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# Nanoparticles Encapsulating Sesame seeds (*Sesamum indicum*) Oil: Physicochemical, antioxidant and enzymatic inhibition properties

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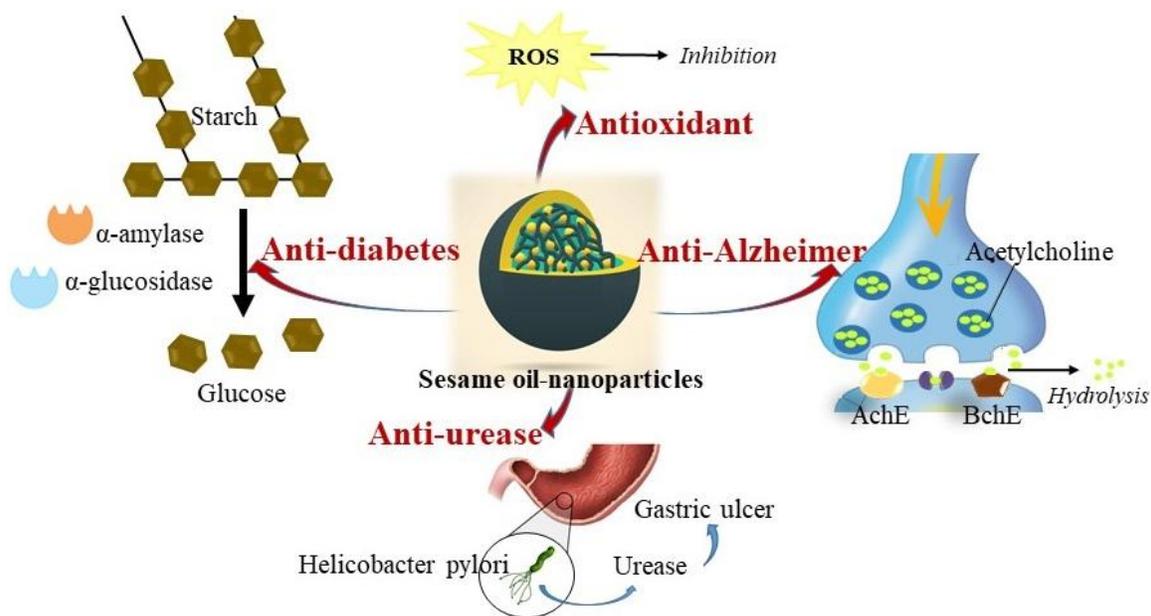
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## Graphical abstract



## Abstract

The present considers the encapsulation of *Sesamum indicum* L. seeds oil obtained by means of the supercritical CO<sub>2</sub> extraction technique in new polymethyl methacrylate based nanoparticles and investigates the *in vitro* antioxidant and enzymatic inhibition effects. The activity of the formulation to inhibit carbohydrate hydrolyzing enzymes (i.e.  $\alpha$ -amylase and  $\alpha$ -glucosidase), cholinesterase (i.e. acetylcholinesterase and butyrylcholinesterase) and urease involved in the management of diabetes, Alzheimer and ulcer, respectively, was investigated. Round nanoparticles with a mean size of  $259 \pm 4$  nm, a zeta-potential ( $\zeta$ ) of  $+78 \pm 4$  mV, and an efficiency of encapsulation of  $99 \pm 1\%$  were obtained. A high antidiabetic effect with IC<sub>50</sub> around 77.72 and 53.6  $\mu\text{g} \cdot \text{mL}^{-1}$  towards  $\alpha$ -amylase and  $\alpha$ -glucosidase, respectively) and moderate antiurease and anti-Alzheimer activities with IC<sub>50</sub> equal to 130.74, 62.21 and 77.73  $\mu\text{g} \cdot \text{mL}^{-1}$  towards acetylcholinesterase, butyrylcholinesterase and urease, respectively, were observed. This study contributes to the development of an effective approach to enhance sesame seeds oil stability and efficiency, provided drug carriers for hydrophobic oil drugs.

