011). Saponins are a class of naturally occurring compounds commonly found in various plant species, known

for their characteristic ability to produce stable foam when agitated in aqueous solutions. The foam test serves as a simple yet effective qualitative method for the detection of saponins in plant extracts.

2.3.5 Test for Tannin Compounds

The Ferric Chloride test was employed to assess the presence of tannin compounds in the extract. Initially, a small amount of the extract was placed in a test tube or a suitable container. Then, a few drops of Ferric chloride solution were added to the extract. Upon the addition of Ferric chloride, the resulting mixture was observed for any color changes or precipitation. Specifically, the absence of a greenish-black coloration indicated the presence of tannins in the extract (Firdouse and Alam, 2011). Tannins are polyphenolic compounds commonly found in plant tissues, known for their ability to form complexes with metal ions such as iron. The Ferric Chloride test serves as a straightforward qualitative method for the detection of tannins based on the formation of a characteristic color change in the presence of Ferric ions.

2.3.6 Test for Phenols Compounds

Phenols compounds were identified using the Ferric chloride test. In this procedure, a 5% neutral ferric chloride solution was prepared and used as the reagent. A small aliquot of the extract was placed in a test tube or appropriate container, followed by the addition of a few drops of the prepared Ferric chloride solution. Upon the addition of Ferric chloride, the resulting mixture was carefully observed for any color changes or precipitation. Specifically, the appearance of a dark green coloration within the mixture indicated the presence of phenolic compounds in the extract (Firdouse and Alam, 2011). Phenolic compounds are a diverse group of secondary metabolites found in plants, known for their antioxidant properties and potential health benefits. The Ferric chloride test serves as a qualitative method for the detection of phenolic compounds based on the formation of a characteristic color change in the presence of Ferric ions.

2.3.7 Test for Steroids Compounds

Steroids were tested by adding 10ml of chloroform into 1ml of the extract, and the mixture was thoroughly mixed to ensure proper dissolution of the components. Following this, an equal volume of concentrated sulfuric acid was added to the chloroform-extract mixture. Upon the addition of sulfuric acid, the resulting solution was carefully observed for any observable changes in color or fluorescence. Specifically, the formation of a red upper layer and the appearance of a yellow or green fluorescence in the sulfuric acid layer were indicative of the presence of steroids in the extract (Kumar *et al.*, 2009). Steroids are a class of organic compounds with a characteristic ring structure, and their presence can be detected based on specific chemical reactions with sulfuric acid. The observed color changes and fluorescence serve as qualitative indicators for the presence of steroids in the extract.

2.3.8 Test for Terpenoids Compounds

Terpenoids were tested by adding 5 ml of chloroform and 2 ml of concentrated H_2SO_4 into 1 mL of the extract. The mixture was then thoroughly mixed to ensure proper dispersion of the components. Following this, the solution was allowed to stand for a brief period to facilitate chemical reactions between the extract and the reagents. During this time, the formation of a reddish-brown coloration at the interface between the chloroform and sulfuric acid layers was carefully observed. This color change served as a qualitative indicator of the presence of terpenoids in the extract (Sheel and Nisha, 2014). Terpenoids are a diverse class of natural compounds characterized by their complex chemical structures, often containing

multiple isoprene units. The observed reddish-brown coloration at the interface is attributed to specific chemical reactions between terpenoids and sulfuric acid, leading to the formation of colored products.

2.4 Determination of Antioxidant Activity

In antioxidant DPPH (2,2-diphenl-1-picryl-hydrazyl-hydrate) free radical scavenging assay (Kumaran and Karunakaran, 2006) where quercetin was used as positive control while DMSO as negative control. Firstly, the crude extract was prepared in different concentrations by two-fold dilution method in DMSO with the concentration of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 mg/ml in 96 well plates. Then, 200µl of methanolic DPPH solution was added into the well and the plate will be incubated for 30 minute and the absorbance was measured at 517 nm with ELISA reader. Free radical scavenging activity was determined according to the equation:

$$\text{Free radical scavenging activity } (\%) = \frac{A_C - A_S}{A_C} \times 100\%$$

Where, A_S is the absorbance of sample and A_C is the absorbance of the negative control.

2.5 Determination of Total Phenolic Contents

The total phenolic contents of plant extract have been performed using the biological methods involving Folin-Ciocalteu reagent and gallic acid (Sigma, Germany). The extract solution (0.1ml) containing 1000µg of extract was added into the volumetric flask, then 46ml of distilled water and 1ml of Folin-Ciocalteu reagent were added and the flask thoroughly shaken. After 5 minutes, 4 ml of solution 7.5% Na_2CO_3 was added and the mixture was incubated for 1 hours. After that, the absorbance was then measured at 760 nm. The solution was repeated tested with the same method for all standard gallic acid solutions (0–1000 mg, 0.1 ml-1) and the standard curve obtained was measured. The total phenolic content of the crude extract was expressed as milligram gallic acid equivalent (GAE) per gram dry weight of extract (Singh *et al.,* 2016).

