

Purification of polysaccharide isolated from “*Matthiola longipetala*”: structure characterization and antioxidant activities

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Abstract

Purpose: In this study, the polysaccharides isolated from *Matthiola longipetala* (PSMT) purified by DEAE-Sepharose chromatography. Two fractions of polysaccharides, PSMT1 and PSMT2 were obtained.

Methods: The PSMT1 and PSMT2 structures and physicochemical properties were investigated using chemical and instrumental analysis, including Fourier transform infrared (FT-IR) spectroscopy, spectrum visible UV, nuclear magnetic spectroscopy (^1H -NMR and ^{13}C -NMR), X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM).

Results: The results of high-performance liquid chromatography (HPLC-MS) showed that the both purified polysaccharides PSMT1 and PSMT2 were mainly composed of rhamnose, arabinose, glucose and galactose in a molar percent in 39.69%, 29.59%, 8.81% and 21.89%, respectively for PSMT1 and 38.5%, 28.71%, 15.1% and 17.676% respectively for PSMT2. The data of thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC) indicated that PSMT1 and PSMT2 had relatively high thermal stability. The result of gel permeation chromatography (GPC) showed that PSMT1 and PSMT2 had a relative molecular weight of 9.779 kDa and 115.004 kDa respectively. Moreover, the in vitro antioxidant activities of PSMT were evaluated by DPPH free radical assay, ferrous iron–chelating, assay and total antioxidant activity. The experimental results showed that PSMT1 and PSMT2 exhibited an appreciable in vitro antioxidant potential.

Conclusion: Our results revealed that PSMT1 and PSMT2, polysaccharides purified from *Matthiola longipetala* could be novel active biomolecules with antioxidant properties, which promoting their application in the food and pharmaceutical fields.

Highlights

- The polysaccharides isolated from *Matthiola longipetala* (PSMT) purified by DEAE-Sepharose chromatography.
- Two fractions of polysaccharides, PSMT1 and PSMT2 were obtained.
- The both purified polysaccharides PSMT1 and PSMT2 were mainly composed of rhamnose, arabinose, glucose and galactose.
- PSMT1 and PSMT2 had a relative molecular weight of 9.779 kDa and 115.004 kDa respectively.
- PSMT1 and PSMT2 exhibited an appreciable in vitro antioxidant potential.

Statement of Novelty

Matthiola longipetala is a plant found in the Tunisian desert, considered non-usable and treated as waste. Given the adverse health effects associated with synthetic antioxidants used in the food and pharmaceutical industries, bioactive molecules of natural origin are now preferred. The present study is

the first to focus on the purification of polysaccharides extracted from *Matthiola longipetala*, with the aim of their use in various fields as natural antioxidants.

1. Introduction

Polysaccharides are naturally occurring biopolymers, formed by units linked together by glycosidic bonds. These molecules have considered as an important class of polymers. They are commonly found in living organisms, animal and plant tissues. Polysaccharides are used in pharmacy to stabilize a variety of drug supplements and in food fields for the preservation of food products.[1] [2].

The use of these compounds is encouraged by the fact that they are non-toxic and possess various biological activities which are basically related to their structural properties such as chemical composition, degree of branching, bond type, tertiary structure and molecular weight. Depending on their structure, polysaccharides from plants can play the role of antioxidant [3], antimicrobial [4], antitumor [5], anticoagulant [6, 7] and thus prevent many chronic diseases such as stroke, cancers and diabetes.

Many polysaccharides of various plant origins have been prepared. In a previous work, we prepared and characterized the crude polysaccharide of *Matthiola longipetala* (PSMT). The obtained results prove that this polysaccharide had an antioxidant activity and interesting technical-functional properties. It has been shown to be effective in maintaining meat stability by significantly preventing oxidation and bacterial contamination [4].

The structure of polysaccharides varies depending on the type of monosaccharide composition, glycosidic bonds, chain length, molecular weight and branching degree [8] [9]. Moreover, structural analysis and molecular weight of new polysaccharides are very important to better understand the structure-functional properties relationship [10–12].

To our knowledge, the present study is the first to be devoted to the purification and structural characterization of the polysaccharides extracted from *Matthiola longipetala* (PSMT). These molecules are purified by Cellulose DEAE-52 column and their structural properties are investigated using chemical and instrumental techniques such as FTIR spectrum, HPLC-MS, nuclear magnetic resonance (NMR), X-ray diffraction and scanning electron microscopy. Their antioxidant activity is also evaluated.

2. Materials and methods

2.1. Chemicals and reagent

1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), ethylene-diamine tetra-acetic acid (EDTA), sodium phosphate, ammonium molybdate, ferrozine, monosaccharide standards including arabinose (Ara), fructose (Fru), glucose (Glu), galactose (Gal), mannose (Man) rhamnose (Rha), xylose

(Xyl), trifluoroacetic acid (TFA), 1-phenyl-3-methyl-5-pyrazolone (PMP), chloroform, and PEG standards were obtained from Sigma Chemical Co.

2.2. Plant material

M. triloba leaves were collected from Kebili (Tunisia) between Mars and June 2019. The species of the plant was certified by the Plant Biology Laboratory of Faculty of Sciences of Sfax (Tunisia). The raw material was dried at room temperature for at least one week and was further crushed to obtain a fine powder, and, then, stored in glass bottles at room temperature [4].

2.3. Isolation and purification of PSMT1 and PSMT2:

The crude PSMT polysaccharide extract (2 g) was dissolved in 30 ml of deionized water and centrifuged at 5000 rpm for 20 min to remove insoluble compounds. The supernatant containing soluble polysaccharide, was applied to a DEAE-Sepharose Fast Flow column (25 mm × 30 cm) and eluted with 200 mL of deionized water, followed by step elution with 0.2, 0.4, 0.6, 0.8 and 1 mol/L of NaCl at a flow rate of 3 ml/min. Four milliliters aliquots are collected, and the carbohydrates are measured in each of them by colorimetric method using phenolsulfuric acid procedure [1]. Two peaks named PSMT1 and PSMT2 were obtained. Fractions of each peak are combined, dialyzed in a dialysis bag against purified water for 72 hours, then freeze-dried.

2.4. Physicochemical analysis

The phenol–sulphuric acid method was used to determine the carbohydrate content in purified PSMT1 and PSMT2 using D-glucose as the standard [13]. Soluble proteins content of PSMT1 and PSMT2 were quantified by colorimetric assay reported by Lowry et al. (1951) using bovine serum albumin (BSA) as the standard [14]. Total uronic acid content was evaluated according to Filisetti-Cozzi & Carpita. (1991) [15], using D- glucuronic acid as the standard.

2.5. Infrared spectroscopy

The PSMT1 and PSMT2 powder were analyzed by FTIR (Nicolet Nexus FT-IR spectrometer) equipped with an attenuated reflection accessory (ATR) containing a diamond/ZnSe crystal, at room temperature (25°C) in the spectral range frequencies of 600–4000 cm^{-1} . For each spectrum, 32 scans of interferograms were averaged and the spectral resolution was 4 cm^{-1} . Data analysis and treatment was carried out by using the OMNIC Spectra software (ThermoFisher Scientific).

2.6. ^1H -NMR and ^{13}C -NMR analysis

^1H -NMR and ^{13}C -NMR spectra of PSMT1 and PSMT2 were recorded on a Bruker Avance III– 600 MHz 400 spectrometer (Bruker Biospin AG, Fallanden, Switzerland) at 25°C. PSMT1 and PSMT2 were dried in a vacuum over P_2O_5 for 48 h, and then 20 mg of each sample was dissolved in 1 ml of 99% deuterium oxide (D_2O). Analysis of data was carried out using 2 software. Chemical shifts were given in ppm.

2.7. Ultraviolet spectrum and X-ray diffraction

The purified PSMT1 and PSMT2 solutions were prepared by dissolving the sample in distilled water to a final concentration 1 mg/mL. The ultraviolet (UV) absorption spectrum of the samples was recorded at 25°C in the wavelength range of 200 – 800 nm using a UV-vis spectrophotometer (JENWAY/7315, United Kingdom).

