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Chemical composition and antioxidant activity of *Physalis angulata* L. (Solanaceae) and its effect on blood clotting and biofilm formation of some wound bacterial isolates

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Abstract

Physalis angulata a member of the Solanaceae family, is a plant recognized for its significant nutritional and medicinal properties. Widely distributed across tropical and subtropical regions, this plant has garnered attention for its diverse biological activities. The ethanol extract from the plant is being used in this study to investigate its biological and antioxidant properties. The ability of the extract to reduce free radicals was also tested using the compound phenyl-1-picrylhydrazyl). The DPPH method was used because it is a modern color method and is considered the most common and easy to apply, in addition to its speed and superior sensitivity in testing antioxidants for samples of plant extracts, and the values were compared with the value of ascorbic acid IC_{50}. The study also looks at the extract's ability to prevent the formation of biofilms by different pathogens, including *E. coli* and Staphylococcus strains that were isolated from community labs and Ramadi Teaching Hospital. The ethanol extract demonstrated a noteworthy ability to inhibit the formation of cellular membranes in staphylococcal bacteria and *E. coli*. The efficacy of the extract at different concentrations (150, 200, 250 mg/mL) was also assessed, revealing that a concentration of 250 mg/mL resulted in significant inhibition rates (74.3% and 61.45% for *E. coli*). Furthermore, the study explores the impact of ethanol extract on blood coagulation. At high concentrations (150, 200, 250 mg/mL), the extract exhibited properties akin to aspirin and warfarin, extending the clotting times in both total and partial coagulation tests (Prothrombin time, Partial thromboplastin time) at lower concentrations (100, 50, 10 mg/mL), the extract appeared to promote blood clot formation, suggesting a concentration-dependent effect on hemostasis. This dual action positions *P. angulate* as a potential therapeutic agent, warranting further investigation into its biomedical applications.

Keywords

Antioxidant activity; biofilm formation; blood coagulation management; chemical composition; *Physalis angulata* L.

Introduction

The plant *Physalis angulata* belongs to the Solanaceae family and contains more than 120 species that may be perennial or herbaceous. It is a dense herbaceous annual plant up to about 50cm tall. It has bell-like flowers of a yellow color, and its fruits resemble a balloon hanging down, and the fruits are edible. It grows in areas where the weather is temperate and warm. This plant is distributed worldwide in tropical, subtropical, and coastal regions

such as South America. It is known by different names such as kamabo, wild tomato, winter cherry, golden raspberry, etc (1). The plant also contains compounds Such as alkaloids, glycosides, terpenoids, saponins, flavonoids, tannins, and withanolides (2); it has immunomodulatory and immunostimulating activity, anti-tumor, anti-inflammatory and anti-malarial activity and has a significant effect on microbes (3). It is used in Japan as an antipyretic. It is used to treat many ailments and diseases of the intestines and gastrointestinal tract, such as ulcers, boils, wounds, etc. Studies have proved that this *Physalis angulata* plant offers a lot of therapeutic substances, such as antiallergic, asthmatic, anti-Leishmania, anti-malarial, and immunomodulatory activity (4).

Withanolides are steroids found in Physalis. Physalis is a great source of Vitamin C, Beta Carotene, and Vitamin K, which support wound healing and a healthy immune system, and Withanolides can help combat inflammatory diseases (5). Medicinal plants have protective properties resulting from their antioxidant activities and are anti-hypercholesterolemic and anti-cardiovascular diseases (6). Flavonoids are anticoagulants that have a role in vasodilation and have antioxidant activity (7), protecting against blood clots. *E. coli* causes many diseases. Diseases, including septicemia and bacteremia, are caused by the possession of many virulence factors, which are common in humans and animals (8). *S. epidermidis* is widely present and is negative for the blood coagulation test. It is an opportunistic pathogenic bacterium that causes diseases. *P angulata*, also known as the "Ciplukan" plant, was discovered to have antimicrobial properties. Many studies have proven its ability to prevent the growth of bacteria and fungi. Essential oils extracted from various parts of the plant be effective against a range of microorganisms, including *Candida albicans, C. stellate, C. torulopsis, K. pneumoniae, B. subtilis, S aureus*, and Pseudomonas aeruginosa (9,10). Physalin B, a compound found in the plant, has been specifically linked to antimicrobial activity against Staphylococcus aureus and other bacteria. (11,12). These results support the traditional use of *Physalis angulata* in treating of pathological conditions. Consequently, the objective was to investigate the chemical composition, antioxidant capacity, and their impact on bacteria, growth, and some blood characteristics, such as their influence on the clotting factors par and part.

