Palestinian Medical and Pharmaceutical Journal (PMPJ). 2024; 9(2): 137-144

Antioxidative and Antidiabetic Investigations of the *aqueous extract* of *Pelargonium graveolens* Grown in Palestine

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Received: (1/2/2023), Accepted: (11/7/2023), Published: (1/6/2024)

ABSTRACT

Plants have been used for many healing aims since outdated times, and to this day, many essential pharmaceutical dosage forms originated from herbal products. The utilization of herbal products recognized by therapeutic agents in modern healthcare systems has increased over the past two decades. The present biological screening study employed reference methods to estimate Pelargonium graveolens four extracts from the Nablus governorate in Palestine. The outcomes affirmed that the four extracts of the *P. graveolens* plant have potent antioxidant activity compared with Trolox. The aqueous extract has very potent activity, followed by acetone extract in comparison with Trolox (((s)-(-)-6 hydroxy -2, 5, 7, 8-tetramethychroman- 2-carboxylic acid) with half maximal inhibitory concentration (IC_{50}) doses of 2.29±0.03, 6.48±0.71and 2.88±0.15 µg/ml, respectively. Besides, all the current plant four extracts have α-amylase inhibitory activity in a dose-dependent manner. The results showed that the aqueous extract has potent anti-α-amylase inhibitory activity with an IC_{50} value of 20.89±0.98 µg/ml, followed by acetone extract with an IC_{50} value of 29.51±0.74 µg/ml, compared with the positive control Acarbose which has an IC_{50} value of 28.84±1.28 µg/ml. Based on the results of this study, herbal formulations with *P. graveolens* aqueous extract have much potential for treating disorders caused by oxidative stress and diabetes.

Keywords: *Pelargonium graveolens*; Four extracts; Oxidative stress; Diabetes.

INTRODUCTION

From ancient times until now, several communities have relied on medicinal plants to suit their healthcare needs (1). Regarding health management, the World Health Organization (WHO) reports that over 80% of people worldwide use herbal drugs, such as plant extracts or their active ingredients (2). Herbal remedies have been employed as an alternative to conventional treatment in poor nations due to the high healthcare expense and lack of adequate healthcare infrastructure (3). However, plant-based pharmaceuticals play a significant role in the healthcare systems of developed nations (4).

Herbal medicine is an essential part of alternative medicine. Different parts of the plant are used in herbal medicine, including the leaves, stems, fruits, seeds, flowers, and roots (5). Chemical substances found naturally in plants are responsible for the medicinal properties of these plants. When these chemicals are introduced into the body, they may cause various physiological and pharmacological effects. However, herbal medicine can be used to prevent and treat different illnesses (6).

High blood sugar levels are the typical symptom of diabetes, a chronic metabolic disorder. Sugar is taken up by living cells from the circulation and stored or utilized as energy due to insulin (7). In the case of diabetes, the human body is either not producing enough insulin or cannot utilize the insulin it produces adequately. Diabetes may cause damage to the kidneys, eyes, nerves, and other organs if it is not treated (8).

An imbalance between free radicals and antioxidants in the human body causes oxidative stress. Oxygen-containing molecules with an uneven number of electrons are known as free radicals (9, 10). Because of their odd number, they can efficiently react with other molecules and respond quickly, which may cause significant chain chemical reactions in the human body. Oxidation is the name for these reactions (11).

Free radicals can cause damage to fatty tissue, DNA, and proteins in the human body when more free radicals are present than antioxidants (12). Because proteins, lipids, and DNA make up so much of the human body, their damage can lead to various diseases over time (13). Accelerated oxidative stress causes neurodegenerative diseases. Some examples are inflammation, diabetes, atherosclerosis, high blood pressure, Parkinson's, and Alzheimer's (14).

