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Phenolic compounds from an Algerian medicinal plant (*Pallenis spinosa*): simulated gastrointestinal digestion, characterization, biological and enzymatic activities

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Abstract

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DOI: 10.1039/D0FO01764G

Pallenis spinosa is a medicinal plant which is used in folk medicine as curative or preventive remedies for various diseases. Individual phenolic compounds from the methanolic extracts of its flowers, leaves and stem were determined by high performance liquid chromatography method (HPLC) and total phenolic contents (TPC) was evaluated by Folin-Ciocalteu assay. The stability and bioactivity (antioxidant activity, micellar cholesterol solubility, α -amylase, and angiotensin converting enzymes (ACE) inhibitory effects) of these extracts in the gastrointestinal environment was determined before and after their protection hydroxypropylmethylcellulose (HPMC) capsules. HPLC analysis revealed the presence of thirteen phenolic compounds with nine flavonoids and four phenolic acids. Except for Kaempferol, the twelve other compounds have not previously detected in the aerial part of the studied plant. Quantification of phenolics by HPLC and Folin Ciocalteu methods revealed that the greatest TPC was detected in the flower extracts (104.31 ± 0.80 and 145.73 ± 0.48 mg EGA/g of extract, respectively). Leaves extracts displayed the best antioxidant capacity against the two tested radicals DPPH and ABTS ($IC_{50}=1.24 \pm 0.03$ and 0.94 ± 0.02 mg/mL, respectively), FRAP assay ($IC_{50}=0.50 \pm 0.02$ mg/mL), α -amylase inhibitory ($IC_{50}=1.25 \pm 0.00$ mg/mL) and angiotensin activity with an inhibitory percent of $30.10 \pm 0.12\%$. The best activity shown by stem extracts was against micellar cholesterol solubility ($67.57 \pm 0.00\%$). A strong decrease in TPC and their bioactivity were observed after the gastrointestinal digestion (GID) in non encapsulated extracts. These results showed that *P. spinosa* is a good source of phenolic compounds and the GID affects significantly their composition, content and bioactivity.

Keywords: *Pallenis spinosa*; phenolic compounds; *in vitro* gastro-intestinal digestion; antioxidant activity; enzymatic inhibitory effects; micellar cholesterol solubility.

1. Introduction

Pallenis spinosa (L.) Cass. is a medicinal plant belonging to the Asteraceae family ¹, distributed throughout the Mediterranean region ^{2,3}. They are widely used in folk medicine as curative or a preventive remedy for various diseases. In fact, leaves, flowers and stems are used as antidiabetics ⁴ and for their anti-inflammatory effects ⁵, in the treatment of gastralgia, circulatory, respiratory and oral infections ⁶. In the western region of Algeria, this plant is used to treat skin diseases (eczema), in addition, its flowers are used as an important medication for the treatment of vulnerability as well as bruises and injuries ⁷. The whole plant of *P. Spinosa* is also used as insecticide in the Mediterranean basin ⁸.

The presence of bioactive compounds including flavonoids ⁹, sesquiterpenes ^{10, 11}, essential oils ¹² and carotenoids ¹³ in the *P. spinosa* extracts has been reported in the literature. Phenolics are the main active compounds synthesized by plant that have a beneficial effect on human health ^{14, 15}. Indeed, these compounds are known for their multiple biological effects, including antioxidant effect, by acting as free radical scavengers or prevent their formation, and decreasing the neurodegenerative disorders that occur in Parkinson's and Alzheimer's diseases, due to their ability to reduce inflammation and oxidative stress ^{16, 17}. For their effects on human health, research on bioactive compounds (polyphenols, carotenoids, lipids, sugars, proteins, vitamins and minerals) derived from plants have been focused on their application in the food, pharmaceutical and cosmetics industries. However, their effectiveness depends closely on their stability and bioavailability in the environment in which they are used ^{17, 18}. Indeed, only a small proportion are available after their oral administration, due to the various factors such as, instability under gastrointestinal tract conditions (pH, enzymes, presence of other nutrients) ¹⁹, and low permeability and/or solubility within the gut, limit the release of these compounds within the body and consequentially decrease the potential health benefits ¹⁸. Therefore, *in vitro* GID procedure which is mimicking the physio-chemical and the

biochemical factors of the upper gastrointestinal tract were widely used^{14, 20}. In addition, it was shown previously that the encapsulation of polyphenols can prevent their degradation and improving their bioavailability^{17, 18}. This approach enhance product stabilization by minimizing interactions with other dietary ingredients to improve bioavailability, or to achieve controlled polyphenol release during digestion^{19, 21}. In addition, encapsulation methods enhance the bioactivity by protecting the polyphenols like condensed tannins, quercetin and phenolic acids from the hostile conditions of the upper gastrointestinal tract such as pH, temperature, and degree of polymerization^{19, 22}.

To the best of our knowledge and among the reports that we have consulted, there is only one study on the identification of phenolics from the aerial parts of *P. spinosa* plant, where derivatives of four flavonoids have been reported, four studies on its other bioactive substances (sesquiterpeens, essential oils, carotenoids)⁹⁻¹³ and only two on its antioxidant activity and phenolic contents (Folin assay)^{23, 24}. However, *P. spinosa* is cited much more (seven reports) in ethnobotanical studies^{3, 4, 7, 25-28}. Even more, no studies have been conducted on the its other biological activities.

As the proposed plant is used as an herbal tea for oral administration (as decoction and infusion)^{25, 26}, their bioactive compounds (phenolics) are subjected to the gastrointestinal environment. Based on these evidences, the objectives of the present study are: (1) the identification of the phenolic compounds of the aerial parts of *P. spinosa* extracts and the evaluation of their antioxidant activity and their effect on two enzymes involved in metabolic diseases (α -amylase, angiotensin converting enzyme) as well as on micellar solubility of cholesterol; (2) the effect of *in vitro* GID on the phenolic compounds and their activities before and after their protection in HPMC capsules.

2. Materials and methods

2.1. Chemicals and reagents

101 Methanol, ethanol, glacial acetic acid and water were obtained from Merk (Darmstadt,
102 Germany). Sodium carbonate anhydrous (NaCO_3), sodium acetate anhydrous (CH_3COONa),
103 Sodium tetraborate decahydrate ($\text{Na}_2\text{BuO}_7 \cdot 10\text{H}_2\text{O}$), potassium peroxodisulfate ($\text{K}_2\text{S}_2\text{O}_8$), iron
104 (III) chloride anhydrous (FeCl_3), ammonium carbonate $(\text{NH}_4)_2\text{CO}_3$, potassium chloride (KCl),
105 sodium chloride (NaCl), sodium hydrogen carbonate (NaHCO_3), magnesium chloride (MgCl_2
106 $6\text{H}_2\text{O}$), potassium phosphate monobasic (KH_2PO_4) and calcium chloride dried granular
107 (CaCl_2) were purchased from Carlo Erba Reagenti (Rodano, Milano, Italy). Folin-Ciocalteu,
108 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzo-thiazoline-6-sulfonic
109 acid) diammonium salt (ABTS) and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-
110 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), α -amylase from porcine pancreas,
111 bile salts, pepsin from porcine pancreas, angiotensin converting enzymes (ACE), hippuryl-L-
112 histidyl-L-leucine (HHL), were purchased from Sigma Aldrich (st. Louis, Missouri, USA).
113 Standards of ferulic acid, gallic acid, chlorogenic acid, kaempferol, (-) epicatechin, (-)
114 gallo catechingallate, phloridzindihydrate, neochlorogenic acid, ($\pm$)-naringenin, (+)-catechin
115 hydrate, rutin hydrate, quercetin-3-glucoside, kaemferol-3-glucoside (astragalin), were
116 acquired from Sigma Aldrich (Milan, Italy). All solvents were of HPLC-grade. Individual
117 standard solutions of phenolic compounds were prepared at 1 mg/ml in methanol. All
118 solutions were stored at -20°C until use.