Materials and Methods

Plant Collection

The plant material was collected from Al Khaldiya city in Ramadi, Iraq, in June 2023. The plant was identified by the author. Fruits and leaves were dried at room temperature and stored until use.

Preparation of Plant Extract

Fruits and leaves were separated, carefully cleaned, and air-dried. They were processed using an electric grinder to a fine powder once they were totally dry. The leaves and fruits were macerated separately in a heated process to create the extract. Every component was dried in an electric oven several times. After weighing 50 grams of the powdered plant material (leaves and fruits combined) from the physalis angulate plant

150 ml of 70% concentrated ethanol was added. The powdered plant material (fruits and leaves) was cooked in a water bath at 50 - 80 degrees Celsius temperature while macerated in the solvent in a glass flask. After that, the flasks were placed in a shaking incubator. The mixture was filtered through cheesecloth several times, and filtered with gauze several times. After that, the fragrance was taken, and the precipitate was discarded and dried by placing it in Petri dishes inside the oven. Then, the skimming process was carried out to obtain the powder extract formed (13).

Diphenyl picrylhydrazy Scavenging Activity (Change from violet to yellow color)

The scavenging activity of the leaf and fruit extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to determine their free radical scavenging activity as natural antioxidants. The antioxidant activity was measured according to the method described in (14); 250 mg of the plant extract was dissolved in 50 ml of ethanol to obtain a 5 mg/ml concentration. The volume was adjusted to 10 ml with ethanol to achieve a final concentration of 0.5 mg/ml. A standard solution of ascorbic acid was prepared by dissolving 100 mg of ascorbic acid in 25 ml of distilled water, resulting in a final concentration of 10 mg/ml. This served as the stock solution from which 0.1, 0.2, and 0.3 mg/ml solutions were prepared. Sixty microliters of the standard ascorbic acid solution and the plant extract (as a control) were placed in separate tubes. Then, 3 ml of DPPH solution was added to each tube and vortexed thoroughly. The tubes were kept in the dark for 30 minutes, after which the absorbance was measured at a wavelength of 517 nm compared to the DPPH control.

The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\text{DPPH Activity (\%)} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} * 100$$

Effect of the Ethanolic Extract on Blood Coagulation

Five healthy people in the 20–40 age range had blood samples drawn from their venous blood. The samples were collected and centrifuged for 15 minutes at 2000 rpm. After separation, the extract was added to the plasma at a 1:1 ratio. To prepare these concentrations of 10, 50, 100, 150, 200, and 250 mg/ml, the stock solution, 500 mg/ml, was made by diluting distilled water; to enable the plasma and extract to combine, the samples were incubated in a rotary mixer for five minutes at 37°C. After that, they were prepared to detect intrinsic and extrinsic coagulation factors. A plasma sample was retained from each sample to serve as a reference and point of comparison (15).

PT and PTT Assays

A glass tube is filled with 100 microliters of plasma, and the obtained extract concentration (10, 50,100,150,200,250 mg/ml). It is then incubated at 37°C for five minutes. After adding 200 microliters of PT solution, the timer is set to

begin immediately. Seconds are used to measure the clotting time. 100 microliters of extract-treated plasma and 100 microliters of PTT solution at 37°C are added for the PTT test. The clotting time is expressed in seconds, and the timer is set to begin immediately. Every produced concentration of plasma treated with the leaf ethanolic extract, as well as every sample, goes through this process again.

Antibiofilm Activity of Plant Extract against Wound Bacteria

A stock solution is prepared from the powder extract powder by dissolving 5 g of it in 25 ml of dimethyl sulfoxide solution (DMSO) and placing it in the rotary mixer to mix it well. Serial concentrations of 150, 200, and 250 mg/ml were prepared (16), and tubes were prepared containing the culture medium to which concentrations of the ethanolic extract of the leaves of the P. angulate plant were added. The bacterial suspension was inoculated into a medium made from the heart and brain marrow. These tubes were incubated for 24 hours at a temperature of 37° C, and then 200 microliters of the culture were added to etch the microtiter plate with three replicates in rows. Verticality for each type of bacterial isolates under study, which is considered the control treatment, then add 200 microliters of the culture of bacteria growing in the culture medium containing the extract, then incubate at a temperature of 37°C for 24 hours.