## Keywords

Sesame seeds oil, nanoparticles, encapsulation, antioxidant, enzymatic inhibition.

## 1. Introduction

Since time immemorial, plant based remedies have been the backbone of traditional medicine for the betterment of people worldwide. Plants constitute an inexhaustible potential that can be exploited in the identification, development, synthesis, etc. new substances intended for the production of effective drugs or reliable drug leads against a large number of types of diseases [1, 2]. Basing on information from the World Health Organization (WHO), traditional medicine is used by 70 to 95% of the contemporary world population for primary health purposes [3]. This renewed interest is essentially based on the strength of innovation in the pharmaceutical industry field to meet the needs of an ever more demanding market in terms of cost, accessibility and efficiency. Indeed, traditional medicine can be easily afforded, is closer to the patient's ideology and more accessible to the public comparatively to drugs of synthetic origin [4]. Despite the increase of the popularity of the natural products, the biodiversity of the world has been considered for essential biological activities in just a proportion of less than 10% [5], the remaining part still to be discovered and identified.

*Sesamum indicum* L., currently known as sesame is from the family of Pedaliaceae. It is greatly used for various medicinal, nutritional and industrial applications [6–8]. Sesame seeds have been shown to be effective as antioxidant [9,10], antimicrobial [10], hypolipidemic [11], hepatorenal protective [12], antidiabetic [13], anti-inflammatory [14] and analgesic [15]. It has been recently reported that sesame seeds exhibit a potential to protect from COVID-19 disease [16]. Sesame seeds contains 47 to 55% of oil and is considered to be one of the main oilseed crops worldwide [17]. Sesame oil is generally involved in the preparation of food, cosmetics, and pharmaceuticals formulations [18].

However its possible oxidation in environmental stresses induced by the presence of oxygen, trace metal, light and humidity due to a chemical structure characterized by an unsaturated bond [19]. Rustan and Drevon reported that polyunsaturated fatty acids are chemically unstable and are may be affected by loss of volatile compounds, oxidation, polymerization and isomerization upon exposure to several factors such as moisture, light, heat, oxygen and metallic ions [20]. As reported by Omar et al., the oxidative stability of sesame oil after a storage of two months might be affected by the antioxidant activity of punicalagin so that an addition of this phenolic compound to sesame oil significantly inhibited the formation of thiobarbituric acid reactive substances and decreased P-ansidine value and total oxidation index as compared to sesame oil alone [19]. The oxidative deterioration of the polyunsaturated fatty acids affects negatively on oils nutritional quality and shelf life and in many cases, can form toxic compounds [21]. Attention is now progressively paid to improve the vegetable oils stability [22,23]. Nanoencapsulation strategy arises as an effective and feasible solution to prevent the deterioration of polyunsaturated fatty acids during industrial processing, since it avoids the direct contact with the common environmental stressing factors. The incorporation of vegetable oils in polymeric nanoparticles (NP) can be a promising approach to enhance their properties like water solubility, stability, bioavailability and biological activity [24,25]. Several research works have been recently concerned by the encapsulation of plant oils in polymeric NP [26–30]. In a previous study, a nanosystem made from a new Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammoniumethyl methacrylate chloride) (PMMA) (Eudragit<sup>®</sup> RS100) had been designed as a carrier for vegetable oils [29]. To the best of our knowledge this study is the first one to consider the encapsulation

of sesame seeds oil in PMMA nanoparticles, assessing the antioxidant, anti-Alzheimer, antidiabetic and anti-urease potentials. A priori, supercritical CO<sub>2</sub> extraction process, an innovative and ecofriendly technique, was used as an alternative to organic solvent extraction to extract sesame oil. The analyses of the extracted oil were performed by means of gas chromatography with a flame ionization detector (GC–FID). The encapsulation of oil in PMMA-NP was followed by its characterization by dynamic light scattering, scanning and transmission electron microscopy, Fourier transform infrared spectroscopy (FTIR) and stability study. Finally, the investigation of *in vitro* antioxidant and enzyme inhibition activities for sesame oil in both free and encapsulated forms was performed and compared to that of standard drugs.

