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ORIGINAL ARTICLE

Functional and Novel Foods

Characterization and Comparative Assessment of the Antioxidant, Antidiabetic, and Antibacterial Potential of Polysaccharide Fractions Extracted from *Arbutus unedo* L. Fruit

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ABSTRACT

Background: *Arbutus unedo* L., commonly referred to as the "strawberry tree", is a Mediterranean species esteemed for its wealth of biologically active fractions and phytochemical compounds. Historically, the fruits, leaves, and roots of *A. unedo* have been utilized extensively for both ethnomedicinal and culinary applications.

Objectives: The aim of this study was to characterize the structural properties of polysaccharides derived from *Arbutus unedo* L. and to systematically evaluate their antioxidant, antidiabetic, and antibacterial properties.

Methods: Polysaccharide fractions were isolated from the fruit and characterized via Fourier-Transform Infrared (FTIR) spectroscopy. The antioxidant potential was determined by DPPH radical scavenging, Ferric Reducing Power (FRP), and Total Antioxidant Capacity (TAC). The antibacterial activity was assessed through the disk diffusion method, while the antidiabetic potential was evaluated *in vitro* via the inhibition of porcine pancreatic α-amylase.

Results: Two distinct polysaccharide extracts, designated *A. unedo* P1 and *A. unedo* P2, were obtained, revealing heterogeneous chemical profiles. Notably, *A. unedo P2* exhibited a higher total phenolic content than *A. unedo* P1. Conversely, *A. unedo* P1 demonstrated a stronger inhibitory effect against α-amylase (IC_{50} = 1.97 ± 0.22 mg/mL) than *A. unedo* P2 (IC_{50} = 5.66 ± 0.43 mg/mL). Similarly, *A. unedo* P1 demonstrated greater antioxidant capacity in the DPPH assay (IC_{50} = 0.051 ± 0.002 mg/mL) and higher ferric reducing assays (IC_{50} = 0.547 mg/mL) compared with *A. unedo P2* (IC_{50} = 0.395 ± 0.03 mg/mL and 3.56 mg/mL, respectively). Additionally, *A. unedo* P1 manifested substantial antibacterial activity.

Conclusion: Despite its lower phenolic concentration, the *A. unedo* P1 fraction exhibited superior antioxidant, antidiabetic, and antibacterial properties compared to *A. unedo* P2. These findings underscore the potential of *A. unedo* P1 as a high-value natural bioactive ingredient for nutraceutical formulations and functional food applications.

Keywords: *Arbutus Unedo* L.; Polysaccharides; Antibacterial Activity; Alpha-Amylase Inhibition; Strawberry Tree, Antioxidant Potential.

Article Information

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1 INTRODUCTION

Arbutus unedo L., an evergreen shrub belonging to the Ericaceae family, is widely distributed throughout the circum-Mediterranean region, including western, central, and southern Europe, northeastern Africa, the Canary Islands, and western Asia (Kim, 2012; Oliveira *et al.*, 2011; Torres *et al.*, 2002). This species has garnered escalating scientific and commercial interest due to its multifaceted traditional, industrial, phytochemical, and pharmacological applications (Morgado *et al.*, 2018).

The fruits of *A. unedo* are extensively utilized across diverse traditional and industrial sectors. Their chemical composition is predominantly characterized by carbohydrates, representing approximately 70 – 80% of the dry weight, complemented by a substantial dietary fiber fraction ranging between 10 and 30%. In addition, minor proportions of proteins (1 – 9%) and lipids (2 – 3%) contribute to the nutritional value of this Mediterranean fruit (Morales and Cilla, 2022). Both the fruits and leaves of the strawberry tree constitute valuable sources of essential mineral elements, vitamins, and a broad spectrum of bioactive phytochemicals that contribute to their nutritional and functional properties. Due to their favorable chemical composition, the consumption of *A. unedo* fruits has witnessed a recent resurgence in popularity (Zlabur *et al.*, 2020). Moreover, *A. unedo* tree represents a significant source of novel compounds possessing strong antioxidant capacities (Zitouni *et al.*, 2021), primarily attributed to a high concentration of flavonoid (El Haouari *et al.*, 2021; Fortalezas *et al.*, 2010).

