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Exploring the Anti-Oxidative and Enzyme-Modulatory Potential of Opopanax Hispidus Extracts for Natural Diabetes Management

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and oxidative stress, contributing to long-term complications. Opopanax hispidus (Apiaceae) is a perennial aromatic plant used in traditional medicine, yet its antidiabetic potential remains largely unexplored. This study evaluated the antioxidant and α-glucosidase inhibitory activities of aqueous and methanolic extracts of O. hispidus aerial parts. Phenolic and flavonoid contents were analyzed by HPLC, identifying kaempferol, rutin, luteolin, chlorogenic acid, and p-coumaric acid. Antioxidant activity was assessed via DPPH and ABTS radical scavenging assays, showing higher DPPH activity for the aqueous extract (87.55% at 5 mg/mL) than the methanolic extract (65.39%), while both exhibited similar ABTS inhibition (~87–88%). In vitro α-glucosidase inhibition revealed dose-dependent activity, with the methanolic extract reaching 67.18% inhibition at 5 mg/mL and the aqueous extract showing more consistent inhibition across concentrations (59.88% at 5 mg/mL). These findings indicate that O. hispidus extracts are rich in bioactive phenolics and display strong antioxidant and moderate α-glucosidase inhibitory effects, suggesting potential as natural agents for managing postprandial glycemia. Further in vivo studies are warranted to clarify the molecular mechanisms and therapeutic relevance of these extracts.

Key Words: *Opopanax hispidus, antioxidant, α-glucosidase, diabetes mellitus, phenolic compounds.*

1.INTRODUCTION

Throughout human history, herbal resources provided by nature have been used in the fight against disease, and traditional treatment methods used long before modern medicine formed the basis of this approach (Fabricant et al., 2001). Despite the widespread use of modern pharmacotherapeutic agents today, medicinal plants retain their value as an important component of both complementary treatment methods and modern drugs. According to World Health Organization data, approximately 80% of the world's population still relies on herbal products to meet their basic health needs, and traditional plant-based treatments are a strong choice for both economic and cultural compatibility reasons (Fabricant et al., 2001; Izah, 2024).

One of these traditional areas of application is Diabetes mellitus (DM), which has become a global pandemic. Diabetes is a chronic disease characterized by disorders in carbohydrate, fat, and protein metabolism, resulting from absolute or relative insufficiency in insulin secretion and insulin

resistance, with both genetic and environmental factors playing a role (Govindappa, 2015). According to international data, 463 million adults worldwide were living with diabetes in 2019, and this number is projected to reach 700 million by 2045 (Saeedi et al., 2019). This rapid increase in the prevalence of diabetes indicates that the disease has become not only an individual health problem but also a major global public health burden.

Medicinal plants are important natural sources with antidiabetic potential due to the bioactive compounds they contain, such as phenolics, flavonoids, terpenoids, alkaloids, coumarins, tannins, and saponins. Many mechanisms of action have been identified for these phytochemical compounds, including insulin-like activity, protection and regeneration of pancreatic β-cells, reduction of glucose absorption, protective effect against hepatopancreatic damage, increased peripheral glucose utilization, and inhibition of various enzymes involved in glucose metabolism (Yatoo et al., 2017). Furthermore, the role of plant antioxidants in reducing oxidative stress, which is commonly seen in diabetes, is critical, as they slow the progression of diabetes-related complications by neutralizing free radicals (Khan et al., 2015).

In this context, Opopanax hispidus is a perennial aromatic plant species belonging to the Apiaceae family, which holds an important place in the flora of Türkiye. The Apiaceae family contains approximately 3000 species and includes many medicinal plants that have an important place in traditional medicine (Ghasemia and Habibia, 2014). Known in Türkiye by various names such as “sarı ot,” “çörtük,” “kaymacık,” and “kekire,” O. hispidus is noteworthy for its ethnobotanical uses and rich pharmacological properties. The plant has been reported to possess antioxidant and antimicrobial activity; its stems and leaves have been traditionally used for conditions such as hemorrhoids, female infertility, and blood purification (Çölçimen et al., 2019). Furthermore, the literature indicates that the plant is rich in secondary metabolites such as phenolics, coumarins, and flavonoids, and that these compounds possess potent bioactivities (Matejic et al., 2015; Babacan et al., 2023).