119 2.2. Preparation of samples and phenolic extracts

120 2.2.1. Preparation of samples

121 The aerial parts of *P. spinosa* have been harvested from the same field, at the full flowering
122 period during the month of May 2018 from Chabet-el-Ameur, Boumerdes region (central
123 northern of Algeria). Plant materials were washed with water to remove dust and then dried at
124 room temperature for three weeks. After that they were ground by using an electrical grinder
125 (IKA model A₁₁ Basic) in order to obtain a homogeneous fine powders. The powders were

passed through standard sieve (diameter: 125 μm) and stored in airtight bottle under darkness until use.

2.2.2. Extraction of phenolics

Phenolics compounds were extracted using the method reported by Tenore, et al.²⁹ with some modifications. Briefly, 1 g of dried powder, of each part of *P. spinosa* plant (flower, leave and stem), was added to 20 mL of extraction solvent 80% methanol containing 1% formic acid (v/v); the mixture was vortexed for 2 min, sonicated for 10 min followed by stirring at medium speed for further 10 min. This procedure was repeated once more using the same solvent. After centrifugation (HermleLabortechnik GmbH, Model Z206A, Wehingen, Germany) at 6000 rpm for 10 min, the two obtained supernatants were combined. Subsequently, the supernatant was filtered under pressure by using Buchner funnel through Whatman paper filter 43-48 μm and the methanol solvent evaporated using a rotary evaporator, then the extracts were frozen at -80°C and lyophilized.

2.2.3. Encapsulation of extracts

Capsules (HPMC) were filled with 1 g powder of crude lyophilized extract of each aerial part. After that, they were preserved for *in vitro* simulated GID.

2.2.4. *In vitro* gastrointestinal digestion of extracts

The *in vitro* GID procedure developed by Minekus, et al.³⁰, was selected as a starting part, with minor modifications. This method involves three steps (salivary, gastric and intestinal). In short, 1 g of crude and coating extracts was dissolved in 700 μL of simulated salivary fluid (SSF) (Table1), then 100 μL of α -amylase (1500 U/mL) solution, 5 μL CaCl_2 (H_2O) and 195 μL distilled water were added. The mixtures were incubated at 37°C for 2 min in a continuous stirring. After oral digestion, they were mixed with 1.5 mL of SGF (Simulated Gastric Fluid)

(Table 1) solution, 320 μL of pepsin solution (25000 U/mL), 1 μL of 0.3 M $\text{CaCl}_2(\text{H}_2\text{O})_2$ and 139 μL of distilled water. Then, the pH was adjusted to 3.0 using 6 M HCl solution, the mixtures were maintained in the oven at 37 $^\circ\text{C}$ under stirring for 2 h. Subsequently, the obtained suspensions were mixed with 2.2 mL of SDF (Simulated Duodenal Fluid) solution (Table 1), 1.0 mL of a pancreatin enzyme solution (800 U/mL), 0.5 mL of bile salts (160 mM), 8.0 mL of 0.3 M $\text{CaCl}_2(\text{H}_2\text{O})_2$, and 262 μL of water. The pH was adjusted to 7.0 using 1 M NaOH solution, and then kept at 37 $^\circ\text{C}$ for 2 h. Exceeded the incubation period; the mixtures were centrifuged at 6000 rpm for 10 min at 25 $^\circ\text{C}$. The supernatant was collected and filtered under pressure by using Buchner funnel through Whatman paper filters 43-48 μm . Finally the filtered extracts were frozen at -80°C and then lyophilized.

2.3. Evaluation of antioxidant activity

The antioxidant activity of the crudes extracts before and after digestion (encapsulated and no encapsulated) was carried out according to three methods: the reduction of iron FRAP and scavenging of the free radicals DPPH and ABTS, using UV/VIS spectrophotometer (PG instruments Limited- Model T 80+). The results were expressed in terms of IC₅₀ (mg/mL) values. Negative control was prepared ((MeOH: HCOOH 9:1(v/v))). Before analysis, all samples were diluted with methanol (MeOH:HCOOH 9:1(v/v)) and filtered by using membrane filtration (0.2 μm).

2.3.1. Scavenging ability of DPPH

The scavenging activity of the DPPH (2,2-diphenylpicrylhydrazyl) radical was carried out according to Shanmuganathan and Karthikeyan ³¹. 1 mL of 0.002% (w/v) DPPH methanol solution was added to 200 μL of extract (at different concentrations). After stirring, the tubes were placed in the dark at room temperature during 10 min. The absorbance of the samples was recorded by UV-spectrophotometer at 517 nm wavelength. Finally, the percentage of antioxidant activity was determined according to the following equation:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where: A_{sample} was the absorbance of extract, $A_{control}$ the absorbance of the DPPH solution. Results were expressed as IC50 value (mg/mL) which is determined as the concentration of extracts causing inhibition of 50% of DPPH radical, a lower IC50 value corresponds to a higher antioxidant activity.

2.3.2. Scavenging ability of ABTS radical

For testing the antioxidant activity of extracts against ABTS radical, the modified method of Re, et al.³² was used. As described below, after incubation of the mixture (7 mM ABTS stock solution and 2.45 mM potassium persulfate for 4-16 h), radical cation (ABTS^{•+}) was prepared and diluted in ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. Then 1 mL this solution was added to 100 μ L of the tested samples and vortexed thoroughly. The reactive mixture was incubated in the dark at room temperature for 2.5 min and the absorbance was recorded at 734 nm. The percentage of antioxidant activity was determined according to the following equation:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where: A_{sample} was the absorbance of extract, $A_{control}$ the absorbance of the DPPH solution. Results were expressed as IC50 value (mg/mL) which is determined as the concentration of extracts causing inhibition of 50% of ABTS radical.

2.3.3. Ferric reducing power

Reducing power was assessed by FRAP assay according to Thaipong, et al.³³ with some modifications. Stock solutions were prepared (included 300 mM acetate buffer of pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl₃ solution in distilled water). The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution,

and 2.5 mL of FeCl₃ solution. In a reaction tube, 150 µL of extracts were allowed to react with 2850 µL of the FRAP solution for 4 min at room temperature in the dark. The absorbance was determined at 593 nm. Results were expressed as IC₅₀ value (mg/mL).

2.4. *In vitro* micellar solubility of cholesterol

Micellar solubility of cholesterol of all extracts was determined according to the method of Ogawa, et al.³⁴, with some modifications. In brief, a bile salt micellar mixture, containing 2 mM of cholesterol, 2.4 mM of phosphatidylcholine, 6.6 mM of sodium taurocholate salt, 132 mM of NaCl, and 15 mM of sodium phosphate at pH 6.8, was prepared by sonication for 30 min and then incubated overnight at 37°C. Exceeded the preservation time, 100 µL of various extracts were added to the 3 mL of micellar solution and incubated for 1 h at 37 °C. The mixture was centrifuged at 16000 rpm for 20 min and the supernatant collected for the determination of cholesterol using total cholesterol test kits.