The medicinal efficacy of *A. unedo* derivatives has been substantiated by several studies, demonstrating antiviral activity against influenza and HIV, largely mediated by its diverse bioactive secondary metabolites (Ge *et al.*, 2014; Junior *et al.*, 2013; Saha *et al.*, 2010). Furthermore, its consumption is associated with a reduced risk of cardiovascular diseases (Mosele *et al.*, 2016; Ramires *et al.*, 2024). Emerging evidence suggests that *A. unedo* extracts may facilitate glycemic regulation through the inhibition of key digestive enzymes, involved in carbohydrate metabolism (Nunes, 2017). Additionally, the integration of such wild fruit into the human diet has been demonstrated to augment endogenous antioxidant defenses and optimize nutrient absorption (Trichopoulou *et al.*, 2000).

Plant-derived polysaccharides represent a major class of natural macromolecules widely distributed in both edible and medicinal species. In recent years, these biopolymers have received considerable scientific interest due to their broad spectrum of biological activities, including antioxidant, anti-inflammatory, immunomodulatory, and antidiabetic effects. Their low toxicity and superior biocompatibility have further solidified their status as desirable therapeutic agents (Liu *et al.*, 2015; Wang *et al.*, 2022). These biological effects are intrinsically dictated by their structural architecture—specifically molecular weight, monosaccharide composition, and glycosidic linkage patterns—which govern their interactions with biological systems (Mohammed *et al.*, 2022). Consequently, plant polysaccharides are currently at the forefront of research in the functional foods, nutraceuticals, and biomedical sectors. Advancements in extraction technologies and structural elucidation methods, have revealed complex structure–activity relationships, supporting their potential as multifunctional agents in disease prevention and management (Chen *et al.*, 2024; Zahran, 2024).

The present study aims to chemically characterize the polysaccharides extracted from *Arbutus unedo* fruits. Furthermore, their antibacterial efficacy, antioxidant capacity via *in vitro* assays, and antidiabetic potential—evaluated through the inhibition of enzymes relevant to glucose digestion—were investigated, highlighting their potential as natural bioactive constituents for functional and pharmaceutical applications.

2 Material and Methods

2.1 Plant Material Sampling

Mature fruits of *Arbutus unedo* L. were harvested in December 2023 from the Ain Fezza (Tizi) region in Tlemcen, Western Algeria. Only undamaged specimens at the optimal stage of maturity were selected. The samples were thoroughly washed with distilled water to remove exogenous debris, and air-dried at ambient temperature (22 – 25 °C) for ten days until a constant mass was attained. The desiccated fruits were stored in airtight, light-protected containers at room temperature to preserve biochemical stability. Botanical authentication was performed by a specialist taxonomist at the Department of Ecology and Environment, University of Tlemcen, Algeria.

2.2 Extraction of Polysaccharides

Two distinct polysaccharide fractions, designated as *A. unedo* P1 and *A. unedo* P2, were isolated following the methodologies delineated by Medjdoub *et al.* (2025) with minor modifications:

- ***Preparation of A. unedo P1***: Ten grams of pulverized fruit powder were decocted in 100 mL of distilled water for one hour. The resulting extract was filtered through a multilayer muslin cloth to remove solid residues. Polysaccharide precipitation was induced by adding an equal volume of chilled ethanol to the filtrate, followed by a 24-hour incubation period. The precipitate was recovered via centrifugation at 4000 rpm for 10 minutes, rinsed with ethanol, and dried at 45 °C. The final product was ground into a homogeneous fine powder using a glass mortar and stored for subsequent analysis.
- ***Preparation of A. unedo P2***: The extraction procedure remained identical to that of P1, with the exception of a pre-treatment step: the fruit material was macerated in acetone for 48 hours, with regular solvent replacement, until complete decolorization was achieved.

2.3 Physicochemical Characterization

2.3.1 Quantification of Primary Constituents

- ***Protein Content:*** Determined via the Biuret method, based on the formation of a violet coordination complex between copper ions and peptide bonds in an alkaline medium. Absorbance was measured at 540 nm, using bovine serum albumin (BSA) as the calibration standard.
- ***Total Carbohydrates***: Quantified through the phenol-sulfuric acid method (DuBois *et al.* 1956). Samples were reacted with phenol and concentrated sulfuric acid, with absorbance recorded at 490 nm against a glucose reference curve.
- ***Total Phenolic Content (TPC)***: Evaluated through the Folin–Ciocalteu reagent according to Rjeibi *et al.* (2019). The reaction mixture was incubated in the dark, and the optical density was measured at 765 nm. Results were expressed as gallic acid equivalents (GAE).