## **2. Material and methods**

### **2.1. The Sesame plant material**

The seeds of *Sesamum indicum* L. used were purchased from an herbs store (Setif, Algeria). After washing the seeds were ground by means of a grinder (Sunbeam Osterizer blender, Boca Raton, USA). In order to obtain particles with a diameter around 0.3 mm, the seeds were sieved and the resulting powder was kept in glass bottles under a vacuum desiccator ready for extraction experiments.

### **2.2. Chemical reagents**

Carbon dioxide of 99.5% purity was supplied by SIDAL SPA Air liquid (Algeria) and Eudragit<sup>®</sup> RS100 was purchased from EVONIK (EVONIK, Germany). Acarbose, galantamine, thiourea, butylhydroxyanisole (BHA), 4-nitrophenyl $\alpha$ -D-glucoopyranoside, starch powder, 5,5'-dithiobis(2-nitrobenzoic) acid, acetylthiocholine iodide, S-butrylthiocholine iodide, DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2' -azino-bis

(3-ethylbenzothiazoline-6-sulfonic acid), potassium ferricyanide, trichloroacetic acid, iron (III) chloride, phenanthroline and urea were all supplied by Sigma Chemical Co. (Germany).

For enzymatic studies, electric eel acetylcholinesterase (Type-VI-S, EC 3.1.1.7, 827,84 U/mg), horse serum butyrylcholinesterase (EC 3.1.1.8, 7,8 U/mg), *Aspergillus oryzae*  $\alpha$ -amylase, *Saccharomyces cerevisiae*  $\alpha$ -glucosidase (Type I,  $\geq 10$  units/mg protein) and Jack bean urease were all obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Stern-heim, Germany). Potassium iodide was purchased from Biochem Chemopharma and all other reagents and chemicals were of analytic grade. Milli-Q system was used to provide deionized water for all experiments.

### **2.3. Supercritical CO<sub>2</sub> extraction of sesame oil**

A total mass of 100g of sesame powder was fed to a dynamic pilot-plant (Separex 4343, type SF2, Champigneulle, France), to undergo a supercritical fluid extraction using 99.99% pure supercritical carbon dioxide as the carrier solvent. The operating parameters of extraction and separation temperature, pressure, extraction time and CO<sub>2</sub> flow rate were set at 40 and 30°C, 250bar, 180 min and 50g.min<sup>-1</sup>, respectively. The oil was collected and kept at 4°C ready for analysis (**Figure in Supporting Information**).

### **2.4. Gas chromatography analysis of fatty acids**

The analysis of sesame oil was investigated using an Agilent 6890 N apparatus equipped with a flame ionization detector (FID) and a split/splitless injector. Helium was the carrier gas with a flow rate of 1.3 mL.min<sup>-1</sup>. The first injection was conducted on an INNOWAX column (60 m  $\times$  0.32 mm, 0.50- $\mu$ m film thickness) according to the following temperature program: 2min at 60°C, an increase up to 245°C at a rate of

2°C/min and then constant for 30 min. An HP-1 (Agilent) column (50 m × 0.32 mm, 0.50-µm film thickness) was used for a second injection with an initial temperature of 80°C for 8 min, a first increase up to 220°C at 2°C/min and then a second one up to 310°C at 10°C /min and finally constant isothermally at 310°C for 20 min. The temperatures of the injector and the detector were maintained in both cases at 250°C. Before GC analysis, the derivatization of fatty acids to fatty acid methyl esters was performed by boron trifluoride (BF<sub>3</sub>)/methanol (10% BF<sub>3</sub>), followed by the injection of 1µl of the solution in split mode (1/60) into the chromatograph column.