Alpha-amylase and alpha-glucosidase inhibitors used in diabetes treatment today slow down carbohydrate digestion, thereby helping to control postprandial hyperglycemia. However, a significant portion of current hypoglycemic drugs are associated with gastrointestinal side effects and the risk of hypoglycemia (Wasana et al., 2021). Therefore, research into plant-derived natural enzyme inhibitors is important for the development of safer and more effective alternative treatments. It is known that plant foods rich in polyphenols support glycemic control by inhibiting α-amylase and α-glucosidase enzymes (Nair et al., 2013).

In light of this information, considering both its ethnobotanical history and potential bioactive compounds, the antioxidant and antidiabetic potential of Opopanax hispidus should be evaluated using scientific methods. The fact that the plant has not been investigated from a modern pharmacological perspective makes it an important natural candidate for new antidiabetic and antioxidant agents.

The ultimate aim of this study is to comparatively evaluate the antioxidant and α-glucosidase inhibitory activities of aqueous and methanolic extracts of O. hispidus and to reveal the plant's antidiabetic potential based on the findings.

2. Materials and Methods

2.1. Plant Material and Collection

The plant material (Opopanax hispidus) used in this study was collected by Sifa Karakas from the Patara region, located near Kaş in Antalya, southwestern Türkiye (36°15′23.5″N, 29°19′02.9″E). The leaf parts of the plant were used for extract

preparation and subsequent nanoparticle synthesis. The collected plant samples were air-dried under appropriate laboratory conditions and processed into dried plant material for further analyses.

2.2. Preparation of Plant Extracts

2.2.1. Aqueous Extract

A total of 2 g of dried plant material was added to 300 mL of distilled water. The mixture was heated in a microwave oven (900 W) for 2 minutes and then allowed to cool at room temperature for 1 hour. The extract was filtered using Whatman No.1 filter paper and subsequently centrifuged at 4500 rpm for 5 minutes. The supernatant was collected and stored at −20°C until further use.

2.2.2.Methanolic Extract

For methanolic extraction, 2 g of plant material was mixed with 300 mL of pure methanol (Honeywell-Riedel-de Haen, Cat No: 24229). The mixture was incubated at room temperature in the dark for 48 hours. After incubation, the extract was filtered through Whatman No.1 filter paper and stored at −20°C until analysis.

2.3. HPLC Analysis of Phenolic Compounds

The phenolic composition of aqueous and methanolic extracts was determined using high-performance liquid chromatography (HPLC) at the Scientific and Technological Application and Research Center (BILTEKMER), Burdur Mehmet Akif Ersoy University.

Analyses were performed using a Shimadzu HPLC system equipped with LC-20AT and LC-20AD pumps, SIL-20AC HT autosampler, CTO-10ASVP column oven, SPD-M20A diode array detector (DAD), RID-10A refractive index detector, and RF-20A fluorescence detector. Prior to analysis, samples were filtered through a 0.45 µm membrane filter. Chromatographic separation was achieved using a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of (A) water containing 0.1% formic acid and (B) methanol or acetonitrile, with gradient conditions optimized experimentally. The flow rate and analysis duration were adjusted according to the experimental setup, and the injection volume was set to 10 µL. Detection of phenolic compounds was carried out using the DAD detector at appropriate wavelengths (e.g., 280 nm). Identification and quantification were performed by comparing retention times and peak areas with those of commercial standards. Results were expressed as mg/g extract.