The XRD patterns of purified samples were recorded by a Bruker D5000 ray diffractometer with a radiation source of Cu K α . Measurements were made from 7 to 40° at a scanning rate of 1°/min, a voltage of 40 KV and a current of 20 mA.

2.8. Thermal properties:

Thermogravimetric analysis (TGA) was carried out on Q500 high resolution (TA Instruments) as described by Hajji et al [1]. Each sample (PSMT1 et PSMT2) (1.5 mg) was placed in an aluminum oxide pan and heated within a temperature range of 30–700°C at a heating rate of 10°C/min under nitrogen atmosphere. The DSC (Q20 Modulated, TA Instruments) analysis was performed to use 5 mg of purified PSMT1 and PSMT2 samples.

2.9. Molecular weight determination

The weight-average molecular mass (M_w) and polydispersity (M_w/M_n) of the purified samples PSMT1 and PSMT2 were determined by gel permeation chromatography (GPC) method [16]. Average molecular weights (M_n) and dispersities ($\mathcal{D}$) were determined using size exclusion chromatography (SEC) on a Shimadzu Prominence system (Shimadzu Corp, Kyoto, Japan). This system is equipped with a PL aquagel-OH guard column (Agilent, 8 μ m, 50 x 7.5 mm), a PL aquagel-OH 40 column (Agilent, 8 μ m, 300 x 7.5 mm), two PL aquagel-OH 30 columns (Agilent, 8 μ m, 300 x 7.5 mm) and a Shimadzu RI detector 20 A. The mobile phase was a phosphate buffer (0.2 M NaNO₃, 0.01 M NaH₂ PO₄, pH 7) with a flow of 1 mL.min⁻¹ at 35°C. PEG standards were used for calibration and polymers characteristics obtained expressed according to those standards.

2.10. Monosaccharides composition analysis

Briefly, 2 mg of each purified fractions (PSMT1 and PSMT2) was hydrolyzed with TFA (4M, 2mL) in ampoule (5mL). The ampoule was kept in an oil bath at 110°C for 8 h. The samples pH was neutralized to about pH 7.0 with NaOH (3.0 M). Next, the hydrolyzed solutions of PSMT and seven monosaccharides' standards were mixed with 3.0 M aqueous NaOH (500 μ L) and 500 μ L of 1-phenyl-3-methyl-5-pyrazolone (PMP) (0.5 M in methanol), followed by reaction for 30 min at 70°C. Each mixture was cooled to room temperature and neutralized with 500 μ L of HCL (0.3M), then dissolved in chloroform (1 mL). After centrifuging, the organic phase was carefully discarded three times.

The analysis of monosaccharides containing PMP-labeled was realized on SYNAPT G2-S (Waters Corporation, Manchester, UK) equipped with an ESI source was employed. High resolution electrospray ionization mass spectrometry (HR-ESI-MS) was acquired in positive or negative ion mode. The analytical column used was Kinetex C18 de 100 mm*2.1 mm – 1.7 μ m (T° = 30°C). The conditions carried out on: capillary voltage 3000 V; cone voltage 20V, dry gas temperature 140 °C, desolvation temperature =

450°C, dry gas flow, 1000 L.h⁻¹ and nitrogen as nebulizer gas, Pressure = 6.5 bars. The standard sugars used were Arabinose, Fructose, Galactose, Glucose, Mannose, Rhamnose and Xylose.

2.11. Scanning electron microscopy (SEM)

The microstructure of samples (PSMT1 and PSMT2) was examined by using Hitachi S4800 scanning electron microscopy (SEM). Samples were sputter coated prior to analysis.

2.12. Antioxidant activity

2.12.1 DPPH• radical scavenging capacity

The antioxidant activity of PSMT1 and PSMT 2 was evaluated by DPPH radical scavenging ability [17]. The samples at different concentrations (0.5-3 mg/mL) were incubated with DPPH• solution (0.2 mmol/L in ethanol). Absorbance was recorded at 517 nm UV-vis spectrophotometer. The used standard was BHA. The DPPH radical scavenging capacity was calculated using the following equation:

$$\% \text{ DPPH scavenging} = [(A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}}] \times 100$$

Where A_{cont} is the absorbance of the control reaction (without addition of the sample), A_{test} was the absorbance of PSMT1 and PSMT2 solution in the reaction mixture. Three replicates were performed for each test sample.

2.12.2 Metal iron chelating assay

The chelating activity of PSMT was evaluated as described by Carter.1971 [18]. It was determined by estimating the decrease in red color of complex (Fe^{2+} -ferrozine) at 562 nm.

The EDTA was used as positive control. The percentage of the chelating ability was assessed using the following equation.

$$\text{Ferrous ion - chelating activity (\%)} = [(A_{\text{cont}} - A_{\text{test}}) / A_{\text{cont}}] \times 100$$

Where A_{cont} is the absorbance of the control reaction (without addition of the sample), A_{test} was the absorbance of PSMT1 and PSMT2 solutions in the reaction mixture. Three replicates were performed for each test sample.

2.12.3 Determination of Total Antioxidant Activity

The total antioxidant activity of PSMT1 and PSMT2 was evaluated according to Prieto et al. (1999) [19]. Briefly, 1 mL of fucoidan solution at different concentrations (0.5, 1, 1.5 and 2mg/mL) was mixed with 1 mL of standard reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium

molybdate). The different mixtures were incubated at 95°C and the absorbance read at 695 nm. BHA was used as a reference compound.

2.13 Statistical analyses

Statistical analyses were performed with SPSS ver.17.0, professional edition. The mean differences between tests were examined by Duncan test and compared by one-way analysis of variance (ANOVA). Differences were considered significant at p-value < 0.05. All tests were carried out in triplicate.

3. Results and discussion

3.1. Isolation and purification of polysaccharides

The PSMT solution was loaded into DEAE-Sepharose column and was then eluted with NaCl gradient from 0 to 1 mol/L. As indicated in Fig. 1, tow fractions named PSMT1 and PSMT2 were obtained with 1.8% and 0.8% yield, respectively. The purification yield of both fractions was relatively low compared to polysaccharides fractions ATPS-PP-1 (39.1%) and ATPS-PP2 (53.7%) purified from Pumpkin [20].The PSMT1 was eluted with deionized water and the PSMT2 was eluted with 0.4 mol/L NaCl solution.

3.2. Physicochemical analysis

The total sugar contents of PSMT1 and PSMT2, evaluated by phenol-sulfuric acid method, were 87.66% ±1.93 and 83.58% ±1.8, respectively, which were similar with MDP-1 (89.6%) and MDP-2 (86.7%), fractions purified from *Mycena dendrobii*- [21]. The protein content was approximately 2.1% ±0.23 for PSMT1 but not detected for PSMT2. This result showed that PSMT1 was a potential protein conjugate polysaccharide. The uronic acid content of PSMT1 and PSMT2 were 12.53% ±0.83 and 4.13%±0.53 respectively (**Table 1**).

Table 1: The content of carbohydrate, protein and uronic acid of PSMT1 and PSMT2

	PSMT1	PSMT2
Carbohydrate (%)	87.66 ± 1.93 ^a	83.58 ± 1.8 ^b
Proteins (%)	2.1 ± 0.23 ^a	ND
Uronic acid (%)	12.53 ± 0.83 ^c	4.13 ± 0.53 ^a

Nd: not detected

Different letters (a, b and c) for each row indicated significant difference (p < 0.05)

3.3. FTIR spectroscopy analysis

The FT-IR spectrum of the purified samples PSMT1 and PSMT2 from 500 to 4000 cm^{-1} was presented in Fig. 2a **and b**. The FT-IR spectra of PSMT1 and PSMT2 displayed an intensive peak at around 3354 cm^{-1} attributed to the stretching vibration of hydroxyl group (O-H). The relative weak absorption peak near 2924 cm^{-1} described to C-H stretching vibration [21]. The absorption peak around 1645 cm^{-1} for both PSMT1 and PSMT2 was attributed to the presence of carboxylic group as was found in uronic acids [22]. The peaks located at 1641 cm^{-1} and 1423 cm^{-1} for both samples were attributed to the asymmetric and symmetric stretching of the carboxylate anion group (C = O), respectively [23]. The strong peaks for each sample in around 1072 cm^{-1} and 1042 cm^{-1} were attributed to the presence of C-O-C and C-O-H stretching vibration, which were ascribed to pyranose ring [24]. The tow purified samples appeared no absorption peaks at 1541 cm^{-1} , indicating the absence of protein [25]. The weak peaks in the range of 900 cm^{-1} and 800 cm^{-1} showed that both α and β -configuration exist in PSMT1 and PSMT2 [26].