## **2.5. Preparation of PMMA loaded nanoparticles**

The preparation of PMMA-NP was performed using a protocol that was already reported [29] using the nanoprecipitation technique [31]. Through this, 220mg of PMMA was dissolved in 12.5mL of acetone; then mixed with different amounts of sesame seeds oil (15, 30, 60, 120, 240 and 300 mg). After that, the resulting organic phase was added to the aqueous phase consisting of 25 mL water, under magnetic stirring (500 rpm) and ambient temperature (**Figure in Supporting Information**). A total evaporation of acetone was carried out subsequently using Buchi Rotavapor R-124<sup>®</sup> (10°C, 25rpm). For blank nanoparticles, the same protocol was repeated without adding vegetable oil.

## **2.6. PMMA loaded NP Characterization**

### **2.6.1. Size and zeta potential**

The determination of the particle size diameter was performed by the dynamic light scattering (DLS) technique, using the determined mean size (Z-average size) at 25°C by means of a Malvern Zetasizer Nano ZS 200 (Malvern Instruments, UK). The dilution of the colloidal dispersions in 1 mL of distilled water was performed before each size

measurement. For zeta ( $\zeta$ ) potential, DLS measurement was carried out in a clear disposable zeta cell with the same instrument after dispersion in 1mM NaCl solution and at pH around 6.5. All the measurements were triplicated and values were expressed as means  $\pm$  standard deviations (SD).

### 2.6.2. Efficiency of encapsulation and oil loading

For the determination of the encapsulation efficiency (EE%) and the oil loading ( $\text{mg}\cdot\text{mg}^{-1}$  of polymer), a separation of the encapsulated sesame oil into the nanoparticles within the colloidal dispersion, was necessary. Thus, a centrifugation for 30 min and at 15000 rpm of the provided dispersion. Then, the dissolution of the precipitated nanoparticles in chloroform, was followed by filtration and analysis by ultraviolet spectrophotometer UV-1800 (Shimadzu, Japan) at  $\lambda = 274.8$  nm. **The analytical method has been validated in terms of linearity, precision and repeatability. The standard curve equation was found to be  $Y = 0.2466X - 0.0046$  with good regression coefficient ( $R^2 = 0.9992$ ). The oil recoveries at different concentrations (1, 2 and 4mg/ml) were more than 99%, whereas the relative standard deviations (RSD) for the interday and intraday precisions were below 10.0%. The limit of detection (LOD) and quantification (LOQ) values were found to be as 0.13 and 0.46 mg/mL, respectively (Supporting Information).**

The determination of EE% and oil loading was by means of the following equations:

$$\text{Encapsulation efficiency (EE \%)} = \frac{\text{Amount of encapsulated sesame oil}}{\text{Total amount of sesame oil}} \times 100 \quad (1)$$

$$\text{Oil loading (mg}\cdot\text{mg}^{-1}\text{)} = \frac{\text{Encapsulated sesame oil amount}}{\text{Total Polymer amount}} \quad (2)$$

### 2.6.3. Scanning electron microscopy

The evaluation of the nanoparticles morphology was performed by scanning electron microscopy (SEM) using a FEI Quanta 250 FEG microscope, operating at 15 kV. A

spreading of five microliters of diluted aqueous suspension of NP on a flat steel holder was followed by drying at room temperature. The grids were analyzed after a coating under vacuum by cathodic sputtering with copper.

#### **2.6.4. Transmission electron microscopy**

The transmission electron microscopy (TEM) was used to observe the morphology of the obtained NP using Philips CM200 EFG microscope. The procedure consisted in spreading one drop of colloidal dispersion onto a microscope grid with a copper support covered with carbon, followed by drying at room temperature for TEM analysis under 120 kV acceleration voltage.

#### **2.6.5. Fourier transform infrared spectroscopy**

The determination of the encapsulation and the distribution of sesame oil in PMM-NP, was carried out by means of Fourier transform infrared spectroscopy (FTIR) analysis using a FTIR spectrometer (Thermo Scientific Technologies FTIR spectrometer with IR MONIC Solution software). The infrared spectra of sesame oil, PMMA, and sesame oil loaded-NP were recorded between 400 and 4000  $\text{cm}^{-1}$  and plotted as transmittance versus wave number.