2.5. Enzyme inhibitory activities

2.5.1. *In vitro* α-amylase inhibitory assay

The inhibitory effect of the extracts of aerial part of *P. spinosa* before, and after the GID on α-amylase were assayed according to the procedure described previously by Ali, et al.³⁵. In brief, 40 µL of extracts diluted with 160 µL of distilled water was made to react with 200 µL of 4 U/mL of α-amylase enzyme. After 11 min incubation at 37 °C, 400 µL of 1% starch solution was added. After 20 min of incubation, 200 µL of dinitrosalicylic acid reagent (96 mM) was added and kept in boiling water bath for 5 min. After that, the mixture was diluted with 2 mL of distilled water. The absorbance was recorded at 540 nm and the percentage of inhibition of α-amylase was calculated as follows:

$$Inhibition(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

222 Where $Abs_{control}$ and Abs_{sample} are the absorbance at 540 nm for the blank and for the sample, respectively. The same procedures were performed simultaneously for the controls and blank, 223 by replacing 40 μ L of extracts with distilled water. IC_{50} defined as the concentration of 224 extract required to inhibit 50% of the enzyme activity was determined in the presence of 225 various concentrations of all extracts. 226

227 2.5.2. *In vitro* ACE-I inhibitory assay

228 The inhibitory properties of the aerial parts of *P. spinosa* before, and after the GID against 229 ACE- I were assayed using the method described by Luz, et al. ³⁶. A volume of 25 μ L (2 230 mg/mL) of all extracts before, and after the GID were added to 50 μ L of 5 mM hippuryl-L- 231 histidyl-L-leucine (HHL) (5 mM in 0.1 M borate buffer pH 8.3 containing 0.4 M of NaCl) 232 and pre incubated for 10 min at 37 °C. After incubation, the reaction was initiated by adding 233 20 μ L of ACE solution (100 mU/mL) and incubated for 60 min at 37 °C. The enzyme 234 reaction was stopped by adding 100 μ L of 1M of HCl. After that, samples were centrifuged at 235 14 rpm for 10 min at room temperature. The enzymatic activity was calculated by quantifying 236 the released hippuric acid (HA) content in the supernatants at 228 nm against a blank solution. 237 The amount of HA was determinate by reverse-phase HPLC (Jasco MD-4016, Italy) using a 238 column Kinetex C18 (250 $\times$ 4.6 mm, particle size of 5 μ m; Phenomenex, Torrance, CA, USA) 239 and detected using a UV-vis detector at 228 nm. The mobile phases were as follows: phase A, 240 water containing 0.05 % trifluoroacetic acid (TFA), and phase B, acetonitrile containing 0.05 241 % TFA. A linear gradient elution program was applied as follows: 0-8 min, 20-50% B; 8-12 242 min, 50-70% B; 12-14 min, 70-20% B; 14-20 min, 20% B. The column temperature was 30 243 °C, the flow rate was 1 mL/min and the injection volume was 20 μ L, the percentage of 244 enzyme inhibition was calculated by the following equation:

245

$$ACE_{\text{Inhibition activity}}(\%) = \frac{HA_{\text{control}} - HA_{\text{sample}}}{HA_{\text{control}}} \times 100$$

Where HA_{sample} is the measured area at 228 nm in the presence of an inhibitor and HA_{control} is the area of the blank solution.

2.6. Bioaccessibility Index percentage (BI %)

In order to evaluate the effect of the composition of the *P. spinosa* extracts (encapsulated and non encapsulated) on the digestion of the phenolic compounds, the bioaccessibility index (BI) for TPC, antioxidant activity, enzyme inhibitory activity and cholesterol inhibitory were calculated as proposed by Neto, et al.³⁷.

–Phenolic compounds bioaccessibility index (PCBI), which indicates the fraction of bioaccessible phenolic compounds from extracts:

$$PCBI = \frac{PC_a}{PC_b} \times 100$$

Where PCa is the content of phenolic after GID and PCb is the content of phenolic before GID.

–Antioxidant activity bioaccessibility index (AABI), which is indicative of bioaccessibility of antioxidant activity from extracts:

$$AABI = \frac{AA_a}{AA_b} \times 100$$

Where AA_a is the antioxidant activity after *in vitro* GID and AA_b is antioxidant activity originally detected in extracts. *AABI* was calculated individually for each result obtained by each assay used to measure the antioxidant activity (DPPH, ABTS and FRAP).

–Enzyme inhibitory activity bioaccessibility index (*EIABI*), which is indicative of bioaccessibility of enzyme inhibitory activity from extracts:

$$EIABI = \frac{EIA_a}{EIA_b} \times 100$$

Where EIA_a is the enzyme inhibitory activity after *in vitro* GID and EIA_b is enzyme inhibitory activity originally detected in extracts of *P. spinosa*. $EIABI$ was calculated individually for α -amylase and ACE-I inhibitor assays.

– Cholesterol inhibitory bioaccessibility index ($CIBI$), which is indicative of bioaccessibility of cholesterol inhibitory from extracts:

$$CIBI = \frac{CI_a}{CI_b} \times 100$$

Where CI_a is the cholesterol inhibitory after *in vitro* GID and CI_b is cholesterol inhibitory originally detected in extracts.

For each part, the percentage of improvement of bioaccessibility was calculated by the subtraction of the percentage of bioaccessibility index in encapsulated extracts of *P. spinosa* of each assays for that of no encapsulated as follows:

$$\%_{\text{Improvement or reduction of bioaccessibility}} = BIEE - BINEE$$

This measurement represents an estimation of the improvement of bioaccessibility of compounds for absorption by tissues.

2.7. Analysis of phenolics by HPLC

Analytical separation of phenolic compounds was carried out employing a Jasco HPLC system (Tokyo, Japan) consisting of a Jasco RHPLC pump (PU-4180), a Jasco Photo Diode Array Detector (MD-4010), Jasco Intelligent Sampler (AS-2055 Plus), Jasco Column Oven (CO-4061), Jasco Bottle Stand (BS-4000-1), Jasco LC-Net II/ADC interface box and a Chrom NAV Control Center software attached to a computer system. Analytical separation of phenolic compounds was carried out on a Kinetex column C18 (250 × 4.6 mm; 5 µm) (Phenomenex, CA, USA). 100 mg of three types of samples (Before digestion: crude lyophilized extracts, after digestion uncoated and after digestion coated extracts) of each aerial part of *P. spinosa* dissolved in 2 mL 99: 01 methanol: HCOOH and filtered through

0.20 μm syringe filter (Phenomenex). The analysis was carried out at flow rate, injection volume of the samples and column temperature were 1 mL/min, 20 μL and 30° C, respectively. Elution through the column with a gradient mobile phase consisting of A (redistilled water: formic acid in ratio 98:2) and B (redistilled water: ACN: formic acid in ratio 49.75:49.75:0.5) and the solvent gradient was programmed as follows: 10% B at 0 min, increasing from 0-50 min to 55%, 50-65 min 55-95% B, 65-70 min 10% B. The corresponding phenolics chromatogram was recorded at a wavelength of 280 nm. Identification of phenolics compounds were confirmed by comparing their retention time with that of the standards. The peak area of standard curves of the corresponding phenolics at a wavelength of 280 nm was used for their quantification.

2.8. Quantification of phenolics by colorimetric method

The quantification of total phenolics was made by the methodology of Singleton and Rossi³⁸. The TPC was calculated by using a standard curve made with gallic acid and expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g Ext). Crude extracts before and after digestion (coated and uncoated) were tested.

2.9. Statistical analysis

Statistical analysis were performed with the one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test in order to assess the significant differences between a pair of means using JMP 10 software from SAS Institute Inc (Cary, NC, USA). All experimental results are expressed as mean $\pm$ standard deviation (SD) of three replications. The level of ($p \leq 0.05$) was considered statistically significant.

3. Results

3.1. Extraction yield

The average yield of the lyophilized material of flowers, leaves and stems extracts of *P. spinosa* were about 26.6 %; 20.4 % and 14.8% (w: w), respectively.

3.2. Determination of phenolic compounds by HPLC

HPLC chromatograms of the representative extract (flower extracts) are illustrated in Figure 1 and the chromatographic data of the phenolic compounds identified in different parts of the plant (Flowers, leaves and stem) are summarized in Table 2.