After the end of the incubation period, the holes are washed with physiological saline water to get rid of non-adherent bacterial cells; then, they are dyed with crystal violet dye at a concentration of 1% for 5 minutes. Then, the holes are washed three times to remove the dye and left to dry at room temperature (17).

To extract the dye from the adherent bacteria, 160 microliters of glacial acetic acid were added. The adherent bacterial cells' and the dye's optical density (OD) were measured using an ELISA reader at a wavelength of 360 nm to ascertain the isolates' effectiveness in producing biofilms. This was compared to the bacterial cells treated with the plant extract to calculate the percentage inhibition of bacterial adhesion using the following equation. (18):

Percentage inhibition of biofilm (%) = 1 - OD (treated) / OD (control) * 100%

Statistical Analysis

The data were analyzed using a one-way, completely randomized design (C.R.D.). The differences between means were tested using the L.S.D. test at a significant level of 0.05. The data were analyzed using SPSS statistical software, and Excel was used to present the results (19).

Results

Chemical Composition of the Plant

Physalis angulata fruits and leaves have been subjected to a phytochemical analysis, which has revealed the presence of several secondary metabolites, including alkaloids, glycosides, flavonoids, tannins, and phenols (4). Additionally, it includes phytol, an unsaturated branched-chain terpene that is thought to be an effective sterilizing agent because most studies have shown that it may stop the growth of a variety of microorganisms, including fungus and bacteria (20), Table 2.

Antioxidant Activity

The results of the DPPH assay of the fruit and leaf extracts of *P. angulata* showed significant radical scavenging in their ability to capture free radicals. Ascorbic acid was used as a standard for comparing values. It has been mentioned by (21) that plant-derived antioxidants such as tannins, phenolic acids, flavones, flavonoids, and other compounds are considered natural scavengers of free radicals that protect living cells. The ethanolic extracts of the leaves and fruits of *P. angulata* had good ability for both assays.

The ethanol extract of leaves showed radical scavenging activity of 93% over a standard value for ascorbic acid of 88% in DPPH assays, and the ethanol extract of fruits showed radical scavenging activity of 60% over a standard value for ascorbic acid with 88% in DPPH assays as shown in (Fig. 1)

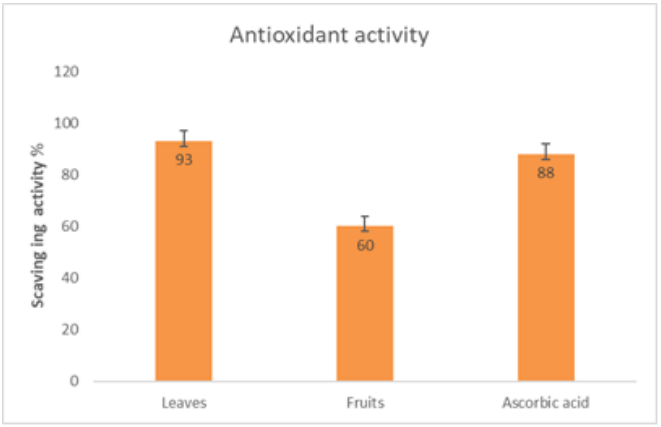


Fig. 1 Antioxidant activity of the fruit and leaf extract of the *P. angulata* plant. DPPH radical scavenging ability.

All results are presented as mean. Studies by (22) have confirmed that the antioxidant activity of the ethanolic extract can be attributed to the plant's content of phenols and flavonoids, which are known for their effectiveness as antioxidants. These compounds are among the most effective in displacing free radicals by donating hydrogen atoms through their hydroxyl groups.

Effect of the ethanolic extract of *P. angulata* leaves on blood clotting factors.

The findings demonstrated that at doses of 250, 200, and 150 mg/ml, the ethanolic extract of *Physalis angulata* leaves significantly extended both the whole and partial clotting times ($p < 0.05$). At these concentrations, the measurements varied from 138-29, 130-36, and 150-41 seconds, in that order. However, the extract shortened the clotting time to 10, 50, and 100 mg/ml doses. At these concentrations, the measurements varied from 23-19, 23-18, and 18-15 seconds, in that order. The treated samples' total clotting time (PT - Prothrombin time) test findings, which varied from 16 to 14 seconds, were noticeably longer than the control samples (Fig. 2&3 and Table 1).