Pelargonium graveolens (*Geranium rosa),* commonly known as Scented Geranium or Rose Geranium, belongs to the Geraniaceae family. It is 1–2 feet tall with opposite leaves, long petioles, and deeply incised lobes. The leaves are very fragrant and covered with a soft pubescence. The flowers are red or pink. The plant originates in Africa and is now cultivated in many temperate countries (15, 16).

In recent years, *Pelargonium* species have been used to treat intestinal illness, wounds, and respiratory problems. Respiratory and cold medications usually contain *Pelargonium* oil and have been used in Europe and the United States (17). In several studies, *Pelargonium* oil was used to help with hormonal balance, liver and kidney dysfunction, and to remove toxins from the liver that may affect the body's overall health. Besides, it has other advantages, like supporting the digestive and nervous systems (18). *In conventional medicine, P. graveolens has been used* to relieve hemorrhoids and in the worldwide perfumery, cosmetic, and aromatherapy industries (19).

Therefore, the present study pointed to investigating *P. graveolens* four extracts with various portions of polarities for their antioxidant and α-amylase inhibitory activities.

MATERIAL AND METHODS

Plant material

The aerial parts of *P. graveolens* were collected in July 2021 from the Nablus governorate in Palestine. Afterward, the sample was botanically characterized at the An-Najah National University (ANNU) Pharmacognosy Laboratory. The dried plant is kept under the voucher specimen code (Pharm-PCT-2779). The collected parts were dried in the shade at room temperature until full dryness and then ground coarsely for further use (20).

Instruments

UV-Visible Spectrophotometer (Jenway 7135, England), filter papers (Whitman no.1, USA), shaker device (Memmert shaking incubator, Germany), rotary evaporator (Heidolph VV2000 Heidolph OB2000, Germany), grinder (Moulinex model, Uno, China), balance (Rad wag, AS 220/c/2, Poland), and freeze dryer (Mill rock technology BT85, China).

Chemicals

Methanol, n-hexane, and acetone were purchased from LobaChemie (India). (DPPH) 2, 2-Diphenyl-1-picrylhydrazyl, and Acarbose were obtained from Sigma-Aldrich (Germany). DMSO (Dimethyl sulfoxide) was brought from Riedeldehan (Germany). In addition, Trolox ((s)-(-)-6 hydroxy -2, 5, 7, 8-tetramethychroman- 2-carboxylic acid) was obtained from Sigma-Aldrich (Denmark). α-Amylase (Sigma, Mumbai, India), DNSA (3,5-dinitrosalicylic acid) reagent (Sigma, LA, USA), and Acarbose (Sigma, St. Louis, USA).

Extraction method

The pulverized *P. graveolens* plant material was extracted thoroughly using four solvent fractionation methods: methanol, water, acetone, and hexane. In a nutshell, 200 g of powdered seeds were put in an Erlenmeyer flask, extracted individually with 2 L of each solvent, and shaken for 72 h at 25 °C, and the power was adjusted at 400 rpm. A suction filtering technique was used to filter each solvent. After that, the organic extracts were maintained in an oven at 30 °C for full evaporation of the solvents, whereas the aqueous fraction was dried for 48 h utilizing a Millrock Technology lyophilizer. For subsequent usage, each portion was kept in the refrigerator at a temperature of 6 °C (21).

Antioxidant capacity assay

The *P. graveolens* extracts solution was diluted to maintain concentrations of 0, 2, 3, 5, 7, 10, 20, 30, 40, 50, and 80 µg/ml using methanol as solvent. Each test tube contained 1 ml of each concentration and was labeled properly. One ml of methanol and one ml of 0.002% methanol DPPH solution were added to each test tube to prepare 3 ml as the final volume inside each test tube (caution: the

preparation steps should be done with minimum light exposure because DPPH is light sensitive). The samples were incubated for 30 min in a dark place, and the spectrophotometer device determined their optical densities at a wavelength of 517 nm. The same procedures were repeated for the positive control drug, Trolox. The equation used in this analytical study to calculate the inhibition percentage is shown below:

% DPPH inhibition = (AB−AE)/AB×100%

AB is the recorded absorbance of the blank solution; AE is the recorded absorbance of the *P. graveolens* sample solution.