2.4. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging method. This is assay is based on the ability of antioxidant compounds to donate hydrogen atoms or electrons to the stable DPPH free radical, resulting in its reduction and a decrease in absorbance at 517 nm. The extent of discoloration reflects the free radical scavenging capacity of the tested samples. A stock solution was prepared by dissolving 4 mg of DPPH in 100 mL methanol. Sample solutions at concentrations of 5, 2.5, and 1.25 mg/mL were prepared. For each test, 500 µL of sample was mixed with 500 µL of DPPH solution. Ascorbic acid was used as the positive control and was prepared in methanol at the same concentrations as the test extracts (5, 2.5, and 1.25 mg/mL). The positive control was subjected to the same experimental procedure and incubation conditions as the extract samples. Methanol alone was used as a blank. All mixtures were incubated at 38°C for 30 minutes in the dark. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. All experiments were performed in triplicate. Radical scavenging activity was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = [(A_o - A_1) / A_o] \times 100$$

A_o (Control absorbance): Absorbance of the negative control (reaction mixture without sample).

A_1 (Sample absorbance): Absorbance of the reaction mixture containing the test sample.

2.5. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Assay

The ABTS radical scavenging activity was determined using the ABTS assay. This assay is based on the ability of antioxidant compounds to neutralize the ABTS•+ radical cation through electron or hydrogen atom transfer reactions, leading to a reduction in the characteristic blue-green color of the radical and a corresponding decrease in absorbance at 734 nm. The magnitude of absorbance reduction is proportional to the antioxidant capacity of the tested samples. ABTS solution was prepared by dissolving 0.019 g ABTS in 5 mL distilled water. Potassium persulfate solution was prepared separately and mixed with ABTS solution, followed by incubation in the dark at room temperature for 16 hours. The resulting solution was diluted with methanol to obtain an appropriate absorbance level. Sample solutions (5, 2.5, and 1.25 mg/mL) were mixed with ABTS solution and incubated in the dark for 12 minutes. Ascorbic acid, prepared in methanol at concentrations of 5, 2.5, and 1.25 mg/mL, was used as the positive control and analyzed under the same experimental conditions as the extract samples. Absorbance was measured at 734 nm. All measurements were conducted in triplicate. Radical scavenging activity was calculated using the same formula as described for the DPPH assay.

2.6. α-Glucosidase Inhibition Assay

α-Glucosidase inhibitory activity was determined using enzyme inhibition assays with Saccharomyces cerevisiae-derived enzyme. Reaction mixtures containing sample (50 µL), phosphate buffer (100 µL, 0.1 M, pH 6.8), and enzyme solution (50 µL, 1 U/mL) were pre-incubated at 37°C for 10 minutes. Then, 50 µL of p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate was added and incubated at 37°C for 30 minutes. The reaction was terminated by adding 100 µL of 0.2 M Na_2CO_3. The release of p-nitrophenol was measured at 405 nm using a spectrophotometer. Acarbose was used as the positive control and was prepared in phosphate buffer at the same concentrations as the test extracts (5, 2.5, and 1.25 mg/mL). The positive control was evaluated using the same reaction conditions and incubation times applied to the extract samples. All experiments were conducted in triplicate, and inhibition percentage was calculated using the following formula:

$$\text{Inhibition (\%)} = [(A_o - A_1) / A_o] \times 100$$

A_o (Control absorbance): Absorbance of the negative control (reaction mixture without sample).

A_1 (Sample absorbance): Absorbance of the reaction mixture containing the test sample.

3. Results

3.1. Bioactive Compound Content of Extracts

The phenolic and flavonoid contents of aqueous and methanolic extracts of Opopanax hispidus were analyzed using HPLC. The reported values correspond to the quantified amounts of individual compounds in the analyzed extract samples submitted for HPLC analysis. Therefore, the results reflect the relative abundance of the detected compounds in the aqueous and methanolic extracts rather than their concentrations in the original plant material.