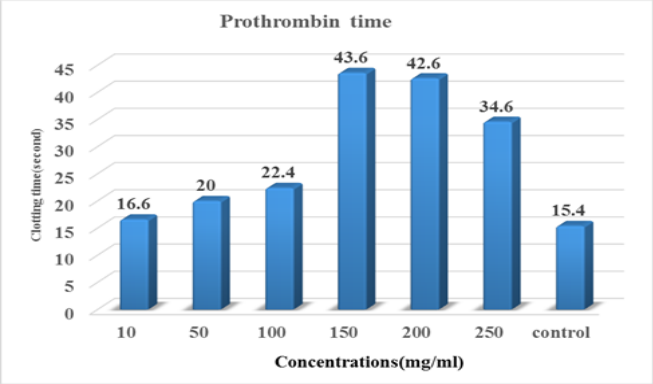


Fig 2. Effect of leaf extract on prothrombin total clotting time

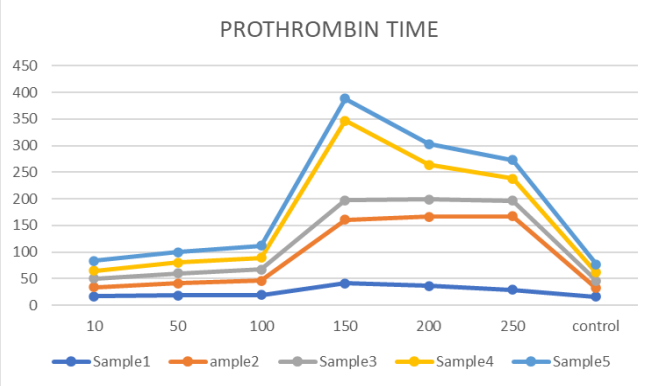


Fig 3. Effect of the ethanolic extract of the leaves on the factors of total clotting time (PT).

Table 1 : Percentages of inhibition of the formation of the life membrane of the species of wound bacteria using the ethanol extract of the leaves of the harankash plant at a concentration of 150, 200, 250) mg/mL.

Bacteria isolates	Biofilm Inhibition %	concentration of 150 mg\ml	Control
S. aureus	50.3	0.081	0.163
S. epidermidis	74.3	0.065	0.253
E. coli	61.45	0.074	0.192
P. aeruginosa	45.92	0.073	0.135
Mean	57.9925	0.07325	0.18575
SD	12.68606	0.006551	0.050513
SE	6.343032	0.003276	0.025257
Bacteria isolates	Biofilm Inhibition %	concentration of 200 mg\ml	Control
S. aureus	61.96	0.062	0.163
S. epidermidis	75.89	0.061	0.253
E. coli	63.54	0.070	0.192
P. aeruginosa	48.89	0.069	0.135
Mean	62.570	0.066	0.186
SD	11.044	0.005	0.051
SE	5.522	0.002	0.025
Bacteria isolates	Biofilm Inhibition %	concentration of 250 mg\ml	Control
S. aureus	61.96	0.062	0.163
S. epidermidis	76.28	0.06	0.253
E. coli	59.89	0.077	0.192
P. aeruginosa	56.29	0.059	0.135
Mean	63.605	0.065	0.186
SD	8.769	0.008	0.051
SE	4.384	0.004	0.025

The partial clotting time (PTT- Partial thromboplastin time) test findings indicated that the clotting time was prolonged at the high doses (150, 200, and 250 mg/ml). The values varied from (43-172), (47-195), and (56-180) seconds, in that order. In comparison to the control sample, which yielded a clotting time ranging from (40-32) seconds at a level of $P < 0.05$, the values at the doses of (10, 50, and 100) mg/ml ranged from (52-31) seconds, (100-35) seconds, and (59-31) seconds, respectively (Fig 4 & 5 and Table 2).