2.6 Determination of Total Flavonoids Contents

The total flavonoid content was measured with an aluminum chloride colorimetric assay. An aliquot (1 ml) of extracts or a standard solution of (+)-catechin (20, 40, 60, 80, and 100 mg/l) were added slowly into a 10 ml volumetric flask that contain 4 ml of distilled deionized water (dd H_2O). After that, 0.3 ml 5% of $NaNO_2$ was added into the flask. After 5 minutes, 0.3 ml 10 % $AlCl_3$ was added. At the sixth minute, 2 ml of 1M NaOH need to be added and the total volume was made up to 10 ml with the addition of H_2O. Then, mix the solution well, and the absorbance against a prepared reagent blank at 510 nm was measured with an UV-VIS Spectrophotometer λ5. The data of total flavonoid contents of the sample was expressed as milligrams of (+)-catechin equivalents (CE) per 100g dry mass (mg CE/100 G DW). Lastly, analyzed all samples in duplicate (Atanassova *et al.,* 2011).

2.7 Determination of Antibacterial Activity

The antibacterial activities of the *Pereskia bleo* extract were determined by the standard agar disc diffusion assay using Muller Hinton agar (Li *et al.,* 2000). Several species of Gram-positive bacterial strains (*Bacillus* sp., *Staphylococcus epidermidis, Staphylococcus aureus*) and Gram-negative bacterial strains (*Escherichia coli, Salmonella* sp., *Pseudomonas* sp.) were used for this test.

The Muller Hinton agar (MHA) and nutrient agar (NA) were prepared separately by suspended the medium into 1L of distilled water. Both solutions were autoclaved for 2 hours for sterilization and to dissolved it completely. The medium was allowed to harden and

stored in 4°C freezers. Then, the bacterial strains were subcultured on the surface of nutrient agar plates and let incubated at 37°C for overnight. The stock solution of the extract then was prepared by dissolved 0.001g of the sample in 1ml of DMSO. Then, 2 types of sample concentration were prepared. Firstly, for 100µg/ml concentration, 0.1ml of stock sample was added to 0.9ml DMSO. Next for 500µg/ml concentration, 0.5ml of stock sample was added into 0.5ml DMSO and kept both of the samples in centrifuge tubes.

For the preparation of bacteria suspension. Firstly, 0.9% of NaCl was prepared by weight 0.9g of NaCl into 100ml volumetric flask and 100ml of distilled water was added to the NaCl. Next, 5ml of the prepared 0.9% NaCl was poured into 6 separate test tubes. Then, 1-3 colonies of chosen bacteria were picked from the pate and dissolved in 0.9% NaCl. The turbidity was compared with 0.5 standard McFarland.

After that, the suspension prepared was swabbed thoughtfully on MHA plates by using the sterile cotton swab. 20µL of the 100µg/ml and 500µgml samples were pipetted onto sterile paper discs (6mm in diameter, Whatman No. 1) and let dry under sterile conditions. Tetracycline antibiotic disc was used as positive control since it effective against Gram-positive and Gram-negative bacteria. The plates were then incubated at 37°C for overnight. The zone of inhibition was observed and measured. The antibacterial activity was indicated by the presence of the clear inhibition zones around the disc.

3. Results and discussion

The crude extract obtained in this study is 43.70 g and shows the presence of glycoside, alkaloid, flavonoid, saponin, tannin, steroid, and terpenoid. The results were consistent with previous studies. The presence of various phytochemical compounds in Pereskia bleo flowers can be attributed to several factors. These compounds serve biological functions within the plant, such as defense against pathogens, herbivores, and environmental stressors. Over time, plants have evolved mechanisms to produce secondary metabolites like alkaloids and terpenoids to adapt to their environment, enhancing their chances of survival in challenging conditions. Additionally, some compounds, like flavonoids and terpenoids, can attract pollinators, contributing to the plant's reproductive success. Many of these phytochemicals also possess medicinal properties and are used in traditional medicine for various therapeutic purposes. Moreover, certain compounds, such as steroids and terpenoids, may act as chemical signals within the plant or between plants, regulating growth, development, and defense responses, and contributing to the overall health and survival of the plant. Loganayaki et al. (2013) further emphasizes that certain secondary metabolites, including flavonoids, glycosides, alkaloids, and saponins, are crucial to prevent the undesirable oxidation of free radicals.