The analysis revealed that both extracts contained kaempferol, p-coumaric acid, chlorogenic acid, rutin, and luteolin. The amounts of these compounds varied considerably depending on the extraction solvent. In the aqueous extract, kaempferol (7266.36), luteolin (7150.45), and rutin (6718.43) were the predominant compounds, whereas in the methanolic extract, rutin (65176.85) and luteolin (13034.93) were detected at substantially higher levels. Methanolic extraction resulted in higher levels of rutin, luteolin, p-

Table 1. *HPLC quantification results of major phenolic and flavonoid compounds detected in aqueous and methanolic extracts of Opopanax hispidus.*

Compound	Aqueous extract	Methanolic extract
Kaempferol	7266.36	596.66
Luteolin	7150.45	13034.93
Rutin	6718.43	65176.85
p-Coumaric Acid	332.38	1674.26
Chlorogenic Acid	63.37	469.73

∗Values represent HPLC quantification results obtained from the analyzed extract samples.

Table 2. *DPPH Free Radical Scavenging Activities (% Inhibition) of Opopanax hispidus Extracts and Ascorbic Acid*

	5 mg/mL (%)	2.5 mg/mL (%)	1.25 mg/mL (%)
Ascorbic acid*	98.49	98.86	95.47
Aqueous extract	87.54	82.64	79.62
Methanolic extract	65.39	44.48	5.32

Data are expressed as the mean percentage of DPPH cation radical inhibition.
** Ascorbic acid was used as a positive control.*

coumaric acid, and chlorogenic acid, while kaempferol was more abundant in the aqueous extract. These findings suggest that solvent polarity influenced the extraction efficiency and phytochemical profile of the obtained extracts (Table 1).

3.2. Antioxidant Activity

The antioxidant activities of the aqueous and methanolic extracts of O. hispidus were evaluated using DPPH and ABTS assays, and compared with ascorbic acid.

3.2.1. DPPH Radical Scavenging Activity

The DPPH assay showed that the aqueous extract exhibited higher radical scavenging activity than the methanolic extract. At 5 mg/mL, the aqueous extract achieved 87.55% inhibition, while the methanolic extract showed 65.39%. As the concentration decreased, the aqueous extract maintained relatively high activity, whereas the methanolic extract displayed a sharp decline, reaching only 5.32% inhibition at 1.25 mg/mL. Ascorbic acid, used as a positive control, showed strong inhibition (95–99%) across all concentrations (Table 2, Figure 1).

3.2.2. ABTS Radical Scavenging Activity

For the ABTS assay, at 5 mg/mL, the aqueous extract showed 87.39% inhibition and the methanolic extract 88.40%. As the concentration decreased, the inhibitory effects of both extracts declined, with 1.25 mg/mL showing 29.98% and 30.02% inhibition, respectively. Ascorbic acid exhibited 92–95% inhibition at all tested concentrations (Table 3, Figure 2). These findings indicate that both extracts are effective antioxidants at higher concentrations, but their

Table 3. *ABTS Radical Scavenging Activity (% Inhibition) of Opopanax hispidus Extracts and Ascorbic Acid*

	5 mg/mL (%)	2.5 mg/mL (%)	1.25 mg/mL (%)
Ascorbic acid*	94.89	93.98	92.89
Aqueous extract	87.38	58.68	29.98
Methanolic extract	88.39	58.56	30.01

*Data are expressed as the mean percentage of ABTS cation radical inhibition. *Ascorbic acid was used as a positive control.*

Table 4. *α-Glucosidase Inhibitory Activities (%) of Aqueous and Methanolic Extracts of Opopanax hispidus*

	5 mg/mL (%)	2.5 mg/mL (%)	1.25 mg/mL (%)
Acarbose*	93.08	71.57	66.39
Aqueous extract	59.88	54.44	42.47
Methanolic extract	67.17	54.83	31.12

*Data represent the percentage of α-Glucosidase enzyme inhibition. *Acarbose was used as the standard reference inhibitor (positive control).*