2.3.2 pH and Spectroscopic Analysis

The pH of the polysaccharides was determined in 1% (w/v) aqueous solutions employing a calibrated pH meter, as previously described by Rjeibi *et al.* (2020). Structural

characterization was conducted via Fourier-transform infrared (FTIR) spectroscopy utilizing an Agilent Cary 600 Series spectrometer equipped with an Attenuated Total Reflectance (ATR) module. Solid samples were analyzed directly without further preparation, according to the technique outlined by Vandanjon *et al.* (2023).

2.4 Antibacterial Efficacy

The antibacterial activity of *A. unedo* P1 was evaluated against a panel of seven reference strains: three Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 49452) and four Gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Klebsiella pneumoniae* ATCC 700603).

Aromatogram profiles were established according to Benaissa *et al.*, (2024) employing five concentrations (6.25 to 100 mg/mL) via the disk diffusion assay as described by Larif *et al.* (2015). Standardized bacterial suspensions (0.5 McFarland) were inoculated onto Mueller–Hinton agar (Fluka Biochemika, Spain). Sterile disks (6 mm) were impregnated with 20 µL of the extract. Following a 24-hour incubation at 37°C, inhibition zones (mm) were measured and compared against Gentamicin (10 µg) as a positive control. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration exhibiting total visual inhibition of growth.

The resulting bacterial suspensions were adjusted to 0.5 McFarland standards, corresponding to an absorbance of 0.08–0.13 at 625 nm (approximately 10^8 CFU/mL). The inoculum was then evenly spread on Mueller–Hinton agar plates using a sterile swab.

All experiments were performed in triplicate. The minimum inhibitory concentration corresponds to the lowest concentration of *A. unedo* polysaccharides at which bacterial proliferation is totally inhibited. MIC values were determined using the endpoint method (growth/no growth) and the standard deviations were not calculated.

2.5 Evaluation of Antioxidant Potential

2.5.1 Ferric Reducing Antioxidant Power (FRAP)

The FRAP was assessed by the reduction of potassium ferricyanide to ferrocyanide, with absorbance recorded at 700 nm (Chekroun *et al.*, 2015). Concisely, 1 mL of the extract was combined with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% $K_3[Fe(CN)_6]$. Upon 20 minutes incubation at 50 °C, 1 mL of trichloroacetic acid (10%) was introduced and centrifuged at 3000 rpm for 10 minutes. One milliliter of supernatant was combined with 1 mL of distilled water and 200 µL of 0.1 % $FeCl_3$ solution. Absorbance was recorded at 700 nm. Higher absorbance values reflect greater reducing capacity. EC_{50} was defined as the concentration yielding an absorbance of 0.5. In this assay, ascorbic acid served as the reference control.

2.5.2 DPPH Radical Scavenging Activity

Antioxidant potential was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The maximum absorbance of the DPPH radical occurs at 517 nm. Antiradical activity of *A. unedo* polysaccharides was measured as described by Molyneux (2004). Sample solutions, serially diluted in methanol, were mixed in equal volume with 0.152 Mm DPPH solution. Consequently, incubation of the preparation was carried out for 30 minutes. The reference standard ascorbic acid and results were reported as IC_{50} measurement (the half-maximal inhibitory concentration of DPPH radicals). The DPPH scavenging was estimated employing equation (1),

$$\% = 100\frac{A_0 - A_1}{A_0} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where: A_0: absorbance of the control; A_1 absorbance of the sample or standard solution.

2.5.3 Total Antioxidant Capacity (TAC)

The phosphomolybdenum technique was employed to evaluate the total antioxidant capacity of *A. unedo* polysaccharides. The assay relies on electron transfer, leading to the reduction of ammonium molybdate and the formation of a greenish phosphate–molybdate complex (Prieto *et al.*, 1999).

A volume of 0.2 mL of each fraction was homogenized with 2 mL of reactant (sulphuric acid H_2SO_4 0.6 M, sodium phosphate Na_3PO_4 28 mM and ammonium molybdate 4 mM). The samples were maintained at 95°C for 90 minutes. After the solutions cooled, absorbance readings were taken at 765 nm with the blank as reference.

The TAC was expressed as milligrams of ascorbic acid equivalents per gram of polysaccharide extract (mg AAE/g E).