3.4. ^1H -NMR and ^{13}C -NMR analysis

Structural analysis of complex polysaccharides. The ^1H NMR and ^{13}C NMR spectra of PSMT1 and PSMT2 are shown in **Fig. 3**. Most chemical shifts were ranged from δ 3.0 to δ 5.4 ppm, which was the specific feature of polysaccharides in ^1H NMR spectrum [27]. As presented in **Fig. 3a and b**, the ^1H NMR spectra of the tow fractions showed that the chemical shifts for some protons occurred around 5.0 ppm suggesting α - and β -configuration both existed in the fractions. PSMT 1 and PSMT 2 spectrum exhibited a set of wide and intense signals between δ 3.0 and δ 5.0 ppm corresponding CH-O and CH₂-O sugar groups [1]. Chemical displacement at 4.38, 4.08, and 3.62 ppm was generated by the H-5, H-4 and H-2 of $\rightarrow$ 4) α -GalA (1 $\rightarrow$). The hydrogen signal peak at 5.26 ppm presented in the spectrum of PSMT1 and PSMT2 indicated the presence of α -Ara (1 $\rightarrow$) [28].

The ^{13}C NMR spectrum of PSMT1 and PSMT2 was presented in a specific region ranging from δ 60–108 ppm which indicated that the both of samples were heteropolysaccharides as it was shown in the data of monosaccharides composition. The signal observed at 59.6 ppm showed the non-reducing terminal of α -Ara, and signal at 98.75 ppm indicated the presence of $\rightarrow$ 2) α -Rha (1 $\rightarrow$) [29] (**Fig. 3c and d**). The resulted data indicated that the α and β anomeric configuration existed as showed by ^1H NMR spectrum.

3.5. UV spectrum and X-ray diffraction analysis

PSMT1 and PSMT2 were recorded in the UV visible absorption spectrum (200–800 nm). The results indicated in Fig. 4a **and b**. exhibited a wide absorbance peak at 200 nm, consequently, PSMT1 and PSMT2 were specified as polysaccharides. For the UV spectra of PSMT1 at 280 nm, weak absorption was observed, indicating the trace of protein that could be conjugated. While, for PSMT2, there is no absorbance at wavelength of 280 nm, wish showing the absence of protein impurities in PSMT2. This finding was in accordance with result apported by (Hu et al., 2019) wish exhibited the presence of glycoproteins in the fractions CMKP-1 and CMKP-2 purified from *Carex meyeriana Kunth* [30].

XRD analysis is a method applied for determination of polysaccharides crystalline structure. The data of X-ray diffraction spectrum of PSMT1 and PSMT2 were illustrated in Fig. 4c **and d**. The diffractograms

recorded between 0° and 80 ° showed low crystallinity of polymers and indicated major crystalline reflection at 13.167 ° for PSMT1 and 12.936 ° for PSMT2. These results were in accordance with those reported by Hamed et al. (2020) and Qian et al. (2009) [31]. The crystallinity of materials affected various physical proprieties including tensile strength, flexibility, solubility, swelling and viscosity. These proprieties are dependent on order degree within the material [31].

3.6. Thermal proprieties

The thermal analysis TGA and DSC were important for bioactive biomolecules to determine the thermostability, melting temperature (T_m) and molecular weight changes. TGA thermograms of PSMT1 and PSMT2 were presented in Fig. 5. The PSMT1 and PSMT2 TGA profiles indicated two distinct stages of weight- loss. In the first one, a mass loss of approximately 12.34% occurred between 25°C and 150°C for PSMT1 and 16.42% covered from 24°C to 125°C for PSMT2 which mainly attributed to the evaporation and desorption of water in the PSMT1 and PSMT2 samples [32]. The second stage for PSMT1 observed between 154°C and 500°C was attributed with weight loss of 77.47% (Fig. 5a). Regarding the PSMT2, the second stage occurred between 150°C and 550°C with a weight loss of 69.86% (Fig. 5b) This may be caused by the thermal decomposition of polysaccharides, the change of functional groups and depolymerization of structure[33]. DSC was further used to determinate the occurrence of exothermal or endothermal changes with an increased temperature [34]. As shown in Fig. 5c **and d**, the first transition was observed between 62°C and 186°C with a major pic at 154°C for PSMT1 and 150°C for PSMT2 which can be attributed to evaporation of free water present in PSMT1 and PSMT2 (T_e). Regarding PSMT1, the second endothermic peak was obtained at temperature range 196 -220°C with a peak at 210°C but for PSMT2, the peak was found at 212°C which can be associated to melting temperature (T_m) of pure polysaccharides. These results are in agreement with previous studies reported by Arab et al. (2021) [35].

3.7. Molecular weight analysis by

The GPC profiles of the purified samples PSMT1 and PSMT2 are presented in Fig. 6. The data showed that the Mw of PSMT1 and PSMT2 was 9.779 kDa and 115.004 kDa with the retention time of 24.043 min and 23.07 min respectively. The Mw of PSMT1 was higher than the previously reported LMw-PGP isolated from *Platycodon grandiflorus* (1.9 kDa) [36] and SCPP1 purified from *Schisandra chinensis* (3.4 kDa) [36] but lower than reported polysaccharides AGP1 extracted from *nethum graveolens* seeds (2.1×10^4 Da) [34] and GFP-W1 purified from *Grifola frondos* (9.161×10^5 Da) [37]. The Mw of PSMT2 was comparable to polysaccharides SUE purified from sulfate *Ulva* (178 kDa) [38], but lower than reported polysaccharides extracted from *Auricularia auricular* (2.77×10^4 Da) [39] and *Gentiana scabra* (3.8×10^4 Da) [40]. In this regard, molecular weight is an important factor for associated with polysaccharide bioactivity, as low molecular weight polysaccharides more readily permeate the cell membrane and exert effects in vivo than those of higher molecular weight [41].

3.8. Monosaccharides composition of PSMT1 and PSMT2:

The monosaccharides composition was determined by HPLC-MS by comparing the retention time against standards. As shown in **Fig. 7a and b**, the PSMT1 and PSMT2 were both composed of rhamnose, arabinose, glucose and galactose in a molar percent in 39.69%, 29.59%, 8.81% and 21.89%, respectively for PSMT1 and 38.5%, 28.71%, 15.1% and 17.67%, respectively, for PSMT2. Fructose, Mannose and xylose were not detected. These data indicated that PSMT1 and PSMT2 were a heteropolysaccharides with rhamnose as the major monosaccharide ingredient. Previous study performed on polysaccharides ATPS-PP-1 and ATPS-PP-2 purified from Pumpkin also revealed heterogeneous compositions but with glucose as the major sugar compound [20]. Comparable results were reported in the case of polysaccharides purified from *Grateloupia lithophila* indicated rhamnose (95.88%) as the major monosaccharide constituent but with absence of glucose and arabinose and presence of fructose (2.21%) and xylose (1.13%) [42]. Differently, purified polysaccharide from *Periploca laevigata* exhibited higher contents of glucose (62.9%), galactose (19.4%), mannose (11.8%) and glucuronic acid (5.9%) [1].

3.9. Microstructural analysis of PSMT1 and PSMT2 by SEM:

Scanning electron microscopy is a commonly technique to determine the structural morphology of polymers. The morphologies images of PSMT1 and PSMT2 were shown in Fig. 8. The SEM revealed that the morphology of PSMT1 and PSMT2 mainly presented a spongy porous structure. The porous surface of PSMT1 and PSMT2 was comparable to polysaccharides puied from banana peel waste [43].