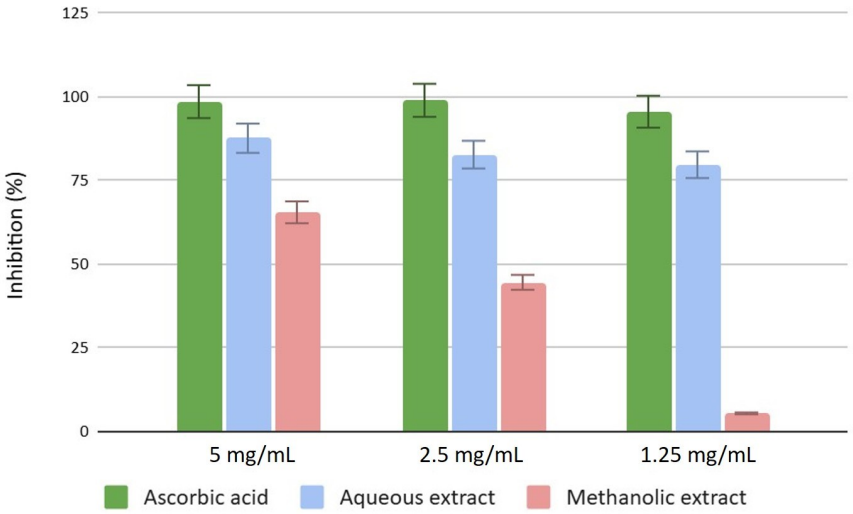

Figure 1. *DPPH Radical Scavenging Activity. DPPH scavenging activity (%) of aqueous and methanolic Opopanax hispidus extracts at different concentrations, compared with ascorbic acid. Measurements were performed in triplicate.*

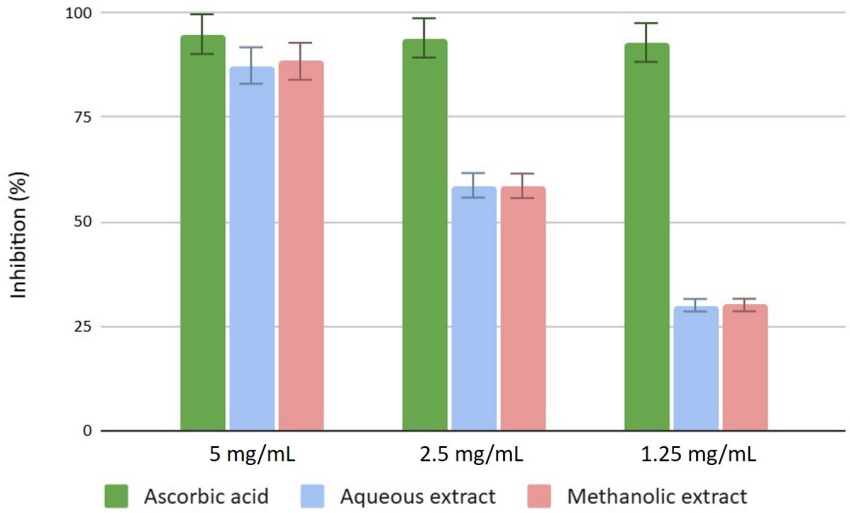

Figure 2. *ABTS Radical Scavenging Activity. ABTS scavenging activity (%) of aqueous and methanolic Opopanax hispidus extracts at different concentrations, compared with ascorbic acid. Measurements were performed in triplicate.*