Compounds identified before GID: In total thirteen compound were detected, they can be classified into two groups corresponding to flavonoids and phenolic acids. The first group (flavonoids) being the predominant one and contains nine compounds (epicatechin, epigallocatechin, phlorizin, kaempferol, catechin, rutin, naringenin, apigenin and apigenin glucosid) while the second group contains four phenolic acids (Gallic, neochlorogenic, chlorogenic and ferulic acids). With the exception for kaempferol which was already reported in the aerial part of the studied plant ³⁹, all other compounds have not previously detected. Indeed, catechin, the major phenolic compound in *P. spinosa* extracts with highest content, was detected at a concentration of 53.31, 28.92 and 18.57mg/g for flower, leaves and stems extracts, respectively. Rutin and naringenine, second major compounds, were detected only in flowers and leaves extract (16.30 and 16.25 mg/g, respectively), gallic acid was detected at a concentration of 12.18, 7.84 and 5.56 mg/g in flower, leaves and stems extracts. Kaempferol, which was already reported in the literature, was detected only in flower extracts at a concentration of 8.21 mg/g, ferulic acid was detected in stem extract with a concentration of 4.69 mg/g and apigenin glucosid with a concentration of 3.97 and 3.90 mg/g in flower and stem extracts, respectively (Table.2). For other compounds including epicatechin, epigallocatechin, phlorizin, apigenin, neochlorogenic acid and chlorogenic acid, they were detected in all extracts but at low concentration.

Compounds identified after GID: a strong decrease in gallic acid content was observed in stem extract (71.64%), followed by that of flowers (64.23%) and leaves (50.03%). Furthermore, other compounds such as epicatechin, epigallocatechin, phlorizin, kaempferol, catechin, rutin, naringenin, apigenin, neochlorogenicacid, apigenin glucosid were significantly reduced ($p \leq 0.05$).

Results of PCBI were evaluated by using a simulated GID. The use of HPMC capsules allowed us to know the effect of GID conditions on the bioactive compounds. Results of the PCBI of individual phenolics from different extracts were presented in Table 3. Compared to the no encapsulated extracts, PCBI was increased after GID in the encapsulated samples by 6.93%, 7.84% and 7.75% in the flowers, leaves and stems samples, respectively. The content of neochlorogenic acid, epigallocatechin, catechin, kaempferol, naringenin, epicatechin and rutin were also increased (neochlorogenic acid increased by 41.82%, 29.93% and 20.23%, respectively, in flowers, stems and leaves. Also, content of naringenin and epigallocatechin increased by 48.13% and 15.61, respectively, in flowers and leaves; whereas rutin and kaempferol were increased by approximately 14.15% and 19.59% in stem extract. We detected also an increase in catechin and epicatechin levels by 12.59%, 13.07% in leaves and 5.23%, 62.79% in stem, respectively, while the catechin level increased by 8.63% in flowers.

3.3. Total Phenolic content and antioxidant activity

3.3.1. Total phenolic content

The results of TPC in the three extracts of *P. spinosa* before and after *in vitro* GID are reported in table 4. Extracts from flowers contains the higher amount of TPC compared to the leaves and stems extracts (145.73 $\pm$ 0.48, 115.58 $\pm$ 0.06 and 83.04 $\pm$ 0.81 mg GAE/g of extract, respectively).

Results of PCBI show that the lower contents are observed in no encapsulated samples submitted to GID. The PCBI was reduced with a percentage of 10.09%, 8.69% and 3.85% compared to encapsulated extracts for leaves, flowers, and stems, respectively.

3.3.2. Antioxidant activity

The results of antioxidant activity expressed as IC_{50} (mg/mL) values are shown in Table 4. Extracts from flowers, leaves stems showed a good activity against DPPH and ABTS radicals ($IC_{50} = 1.24 \pm 0.03$, 1.68 ± 0.05 and 1.68 ± 0.05 , respectively and 0.94 ± 0.02 , 1.27 ± 0.02 and 1.09 ± 0.01 , respectively), they showed also an interesting reducing power ($IC_{50} = 0.50 \pm 0.02$, 0.91 ± 0.03 , 0.69 ± 0.02 , respectively). The antioxidant effect of all extracts is compared to those of two positive controls (BHT and Trolox). *P. spinosa* extracts shows for both DPPH and FRAP assays, higher activity than that exhibited by BHT but less than that of Trolox. As for their scavenging effect against ABTS radical, all extracts have displayed lower activity.

Results of AABI presented in table 4, shows the negative effect of the GID on the antioxidant activity of all extracts. Regarding the DPPH radical scavenging activity of flowers, leaves and stems extracts, their IC_{50} (mg/mL) values were about 3.75 ± 0.01 ; 5.69 ± 0.20 and 6.46 ± 0.04 for digested encapsulated samples, respectively, and 4.43 ± 0.11 ; 13.24 ± 1.16 and 7.16 ± 0.08 for digested no encapsulated extracts, respectively, whereas those of the undigested samples, they were about 1.24 ± 0.03 ; 1.68 ± 0.05 and 1.46 ± 0.03 , respectively. The same results were observed for the ABTS radical, and the highest activity belongs to the undigested extracts, followed by the encapsulated digested samples and non encapsulated digested samples. Concerning the reducing power (FRAP assay), similar results about the effectiveness of different extracts were obtained compared to the two previous tests; all extracts (flowers, leaves and stems) have exhibited significant reduction potential. Indeed, a strong increase of bioaccessibility of the scavenging effect of ABTS radical is observed in the

encapsulated extracts (40.22%, 29.34% and 14.48%) followed by that of DPPH[•] scavenging activity (28.13%, 20.04% and 11.44%) and that of FRAP (11.56%, 9.30% and 4.67%) for flowers, leaves and stems, respectively. The results of this study confirm the negative effect of GID on the bioactivity of extracts of *P. spinosa*.

3.4. Inhibitory effect of *P. spinosa* extracts against α -amylase

The inhibition of the α -amylase, enzyme that generates dietary glucoses from carbohydrate, is an important indicator in assessing the efficiency of natural compounds in suppressing hyperglycemia. Therefore, this study examined the effect of *P. spinosa* extracts on the activity of this enzyme. The result shows that all extracts displayed a strong activity, at 10 mg/mL, with high inhibition percent ranged between 68.54 ± 1.32 and 91.21 ± 0.40 . The IC₅₀ value (mg/mL) of different extracts were calculated, flowers and stems extracts shows higher activity (1.25 ± 0.00 and 1.89 ± 0.05 , respectively) compared to that of leaves (3.55 ± 0.09). As we can see (Table 5) the inhibitory effect on α -amylase activity of extracts of *P. spinosa* is lower than the positive control (acarbose) with an IC₅₀ value of 0.04 ± 0.00 . The enzyme inhibitory activity bioaccessibility index (EIABI) were also determined, the activity of α -amylase was reduced after GID (8.96%, 5.73%, and 5.28% for flowers, stems and leaves extracts, respectively).

3.5. Inhibitory effects against angiotensin converting enzyme

Nowadays, ACE inhibitor drugs (Captopril, Lisinopril, Enapril) are widely used, to control the renin angiotensin aldosterone system which causes hypertension. Notwithstanding, these drugs have shown satisfactory antihypertensive effects. So, natural source are an alternative to use as a complement to synthetic ACE inhibitors. As illustrated in Table 6, all extracts showed an ACE inhibition percent less than 50% at 2 mg/mL.

Regarding the EIABI (Table 6), the comparison between the encapsulated and no encapsulated samples after GID showed a drastic reduction of activity ranged between 71.66 to 36.92% for the different typologies of extracts.