3.10. Antioxidant activities of PSMT1 and PSMT2

The assay of free radical scavenging was evaluated in order to investigate the antioxidant activity of both PSMT1 and PSMT2 fractions purified from the crude polysaccharide PSMT. As shown in Fig. 9a **and b**, all concentrations of the PSMT1 and PSMT2 solutions indicated obvious radicals DPPH scavenging ability in a concentration-dependent manner. PSMT1 exhibited an interesting radical scavenging ($IC_{50} = 0.87$ mg/mL) and reached $73.5\% \pm 0.29$ at a concentration of 5 mg/mL (Fig. 9a) wish was higher than SPC-60, SPC-90 SPC-80 and SPC-70, polysaccharides purified from *Sagittaria sagittifolia* L showing radical scavenging activity of 45,12%, 46,53%, 47,61% and 50,23% respectively at 5 mg/mL [44]. However, PSMT1 exhibited lower activity compared with polysaccharide purified from *Sophorae tonkinensis* ($IC_{50} = 0.084$ mg/mL) [45]. BHA indicated a strong activity $97\% \pm 3$ for a concentration of 0.5 mg/mL. The DPPH scavenging ability of PSMT2 was found to be $59.5\% \pm 0.16$ at 5mg/mL ($IC_{50} = 4$ mg/mL) (Fig. 9b), revealing that the scavenging ability of PSMT2 was lower than CPHP I, a polysaccharide purified from *Cissus pteroclada* Hayata, indicating radical scavenging activity of 87.69% at 1.4 mg/mL [46]. The methodology of extraction and purification, the conformational characteristic and sugar composition of polysaccharides could be caused these differences [34]. The PSMT2 showed lower activity than PSMT1 fraction. It is possible that the presence of protein in PSMT1 might explain potent radical according scavenging activity.

Ferrous ion (Fe^{2+}) chelation may inhibit oxidation reactions by retarding metal-catalyzed oxidation. As recorded in Fig. 9c **and d**, the Fe^{2+} chelating activities of both PSMT1 and PSMT2 fractions and EDTA which increased in a dose-dependent manner. These results proved that both PSMT1 and PSMT2

displayed strong chelating power ($IC_{50} = 0.446$ mg/mL for PSMT1, $IC_{50} = 0.41$ mg/mL for PSMT2). Indeed, at 2mg/mL, the chelating ratio of PSMT1 and PSMT2 was $90\% \pm 3.4$ and $62.4\% \pm 0.93$ respectively, which increased to 100% and 64.8% respectively with concentration of 4 mg/mL.

The Fe^{2+} -chelating ability of both PSMT1 and PSMT2 is higher than polysaccharides extracted from flora mushroom and fenugreek seeds recording 42.68% and 69.4% at 5 mg/ mL, respectively [47].

The total antioxidant activity was determined based on the reduction of Mo (VI) to Mo(V) by treatment of fucoidan and the subsequent formation of green phosphate/Mo(V) complex at acid pH. The water-soluble and fat-soluble antioxidants were analyzed by this test. The total antioxidant activity of fucoidan was determined by phosphomolybdenum test and compared with BHA. The data showed that the activity increased in a dose-dependent manner (Fig. 9e **and f**). The activity of both fractions PSMT1 and PSMT2 was important, however the PSMT1 showed higher total antioxidant activity (56.28 ± 3.01 mol/mL) at 1 mg/mL) than PSMT2 (28.2 ± 1.85 mol/mL) at 1 mg/mL). this result exhibited lower activity compared with polysaccharides isolated from *Spatoglossum asperum* because of its sulfate content [48].

The results of the various used in vitro tests of antioxidant activity show a greater efficacy of PSMT1 than PSMT2. This could be justified by its higher protein content as well as its smaller size and greater richness in uronic acid. Indeed, previous studies have reported that relatively low molecular weight and relatively high uronic acid content increase the antioxidant activity [49] [50].

Conclusion

In this study, the water-soluble polysaccharides (PSMT1 and PSMT2) were obtained by purification of crude polysaccharides isolated from *Matthiola longipetala*, using DEAE-Sepharose chromatography. The characterization of purified polysaccharides PSMT1 and PSMT2 was investigated using Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (X-RD), High Performance Liquid Chromatography (HPLC-MS), Scanning Electron Microscopy (SEM), spectrum visible UV and nuclear magnetic resonance (^{13}C and 1H NMR). The both fractions were composed of rhamnose, arabinose, glucose and galactose. According to TGA and DSC analysis, PSMT1 and PSMT2 indicated a high thermal stability. In addition, PSMT1 and PSMT2 performed a strong antioxidant activity. Overall, the results suggested that purified polysaccharides (PSMT1 and PSMT2) from *Matthiola longipetala* could be used as source of bioactivities in the medical and food industries.

Declarations

Author Contributions

Conceptualization, methodology, S.E., I.B., E.P., V.D., S.L., formal analysis, S.E., S.L., resources, S.L., M.H., data curation, S.L.; writing—review and editing, S.E., A.B., M.H., visualization, A.B., M.H., All authors have read and agreed to the published version of the manuscript.

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Figures

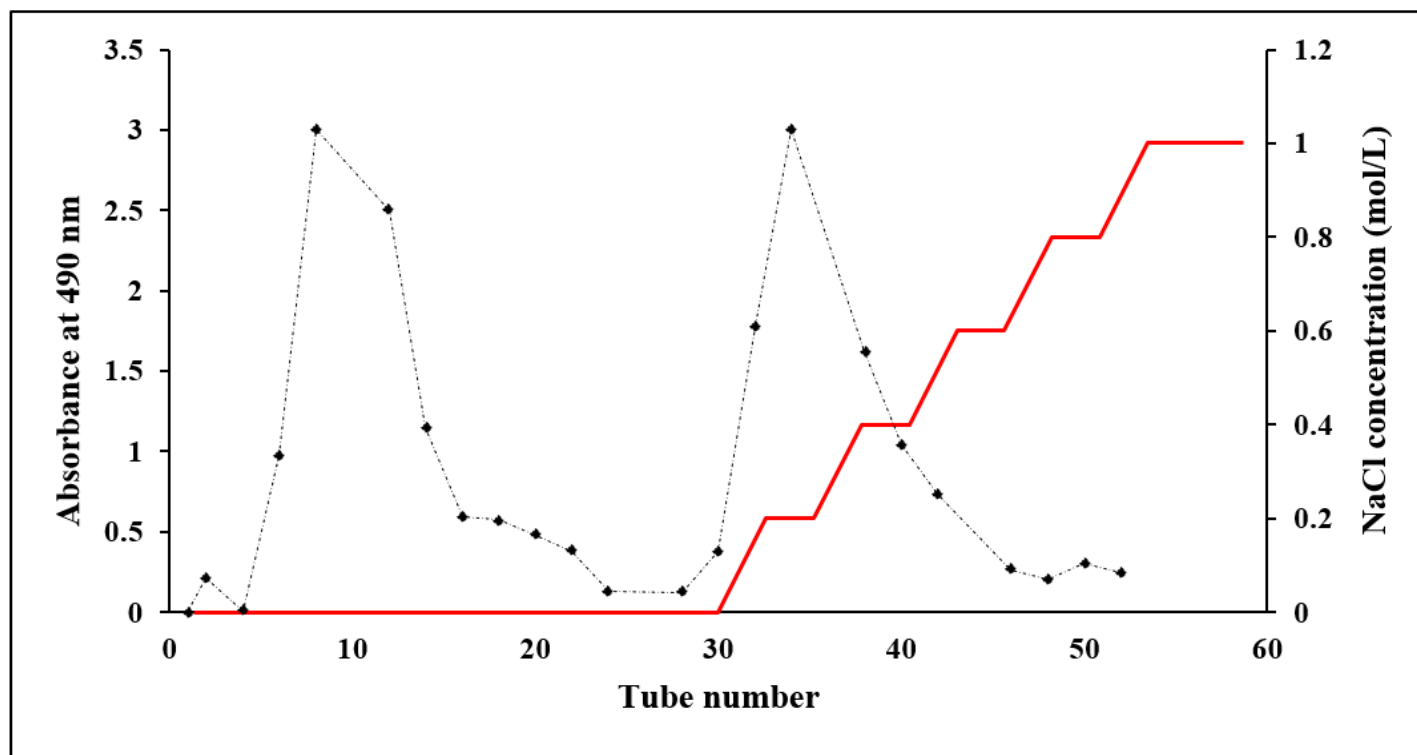


Figure 1

The elution profile of crude polysaccharides PSMT isolated from *Matthiola longipetala* leaves on a DEAE-Sepharose Fast Flow column (25 mm × 30 cm) eluted with 200 mL distilled water and NaCl solution (0 to 1 mol/L) step gradient at a flow rate of 3 mL/min, the volume of each aliquot collected was 4 mL.