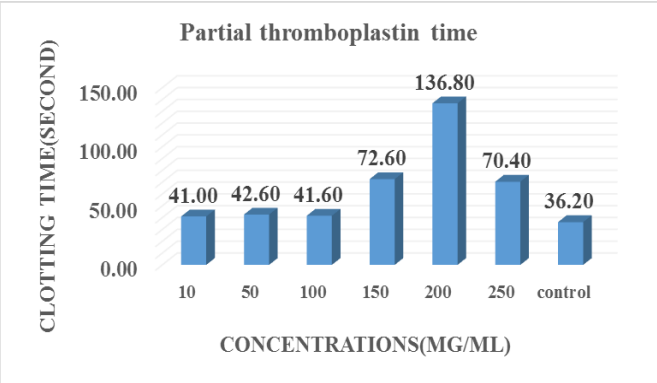


Fig 4. Average effect of leaf extract on thromboplastin partial clotting time

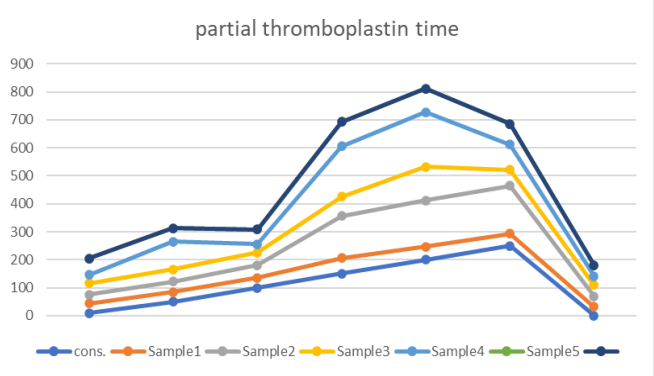


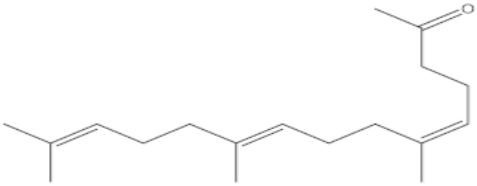
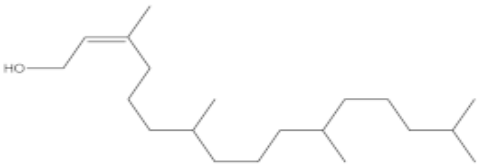
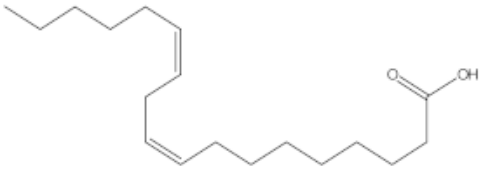
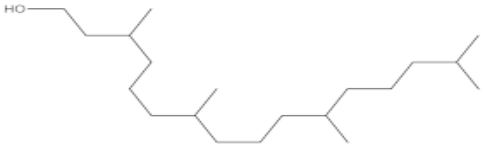
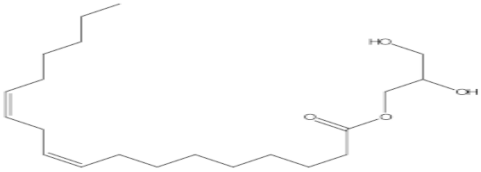
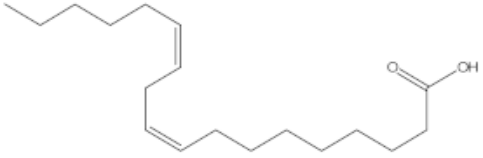
Fig 5: Effect of the ethanolic extract of the leaves on the factors of partial coagulation time (PTT).

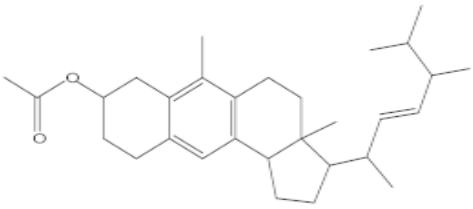
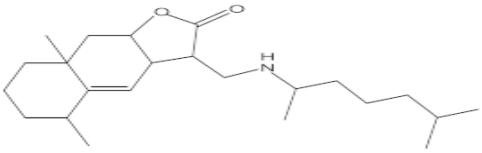
Consuming a large number of medicinal plants has been demonstrated in previous research to lower the chance of getting various illnesses in general and cardiovascular disorders in specific. They also mentioned the anticoagulant properties of alcoholic and watery plant extracts (23) and that plants are recognized for their high protein and carbohydrate content, both of which have been demonstrated to have biological effects on blood coagulation. Isolated from a variety of plant species, sugars, including pectin and hemicellulose, have demonstrated anti-thrombin and anti-thrombosis properties (24).

According to earlier research, medicinal herbs have an anticoagulant effect that helps cure illnesses involving venous and arterial thrombosis.