3.6. Inhibitory effects on micellar solubility of cholesterol

The results in table 6 describe the inhibition percentage of different extracts (undigested and digested flowers, leaves, and stems) of *P. spinosa* on cholesterol micellization. As for inhibitory enzyme assays (α -amylase and ACE), the most active extracts were found before GID. At 0.6 mg/mL, extract from stems, flowers and leaves have exhibited a strong inhibitory activity on cholesterol micellization ($67.57 \pm 0.00\%$, $63.51 \pm 1.91\%$ and $60.81 \pm 5.73\%$ respectively).

The IABI were presented in table 6, Similarly to other biological activities, the lower cholesterol micellization inhibitory activity were found in the no encapsulated samples after GID with 39.19%, 36.49% and 8.11% in stems, flowers and leaves extracts, respectively. Whereas, the encapsulation greatly improved the inhibitory activity of cholesterol micellization leading an improvement ranging from 23.40 to 53.99% of cholesterol micellization compared to no encapsulated extracts.

4. Discussion

Pallenis spinosa is not well known outside the Mediterranean region and few studies about its chemical composition and biological activities are available in the literature. As mentioned above, most of data published on this plant are in ethnobotanical reports, hence the interest to investigate in the phytochemistry and bioactivity of its compounds, which could help to increase the acknowledgment about it and confirm its traditional uses by the Mediterranean population. The results obtained by Folin Ciocalteu method shows that extracts before GID contains higher TPC (Table 4). The comparison of our results with those reported

in the aqueous and methanolic extracts of the studied plants conducted by Khettaf, Belloula and Dridi ²⁴ (71.59 $\pm$ 4.44 and 125.71 $\pm$ 59.00 mg GAE/g extracts, respectively) and Alali, Tawaha, El-Elimat, Syouf, El-Fayad, Abulaila, Nielsen, Wheaton, Falkinham and Oberlies ²³ (13.5 mg GAE/g dry weight and 10.5 mg GAE/g dry weight, respectively) is not possible as these values correspond to the content of the whole aerial part and ours correspond to the separate aerial parts.

Concerning the results of antioxidant activity of all extracts before GID, they have exhibited an interesting scavenging effect on DPPH radical, expressed in term of IC50 value (mg/mL), but less than those reported by Khettaf, Belloula and Dridi ²⁴, who found an IC50 value of 49.33 $\pm$ 3.21 μ g/mL in the methanolic extract, which is lower than that found in the present study. In the other hand, Alali, Tawaha, El-Elimat, Syouf, El-Fayad, Abulaila, Nielsen, Wheaton, Falkinham and Oberlies ²³ have evaluated the DPPH scavenging effect of methanolic extract of *P. Spinosa*, but we cannot compare their results with those obtained in the present study because they have expressed them in term of Trolox equivalents/g of dry weight, in contrary, our results are expressed in term of IC50 value (mg/mL).

Pallenis spinosa has been reported for its antidiabetic effect, in this study we have tried to evaluate the effect of extracts from its aerial parts (flowers, leaves and stem) on α -amylase, an important indicator in assessing the efficiency of natural compounds in suppressing hyperglycemia. The results obtained are interesting, as long as the IC50 values (mg/mL) are relatively low (1.25 $\pm$ 0.00; 3.55 $\pm$ 0.09 and 1.89 $\pm$ 0.05, respectively). Previous findings revealed that extracts from plants which are rich in phenolic compounds (phenolic acids and flavonoids) efficiently inhibited *in vitro* α -amylase enzyme ⁴⁰⁻⁴², and Kaempferol, catechin (major compound) and epicatechin may be involved in this activity ^{16, 43}.

Concerning the inhibition of α -amylase enzyme mechanism, previous study have reported that this inhibition results from polyphenol-enzyme binding interactions ⁴⁰⁻⁴². Indeed,

the interaction between proteins and polyphenols is obvious, it is used in the detection and quantification of polyphenols in the colorimetric assays, such as the determination of tannin contents in plant extracts (fruits, vegetables and medicinal plants) using as standard protein bovine serum albumin (BSA). The phenolic compounds react by establishing a covalent bond with proteins enzymes thus inducing the blocking of the side chains of the amino acids ⁴¹ or by conformational changes of the enzyme molecule (reversible or irreversible inhibition) ⁴². The direct evidence for spatial structural changes has been suggested as the degree alterations of secondary structures of the enzyme ⁴⁴.

The basic factor of cardiovascular diseases is atherosclerosis, which is due largely to an increase in the activity of the acetylcholine esterase enzyme (ACE) ⁴⁵, a widely distributed zinc-dependent metalloproteinase, that converts angiotensin I to angiotensin II ⁴⁶, its catalytic active site has zinc ion, which is essential for enzyme activity ⁴⁷. One of the promising bio- functionality reported for phenolic compounds is their inhibitory effect on ACE ⁴⁸. The effect of plant extracts against this enzyme may be attributed to the presence of flavonoids mainly flavonols such as kaempferol and rutin (quercetin derivatives) ⁴⁵ or by their synergism ⁴⁶. Results of the HPLC analysis indicates the presence of these phenolics in the aerial plant of *P. spinosa* extracts, with flavonoids as major compounds).

One of the principal step in the absorption of dietary cholesterol is micellar solubilization ⁴⁹, involving secreted bile which consists mainly of phosphatidylcholine and bile salts ⁵⁰. Indeed, the reduction of cholesterol absorption by reducing the solubility of cholesterol micellization in the intestinal lumen is a new target site of intervention for the treatment of hyperlipidemia and obesity ⁴⁹. Various plant extracts have been reported to inhibit cholesterol micellization. Aissata, Wenping, Lamine, Barra and Sidikiba ⁴⁹, Narai-Kanayama, Saruwatari, Mori and Nakayama ⁵⁰, have suggested that phenolics from tea (tea catechins) decrease the micellar solubility of cholesterol and the effect induced by catechins

with a galloyl moiety can be a major cause of the inhibition of cholesterol absorption ⁵¹. In the other hand, Ontawong, et al. ⁵² have reported that coffee fruit extracts rich in polyphenols showed a lipid lowering effect. In their study, Aissata, Wenping, Lamine, Barra and Sidikiba ⁴⁹ showed that edible plant extracts (Dendrobium Nobile, Hawthorn, Wolfberry) have the ability to delay digestion and fat absorption through gastrointestinal mechanisms inhibition of cholesterol micellization. Likewise, Ontawong, Duangjai, Muanprasat, Pasachan, Pongchaidecha, Amornlerdpison and Srimaroeng ⁵² have reported that *Spirogyra neglecta*, edible freshwater macroalga, extract could inhibit cholesterol absorption and Chatatikun and Kwanhian ⁵³ indicated that Nipa palm vinegar delay the digestion of a high-fat diet and absorption through small intestine through the inhibition of solubility of cholesterol micelles. It has been previously reported that gallic acid, catechin, and epicatechin from grape seeds extract ⁵³ and epigallocatechin from tea extract ⁵⁴ inhibit the solubility of cholesterol micelles by results in delaying the absorption of cholesterol. Also gallic acid, isoquercetin, quercetin, catechin, and rutin from nipa palm vinegar have been reported as main active compounds with possible cholesterol-lowering effects ⁵³. In the current study, the inhibitory effect of different parts of *P. spinosa* on cholesterol solubility may be due to the action of their phenolic contents. As we can see (Table 2), it could be hypothesized that phenolic compounds including gallic acid, catechin, and rutin from *P. spinosa* extracts may be the main active compounds with possible cholesterol-lowering effects. Several reports support that phenolic compounds in the herbal medicines have the ability to inhibit cholesterol micellization by diverse mechanisms. Su, et al. ⁵⁵, Ontawong, Duangjai, Muanprasat, Pasachan, Pongchaidecha, Amornlerdpison and Srimaroeng ⁵² and Duangjai, et al. ⁵⁶ reported that phenolic extracts decreased the *in vitro* micellar solubility of cholesterol into micelles by the precipitation of the complex extracts added-cholesterol leading to the increase of the average size of the formed particles and elimination of cholesterol from the micelles. In the