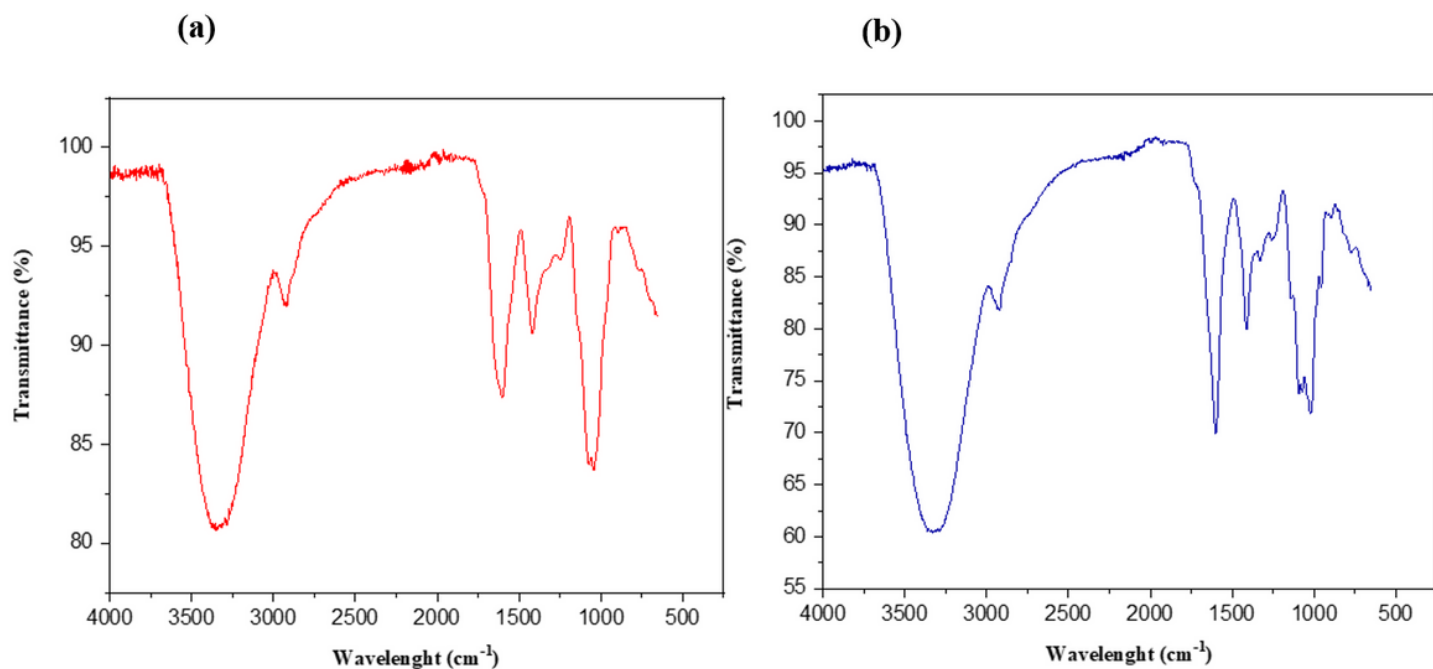


Figure 2

Fourier transformed infrared spectrum of PSMT1 (a) and PSMT2 (b).

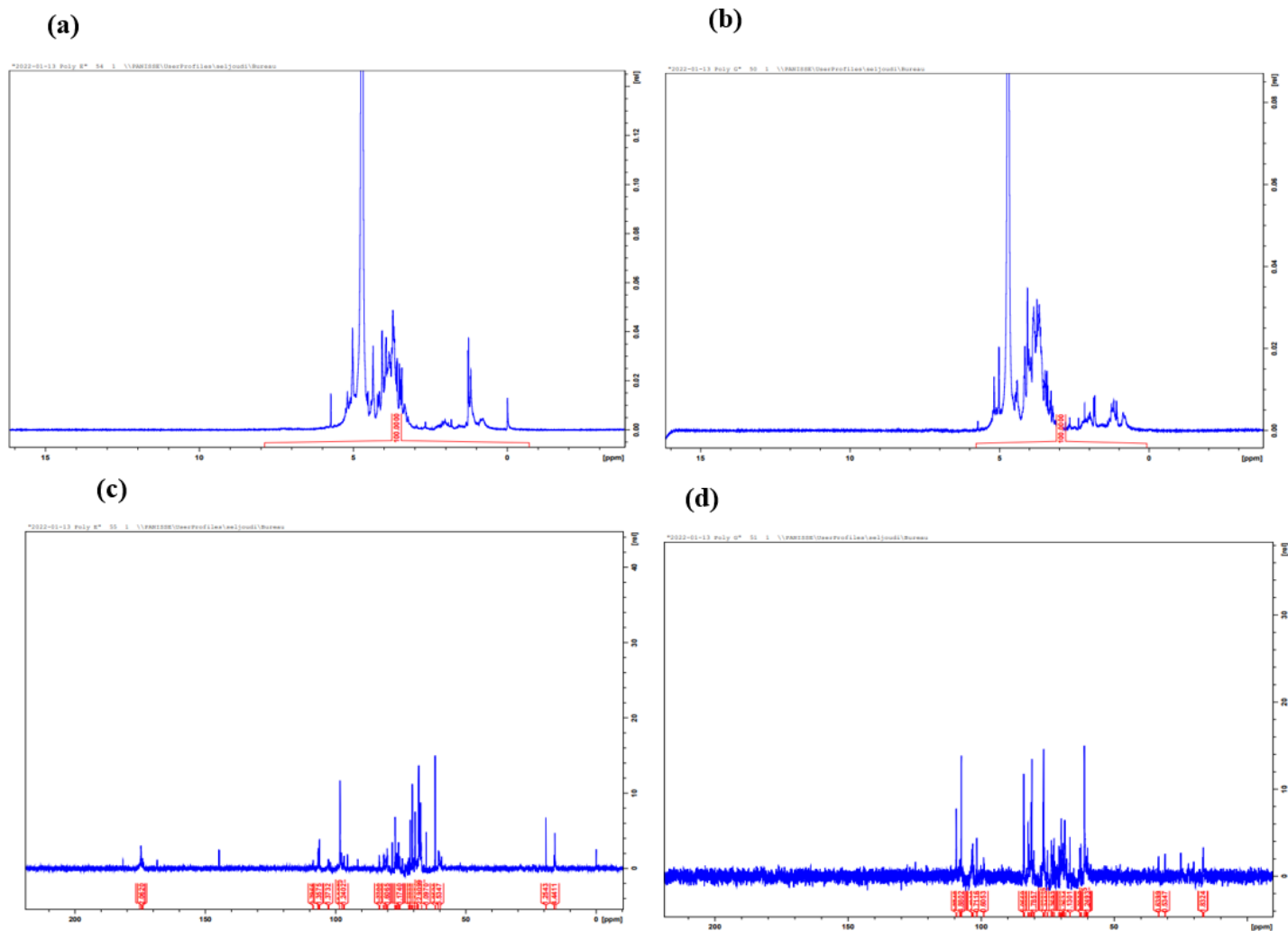


Figure 3

^1H -NMR spectrum of PSMT1 (a) and PSMT2 (b). ^{13}C -NMR spectrum of PSMT1 (c) and PSMT2 (d).