#### **2.7. The study of the stability of PMMA- NP loaded with sesame oil**

Sesame oil loaded-NP were stored for one month at different temperatures of 4, 25 and 40°C and characterized for particle size, zeta potential and EE% after each 10 days. The results were given as mean  $\pm$  SD.

#### **2.8. Antioxidant activity**

The evaluation of the antioxidant activity was performed *in vitro* using four tests: DPPH, FRAP ABTS and phenanthroline. The results were compared to that of a positive control (BHA).

### 2.8.1. DPPH radical scavenging activity assay

The determination of the free radical scavenging activity of DPPH was performed according to the slightly adjusted method previously reported by Blois (1958) and consisted in mixing 40  $\mu\text{L}$  of different concentration sample solutions with a volume of 160  $\mu\text{L}$  of the DPPH solution, followed by an incubation in the dark for 30 min and at ambient temperature. The absorbance reading of the solution was at 517 nm. The calculation of the activity of the DPPH free radical scavenging performed according to the following equation:

$$\text{DPPH Inhibition\%} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (3)$$

The IC<sub>50</sub> “the concentration of the tested sample required to scavenge 50% of free radicals” of all the samples were calculated.

### 2.8.2. ABTS radical scavenging activity assay

The assessment of the scavenging activity of ABTS<sup>•+</sup> (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) followed the slightly modified method described by Re et al. [33]. It consisted in adding 40  $\mu\text{L}$  of the sample solution at different concentrations to 160  $\mu\text{L}$  of ABTS<sup>•+</sup> solution, followed by an incubation during 5 min at ambient temperature. The absorbance reading was at 734 nm and the ABTS<sup>•+</sup> free radical scavenging activity was determined as follow:

$$\text{ABTS Inhibition\%} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (4)$$

### 2.8.3. Ferric reducing antioxidant power assay

The evaluation of the Ferric ion reducing antioxidant power (FRAP) was performed according to the modified method of Oyaizu [34]. Volume samples of 10  $\mu\text{L}$  at different concentrations were mixed with 50  $\mu\text{L}$  of potassium ferricyanide (1%) and 40  $\mu\text{L}$  of phosphate buffer (pH 6.6). After an incubation time of 20 min at 50°C, the addition of 50 $\mu\text{L}$  of trichloroacetic acid (10%) halted the reaction. The absorbance reading was at 700nm after addition of 10  $\mu\text{L}$  of iron (III) chloride (0.1%) and 40  $\mu\text{L}$  of distilled water. The antioxidant activity was evaluated as  $A_{0.5}$  ( $\mu\text{g. mL}^{-1}$ ), hence the concentration corresponding to 0.50 absorbance.

#### **2.8.4. Phenanthroline assay**

Phenanthroline assay was performed as described previously [35] by mixing 10  $\mu\text{L}$  of the sample at different concentrations, 50  $\mu\text{L}$  iron (III) chloride, 30  $\mu\text{L}$  phenanthroline and 110  $\mu\text{L}$  of methanol. The incubation took place in dark for 20 min and at 30°C and the absorbance reading was at 510 nm.

#### **2.9. Enzyme inhibitory activities**

All the measurements were carried out on sesame oil loaded-NP, un-encapsulated sesame oil and un-loaded-NP basing on quantitative colorimetric assays at a National local Research Center in Biotechnology (CRBT, Constantine, Algeria) using a PerkinElmer Multimode Plate Reader EnSpire. The calculation of the IC50 (the concentration at which 50% of the enzyme is inhibited) was performed by using the plot of the percentage of inhibition versus the concentration of the sample. The percentage of enzyme inhibition was calculated as follow:

$$\% \text{ Inhibition} = \frac{A_c - A_s}{A_c} \times 100 \quad (5)$$

Where  $A_c$  is the absorbance of the control and  $A_s$  is the absorbance of the test sample.