other hand, Narai-Kanayama, Saruwatari, Mori and Nakayama ⁵⁰, have report that tea catechins bind strongly to the phosphatidylcholine, containing small vesicles, to modify the micellar structures thus leading to the decrease in the micellar solubility of cholesterol. Ogawa, Hirose, Nagaoka and Yanase ⁵⁴ assume that EGCg reduce the solubility of phosphatidylcholine and cholesterol in micellar solutions and lead to their elimination from the micelles by its interaction with taurocholic acids. In addition the study published by Duangjai, et al. ⁵⁷, mentioned that phenolic compounds could inhibit cholesterol absorption by modifying the size and solubility of cholesterol micelles with involvement of the binding effect of bile acids. In contrast, Kobayashi and Ikeda ⁵¹, suggested that tea polyphenols decreases the micellar solubility of cholesterol via specific interaction with phosphatidylcholine.

Preserving human health and well-being is closely related to the digestion of food, which is source of energy and necessary components, macro- and microelements. The form of the nutritional components released during digestion depends on both initial food properties as well as transformations during the digestive mechanistic and chemical processes ¹⁹. A strong decrease in TPC was observed after the GID, this finding is in agreement with the results of many studies about the effect of GID on the bioavailability of phenolic compounds ^{18, 20, 43}. All of these studies reported a significant reduction in the TPC after GID, which might be associated with the missing of a considerable amount in the insoluble fraction of intestinal digested phenolics ⁵⁸. This lost could be explained by the transition from the acidic gastric to the alkaline intestinal environment, which induce a structural changes in phenolics by the ionization of the hydroxyl groups affected by the pH changes ⁵⁹. Current data suggest that the biological activity of polyphenols is closely related to the pH of the experiment environment^{14, 19}.

Regarding the effect of GID on the antioxidant activity, all samples revealed significant decrease on their activity (DPPH and ABTS radical scavenging activity, FRAP) compared to the extract before GID (Table 4). These results are in accordance with those reported in the literature. Burgos-Edwards, et al.⁶⁰ in their study about qualitative and quantitative changes in polyphenol composition and bioactivity of *Ribes magellanicum* and *R. punctatum*, after *in vitro* GID, have reported that both extracts have shown lower antioxidant activity. Several other researches have reported the decrease of antioxidant activity of phenolic extracts after GID^{43, 61-63}.

The GID had a negative effect on the activity of the three extracts against α -amylase enzyme as shown in Table 5. This is in agreement with earlier reports which reported that the activity of plant extracts towards α -amylase decreased after GID^{15, 43, 61}.

The protective effect of the encapsulation procedure is also confirmed in the present study. After been orally consumed, the chemical structure and the bioactivity of components are changed in the intestinal metabolism¹⁹. Nonetheless, an effective strategy to preserve the health beneficial properties of natural sources is incorporating their extracts rich in polyphenols into polymeric matrices for the improvement of stability and bioavailability⁶⁴. The total phenolic content in the encapsulated extract is significantly higher ($p<0.05$) compared to that found in the no encapsulated ones (Table 3), indicating that encapsulation protects phenolics during the GID. Previous researches have confirmed and demonstrated that encapsulating bioactive compounds could protect them from unfavorable conditions⁶⁵⁻⁷¹.

5. Conclusions

From the current study, we demonstrated that extract from aerial parts of *P. spinosa* are a good source of phenolic compounds with potent bioactivity confirming its traditional use by the Mediterranean population. The HPLC analyzes revealed numerous phenolic compounds among them phenolic acids and several other flavonoids which have never been reported in

this plant. Extract from flowers exhibited the highest TPC and antioxidant activity. All extracts displayed a strong activity against α -amylase enzyme and cholesterol micellization, but a moderate effect on ACE inhibition. The present study confirms the negative effect of the GID on the bioactivité of plant extracts and the protective effect of the encapsulation procedure with respect to phenolic compounds. We can conclude that the objective that we set in this work has been achieved; because the obtained results could help to increase the acknowledgment about the phenolic composition and the bioactivity of phenolic extracts from *P. spinosa* aerial parts. However, for a complete evaluation of bioavailability of these compounds, further *in vivo* studies are required.

Acknowledgements

We wish to acknowledge the Algerian Ministry of Higher Education and Research and the Direction Générale de la Recherche Scientifique et du Développement Technologique (DGRSDT).

Conflicts of interest

There are no conflicts to declare.

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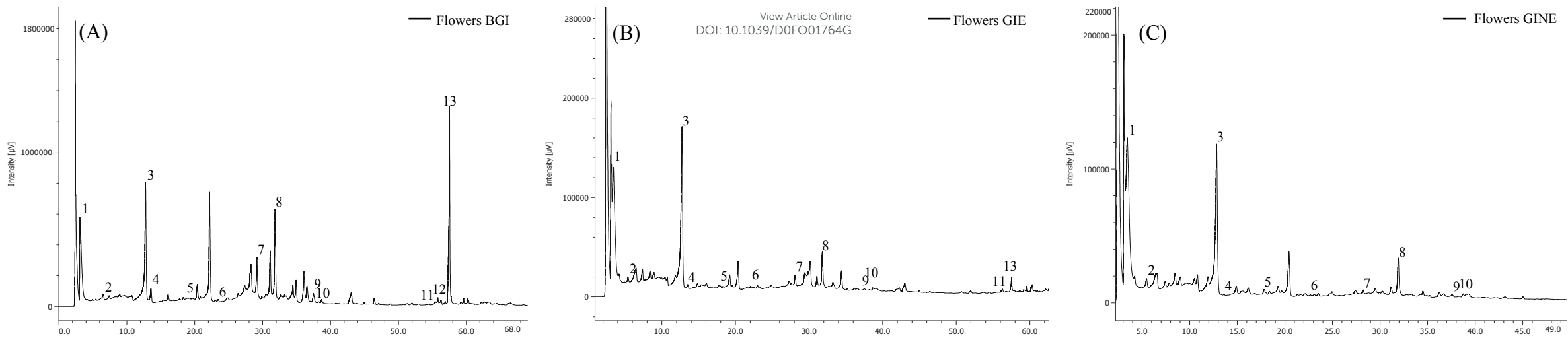


Figure 1. HPLC chromatograms of phenolics compounds at 280 nm of flowers *P. spinosa* of three samples: ((A) before digestion; (B) after digestion encapsulated and (C) nonencapsulated extract): 1, Gallic acid; 2, Neochlorogenic acid; 3, Catechin; 4, Chlorogenic acid; 5, Epicatechin; 6, Epigallocatechin; 7, Ferulic acid; 8, Rutin; 9, Apigenin glucosid; 10, Phlorizin; 11, Naringenin; 12, Apigenin; 13, Kaempferol; BGI, Before Gastro-Intestinal Digestion; GIE, After Gastro-Intestinal Digestion Encapsulated; GINE, After Gastro-Intestinal Digestion Non Encapsulated.

Table 1: Recommended concentrations of electrolytes in Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) ³⁰.

