Peach (Prunus persica L. Batsch) Seed Extract as Antihyperglycemic, Antioxidant and Antibacterial in Alloxan-Induced Diabetic Rats

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Research Article

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Abstract

Peach *(Prunus persica* L. Batsch) seed valorisation is currently seen as an alternative approach to the discovery of bioactive compounds of medicinal interest. In this study, extraction, characterization and biological evaluation of polyphenol rich extracts of peach (*Prunus persica* L. Batsch) seed for antidiabetic and antioxidant potentials were carried out. Phytochemical screening showed selective enrichment of phenolic compounds. HPLC analysis revealed that chlorogenic acid (310.56 µg/mL) was the most abundant compound, followed by 5-hydroxyferulic acid (125.64 µg/mL), gentisic acid (110.21 µg/mL), vanillic acid (102.64 µg/mL) and sinapic acid (100.54 µg/mL). No signs of acute toxicity were observed during dose administration up to 120 mg/kg. The biological activity was studied in alloxan-induced diabetic rats treated with the polyphenol extract (PE) at doses of 40 and 80 mg/kg. The diabetes group was seen to demonstration a significantly higher level of fasting blood surge (FSS) (186.41 ± 5.69 mg/dL), HbA1c (11.12 ± 3.14%) and insulin concentration (13.59 ± 2.69 µIU/mL) than the control rats ($P < 0.001$). The administration of the PE significantly reduced the levels of the biomarkers with the highest dosage resulting in nearly normalized values. In addition, the PE demonstrated antioxidative via reduction of total oxidant status (TOS) (3.92 ± 0.74 to 1.92 ± 0.84 µmol/L) and increase in total antioxidant capacity (TCA), glutathione (GSH), and vitamin E concentrations ($P < 0.001$). Selective antibacterial action on Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), but no such action was noted against Gram-negative. Results from correlation analyses showed that there were high correlations between glycaemic biomarkers and biomarkers for oxidative stress. In summary, the results indicate that PE is a sustainable and affordable source of potent polyphenolic compounds with antihyperglycaemic and antioxidative properties and antibacterial activity.

Introduction

Diabetes mellitus has been found to be one of the rapidly spreading metabolic diseases in the world today and is a matter of major public health concern because of its association with chronic hyperglycemia, oxidative stress, inflammation, and organ dysfunction. According to the International Diabetes Federation, by the year 2045, more than 780 million people would have diabetes worldwide. As a result, there is a need for effective treatment approaches using natural active compounds [1]. The involvement of oxidative stress in the pathogenesis of diabetes complications is attributed to the elevated level of ROS formation, lipid peroxidation, mitochondrial abnormalities, and an impaired antioxidant response system [2].

Plants polyphenols have always attracted the attention of researchers because of their remarkable antioxidant, anti-inflammatory, antihyperglycemic, and cytoprotective activities. In fact, polyphenols influence the processes of glucose metabolism through different ways: for example, blocking the activity of enzymes like α-Glucosidase and α-Amylase, improving insulin sensitivity, preserving pancreatic β-cells, and reducing oxidative stress [3]. Recent investigations have revealed that naturally occurring phenolic acids like chlorogenic acid, vanillic acid, and sinapic acid possess strong antidiabetic effects by affecting glucose transporters and antioxidant signaling pathways [4].

Valorization of peach seed peels is one of the important approaches that have been considered within the context of circular bioeconomy recently. Food processing industries generate significant amounts of waste products in the form of peels, kernels, pomace, and husk. These products are discarded, although they contain many functional phytochemicals. It is possible to recover functional components of agriculture-based wastes while reducing pollution at the same time [5].

Peaches (*Prunus persica* L. Batsch), among the stone fruits, are consumed extensively throughout the world. These fruits are rich in various phytochemicals that can provide health benefits to consumers. Most of the earlier research has concentrated on the extraction of phytochemicals from peach flesh and kernels, while there has not been much work done on the extraction of valuable polyphenols from peach seed peels [6]. It is worth mentioning here that no earlier study has addressed the extraction of polyphenol rich fractions from peach seed peel with an eco-friendly method [7].