2.6 *In Vitro* Alpha-Amylase inhibitory Activity

The antidiabetic potential was evaluated by measuring the inhibition of porcine pancreatic α-Amylase (Medjdoub *et al.* 2025). with minor changes. The reaction was composed of 500 µL of *A. unedo* polysaccharides or acarbose, both prepared in 0.02 M sodium phosphate buffer (pH 6.9), and 500 µL of porcine pancreatic α-amylase (EC 3.2.1.1). The reaction was initiated by incorporating 500 µL of 1% starch solution (prepared in 0.02 M sodium phosphate buffer with 6 mM NaCl, pH 6.9).

The prepared samples were maintained at 37 °C for 15 minutes, the reaction was terminated by adding one milliliter of dinitrosalicylic acid (DNS) reactant. The samples were then heated in a water bath at 100°C for eight minutes, allowed to cool to ambient temperature, and the optical density was taken at 540 nm. A negative control was performed identically, in place of the inhibitor (*A. unedo* polysaccharides or acarbose) with 500 µL of sodium phosphate buffer. α-Amylase inhibition (%) was determined using the equation below (2),

$$\% \text{ of inhibition} = \frac{AC - Ai}{Ac} \times 100 \quad \text{.....(2)}$$

Where: Ac: optical density of negative control; Ai: optical density of reaction in the presence of inhibitor (polysaccharides or acarbose).

The IC_{50} value quantified as the proportion necessary to achieve half inhibition of α-amylase action was determined from the linear plot of inhibition percentage versus inhibitor concentration.

2.7 Statistical Analysis

All experiments were conducted in triplicate, and data were presented as mean ± standard deviation (SD). Statistical significance was determined using Student's t-test, with $p < 0.05$ considered significant. IC_{50} and EC_{50} values were calculated through linear regression analysis.

3 RESULTS

3.1 Characterization of Polysaccharides

The isolation procedures yielded distinct physical forms for the two fractions: *A. unedo* P1 was obtained as a brown solid, whereas *A. unedo* P2 exhibited a white, gel-like consistency. The extraction yields were recorded at 1.45 ± 0.10 and 3.60 ± 0.45 g/100 g of dry plant material for P1 and P2, respectively. The yield for *A. unedo* P2 was significantly higher ($p = 0.01$) representing a 2.5-fold increase compared to *A. unedo* P1.

The physicochemical attributes, summarized in Table 1, indicate that both *A. unedo* polysaccharides exhibited a heterogeneous composition with marked variations. *A. unedo P1* contained a lower concentration of phenolic compounds (12.25%) relative to *A. unedo* P2 (23.01%). While no substantial differences were observed in the concentrations of other primary constituents, the pH values differed considerably; *A. unedo* P1 exhibited a nearly neutral pH (7.2) whereas *A. unedo P2* was distinctly acidic (5.94).

In addition, the FTIR spectra of the extracts (Figure 1 and Figure 2) corroborated these compositional differences. Sharp absorption bands identified in the 1000 – 1100 cm^{-1} region are characteristic of ν(C–O) vibrational stretching, confirming the presence of polysaccharide structures. In contrast, the spectral region below 900 cm^{-1} is attributed to deformation modes, including out-of-plane vibrations of functional groups and aromatic C–H bending vibrations. Key peaks were identified at 1600 cm^{-1}, C-C and C=O, 1410 cm^{-1}, C-OH, 1026 cm^{-1}, O=S=O (Vandanjon *et al.*, 2023).