### **2.9.1. The inhibition activity of alpha amylase**

The evaluation of the inhibitory activity of alpha amylase was carried out according to the iodine/potassium iodide protocol due to Caraway-Somogyi [36]. In 96-well microplate, 25  $\mu\text{L}$  of sample solutions of different concentrations were incubated for 10 min and at 37°C using 50  $\mu\text{L}$  of  $\alpha$ -amylase solution. Then, the reaction was initiated following the addition of 50  $\mu\text{L}$  of a solution of starch (0.1%). Besides, a preparation of a blank was carried out by the addition of sample solution to all reaction reagents without the solution of  $\alpha$ -amylase. After incubation, 25  $\mu\text{L}$  of HCl (1 M) were added to stop the reaction. Finally, the absorbances of sample and blank were read at 630 nm after addition of 100  $\mu\text{L}$  of solutions of iodine-potassium iodide to all the wells. The used positive control was Acarbose.

### **2.9.2. Alpha glycosidase inhibition activity**

The investigation of the inhibitory activity of alpha glucosidase was performed according to a previous slightly modified method [37]. Sample solution volumes of 50  $\mu\text{L}$  at different concentrations were added to an  $\alpha$ -glucosidase solution volume of 100  $\mu\text{L}$  (0.1  $\text{U}\cdot\text{mL}^{-1}$ ) to be incubated during 15 min at 37 °C. This was followed by the addition of 50  $\mu\text{L}$  of p-nitrophenyl  $\alpha$ -D glucopyranoside solution and the incubation of the microplate for 5 min at 37 °C. Acarbose was used as standard and the absorbance reading was at 405 nm.

### **2.9.3. Cholinesterase inhibition**

The determination of Acetyl and butyryl cholinesterase inhibitory activities was performed following the method described in the literature by Rhee et al. [38], basing on Ellman's method [39]. In a 96-well microplate, a volume of 10  $\mu\text{L}$  of a sample solution

was mixed and incubated with 20  $\mu\text{L}$  of acetylcholinesterase ( $5.32 \times 10^{-3}$  U) or butyrylcholinesterase ( $6.85 \times 10^{-3}$  U) solution and a sodium phosphate buffer (pH 8.0) volume of 150  $\mu\text{L}$ , for 15 min at 25  $^{\circ}\text{C}$ . This was followed by the addition of 10  $\mu\text{L}$  of DTNB solution (0.5Mm) along with 10  $\mu\text{L}$  of butyrylthiocholine or acetylthiocholine iodides. The absorbance was read at 412 nm and compared to that of the positive control which was galantamine hydrobromide.

#### **2.9.4. Urease inhibition**

The inhibitory activity of Urease was determined according to the indophenol method as described previously [40]. The incubation of ten microliters of sample solutions was carried out at 30 $^{\circ}\text{C}$  for 15 min at different concentrations using 25  $\mu\text{L}$  of urease solution and 50  $\mu\text{L}$  of urea. This was followed by the addition of phenol (45  $\mu\text{L}$ ) and alkali reagents (70  $\mu\text{L}$ ) to the reaction mixture. The absorbance was measured at 630 nm after an incubation time of 50 min. The used standard compound was Thiourea.

#### **2.10. Statistical analysis**

The data values are denoted as mean  $\pm$  SD and analyzed statistically using XLSTAT software with one-way analysis of variance (ANOVA). For significant difference,  $p < 0.05$  was selected such as criterion.

### **3. Results and discussion**

#### **3.1. Gas chromatography analysis**

The fatty acid composition of the obtained vegetable oil is summarized in **Table 1**. A total of 13 components were identified, representing 98.393% of the total oil weight, of which 81.858% are unsaturated fatty acids and 16.535% corresponding to saturated fatty acids. It is also noted that oleic and linoleic acids were the main components with 40.784

and 39.840% of the total weight, respectively; followed by palmitic acid (10.086%) and stearic acid (5.623%). These results were in agreement with those reported by various works where the predominance of oleic and linoleic acids was observed, but in variable quantities [41–43]. Other compounds appeared as traces in our oil such as: lauric acid, myristic acid, arachidonic acid, linolenic acid, behenic acid, etc.