One such study was conducted by (25) to evaluate the anticoagulant effect of some medicinal plant extracts. The results of the study confirmed that these extracts have an anticoagulant effect.

Table 2: Phytochemical analysis

Retention Time (Min)	Molecular Formula	Molecular Mass g/mol	Peak Area (%)	Compound Name	Structure
49.849	$C_{18}H_{36}O$	268.4780	3.51	Hexahydrofarnesyl acetone	
51.072	$C_{20}H_{40}O$	296.5315	2.055	3,7,11,15-Tetramethyl-2 -hexadecen-1-ol	
58.896	$C_{18}H_{36}O_2$	284.4772	5.908	Octadecanoic acid	
59.848	$C_{18}H_{32}O_2$	280.4464	2.673	9,12-Octadecadienoic acid (Z,Z)-	
57.095	$C_{20}H_{40}O$	296.5315	10.192	Phytol	
62.796	$C_{21}H_{38}O_4$	354.5247	0.281	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	
59.848	$C_{18}H_{32}O_2$	280.4464	2.673	9,12-Octadecadienoic acid (Z,Z)-	
62.906	$C_{21}H_{40}O_3$	340.5419	0.370	Oleic acid, 3-hydroxypropyl ester	

64.095	$C_{21}H_{40}O_3$	340.5415	0.605	Glycidyl stearate	
70.654	$C_{30}H_{44}O_2$	436.6709	0.180	Anthiaergosatn-5,7,9,22-tetraen, 3-acetoxy-	
73.695	$C_{29}H_{50}O_2$	430.7075	0.716	α-Tocopherol	
58.998	$C_{23}H_{39}NO_2$	361.5627	0.747	3-[(1,5-Dimethyl-hexylamino)-methyl]-5,8a-dimethyl-3a,5,6,7,8,8a,9,9a-octahydro-3H-naphtho[2,3-b]furan-2-one	

Anti-biofilm activity of the leaf extract

The results of the study showed that the production of biofilm by the bacterial isolates (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis,* and *Staphylococcus aureus*) used in the experiment gave different readings depending on the absorption of the bacterial isolates used, as shown in (Table 3). The decrease in the absorption values of the bacterial isolates after treatment with the plant extract indicated that the extract could inhibit the adhesion of bacteria in the wells in the assay plate (26).

The results also showed that the leaf extract of *P. angulata* had an effective inhibiting biofilm formation at concentrations of (150, 200, 250) mg/ml. The percentage inhibition of biofilm formation for *S. aureus, S. epidermidis, E. coli,* and *P. aeruginosa* after treatment with a concentration of (150) mg/ml were (45.92, 61.45, 74.03, 50.3) %, respectively. However, at a concentration of (200) mg/ml, the inhibitory percentages for biofilm formation were as follows: (48.89, 63.54, 75.89, 61.96) %. The percentages at a concentration of (250) mg/ml were (56.29, 59.89, 76.28, and 61.96) % for the types of bacterial isolates, respectively.

The study showed that the effectiveness of the extract was at its best at a concentration of (250) mg/ml and that the highest rate of inhibition of biofilm formation was due to *S. epidermidis* at the three concentrations used in the experiment, as shown in Fig. 6.

According to research by (27), bacterial isolates can produce biofilms, which are dependent on the pH (ranging from 5-7.4), temperature (25-37°C), and nutrients that are present in the bacterial environment. Additionally, the study demonstrated a connection between bacteria's pathogenicity and their capacity to produce biofilms, which are the source of several diseases. Plant extracts rich in active chemical components, such as hexadecenoic acid, pentadecanoic acid, phytol, stearic, and oleic acid, are extremely suppressive against numerous bacteria, according to a study by (28) at the Indian University's Department of Biotechnology. Research by (29-32) also demonstrated the antibacterial properties of the polyphenols found in plants, *P. angulata* has a good amount of anti-inflammatory polyphenol, which is one of the natural antioxidants and has various pharmaceutical importance.