Thus, the current study was designed to: (i) develop a green method of extracting polyphenolic (PE) compounds from peach seed peel, (ii) analyze the phytochemicals obtained by using HPLC technique, (iii) assess the anti-hyperglycemic, anti-oxidant and antibacterial effects of the PE on alloxan-induced diabetic rats, (iv) examine the histopathological changes occurring in the pancreas, and (v) elucidate the molecular interaction of phenolic compounds with diabetic enzymes using molecular docking analysis.

This study sought to develop a sustainable approach of producing *Prunus persica* seed peels polyphenols-rich extract (PE) and investigate its effectiveness in treating hyperglycemia and oxidative stress conditions and antibacterial activity.

Materials and Methods

1. Plant Material Collection

Peach seed peels (*Prunus persica* L. Batsch) were collected in Feb 2026 from local agricultural markets in Thi-Qar province, Iraq. The samples were cleaned under distilled water to get rid of any form of contamination and left to dry in the shade at room temperature. These dried samples were pulverized through an electric mill before storing them in tight boxes before extraction.

2. Extraction of Polyphenol-Rich Fraction

Approximately 0.5 kg of powdered peach seed peels were extracted using an eco-friendly solvent extraction system. The material was washed twice with aqueous ethanol at 65 °C followed by treatment with 2% acetic acid solution. The extraction process was conducted under reflux conditions in a thermostatically controlled water bath. The resulting suspension was filtered using a Buchner funnel under vacuum pressure. Subsequently, n-propanol and sodium chloride saturation were applied to improve selective recovery of polyphenolic compounds. The extract was concentrated using a rotary evaporator and stored at 4 °C for further analysis **[8]**. As shown in **Figure 1**.

3. Phytochemical Screening

Qualitative phytochemical tests were conducted for the detection of phenolics, flavonoids, alkaloids, tannins, glycosides, saponins, terpenoids, and carbohydrates according to standard phytochemical protocols **[9]**.

4. HPLC Characterization

C_{18} column has been used in HPLC technique, while the composition of mobile phase included methanol, water, and acetic acid in 40:60 v/v ratios. With a flow rate of nearly 1.4 mL/min, the detection was done at 264 nm wavelength. Phenolic compounds have been discovered using comparisons to conventional substances.

5. Experimental Animals

Female rats (*Rattus norvegicus*), weighing 180-220 g, were taken from the animal house ability in the animal house of Department of Biochemistry, College of Medicine, Thi-Qar University, Al-Nasiriya, Iraq and were kept under laboratory circumstances (25 ± 2°C, 12 h day/night rhythm). Food and water were freely available for the rats during the experiment.

6. Acute Toxicity Study

Acute oral toxicity was evaluated according to OECD guidelines. Animals were divided into four groups and orally administered different concentrations of peach polyphenol extract (PE) (40, 80, and 120 mg/kg). Behavioral changes, mortality, and signs of toxicity were monitored for 72 h.

7. Induction of Experimental Diabetes

Following overnight starvation, 32 female rats were injected with fresh alloxan solution intraperitoneally, at a rate of 150 mg/kg body weight, which was dissolved in a cold 0.1 M citrate buffer solution with a pH of 4.5 to create diabetes mellitus. The next day, the animals were fed when a 5% glucose solution was provided orally to avoid hypoglycemia resulting from injury to the beta cells of the pancreas. After 72 hours following the introduction of alloxan, the glucose levels in the blood were measured. Glucose estimation was carried out using the Glucochek glucometer from Aspen Diagnostic (P) Ltd., which was manufactured using the GOD-POD method **[10]**.