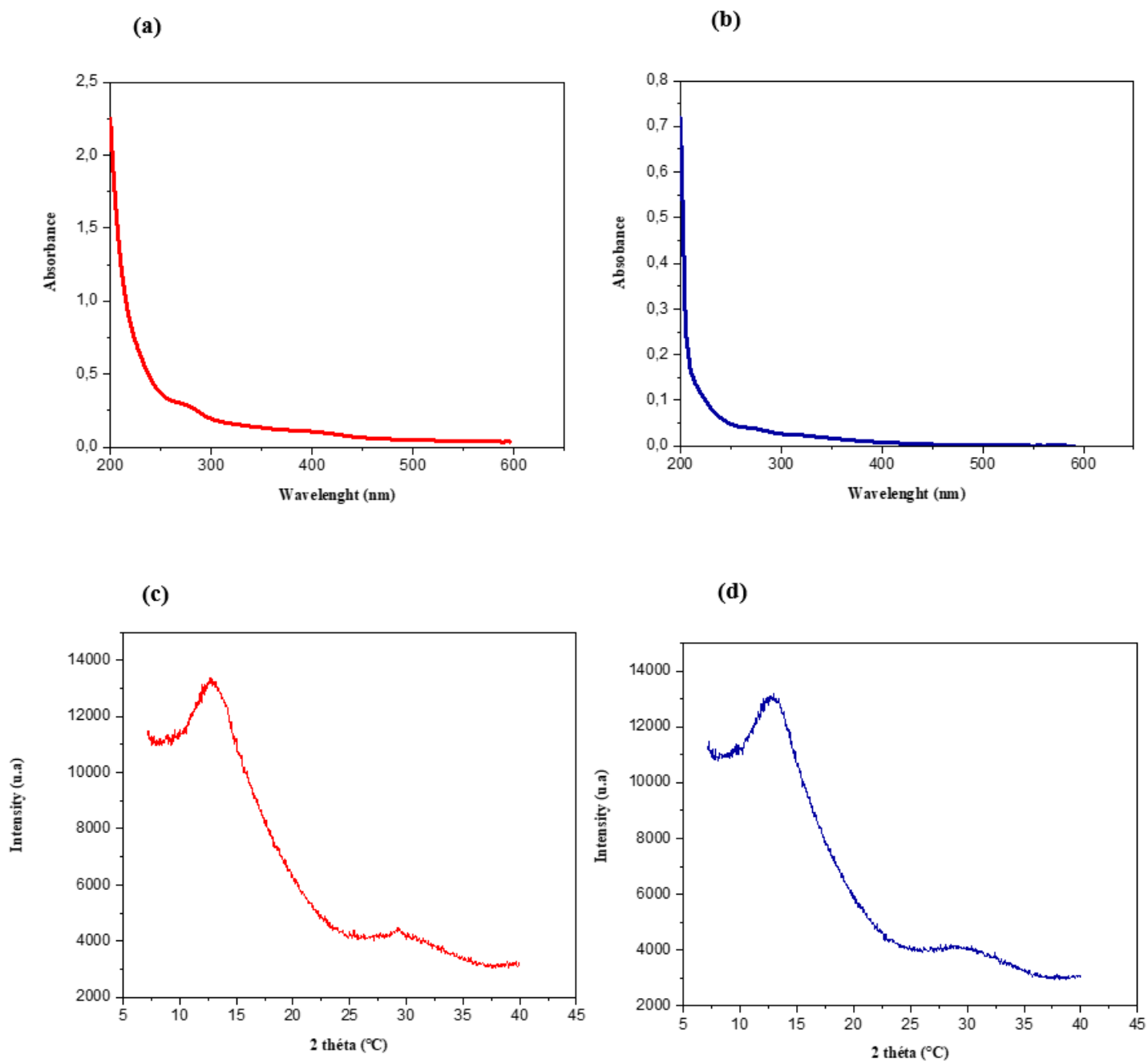
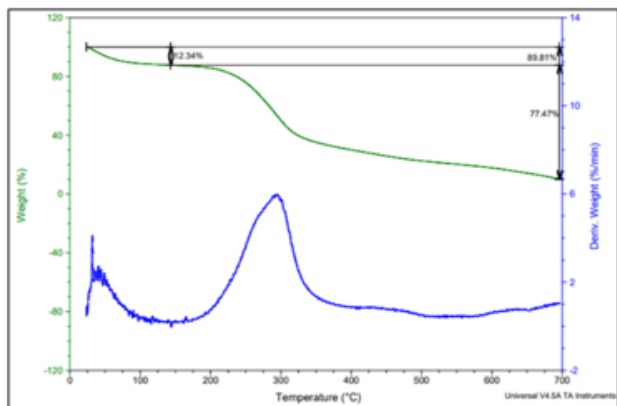
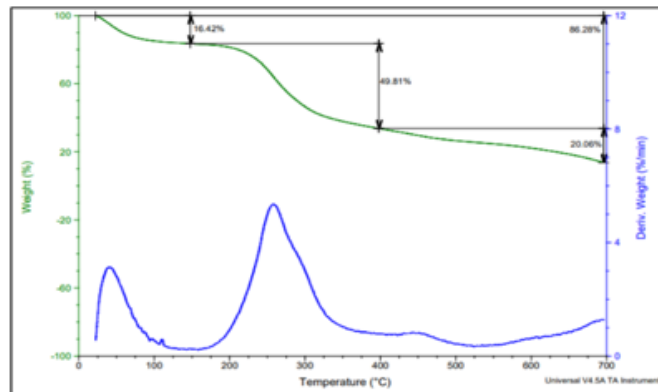
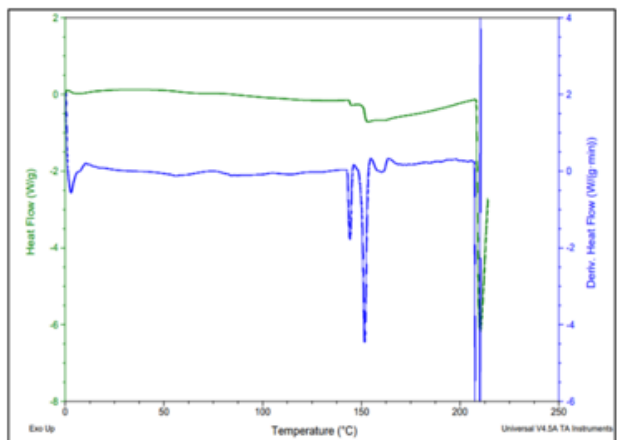
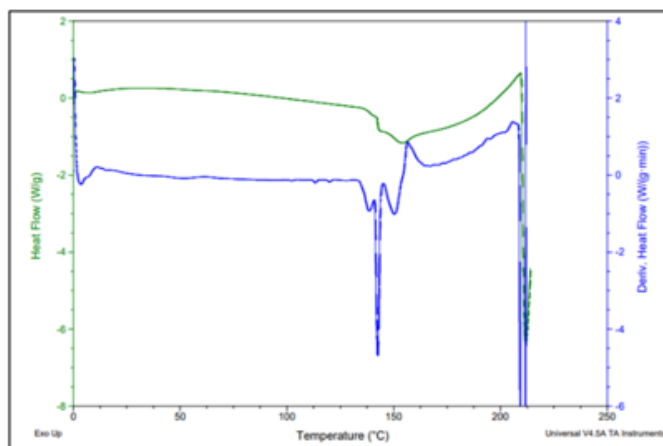


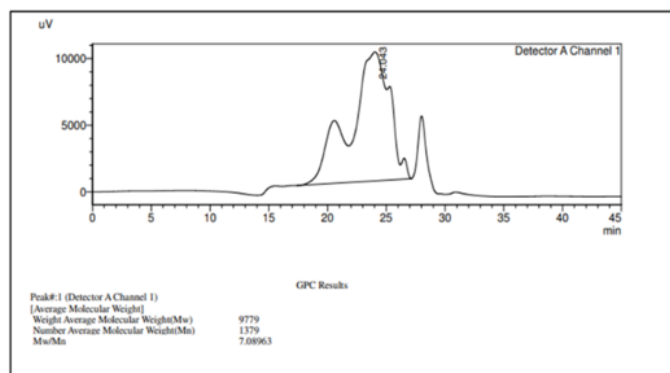
Figure 4

UV-visible absorption spectrum of PSMT1 **(a)** and PSMT2 **(b)** X-ray diffraction pattern of PSMT1 **(c)** and PSMT2 **(d)**