Constituents	g/L	SSF	SGF	SIF
		mL	mL	mL
KCL	37.30	15.10	6.90	6.80
KH ₂ PO ₄	68.00	3.70	0.90	0.80
NaHCO ₃	84.00	6.80	12.50	42.50
NaCl	117.00	0.00	11.80	9.60
MgCl ₂ (H ₂ O)	30.50	0.50	0.40	1.10
(NH ₄) ₂ CO ₃	48.00	0.06	0.50	0.00

Table 2: Content of individual phenolic compounds (mg/g of extract) in of *P. spinosa* extracts

Retention Time (min)		2.93	5.50	11.93	14.40	18.18	23.57	29.20	32.34	38.50	39.19	56.30	57.00	57.64	
Compounds		Gallic acid	Neochlorogenic acid	Catechin	Chlorogenic acid	Epicatechin	Epigallocatechin	Ferulic acid	Rutin	Apigenin glucosid	Phlorizin	Naringenin	Apigenin	Kaempferol	SPC
Flowers	BGI	12.182 ±0.053 ^c	0.593 ±0.000 ^h	53.317 ±0.354 ^a	1.888 ±0.148 ^{fg}	0.593 ±0.000 ^h	1.473 ±0.044 ^g	0.283 ±0.012 ^h	16.303 ±0.076 ^b	1.473 ±0.044 ^g	1.814 ±0.042 ^{fg}	1.500 ±0.274 ^g	16.303 ±0.076 ^b	8.218 ±0.047 ^d	104.310 ±0.805
	GIE	4.754 ±0.024 ^b	0.303 ±0.010 ^{ef}	13.013 ±0.048 ^a	0.203 ±0.002 ^{fg}	0.303 ±0.010 ^{ef}	0.467 ±0.017 ^d	0.039 ±0.000 ⁱ	1.108 ±0.047 ^c	0.467 ±0.017 ^d	0.008 ±0.015 ⁱ	1.104 ±0.054 ^c	1.108 ±0.047 ^c	0.081 ±0.004 ^{hi}	21.612 ±0.017
	GINE	4.358 ±0.295 ^b	0.055 ±0.001 ^{de}	8.413 ±0.001 ^a	0.153 ±0.000 ^{cde}	0.055 ±0.001 ^{de}	0.425 ±0.003 ^c	0.032 ±0.007 ^e	0.272 ±0.013 ^{cde}	0.425 ±0.003 ^c	0.005 ±0.001 ^e	0.382 ±0.004 ^{cd}	0.272 ±0.013 ^{cde}	0.002 ±0.002 ^e	14.382 ±0.313
Leaves	BGI	7.841 ±0.001 ^c	0.608 ±0.014 ^f	28.921 ±0.358 ^a	1.118 ±0.105 ^c	0.608 ±0.014 ^f	1.326 ±0.052 ^c	0.468 ±0.049 ^f	3.821 ±0.037 ^d	1.326 ±0.052 ^c	0.222 ±0.001 ^f	16.254 ±0.238 ^b	3.821 ±0.037 ^d	0.235 ±0.000 ^f	63.507 ±0.017
	GIE	4.624 ±0.026 ^a	0.169 ±0.006 ^{bc}	4.626 ±0.546 ^a	0.149 ±0.001 ^{bc}	0.169 ±0.006 ^{bc}	0.284 ±0.003 ^{bc}	0.041 ±0.002 ^c	0.721 ±0.005 ^b	0.284 ±0.003 ^{bc}	0.024 ±0.001 ^c	0.446 ±0.002 ^{bc}	0.721 ±0.005 ^b	0.022 ±0.003 ^c	11.192 ±0.514
	GINE	3.918 ±0.304 ^a	0.046 ±0.004 ^{de}	0.984 ±0.044 ^b	0.127 ±0.001 ^{de}	0.046 ±0.004 ^{de}	0.077 ±0.010 ^{de}	ND	0.567 ±0.042 ^c	0.077 ±0.010 ^{de}	0.018 ±0.001 ^e	0.412 ±0.012 ^{cd}	0.567 ±0.042 ^c	0.005 ±0.001 ^e	6.214 ±0.299
Stems	BGI	5.568 ±0.050 ^c	0.137 ±0.001 ⁱ	18.571 ±0.037 ^a	0.692 ±0.043 ^h	0.137 ±0.001 ⁱ	1.178 ±0.008 ^g	4.693 ±0.050 ^d	6.383 ±0.065 ^b	1.178 ±0.008 ^g	2.051 ±0.031 ^f	1.444 ±0.068 ^g	6.383 ±0.065 ^b	0.097 ±0.016 ⁱ	46.721 ±0.071
	GIE	1.803 ±0.032 ^a	0.071 ±0.001 ^{ef}	1.639 ±0.037 ^b	0.055 ±0.000 ^{ef}	0.071 ±0.001 ^{ef}	0.087 ±0.001 ^{ef}	0.187 ±0.017 ^e	1.281 ±0.105 ^c	0.087 ±0.001 ^{ef}	0.151 ±0.010 ^{ef}	0.394 ±0.010 ^d	1.281 ±0.105 ^c	0.060 ±0.010 ^{ef}	7.088 ±0.072
	GINE	1.579 ±0.017 ^a	0.030 ±0.001 ^d	0.668 ±0.014 ^b	0.060 ±0.001 ^d	0.030 ±0.001 ^d	0.058 ±0.003 ^d	0.021 ±0.006 ^d	0.378 ±0.007 ^c	0.058 ±0.003 ^d	0.079 ±0.009 ^d	0.464 ±0.015 ^c	0.378 ±0.007 ^c	0.041 ±0.001 ^d	3.466 ±0.009
Linearity range (µg ml ⁻¹)		3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50	3.125–50
Equation		y=38027x +8392	y=21970x +431.4	y=9511x +8867	y=28559 x -17934	y=11304x -1222	y=39530x -9206	y=57529x +25747	y=9241x +3950	y=29826 x +10332	y=29096x +19125	y=10270x-49337	y=11811 x +44186	y=75355x +10170	
R ²		0.999	0.999	0.999	0.999	0.999	0.999	0.997	0.998	0.998	0.999	0.995	0.998	0.999	
LOD		0.0004*	0.0003*	0.0011*	0.0006*	0.0004*	0.0007*	0.0004*	0.0003*	0.0003*	0.0006*	0.0382	0.0031	0.0003*	
LOQ		0.0013	0.0009	0.0036	0.0021	0.0012	0.0022	0.0012	0.0011	0.0012	0.0021	0.1275	0.0103	0.0010	

Results are reported as means ±SD (n=3). Same letters in the same column refers means not statistically different according to ANOVA and Tukey's test. SPC: Sum of Phenolic Content; ND: Not Detected. BGI: Before Gastro-Intestinal Digestion; GIE: After Gastro-Intestinal Digestion Encapsulated; GINE: After Gastro-Intestinal Digestion No Encapsulated. LOD: limit of detection and LOQ: limit of quantification. * Values between the LOD and the LOQ (0.0003 and 3.125), are detected but not quantifiable.

Table 3: Bioaccessibility indexes of phenolics in capsulated and non encapsulated extracts from *P. spinosa* after simulated GID.