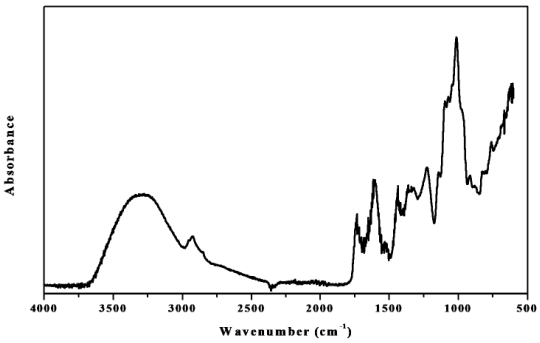

Figure 1. FTIR Spectrum of *A. unedo* P1

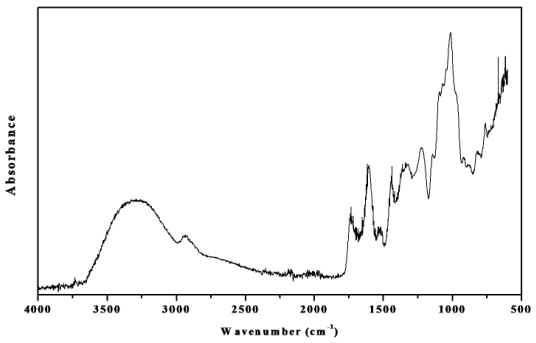

Figure 2. FTIR Spectrum of *A. unedo* P2

Table 1. Chemical Analysis of *A. unedo* Polysaccharides

	T	F	AA	St	S (%)	TPC (%)	P (%)	pH
***A. unedo* P1**	+	-	+	-	39.86 ± 1.00 [a]	12.25 ± 6.06 [b]	36.10 ± 16.07 [c]	7.21 ± 0.026 [d]
***A. unedo* P2**	+	-	+	-	40.14 ± 3.72 [a]	23.01 ± 2.99 [b]	34.30 ± 6.79 [c]	5.94 ± 0.074 [e]
p value					0.91	0.07	0.87	0.0003

Note: T: tannins F: flavonoids; AA: amino acids; St; starch; S; sugar. TPC: total phenolic compounds; P: protein +: Present, -: Totally absent. Measurements were reported as mean± SD (n = 3). Statistical interpretation was conducted using Student's *t*-test. Within the same column differing superscript letters denote statistically considerable variations ($p < 0.05$)

3.2 Antibacterial Efficacy of *A. unedo* Polysaccharides

The antibacterial activity of *A. unedo* P1 was evaluated against a panel of Gram-positive and Gram-negative strains using the disk diffusion method. The results, including inhibition zone diameters and Minimum Inhibitory Concentrations (MICs), are detailed in Table 2. The reference antibiotic, Gentamicin (10 µg), exhibited clear robust inhibitory activity against all tested pathogenes, thereby validating the experimental conditions.

Table 2. Antibacterial Activity of *A. unedo P1*

Bacteria	*A. unedo P1*		
	Diameter of Zone of Inhibition (DD) (mm)	Minimum Inhibitory Concentration (MIC) (mg/mL)	Gentamicin (GN) (mm)
***S. Typhimurium* ATCC 13311**	14 ± 0.57	25	29 ± 0.57
***E. coli* ATCC 8739**	00	-	26 ± 0.57
***K. pneumonia* ATCC 700603**	00	-	21 ± 1
***P. aeruginosa* ATCC 27853**	00	-	22 ±1
***S. aureus* ATCC 6538**	08 ± 1	25	26 ± 1.52
***E. faecalis* ATCC 49452**	10 ± 0.57	25	22 ± 1.52
***B. cereus* ATCC 10876**	00	-	21 ± 0.57

A. unedo P1 exhibited variable antibacterial potency depending on the strain. Among the Gram-positive cohort, *S. aureus* ATCC 6538 demonstrated the lowest susceptibility (8 mm zone), while *E. faecalis* ATCC 49452 exhibited moderate sensitivity (10 mm zone). Regarding Gram-negative bacteria, *S. typhimurium* ATCC 13311 was the most susceptible to the polysaccharide extract, yielding a maximum inhibition zone of 14 mm. The remaining tested strains displayed intermediate levels of susceptibility. Collectivelly, these findings suggest that the polysaccharides extracted via the P1 method possess moderate inhibitory effects against several clinically relevant pathogenes.

3.3 Antioxidant Activity

The antioxidant capacities of the polysaccharide fractions are summarized in Tables 3 and 4. Across all three assays, *A. unedo* P1 demonstrated superior efficacy compared to *A. unedo* P2. Specifically, *A. unedo* P1 exhibited a significantly higher DPPH radical scavenging activity (p = 0.002) DPPH radical with an IC_{50} equal to 0.05 mg/mL compared to *A. unedo P2* with an IC_{50} equal to 0.39 mg/mL. Given that lower IC_{50} value are considered more potent. Similar trends were observed in the TAC assay, however, ferric reducing power exhibited a considerable variations (p = 0.001).

3.4 *In vitro* α-amylase Inhibitory Effect

Table 5 summarizes the inhibitory effects of the extracts on α-amylase activity. *A. unedo* P1 was significantly more potent than *A. unedo* P2 (p = 0.0009), with IC_{50} values of 1.97 mg/mL and 5.66 mg/mL, respectively. These results highlight the potential of *A. unedo* polysaccharides, particularly the P1 fraction, as bioactive agents for the management of postprandial hyperglycemia.