The plant extract's antioxidant half-maximal inhibitory concentration (IC_{50}) was calculated utilizing BioDataFit edition 1.02 (22).

α-Amylase inhibition assay

This method was performed by utilizing the assay modified by McCue and Shetty (23). The following dilutions were prepared: 10, 50, 70, 100, and 500 µg/ml by dissolved plant extract in little milliliters of 10% DMSO and then another dissolved in buffer (0.02 M of Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, at pH 6.9) to give concentrations of 1000 µg/ml. After that, two units/ml of porcine pancreatic α-amylase enzyme solution was freshly prepared in 10% DMSO. A working solution was prepared by mixing 0.2 ml of enzyme solution with 0.2 ml of each hydrophilic extract and then incubated for 10 min at 30 °C. Following the incubation period, 0.2 ml of a freshly prepared 1% aqueous starch solution was added to each working solution. After that, the final solution was incubated for at least 3 min. A 0.2 ml dinitrosalicylic acid (DNSA), a yellow color reagent, was added to stop the reaction. The next step was diluting each working solution with 5 ml of distilled water and then boiling for 10 min in a water bath at 90 °C. The mixture was then cooled to room temperature, and the absorbance was taken at 540 nm. The blank was prepared following the steps above, but the plant extract was replaced with 0.2 ml of the previously described buffer. Acarbose was used as the standard reference, following the same steps used for plant extract.

The following equation was used to calculate the α-amylase inhibitory activity

% α-amylase inhibitory activity = (AB-AE)/ AB*100%

AB: is the absorbance of blank; AE: is the absorbance of *P. graveolens* extracts

Statistical analysis

All the experiments were established in triplicates. The results are given as mean standard deviation (±SD). The results were considered significant when all the obtained p-values were less than 0.005.

RESULTS AND DISCUSSION

Plants have been used for many healing aims since outdated times, and to this day, many essential pharmaceutical dosage forms originated from herbal products. The utilization of herbal products recognized by therapeutic agents in modern health care systems has increased over the past two decades (24, 25).

Antioxidant activity

The antioxidant activity of *P. graveolens* in four extracts from the Nablus governorate in Palestine was screened, and the free radical-scavenging activity was determined by the DPPH assay, which was used as an *in vitro* approach. IC_{50} values assessed the examined samples' ability to inhibit DPPH. They identified the number of antioxidants required to inhibit the radical (DPPH) concentration by 50% and inversely linked it to their antioxidant activities. The assay revealed that the four extracts of the *P. graveolens* plant have significant potent antioxidant activity compared with Trolox. Briefly, the aqueous extract has very potent activity, followed by acetone extract in comparison with Trolox with IC_{50} doses of 2.29±0.03, 6.48±0.71, and 2.88±0.15 µg/ml, respectively, as shown in Table 1 and Fig. 1.

Table (1): Antioxidant activity IC_{50} of *P. graveolens* four extracts and Trolox.

Extracts	Antioxidant activity IC_{50} (µg/ml), ±SD
Hexane	8.511±0.97
Acetone	6.48±0.71
Ethanol	37.15±1.11
Aqueous	2.29±0.03
Trolox	2.88±0.15