Table 3: Statistical analysis of total blood clotting factor PT

cons.	Control (mg/mL)	250 (mg/mL)	200 (mg/mL)	150 (mg/mL)	100 (mg/mL)	50 (mg/mL)	10 (mg/mL)
SD	0.894	5.683	12.818	6.189	2.966	1.871	1.140
SE	0.400	2.542	5.732	2.768	1.327	0.837	0.510
LSD 5%				7.73			
P- value				0.000**			

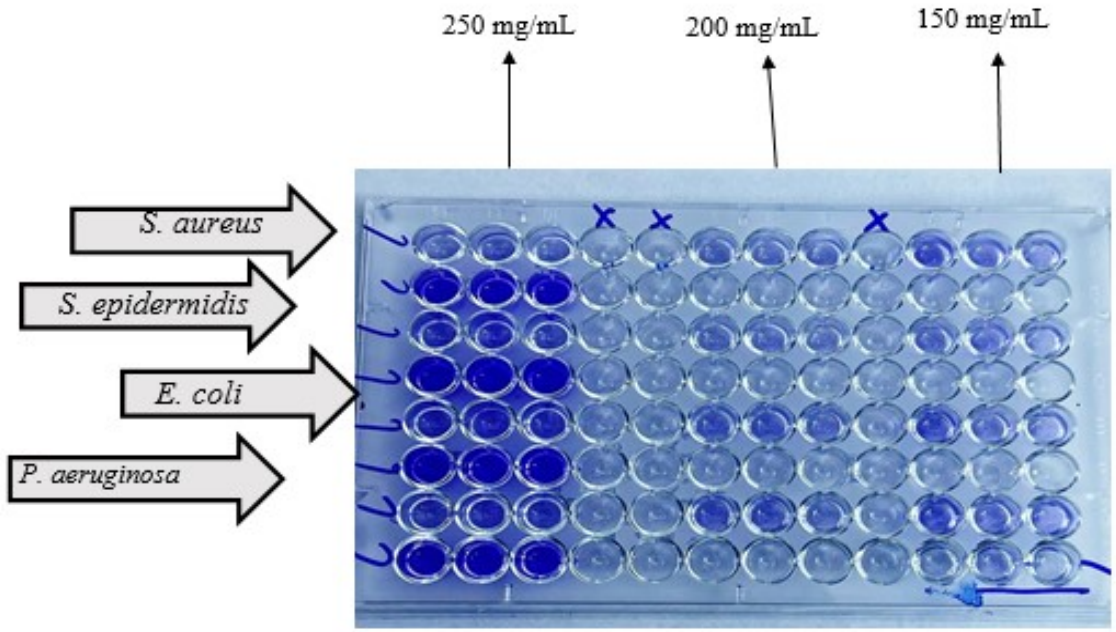


Fig 6. Biofilm formation by wound bacterial isolates using *P. angulata* leaf extract.

Table 4. Statistical analysis of partial blood coagulation factor

cons.	Control	250 (mg/mL)	200 (mg/mL)	150 (mg/mL)	100 (mg/mL)	50 (mg/mL)	10 (mg/mL)
SD	3.90	13.37	43.40	11.74	8.50	6.58	10.89
SE	1.74	5.98	19.41	5.25	3.80	2.94	4.87
LSD 5%				24.24			
P-value				0.000**			

Table 5. Inhibition of membrane biogenesis represents % represent results

Bacterial type	Extract concentrations (mg/ml)		
	150	200	250
Staph. Aureus	50.3± 0.34	61.96± 1.35	61.96± 0.31
Staph. Epidermidis	74.3± 0.29	75.89± 0.06	76.82± 0.02
E. coli	61.45± 0.24	63.54± 0.11	59.89± 0.22
P. aeruginosa	45.92± 0.54	48.89± 0.32	56.29± 0.06

Note: (mean ± SD)

Conclusion

With the leaves having a proportion of 93% and the fruits 60%, *P. angulata's* fruits and leaves demonstrated strong antioxidant activity and the capacity to displace free radicals. By employing the MTP (Micro titration plate) technique, the leaf extract may prevent the growth of biofilms of various bacterial species, including *S. epidermidis*, S. aureus, *E. coli*, and *P. aeruginosa*. It can also influence total and partial clotting factors at 10, 50, and 100 mg/mL doses. It is possible to thin blood using large dosages (150, 200, or 250 mg/mL). After more investigation, it may be utilized as a blood thinner that helps treat blood clotting disorders.

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Authors' contributions

SH performed the experiments, wrote the paper draft, and AT corrected the paper draft, MA: Supervised the extraction experiment, MJ supervised the experiment of the anti-biofilm activity, and MB Supervised the experiment Coagulation tests and analysis of data by OH. All authors agree to be accountable for all aspects of the paper. All authors read the manuscript and approved it.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest

Ethical issues: None

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