8. Experimental Design

Animals were randomly allocated into four experimental groups:

1. Group A: The control group was given 0.4 ml of ion-free water (distilled water).

2. Group B: Received 40 mg/kg of PE.

3. Group C: Received 80 mg/kg of PE.

4. Group D: Received 120 mg/kg of PE.

9. Biochemical Analysis

Blood sugar and antioxidant levels were measured using laboratory animals other than those used in the experiment to detect the toxicity of PE. The animals were divided into four groups, each containing eight female rats, as follows:

1. Group A: The control group was given only 0.4 ml of ion-free water (distilled water).

2. Group B: Eight female rats with diabetes as a pathological control group.

3. Group C: Eight female rats with diabetes were administered 40 mg/kg of PE during the study period.

4. Group D: Eight female rats with diabetes were administered 80 mg/kg of PE during the study period.

Fasting blood sugar (FBS), HbA1c, insulin concentration, TOS, TAC, vitamin E and GSH were measured. **Figure 2** illustrates the current study design.

10. Investigation of the Antibacterial Activity

1. Diffusion disc method

The antibacterial effects of PE on two Gram-positive bacteria, namely *B.subtilis* and *S.saprophyticus*, and two Gram-negative bacteria, namely *E.coli* and *P.aeruginosa*, were evaluated through a study conducted using the bacteria in triplicate. 120 mg/mL of PE was placed on sterile blank discs.

Bacterial inoculum was prepared at an optical density OD_{620}= 0.01 and then evenly distributed on the surface of a Mueller–Hinton agar plate using sterile swabs. Next, the prepared discs were placed onto the inoculum-covered agar plate at standardized distances using sterile forceps. All plates were incubated aerobically at 37°C for 24 hours. Antimicrobial activity was detected by the diameter of the inhibition zones (mm) measured around the disks. Meropenem discs were used as positive controls of antibacterial activity, while distilled water-impregnated discs were used as negative controls.

2. MIC and MBC method

The MIC and MBC of the polyphenolic extract (PE) were obtained by employing the broth microdilution method. Six serial concentrations of the PE (0, 25, 50, 75, 100, and 125 mg/mL) were utilized, whereas chloramphenicol was taken as a positive control. Suspensions of bacteria standardized with OD_{620} = 0.01 were inoculated into microtiter plates that contained various concentrations of the PE. These plates were incubated aerobically at 37°C for 24 h. After incubation, the MIC was obtained as the lowest concentration of the PE at which there is no growth of the bacteria. In determining the MBC, suspensions from wells with no growth of bacteria were transferred to nutrient agar plates without the presence of PE and incubated again at 37°C for 24 h. MBC was then taken as the lowest concentration at which there is no growth of bacteria in agar plates.

11. Permissions for collection

The project was approved by the Ethics and Animal Research Committee at the Department of Biochemistry, College of Medicine, Thi-Qar University, Al-Nasiriya, Iraq (**IRB: 02/2026**). Anesthesia was induced using ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Euthanasia was done when deep anesthesia was attained through cervical dislocation in line with institutional standards for the Declaration of Helsinki regarding animal testing.

12. Statistical Analysis

The values were represented as mean±standard deviation (SD) for data analysis. For statistical analysis of data, the methods adopted included one-way analysis of variance and Tukey's multiple comparison tests. To measure the correlation between the two variables, we used the Pearson correlation coefficient (r). A significance level of $p<0.01$ was used in this study.

Results

Phytochemical Characterization of Peach Seed Peel Extract

The results in **Table** 1 indicate the presence of polyphenolic compounds in the PE, where the results of the polyphenolic compound detection test were positive and the test was negative during the detection of compounds (alkaloids -flavonoids - carbohydrates - glycosides - tannins - saponins - terpenes), indicating that the PE polyphenol extract contains only pure polyphenol compounds.

HPLC analysis showed five polyphenolic compounds in the polyphenol extract PE (gentisic acid, chlorogenic acid, sinapic acid, 5-hydroxyferulic acid, and vanillic acid). **Table** 2 shows the retention time of polyphenol compounds. The above results show that only polyphenol compounds were extracted and identified.

Acute Toxicity Evaluation

The results showed that after administering doses (40, 80 and 120 mg/kg) of polyphenol extract PE, no abnormal symptoms appeared and no deaths were observed in the rats 72 hours after dosing, as shown in **Table** 3.