Antioxidants play a crucial role in minimizing and counteracting the oxidation of molecules within the human body (Hassanbaglou et al., 2012). Oxidant metabolism, essential for cell survival, holds significant value in both the body and food (Rahal et al., 2014). Fig. 1 illustrates the antioxidant activity of Pereskia bleo flower extract. The antioxidant activity was increased with the increment of extract concentration. This is because at higher concentrations of extract means that more antioxidant materials available, more sustained and prolonged action. This extended duration allows antioxidants to continue neutralizing reactive species over a longer period, contributing to increased antioxidant activity. Moreover, As the concentration of antioxidant materials increases, the available reactive species, such as free radicals, may become saturated. Higher concentrations ensure that a larger proportion of these reactive species encounters and reacts with antioxidant molecules, leading to increased overall antioxidant capacity.

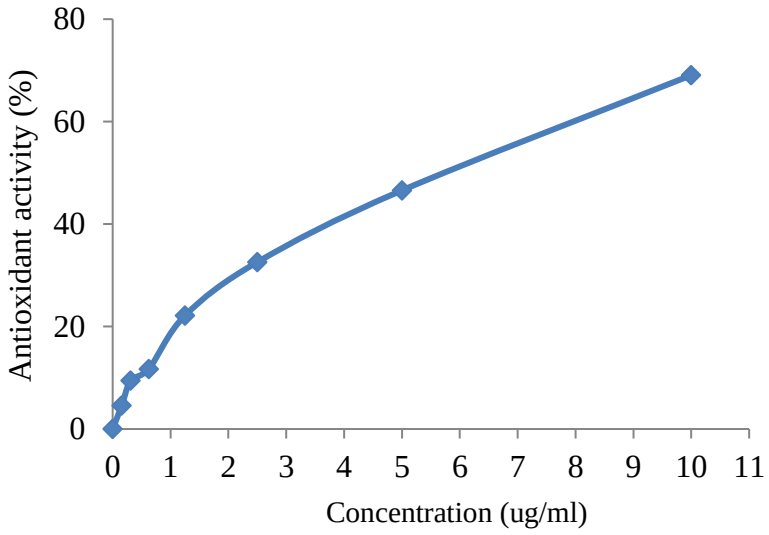

Figure 1: Antioxidant activity of *Pereskia bleo* flower extract.

Fig. 2 shows the gallic acid calibration curve with linear line at intercept. The equation of linear standard curve was y = 0.0115x – 0.009, which y was the absorbance reading at 750 nm and x was a gallic acid concentration in µg /ml. The R squared (r^2) value was 0.9953. Sample of flower of *Pereskia bleo* crude extract was calculated at a concentration 15.059 µg /ml and absorbance 0.165. thus, the total phenolic contents of *Pereskia bleo* flower crude extract was calculated to be 75.295 mg/g. This result is positive outcome in the phenolic content test, affirming the antioxidant potential of the sample as discussed previously.

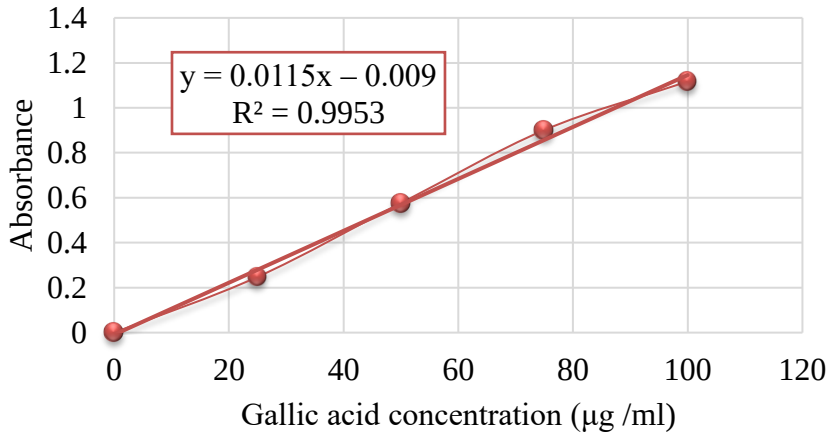

Figure 2 Gallic acid calibration curve.