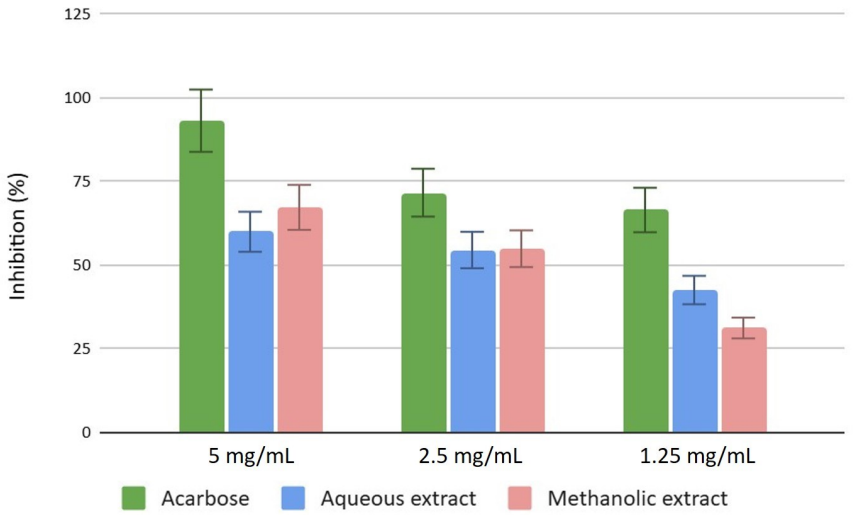

Figure 3. *α-Glucosidase Inhibitory Activity. α-Glucosidase inhibition (%) of aqueous and methanolic Opopanax hispidus extracts at different concentrations, compared with acarbose. Measurements were performed in triplicate.*

radical scavenging capacity diminishes at lower concentrations.

3.3. Antidiabetic Activity

For α-glucosidase inhibition, the aqueous extract showed 59.88%, 54.44%, and 42.48% inhibition at 5, 2.5, and 1.25 mg/mL, respectively. The methanolic extract exhibited 67.18%, 54.83%, and 31.13% inhibition at the same concentrations. Acarbose, the positive control, showed 66–93% inhibition. These results suggest that both extracts have moderate inhibitory activity against α-glucosidase, with the methanolic extract being more effective at higher concentrations and the aqueous extract showing more consistent but slightly lower activity (Table 4, Figure 3).

4. Discussion

Free radicals are chemical species containing at least one unpaired electron in their outer shell, which makes them highly reactive. In biological systems, the most common free radicals and reactive molecules are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are generated during electron transfer reactions involving electron loss or gain (Jomova et al., 2023). Aerobic organisms possess integrated antioxidant systems, including enzymatic and non-enzymatic antioxidants, to mitigate the harmful effects of ROS (Birben et al., 2012). However, when antioxidant defenses are insufficient to suppress radical formation, oxidative stress occurs (Liguori et al., 2018), contributing to various pathological conditions such as cancer, neurological disorders, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma (Birben et al., 2012).

Diabetes mellitus is a chronic disease characterized by elevated blood glucose due to absolute or relative insulin deficiency or impaired insulin action. Oxidative stress has recently gained significant attention because of its profound impact on human health, particularly in the context of diabetes (Caturano et al., 2023). Hyperglycemia in diabetes has been shown to increase free radical production and reduce antioxidant defenses, leading to cellular damage and the progression of complications (Giugliano et al., 1996). Literature data suggest that the redox imbalance in diabetes results from increased radical formation and decreased antioxidant capacity, causing oxidative damage to proteins, lipids, and nucleic acids, which contributes to disease progression (Rahimi et al., 2005). Therefore, oxidative stress is considered a key pathophysiological mechanism in both type 1 and type 2 diabetes.

Given the increasing prevalence of diabetes, inhibiting carbohydrate-digesting enzymes, such as α-amylase and α-glucosidase, has become a promising therapeutic approach. Conventional antidiabetic drugs, including metformin and acarbose, are associated with adverse effects, prompting interest in natural alternatives with higher safety and fewer side effects (Paun et al., 2024). Recent studies highlight the potential of plant-derived bioactive compounds in preventing and treating diabetes and other chronic diseases. Many phytochemicals naturally present in fruits and medicinal plants exert diverse biological effects. Although primarily synthesized for plant defense, these compounds have been reported to exert protective effects on human health (Ahangarpour et al., 2019).

Among the widely distributed phenolic compounds in plants are flavonoids, flavanones, flavonols, isoflavonoids, lignans, phenolic acids, phenolic ketones, phenylpropanoids, quinonoids, stilbenoids, anthocyanins, anthochlors, benzofurans, chromones, coumarins, tannins, and xanthones. Plants of the Apiaceae family are particularly rich in phenolic acids and flavonoids, which confer strong

antioxidant properties (Thiviya et al., 2021). These compounds are closely associated with total antioxidant capacity and contribute substantially to the biological activities of plants (Gulcin, 2020).