Antihyperglycemic Activity

As shown in **Table** 4, there was a significant increase in FBS concentration in diabetic rats (B) when compared to healthy controls (A) ($P < 0.01$). There was a significant decrease in the hyperglycaemic state due to treatment with the polyphenols in groups C and D, especially in group D. Similarly; there was an increase in HbA1c and insulin concentration in diabetic rats (B). At the same time, there was a significant improvement in these values after treatment with the extract, especially in group D ($P < 0.01$).

Antioxidant Activity

As seen in **Table** 5, the findings revealed an increase in TOS in the diabetic group (B) as compared to that of the control group (A) ($P < 0.01$). The administration of the PE led to a decrease in TOS levels in groups C and D, with a maximum reduction in group D. However, there was a marked reduction in TAC in the diabetic rats when compared with the controls. There was a significant rise in TAC levels following administration of the extract, especially group D ($P < 0.01$).

The results presented in **Table** 5 showed a significant reduction in vitamin E and glutathione (GSH) levels in diabetic rats (group B) compared with the control group (A) ($P < 0.01$). In contrast, treatment with the polyphenol extract significantly elevated both antioxidant biomarkers in groups C and D relative to the diabetic group, with the highest improvement and restoration toward normal values observed in group D.

The correlation between the study biomarkers

From the findings in **Table** 6, there was a correlation analysis which showed strong positive correlations between the FBS and HbA1c and TOS. This implied that high blood glucose levels were closely associated with high oxidative stress. On the other hand, there were significant negative correlations between the glycemic parameters and TAC, vitamin E and GSH, showing that hyperglycemia leads to loss of the body's antioxidant defenses. Moreover, there were strong positive correlations between the TAC and vitamin E and GSH levels. This indicated the role played by antioxidants in protection against oxidative damage in diabetes.

Antibacterial Activity

1. Diffusion disc results

As shown in **Table** 7, the activity of the PE was selective against the Gram-positive strains, resulting in inhibition zones of 15.22 ± 0.19 mm for *S. aureus* and 12.45 ± 0.17 mm for *B. subtilis*. Conversely, there were no inhibition zones against the Gram-negative strains *E. coli* and *P. aeruginosa*.

2. MIC and MBC results

The antimicrobial action of **Table** 8 was effective against Gram-positive bacteria, which had an MIC value of 125 and 75 mg/mL and an MBC value of 200 and 120 mg/mL against *S. aureus* and *B. subtilis*, respectively. The comparative evaluation indicated no significant difference between PE and chloramphenicol against the studied Gram-positive bacteria.

Discussion

In this current study, it was observed that the PE obtained from peach (*Prunus persica*) seed peel have significant antihyperglycemic and antioxidant properties in alloxan induced diabetic rats.

Several phenolic acids, such as chlorogenic acid, gentisic acid, sinapic acid, vanillic acid, and 5-hydroxyferulic acid, were detected using HPLC. Of these compounds, chlorogenic acid is found to be

present in the largest amount, which might be able to account for their biological functions **[11]**. It is well documented that chlorogenic acid can improve glucose metabolism by inhibiting glucose uptake, increasing insulin sensitivity, and regulating hepatic glucose production. Additionally, chlorogenic acid has excellent antioxidant properties by scavenging ROS and activating endogenous antioxidant systems **[12]**.

The substantial decrease in FBS and HbA1c concentrations in the treated groups demonstrates the ability of the PE to manage chronic hyperglycemia. Alloxan-induced diabetes involves selective death of pancreatic β-cells via oxidative stress-induced mechanisms **[13]**. The increase in the level of insulin in treated animals implies that the PE possesses protective properties on pancreatic β-cells or improves the secretion and sensitivity of insulin **[14]**. Antihyperglycemic activity has been previously demonstrated for phenolic-rich extracts prepared from agricultural residues **[15]**.

Oxidative stress plays an important role in the development and progression of diabetes mellitus and its complications. The animals that had diabetes in the current study were found to have increased levels of TOS along with decreased levels of antioxidants like TAC, vitamin E, and GSH **[16]**. Administration of peach seed peel polyphenols was able to restore the levels of antioxidants, suggesting strong free radical scavenging and cytoprotective properties. Phenolic acids like sinapic acid and vanillic acid are known to induce antioxidant signaling pathways like Nrf2/ARE **[17]**.