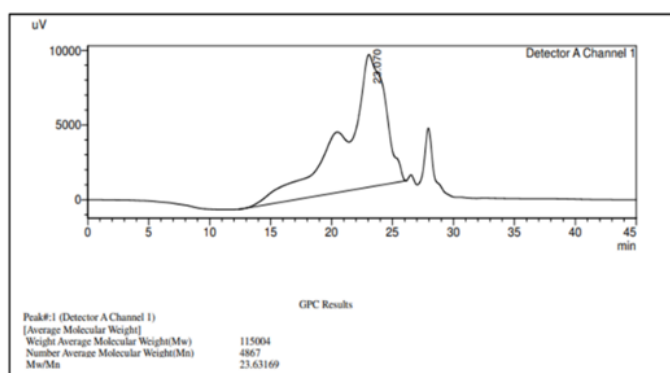
(a)**(b)****(c)****(d)****Figure 5**

TGA thermogram of PSMT1 **(a)** and PSMT2 **(b)**. DSC thermogram of PSMT1 **(c)** and PSMT2 **(d)**

(a)



(b)



(c)

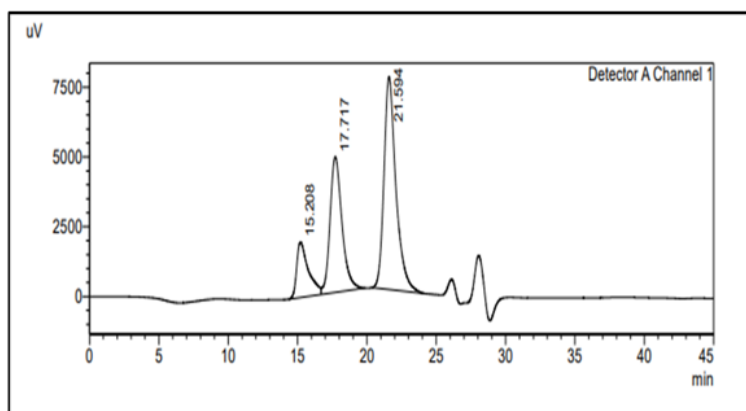
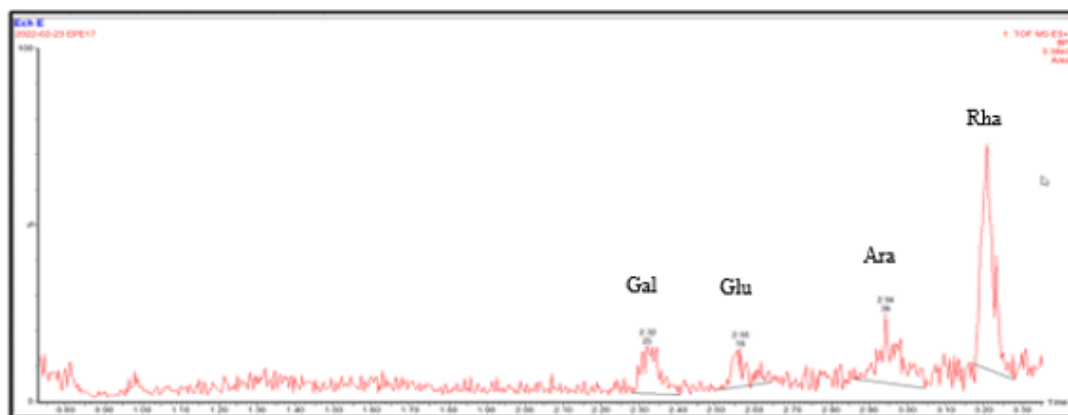
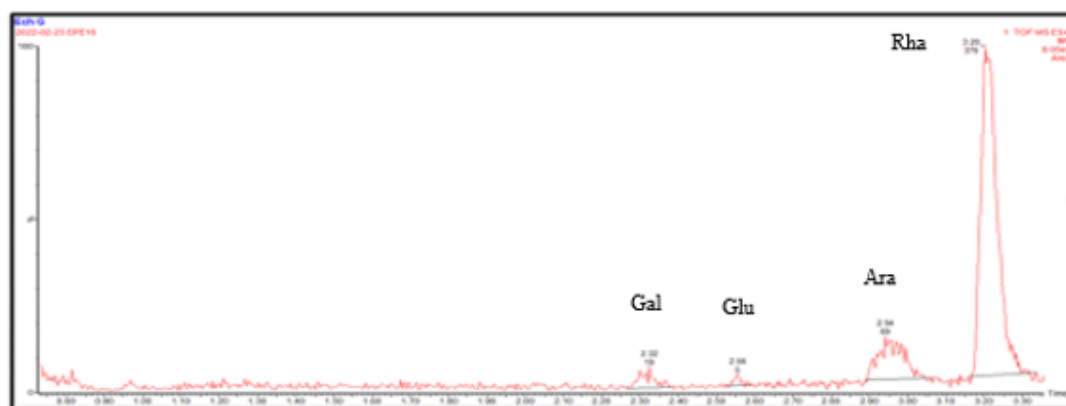


Figure 6

The GPC chromatogram of PSMT1 **(a)**, PSMT2 **(b)** and standards **(c)**



(b)



(c)

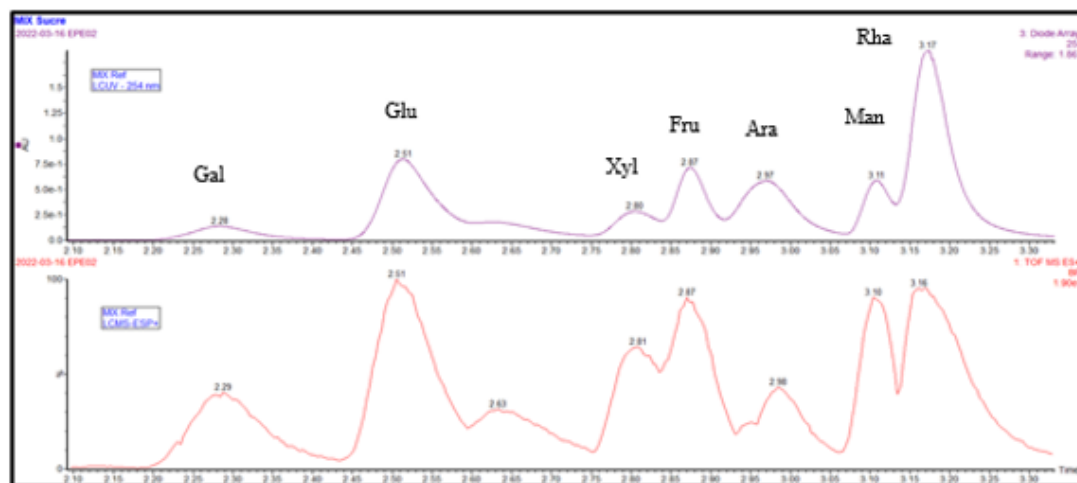
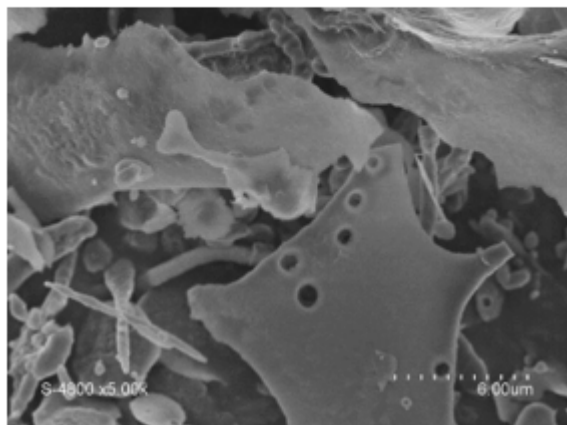


Figure 7

Monosaccharides composition of PSMT1 (a) and PSMT2 (b). HPLC-MS analysis of standard monosaccharides (c)

(a)



(b)