In the present study, the aqueous and methanolic extracts of Opopanax hispidus were analyzed for their phenolic content (HPLC), antioxidant activity (DPPH and ABTS assays), and antidiabetic potential (α-glucosidase inhibition). The findings contribute to the understanding of the plant's pharmacological and phytotherapeutic applications. HPLC analysis identified rutin, luteolin, and kaempferol as major phenolic compounds. Kaempferol was particularly abundant in the aqueous extract, whereas rutin and luteolin dominated in the methanolic extract, reflecting the selective extraction efficiency of different solvents.

Previous reports indicate that Opopanax species are rich in flavonoids and coumarins, responsible for antioxidant, antimicrobial, and anticancer activities (Önder et al., 2020). Our flavonoid profiling aligns with these findings. Moreover, the essential oil from O. hispidus fruits is rich in torulosol and geranyl geraniol acetate, contributing to the plant's biological activities (Matejić et al., 2018). The high content of labdane diterpenes (% 49.09) may explain the plant's cytotoxic, antifungal, anti-inflammatory, and analgesic potential. A newly isolated chalcone derivative also exhibited cytotoxic activity (Taban et al., 2025), suggesting potential anticancer properties.

The DPPH and ABTS assays revealed that the aqueous extract exhibited strong radical scavenging activity (87.55% inhibition at 5 mg/mL), while the methanolic extract displayed limited activity at low concentrations. The high kaempferol content in the aqueous extract likely contributes to its potent antioxidant effect. Both extracts showed similar ABTS inhibition at high concentrations (87–88%) but reduced activity at lower concentrations, indicating dose-dependent antioxidant activity.

Regarding the antidiabetic potential, both extracts inhibited α-glucosidase in a dose-dependent manner. The methanolic extract exhibited a notable inhibitory effect of 67.18% at a concentration of 5 mg/mL, while the aqueous extract showed 59.88% inhibition at the same concentration. These findings indicate that O. hispidus extracts possess the capacity to modulate key carbohydrate-digesting enzymes, suggesting their potential role as natural antidiabetic agents in the management of postprandial hyperglycemia.

Previous studies corroborate our findings: O. hispidus extracts are rich in phenolics and flavonoids, which correlate with antioxidant and enzyme inhibitory activities (Matejić et al., 2015). Phenolic-rich extracts may reduce oxidative stress-associated degenerative diseases and support glucose metabolism. The presence of phenolic compounds also suggests potential antimicrobial and anticancer activities, consistent with prior reports (Matejić et al., 2015; Çölçimen et al., 2020).

Overall, our findings indicate that O. hispidus phenolic and flavonoid constituents can mitigate oxidative stress and regulate glucose metabolism, supporting its traditional use and potential as a natural therapeutic agent.

5. Conclusions

The aqueous and methanolic extracts of Opopanax hispidus were shown to be rich in phenolic and flavonoid compounds, particularly rutin, luteolin, and kaempferol, which contribute significantly to the plant's antioxidant capacity. DPPH and ABTS assays demonstrated strong radical scavenging activity, highlighting the plant's potential in preventing oxidative stress-related diseases.

Enzyme inhibition studies revealed that both extracts inhibited α-glucosidase in a dose-dependent manner, indicating significant potential for regulating postprandial glucose levels and supporting type 2 diabetes management. The

methanolic extract exhibited a more pronounced inhibitory effect at elevated concentrations compared to the aqueous extract. These findings suggest that the bioactive compounds within the extracts may effectively modulate carbohydrate digestion, potentially serving as natural alternatives for controlling glycemic response.

These findings support the pharmacological and phytotherapeutic relevance of O. hispidus, aligning with its traditional use. Further studies are warranted to confirm these findings in in vivo models and to elucidate the underlying mechanisms of action. Overall, O. hispidus extracts may represent a promising natural source of bioactive compounds with antioxidant and antidiabetic potential.

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Conflict of interest

The authors declare that they have no conflict of interest in the publication.

Data availability

The data of this study are available from the corresponding author upon reasonable request.

Ethics Statement

This study was conducted using plant-derived materials and in vitro experimental methods only. No human participants, animals, or clinical samples were involved; therefore, ethical approval was not required.

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