Significantly, the extract showed no signs of acute toxicity at all the concentrations used, further emphasizing its safety aspect and the possibility of its use as an alternative therapy. The absence of any signs of mortality and behavioral changes is in agreement with earlier studies on low toxicity of plant polyphenols **[18]**.

The correlation matrix revealed strong correlations ($p<0.01$) between glucose indices and oxidative stress indices. This is an indicator of the strong relationship between fluctuating glucose level, glucose homeostasis and antidiabetic drugs. FBS, HbA1c and insulin were strongly positively correlated with TOS. This indicates that high levels of blood glucose are associated with oxidative stress and free radical formation **[19]**. However, these strong indicators are strongly correlated with TAC, vitamin E and GSH indices, which indicate their role in antioxidative defense and impact on glucose and insulin secretion **[20]**. Strong negative correlations were also revealed between TOS and TAC, vitamin E and GSH indices, while TAC was positively correlated with vitamin E and GSH, reflecting the complementary role of non-enzymatic antioxidants in oxidative stress reduction. These findings support the pathogenetic theory that chronic hyperglycemia leads to ROS production and impairment of oxidative defense mechanism of oxidation, which could be one of the mechanisms of metabolism and vascular stage of diabetes **[21-23]**.

As seen from the results obtained, the PE showed selective activity against Gram-positive bacteria, namely *S. aureus* and *B. subtilis*, but not Gram-negative bacteria. This discrepancy in bacterial susceptibility can be explained by different structures of the bacterial wall since the presence of the outer lipopolysaccharides layer makes Gram-negative bacteria resistant to phenolic compounds due to

its ability to block their access into bacterial cells. Most likely, the selective antibacterial activity of the extract resulted from the high content of phenolic acids contained therein, such as chlorogenic, vanillic, and sinapic acids, all known for their capability to alter the structure of the bacterial cell wall, inhibit the activity of bacteria enzymes, and cause oxidation damage **[24]**. In addition, quite low MIC and MBC for Gram-positive bacteria indicate the ability of the extract to exert potent bacteriostatic and bactericidal effects on bacteria similar to those provided by common antibiotic preparations **[25]**.

Conclusion

The current study has shown the sustainability of valorization of Prunus persica seed peel as a valuable natural resource of biologically active polyphenolic compounds with pronounced antihyperglycemic, antioxidant and antibacterial. Phenolic-rich extracts obtained via environmentally friendly technology contained chlorogenic acid, gentisic acid, sinapic acid, vanillic acid, and 5-hydroxyferulic acid, which were responsible for the therapeutic effect. Oral administration of PE caused normalization of glycemia in alloxan-induced diabetic rats via FBS and HbA1c reduction and insulin level regulation. Moreover, PE ameliorated oxidative stress through lowering of TOS and upregulation of endogenous antioxidant defence parameters such as TCA, GSH, and vitamin E levels. The lack of toxicity also confirms the safety and applicability of PE in the field of functional ingredients with biological activity. Thus, valorization of peach seed peels is important not only from a biomedical but also from ecological perspective within the sustainable circular bioeconomy concept. The results suggest that fruit industry wastes have high potential as sources of valuable bioactive compounds for development of nutraceutical and pharmaceutical products for diabetes treatment and protection against oxidative stress damage.

Declarations

Acknowledgements

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Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethical considerations

The project was approved by the Ethics and Animal Research Committee at the Department of Biochemistry, College of Medicine, Thi-Qar University, Al-Nasiriya, Iraq **(IRB: 02/2026)**. The guidelines adhered to the ethical principles outlined in the Declaration of Helsinki in relation to animal testing.

Authors' contributions Statement

ABR designed and conducted the research. WAK performed the data management and statistical analyses; ABR reviewed and edited the manuscript for important intellectual content. WAK wrote the manuscript.

Funding statement

No financial assistance was provided for the current research.