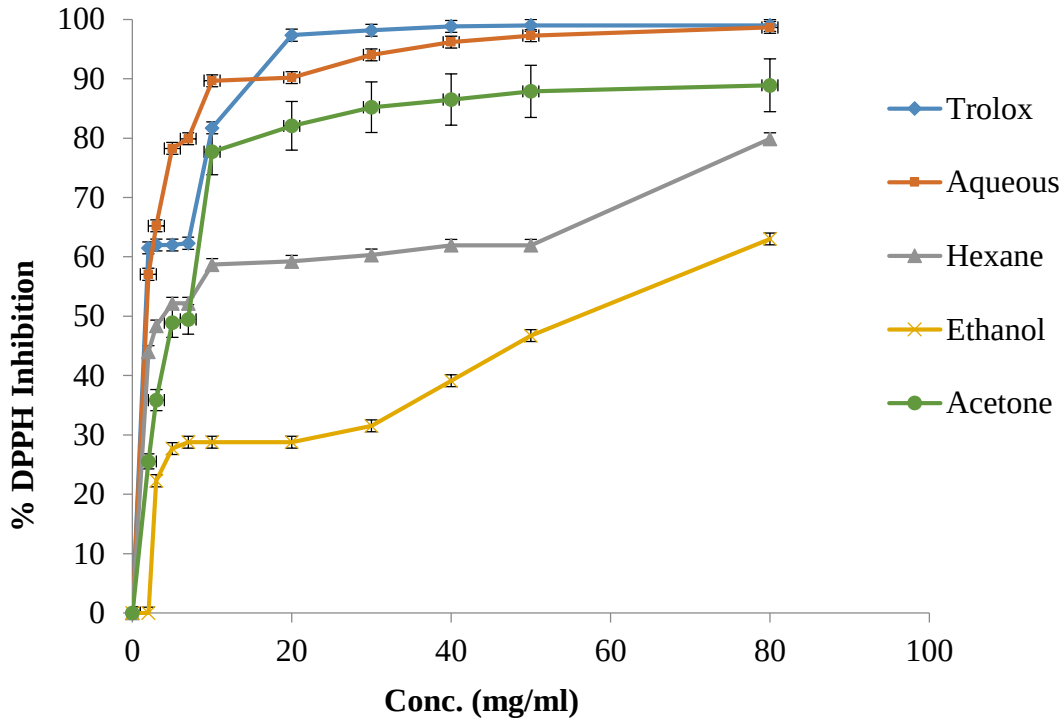

Figure (1): The percentages of DPPH inhibitory activity induced by *P. graveolens* four extracts and Trolox. The experiment was repeated three times, and the results were reported as mean standard deviation (±SD). The obtained p-values were less than 0.005.

α-Amylase inhibition activity

Amylase is an enzyme involved in the digestion of carbohydrates and has many types; one of them is α-amylase produced by the pancreas and salivary glands. One of the ways suggested in treating type II DM is to reduce the absorption of glucose through the intestine and this is achieved via drugs act by inhibiting metabolic enzymes involved in the digestion of complex carbohydrates to glucose, which include α-amylase. This shows good results in decreasing the glucose level in the blood and in the maintenance of the disease; many plants show the different inhibitory effects of the enzyme, which could be a safe alternative and a possible source of new drugs (26).

The α-amylase inhibitory activity by *P. graveolens* four extracts was compared to the positive control Acarbose. Acarbose is a medication used to treat type 2 diabetes. It works by inhibiting the enzymes that break down carbohydrates in the small intestine, which helps slow glucose absorption into the bloodstream. This can help to regulate blood sugar levels and improve glycemic control (27). Like any medication, Acarbose can cause side effects. Some common side effects include

bloating, gas, and diarrhea, which may be caused by the drug's effects on carbohydrate digestion in the small intestine (28). Rare but more serious side effects may include liver problems, severe stomach or abdominal pain, and a decrease in blood sugar levels. It is important to discuss any potential side effects or concerns with a healthcare provider before starting Acarbose (29).

All the current plant four extracts have α-amylase inhibitory activity in a dose-dependent manner. However, the results showed that the aqueous extract has potent anti-α-amylase inhibitory activity with an IC_{50} value of 20.89±0.98 µg/ml, followed by acetone extract with IC_{50} values of 29.51±0.74 µg/ml, compared to the positive control Acarbose which has an IC_{50} value of 28.84±1.28 µg/ml (Fig. 2, and Table.2.) The *P. graveolens* water extract has significant potent α-amylase inhibitory activity, even more, Acarbose which could be an excellent candidate for manufacturing potential antidiabetic herbal supplements.