**Table 1.** Gas chromatography analysis of sesame oil.

N°	Component	Symbol	Formula	Retention index	Quantity (%)
1	Lauric acid	C12 : 0	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	1817	0.272
2	Myristic acid	C14 : 0	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	2024	0.232
3	Palmitic acid	C16 : 0	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	2233	10.086
4	Palmitoleic acid	C16 : 1	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	2261	0.123
5	Margaric acid	C17 : 0	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	2335	0.060
6	Stearic acid	C18 : 0	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	2440	5.623
7	Oleic acid omega 9	C18 : 1	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	2468	40.784
8	Linoleic acid omega 6	C18 : 2	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	2515	39.840
9	Linolenic acid omega 3	C18 : 3	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	2583	0.275
10	Gadoleic acid	C20 : 1	C <sub>20</sub> H <sub>32</sub> O <sub>2</sub>	2645	0.624
11	Arachidonic acid	C20 : 4	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	2670	0.212
12	Behenic acid	C22 : 0	C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>	2851	0.168
13	Lignoceric acid	C24 : 0	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	>3000	0.094
<b>Unsaturated fatty acids</b>					<b>81.858</b>
<b>Saturated fatty acids</b>					<b>16.535</b>
<b>Total</b>					<b>98.393</b>

## 3.2. Nanoparticles characterization

### 3.2.1. DLS measurements

To achieve the required amount of sesame seeds oil to be encapsulated, various amounts had been investigated (15, 30, 60, 120, 240 and 300 mg) and results of DLS analysis are

summarized in **Fig 1**. The average diameter of PMMA-NP increased after vegetable oil encapsulation. This was in accordance with what was reported earlier [29,44]. By increasing the oil amount from 15 to 300 mg, a significant increase in average size occurred from 166 to 300 nm. Similar results were already described [29,44]. It is reported that there is an optimal quantity of plant oils to be encapsulated [45]. In our previous work, the optimum quantity of *Phoenix dactylifera* seeds oil to be encapsulated in PMMA-NP was found to be 120 mg. At higher amounts (more than 120 mg), the colloidal suspension had not a homogenous aspect and a slight increase in particle size was depicted. [29]. Further analysis concerning the correlation between sesame seeds oil amount and the cubic radius of PMMA-NP was carried out in order to determine the optimum quantity to be encapsulated, as demonstrated in **Fig. 2**. According to equation 6, when increasing the amount of polymer/oil in the formulation, the average diameter of the NP increases but their number keeps constant.

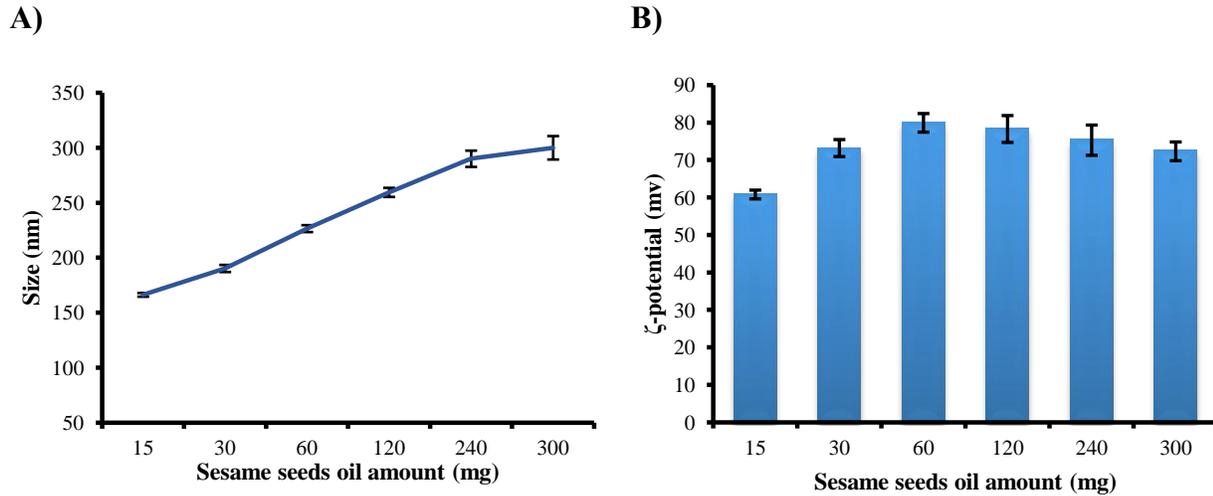
$$R^3 \approx \frac{m_p}{Nb_p} \quad (6)$$

with R the radius of particles,  $Nb_p$  the number of particles and  $m_p$  the amount of polymer or oil.