Declaration of Conflict of Interest

The authors have nothing to disclose concerning potential conflicts of interest.

Consent to publish

Not applicable.

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Tables

Tables 1 to 8 are available in the supplementary files section

Figures

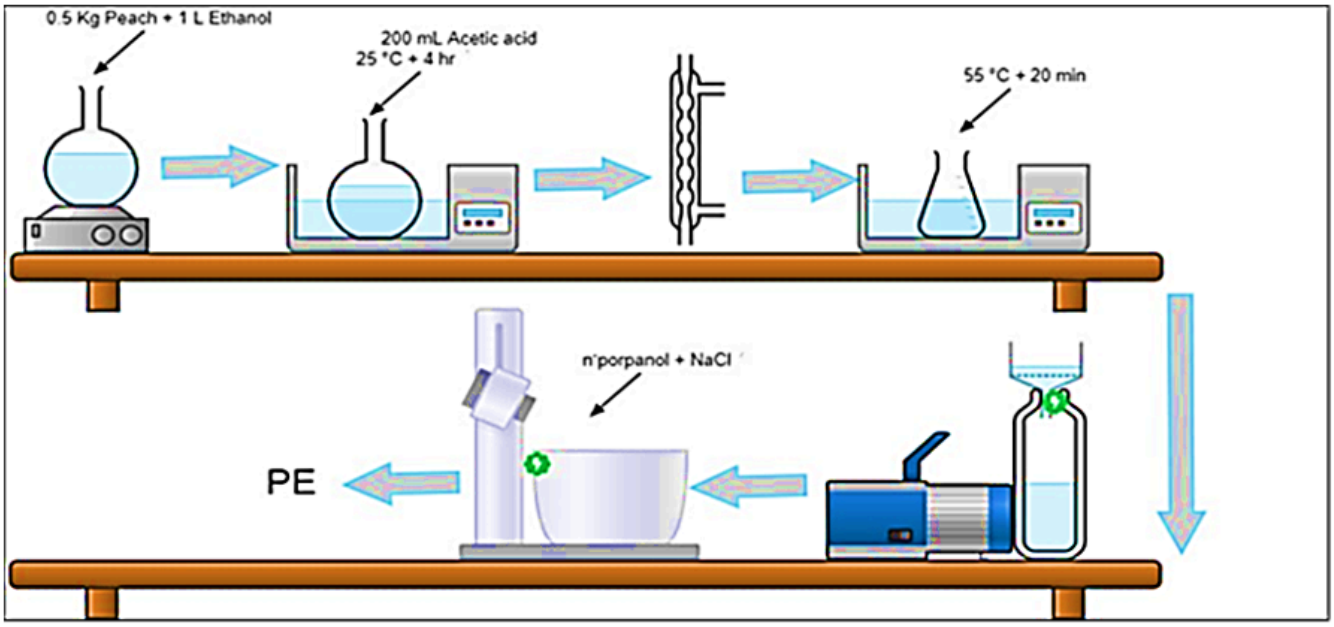

Figure 1

Preparation of PE.