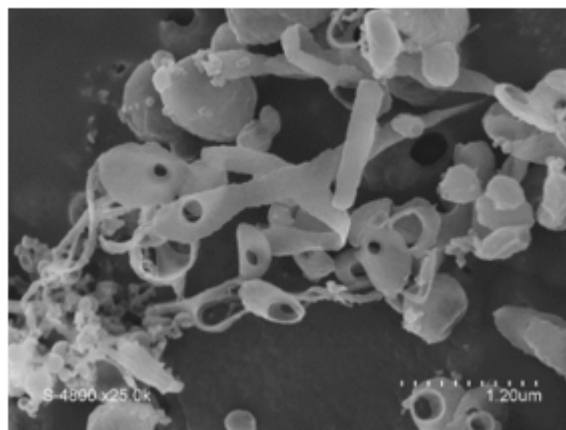
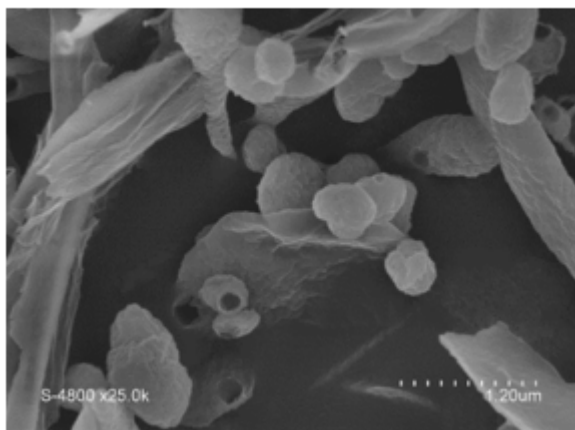
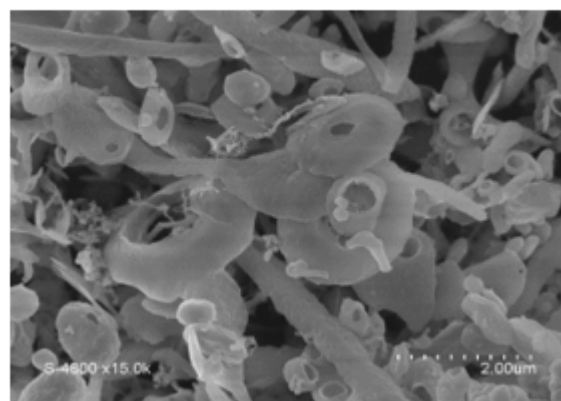
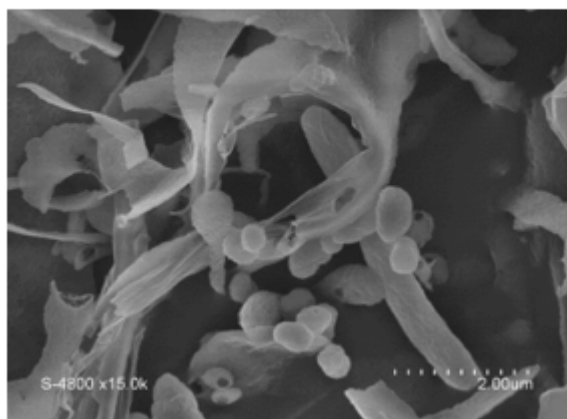
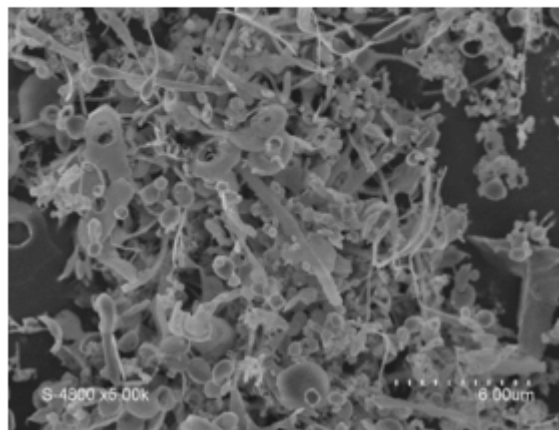


Figure 8

Morphological examination of PSMT1 (a) and PSMT2 (b) using Scanning electron microscopy

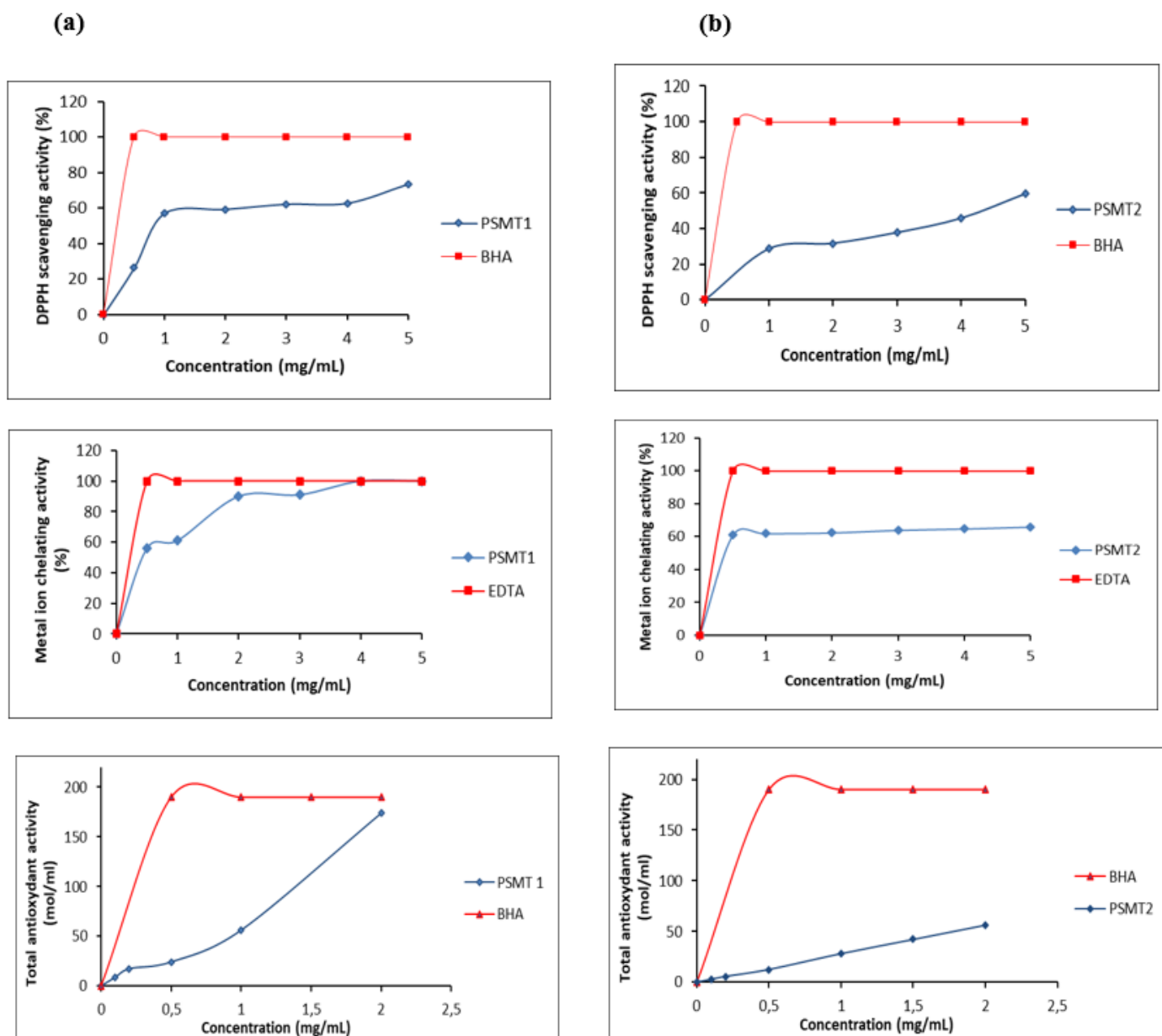


Figure 9

Antioxidant activities of PSMT1 and PSMT2, DPPH radical-scavenging activity of PSMT1 (a) and PSMT2 (b), Metal ion-chelating activity of PSMT1 (c) and PSMT2 (d), (e) Total antioxidant activity of PSMT1 and PSMT2 (f)

Supplementary Files

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