For total flavonoid contents, quercetin was used as a standard to determine the total flavonoid contents in flower of *Pereskia bleo* crude extract. Fig. 3 shows a standard quercetin curve with linear trend line at zero intercept. The linear standard curve equation was y = 0.0307x + 0.0712, where y was an absorbance reading at 430 nm and x was a quercetin concentration in µg /ml. The R-squared (r^2) value was 0.995. Sample of flower of *Pereskia bleo* crude extract was calculated at a concentration 9.477 µg /ml and the total flavonoid content was found to be 7.385 mg/g. This indicates that the sample has a high concentration of flavonoids. The presence of a high concentration of flavonoids in the

sample signifies its richness in these bioactive compounds. Flavonoids are known for their diverse therapeutic properties, including antioxidant, anti-inflammatory, anti-cancer, and cardioprotective effects. Therefore, the detection of flavonoids in the sample suggests that it may offer various health benefits. These benefits could include strengthening the immune system, reducing the risk of chronic diseases, promoting cardiovascular health, and aiding in the management of inflammatory conditions. Moreover, flavonoids are renowned for their ability to scavenge free radicals and inhibit oxidative stress, which plays a crucial role in aging and the development of various diseases. Hence, the presence of flavonoids in the sample underscores its potential as a natural remedy with therapeutic implications for human health and well-being.

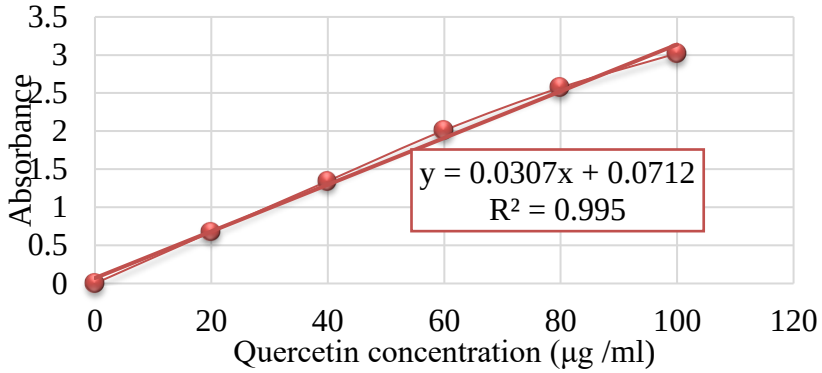

Figure 3 Quercetin calibration curve.

The antibacterial activity of the extract Pereskia bleo flower sample was evaluated against both Gram-negative strains (Salmonella sp., Pseudomonas sp., E. coli) and Gram-positive strains (Staphylococcus aureus, Bacillus sp., Staphylococcus epidermidis) using the disc-diffusion method. Gentamicin served as the positive control, known for its efficacy against both Gram-negative and Gram-positive bacteria. This control was essential for ensuring the accuracy of the assay. The results are summarized in Table 1. The results show that the 500 µg /ml concentration sample have slightly higher potential to inhibit the growth of Gram-negative bacterial compare to 100 µg /ml concentration sample. This is because the interaction between an antimicrobial agent and bacteria can be complex. At lower concentrations, the antimicrobial effect might not be sufficient to cause significant inhibition. However, as the concentration increases, it might reach a threshold where the inhibitory effect becomes more noticeable.

Table 1: Antibacterial activity *Pereskia bleo* flower sample.

Sample Concentration	Inhibition Zone (mm)					
	Salmo sp.	*Pseu sp.*	*E. c*	*S. a*	*Bac sp.*	*S. e*
100 µg/ml	8	N/A	N/A	N/A	N/A	N/A
500 µg/ml	10	7.5	N/A	N/A	N/A	N/A
Gentamicin	15	15	14	17	15	21

N/A = No activity
Bacteria = *Salmo sp.*: *Salmonella sp.*, *Pseu sp.*: *Pseudomonas sp.*, *E. c*: *E. coli*, *S. a*: *Staphylococcus aureus*, *Bac sp.*: *Bacillus sp.*, *S. e*: *Staphylococcus epidermidis.*

4. Conclusions

Pereskia bleo flowers have exhibited a rich array of phytochemical compounds, including glycoside, alkaloid, flavonoid, saponin, tannin, steroid, and terpenoid. These compounds contribute to the diverse biological activities observed in the flowers. Notably, antibacterial assays have revealed significant efficacy against Salmonella sp. and Pseudomonas sp. at concentrations of both 100 µg/ml and 500 µg/ml, indicating the potential of *Pereskia bleo* flowers as potent antibacterial agents, particularly effective against Gram-negative bacteria. Furthermore, the antioxidant activity assessed through DPPH scavenging assays has unveiled robust antioxidant properties within the crude extract of *Pereskia bleo* flowers. This antioxidant potential has shown a direct association with the total phenolic content, which was quantified at 75.295 mg/g. Phenolic compounds present in plants are renowned for their redox properties, rendering them effective antioxidants. This comprehensive analysis underscores the promising medicinal and therapeutic potential of *Pereskia bleo* flowers, paving the way for further exploration and utilization in healthcare and pharmaceutical applications.

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