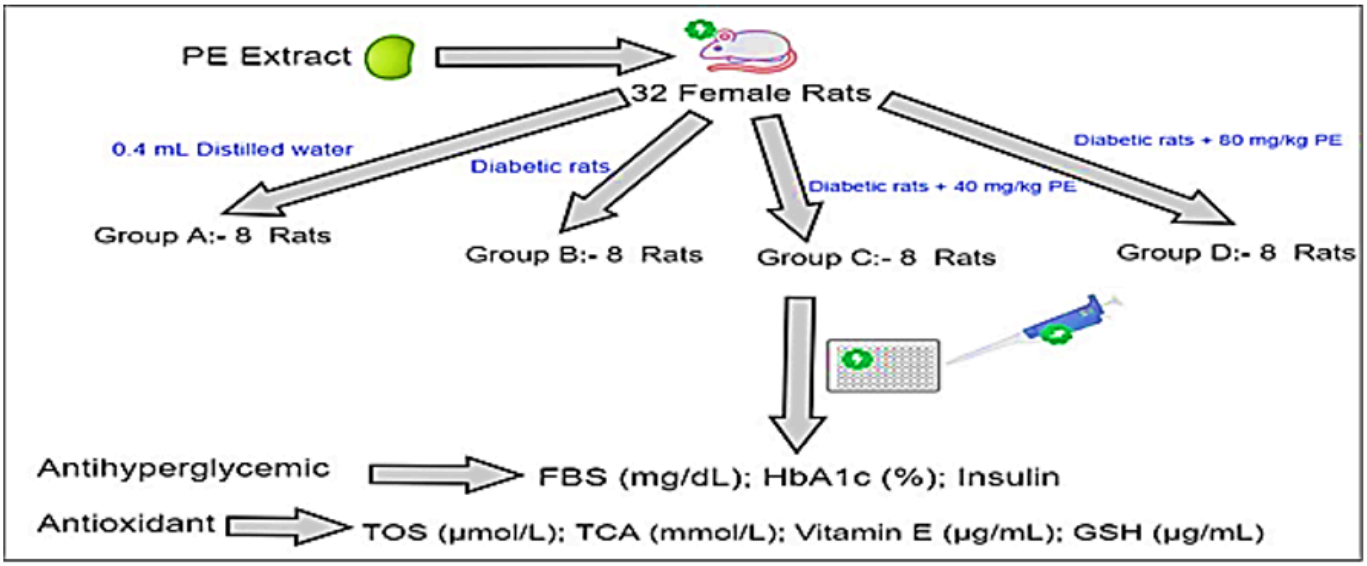

Figure 2

The study protocol.

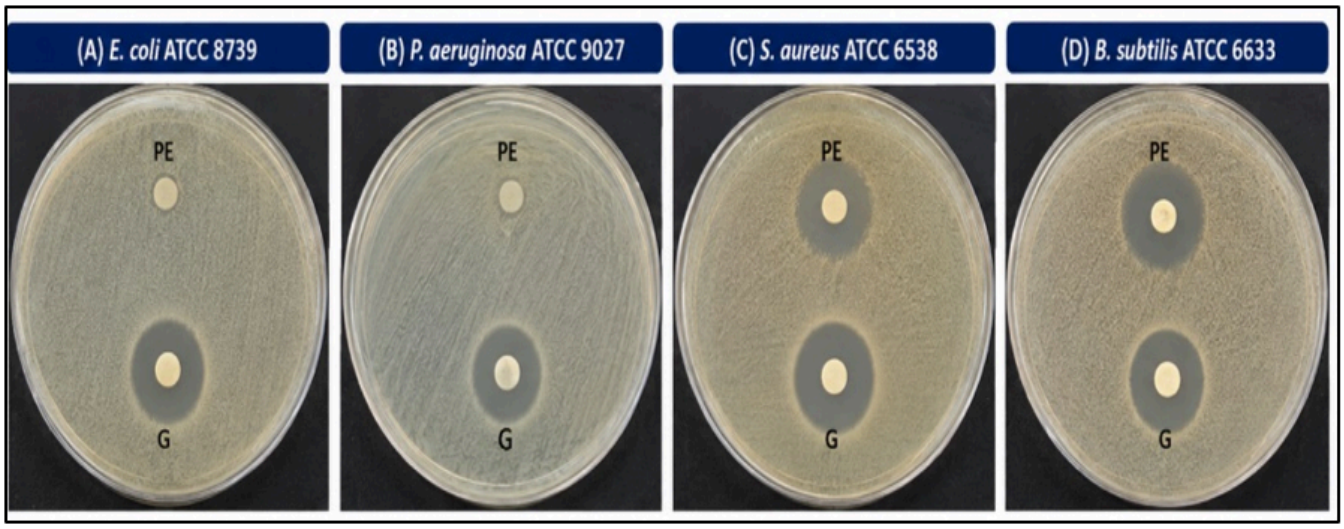

Figure 3

Antibacterial Effectiveness of PE on Gram-positive Bacteria and Gram-negative Bacteria.

Supplementary Files

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