4 Discussion

The extraction yields for *A. unedo* polysaccharides were recorded at 1.45 ± 0.10 and 3.60 ± 0.45 g/100 g of dry plant material for the P1 and P2 fractions, respectively. In a comparative context, Medjdoub *et al.* (2025) reported higher yields of 14.07 ± 2.61 and 4.48 ± 1.01 g/100 g for ethanolic and acetonic polysaccharides derived from the aerial parts of *Zygophyllum geslini*. These disparities suggest that polysaccharides concentrations fluctuate significantly across different plant species and botanical organs.

The results demonstrated a heterogeneous chemical composition for the *A. unedo* polysaccharides, a characteristic that likely supports their diverse biological activities. Such structural and compositional complexity is a character of plant-derived polysaccharides (Soto-Vásquez *et al.*, 2023). For instance, Fu *et al.* (2022) isolated two distinct polysaccharide types from

Table 3. Results of DPPH Radical Scavenging by *A. unedo* Polysaccharides

A. unedo P1 (mg/mL)	DPPH (%)	*A. unedo* P2 (mg/mL)	DPPH (%)	Ascorbic acid (mg/mL	DPPH (%)
0.01	17.67 ± 0.42	0.15	17.06 ± 0.98	0.01	32.13 ± 9.47
0.02	27.35 ± 0.2	0.25	26.09 ± 4.56	0.03	51.56 ± 7.92
0.045	54.52 ± 0.45	0.35	44.36 ± 2.76	0.04	76.94 ± 0.94
0.09	84.75 ± 0.2	0.62	77.42 ± 3.15	0.07	94.68 ± 1.76
IC_{50} (mg/mL)	0.05 ± 0.002[a]	**IC_{50} (mg/mL)**	0.39 ± 0.03[b]	**IC_{50} (mg/mL)**	0.03 ± 0.004

Note: Measurements were reported as mean ± SD (n = 3). Statistical interpretation was conducted using Student's t-test. Within the same line, differing superscript letters denote statistically considerable variations (p < 0.05).

Aconitum carmichaelii characterized by divergent chemical profiles and acidity levels, reflecting the variations observed between our P1 and P2 extracts.

formation, and synergistic interactions with other bioactive metabolites or metal ions. Furthermore, the biological functionality of polysaccharides is heavily influenced by their structural parameters, including molecular weight, degree of

Table 4. Antioxidant Effect of *A. unedo* Polysaccharides (Ferric Reducing Power and Total Antioxidant Capacity)

		A. unedo P1	*A. unedo* P2	Ascorbic Acid
FRP	**EC_{50} (mg/mL)**	0.547 ± 0.036^{a}	3.56 ± 0.20^{b}	0.163 ± 0.003
TAC	**mg AA E/g extract**	100.54 ± 26.36^{c}	98.92 ± 12.36^{c}	/

Note: FRP: Ferric Reducing Power; TAC: Total Antioxidant Activity; Measurements were reported as mean ± SD (n = 3). Statistical interpretation was conducted using Student's t-test. Within the same line, differing superscript letters denote statistically considerable variations ($p < 0.05$).

Table 5. Inhibitory Effect of *A. unedo* Polysaccharides on Porcine α-Amylase Activity

A. unedo P1 (mg/mL)	α-Amylase Inhibition (%)	*A. unedo* P2 (mg/mL)	α-Amylase Inhibition (%)	Acarbose (mg/mL)	α-Amylase Inhibition (%)
0.125	10.26 ± 1.76	0.25	17.98 ± 0.78	0.05	24.44 ± 1.15
0.25	13.48 ± 2.12	0.5	18.35 ± 0.88	0.08	36.17 ± 0.74
0.5	15.72 ± 1.51	1	22.11 ± 3.89	0.13	46.95 ± 5.47
1	29.21 ± 2.07	2	28.94 ± 5.09	0.21	62.54 ± 2.1
IC_{50} (mg/mL)	1.97 ± 0.22^{a}	**IC_{50} (mg/mL)**	5.66 ± 0.43^{b}	IC_{50} (mg/mL)	0.16 ± 0.01

Note: Measurements were reported as mean ± SD (n = 3). Statistical interpretation was conducted using Student's t-test. Within the same line, differing superscript letters denote statistically considerable variations ($p < 0.05$)

Regarding the antibacterial efficacy of *A. unedo* P1, inhibition zone diameters ranged from 0 to 14 mm, depending upon the specific bacterial strain. Conventionally, Gram-positive bacteria are reported to exhibit greater susceptibility to plant extracts than Gram-negative species. This phenomenon is typically attributed to the structural complexity of Gram-negative cell walls, where an outer membrane serves as a semi-permeable barrier against antimicrobial agents (Leus *et al.*, 2023).