Table (2): Anti-α-amylase activity IC_{50} of *P. graveolens* four extracts and Acarbose

Extracts	**Anti-α-amylase activity IC_{50} (µg/ml), ±SD**
Hexane	75.85±0.45
Acetone	29.51±0.74
Ethanol	52.48±1.0
Aqueous	20.89±0.98
Acarbose	28.84±1.28

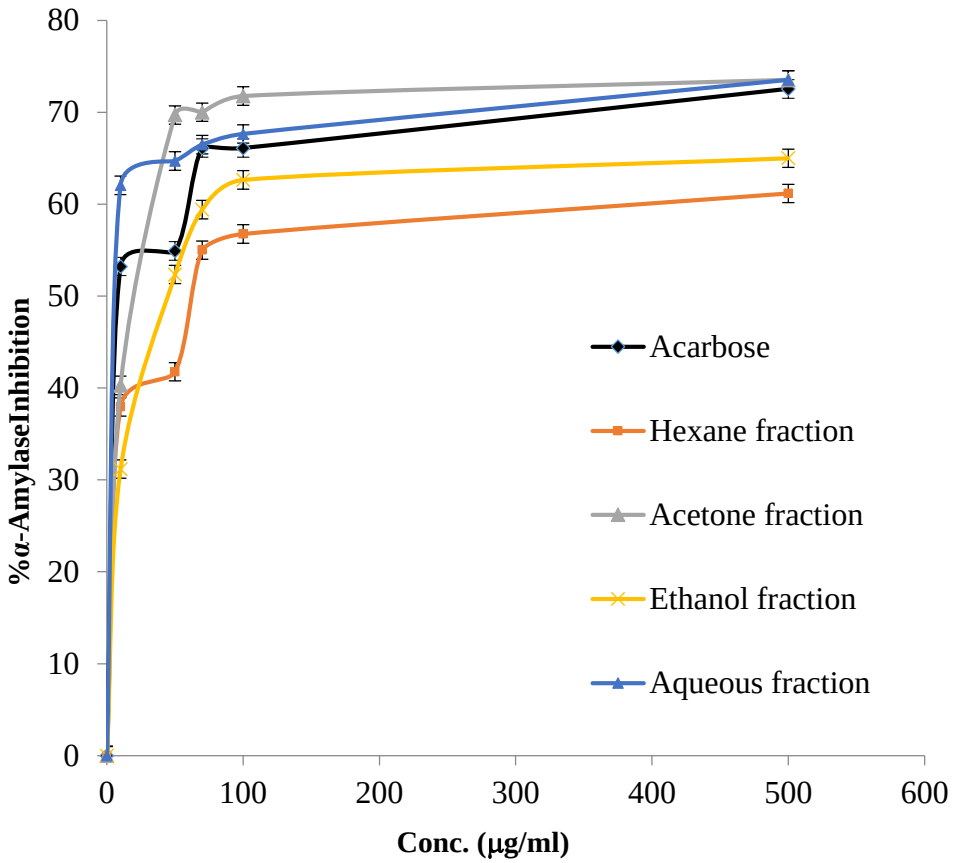

Figure (2): α-Amylase inhibitory activity by *P. graveolens* four extracts and Acarbose. The experiment was repeated thrice, and the results were reported as mean standard deviation (SD). The obtained p-values were less than 0.005.

CONCLUSION

The results showed that *P. graveolens* four extracts have potential antioxidant activity compared with the standard antioxidant drug Trolox and remarkable α-amylase inhibitory activity compared with the antidiabetic drug Acarbose. Based on the results of this study, herbal formulations with *P. graveolens* aqueous extract have much potential for treating disorders caused by oxidative stress and diabetes. They can also be used to make natural drugs or food supplements for the pharmaceutical industry.

FUNDING

This study was not funded

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGMENT

The authors would like to thank An-Najah National University, Department of Pharmacy.

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