Retention Time (min)	Compounds	Bioaccessibility indexes (%)					
		FLOWERS		LEAVES		STEMS	
		GIE	GINE	GIE	GINE	GIE	GINE
2.93	Gallic acid	39.03±0.03	35.77±2.26	58.97±0.35	49.97±3.88	32.37±0.28	28.36±0.56
18.18	Epicatechin	7.62±0.42	10.96±0.60	52.64±0.39	39.68±0.89	67.23±3.46	4.43±0.23
23.57	Epigallocatechin	31.66±0.21	28.87±1.05	21.44±0.64	5.76±0.52	7.35±0.01	4.90±0.28
39.19	Phlorizin	0.43±0.82	0.27±0.04	10.60±0.46	8.30±0.65	7.34±0.62	3.86±0.39
57.64	Kaempferol	0.98±0.06	0.03±0.02	9.38±1.35	2.15±0.33	63.87±21.11	42.88±6.43
11.93	Catechin	24.41±0.25	15.78±0.10	15.99±1.69	3.40±0.11	8.83±0.18	3.60±0.08
32.34	Rutin	6.80±0.26	1.67±0.09	18.86±0.05	14.85±1.23	20.07±1.86	5.93±0.18
56.30	Naringenin	74.50±10.02	25.92±4.98	2.74±0.03	2.53±0.04	27.29±1.95	32.12±0.46
57.00	Apigenin	ND	ND	ND	ND	ND	ND
5.50	Neochlorogenic acid	51.11±1.71	9.29±0.17	27.73±0.33	7.53±0.79	51.36±1.26	22.16±0.53
14.40	Chlorogenic acid	10.78±0.71	8.15±0.66	13.38±1.36	11.40±1.12	7.99±0.50	8.70±0.75
29.20	Ferulic acid	13.95±0.65	11.21±2.12	8.83±0.58	ND	3.98±0.40	0.44±0.12
38.50	Apigenin glucosid	9.06±0.50	0.89±0.06	0.53±0.04	ND	0.36±0.07	ND
	SPC	20.72±0.14	13.79±0.41	17.62±0.81	9.79±0.47	15.17±0.13	7.42±0.03

Results are reported as means ±SD (n=3). BGI: Before Gastro-Intestinal Digestion; GIE: After Gastro-Intestinal Digestion Encapsulated; GINE: After Gastro-Intestinal Digestion Non Encapsulated; SPC: Sum of Phenolic Content.

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Table 4: Antioxidant activity and total phenolic content of extracts from *P. spinosa*

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Samples		IC ₅₀ /DPPH	AABI (%)	IC ₅₀ /ABTS	AABI (%)	IC ₅₀ /FRAP	AABI (%)	(mg GAE/g extract)	PCBI (%)
Flowers	BGI	1.24 ±0.03 ^{ab}	ND	0.94 ±0.02 ^b	ND	0.50 ±0.02 ^a	ND	145.73 ±0.48 ^a	ND
	GIE	3.75 ±0.01 ^{de}	80.26	3.05 ±0.04 ^c	100	2.84 ±0.10 ^{bc}	27.75	31.84 ±0.06 ^d	21.85
	GINE	4.43 ±0.11 ^{ef}	52.12	3.80 ±0.12 ^d	60.77	4.73 ±0.21 ^{cd}	16.18	19.17 ±0.05 ^e	13.15
Leaves	BGI	1.68 ±0.05 ^{bc}	ND	1.27 ±0.02 ^b	ND	0.91 ±0.03 ^{ab}	ND	83.04 ±0.81 ^c	ND
	GIE	5.69 ±0.20 ^{fg}	35.35	3.99 ±0.07 ^d	58.03	5.96 ±0.25 ^d	17.83	15.68 ±0.07 ^f	18.88
	GINE	13.24 ±1.16 ⁱ	15.31	5.41 ±0.21 ^f	28.69	12.548±1.21 ^f	8.53	7.30 ±0.09 ^h	8.79
Stems	BGI	1.46±0.03 ^b	ND	1.09 ±0.01 ^b	ND	0.69 ±0.02 ^{ab}	ND	115.58 ±0.06 ^b	ND
	GIE	6.46±0.04 ^{gh}	28.31	4.61 ±0.07 ^c	36.78	8.59 ±0.67 ^e	10.67	12.16 ±0.02 ^g	10.52
	GINE	7.16±0.08 ^h	16.88	5.74 ±0.32 ^f	22.30	14.30 ±1.19 ^f	6.00	7.71 ±0.04 ^h	6.67
Positive control	BHT	2.80 ±0.00 ^{cd}	ND	0.16 ±0.00 ^a	ND	1.54 ±0.04 ^{ab}	ND	ND	ND
	Trolox	0.032 ±0.00 ^a	ND	0.03 ±0.00 ^a	ND	0.07 ±0.00 ^a	ND	ND	ND

Results are reported as means ±SD (n=3). . Same letters in the same column refers means not statistically different according to ANOVA and Tukey's test. ND: Not Determined. BGI: Before Gastro-Intestinal Digestion; GIE: After Gastro-Intestinal Digestion Encapsulated; GINE: After Gastro-Intestinal Digestion Non Encapsulated.

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856 **Table 5:** Amylase inhibitory activity of extracts from *P. spinosa* before, and after *in vitro* GIDView Article Online
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Samples		α -amylase inhibition		EIABI (%)
		IC ₅₀ (mg/mL)	(%) at 10 mg/mL	
Flowers	BGI	1.25 $\pm$ 0.00 ^b	88.10 $\pm$ 0.57 ^a	ND
	GIE	11.86 $\pm$ 0.40 ^e	25.14 $\pm$ 0.86 ^e	28.54
	GINE	13.60 $\pm$ 0.04 ^f	17.25 $\pm$ 0.34 ^{fg}	19.58
Leaves	BGI	3.55 $\pm$ 0.09 ^d	91.21 $\pm$ 0.40 ^a	ND
	GIE	11.87 $\pm$ 0.22 ^e	37.33 $\pm$ 1.49 ^c	40.93
	GINE	13.35 $\pm$ 0.03 ^f	32.51 $\pm$ 0.29 ^d	35.64
Stems	BGI	1.89 $\pm$ 0.05 ^c	68.54 $\pm$ 1.32 ^b	ND
	GIE	11.33 $\pm$ 0.02 ^e	20.04 $\pm$ 1.09 ^f	29.24
	GINE	13.30 $\pm$ 0.09 ^f	16.11 $\pm$ 0.00 ^g	23.50
Acarbose		0.046 $\pm$ 0.00 ^a	ND	ND

Results are reported as means $\pm$ SD (n=3). Same letters in the same column refers means not statistically different according to ANOVA and Tukey's test at $p < 0.05$. ND: Not Determined. BGI: Before Gastro-Intestinal Digestion; GIE: After Gastro-Intestinal Digestion Encapsulated; GINE: After Gastro-Intestinal Digestion Non Encapsulated.

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Table 6: The effect of *P. spinosa* extracts on the inhibition of cholesterol micellization and angiotensin-1 converting enzyme.

Samples		Cholesterol Inhibition (%) at 0.6 mg/mL	CIBI (%)	ACE Inhibition (%) at 2 mg/mL	EIABI (%)
Flowers	BGI	63.51 ±1.91 ^{ab}	ND	30.10 ±0.12 ^a	ND
	GIE	51.35 ±3.82 ^{bcd}	80.85	16.56 ±0.15 ^c	55.02
	GINE	36.49 ±1.91 ^e	57.46	4.26 ±0.71 ^g	14.15
Leaves	BGI	60.81 ±5.73 ^{ab}	ND	18.10 ±0.02 ^b	ND
	GIE	55.41 ±1.91 ^{abc}	91.12	13.36 ±0.17 ^e	73.81
	GINE	39.19 ±1.91 ^{de}	64.45	0.39 ±0.02 ⁱ	2.15
Stems	BGI	67.57 ±0.00 ^a	ND	14.98 ±0.23 ^d	ND
	GIE	44.59 ±5.73 ^{ede}	65.99	7.41 ±0.13 ^f	49.47
	GINE	8.11 ±0.00 ^g	12.00	1.88 ±0.74 ^h	12.55
Gallic acid		22.16 ±3.06 ^f	ND	ND	ND

Results are reported as means ±SD (n=3). Same letters in the same column refers means not statistically different according to ANOVA and Tukey's test. ND: Not Determined. BGI: Before Gastro-Intestinal Digestion; GIE: After Gastro-Intestinal Digestion Encapsulated; GINE: After Gastro-Intestinal Digestion Non Encapsulated.