Interestingly, the present study observed superior activity against *S. typhimurium*, a Gram-negative bacterium. This finding aligns with observations by Essaidi *et al.* (2023), who reported significant inhibition zones ranging (33 – 40 mm) for *A. unedo* fruit extracts against Gram-negative bacteria such as *Salmonella* species. Conversely, no inhibitory effects were detected against *B. cereus*, *P. aeruginosa*, *E. coli*, or *K. pneumoniae*. These findings are consistent with those reported by Essaidi *et al.* (2023) and Orak *et al.* (2011), both of whom indicated that *A. unedo* extracts may lack efficacy against specific strains, such as *E. coli*. Similarly, Dib *et al.* (2013) noted limited activity against *P. aeruginosa* in methanolic and aqueous extracts of A. unedo roots, although moderate effects were observed against E. coli using aqueous preparations.

The antimicrobial potential of plant polysaccharides is well-documented; for example, Lu *et al.* (2024) demonstrated that litchi pericarp polysaccharides possess Minimum Inhibitory Concentrations (MICs) of 145 µg/mL against *S. aureus*, 205 µg/mL against *E. coli*, 325 µg/mL against *L. monocytogenes*, and 445 µg/mL against *S. typhimurium*, while *Bacillus subtilis* exhibited low susceptibility. Several mechanisms have been proposed to elucidate these effects, including the alterations of bacterial cell membrane permeability, the inhibition of biofilm branching, and the presence of specific functional groups (Aleksanyan *et al.*, 2025). Ledoux and Lacoste (2025) further emphasized that antimicrobial activity results from a complex interplay of the overall chemical matrix rather than the presence of phenolic compounds alone.

In terms of antioxidant potential of *A. unedo* P1 exhibited the most robust activity, a property likely correlated with its phenolic content. Phenolic molecules are recognized for their superior radical scavenging capabilities, which are intrinsically associated with their structural quality (Foss *et al.*, 2022). In comparison to the polysaccharides of *Ficus microcarpa* and *Ficus racemosa* which displayed IC_{50} values against DPPH of 0.13 mg/mL and 0.12 mg/mL, respectively (Muniyandi *et al.*, 2022), *A. unedo* P1 demonstrated higher potency with an IC_{50} of 0.051 mg/mL.

Alpha-Amylase is a critical digestive enzyme that facilitates the hydrolysis of starch and glycogen into absorbable sugars (Riyaphan *et al.*, 2021). Inhibition of this enzyme reduces the absorption of carbohydrates and post-prandial hyperglycemia. While acarbose remains the clinical standard for enzyme inhibition (Oboh *et al.*, 2016), our results indicate a moderate inhibitory effect by *A. unedo* polysaccharides. *A. unedo* P1 was the most effective fraction (IC_{50} = 1.97 mg/mL) compared to P2 (IC_{50} = 5.66 mg/mL). These values indicate a lower potency than the aqueous extract of *Zygophyllum geslini* (IC_{50} = 0.429 mg/mL) as reported by Medjdoub *et al.* (2023).

Polysaccharides have been reported to exhibit antidiabetic activity through inhibition of α-amylase and α-glucosidase. Pillay *et al.* (2025) suggested that these bioactivities arise from specific interactions between polysaccharide functional groups and the enzyme's active or secondary sites. Such inhibition may occur

through competitive, non-competitive, or mixed modes of interaction, illustrating the versatile therapeutic potential of these macromolecules (Ganesan and Xu, 2019).

5 Conclusions

Polysaccharides extracted from *Arbutus unedo* fruits possess distinct chemical profiles and biological activities. Although *A. unedo* P2 fraction exhibited a higher yield and more complex composition, *A. unedo* P1 fraction demonstrated superior antibacterial, antioxidant, and antidiabetic efficacy, highlighting its therapeutic potential. These results emphasize the potential of *A. unedo* polysaccharides as natural bioactive agents suitable for integration into functional foods, dietary supplements, and pharmacological formulations. Future research should prioritize the isolation of specific active components and the elucidation of their molecular mechanisms to fully exploit their nutraceutical and therapeutic value.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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