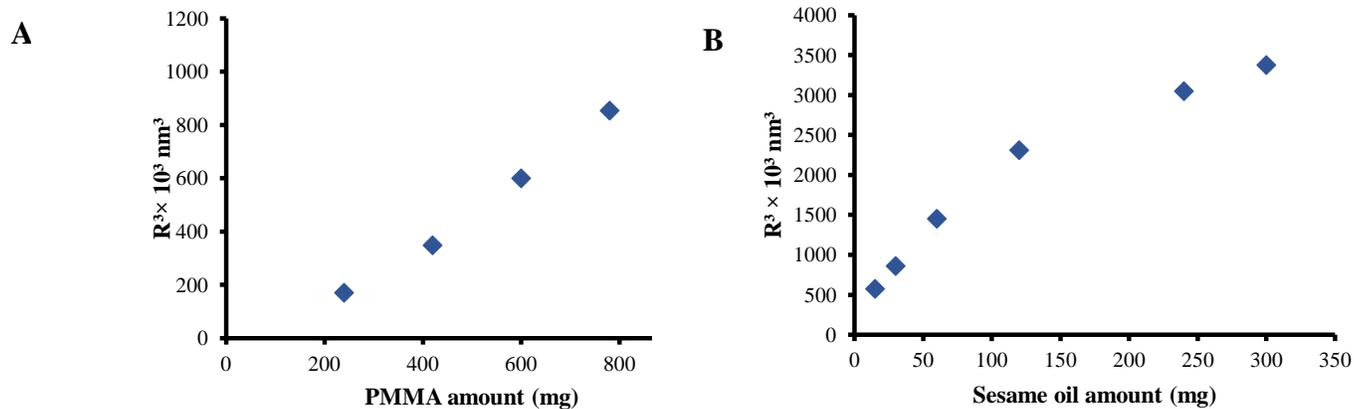
The size increase of an un-loaded NP with the increase of the polymer amount, suggests a correlation between these two parameters (**Fig. 2.A**). It was also showed that the radius of the NP might be affected by the sesame oil amount in the case that this latter increased from 15 to 120mg (**Fig. 2.B**). However greater sesame oil amounts ( $\geq 240$  mg) did not show any increase of PMMA-NP sizes, but new particles were rather generated, as supported by (**Fig. 1**). Therefore an oil amount of 120 mg may be considered as a convenient value in order for the sesame seeds oil to be entrapped.

Further  $\zeta$ -potential analysis was investigated for all the samples and the results were illustrated in **Fig. 1**. Sesame oil loaded-NP exhibited higher  $\zeta$ -potential values as compared to un-loaded NP due to the interaction of the vegetable oil with PMMA polymer. Similar results were found by Froiio et al. when encapsulating *Citrus bergamia* and *Citrus sinensis* essential oils in PMMA-NP [46]. The positive charge of the developed NP is ascribed to the ammonium quaternary groups of PMMA polymer as reported earlier [47].

With respect to EE%, high values were obtained when varying sesame seeds oil from 30 to 120mg; however, lower values were assessed when incorporating more than 120mg of vegetable oil, as illustrated in **Table 2**. The optimal formulation showed a high EE% ( $99 \pm 1\%$ ) with a loading capacity of 0.497 mg oil/mg of PMMA polymer.



**Fig. 1.** Effect of sesame seeds oil amount on (A) particle size, and (B)  $\zeta$ -potential of PMMA based nanoparticles. The results are the mean of three independent experiment  $\pm$  S.D.



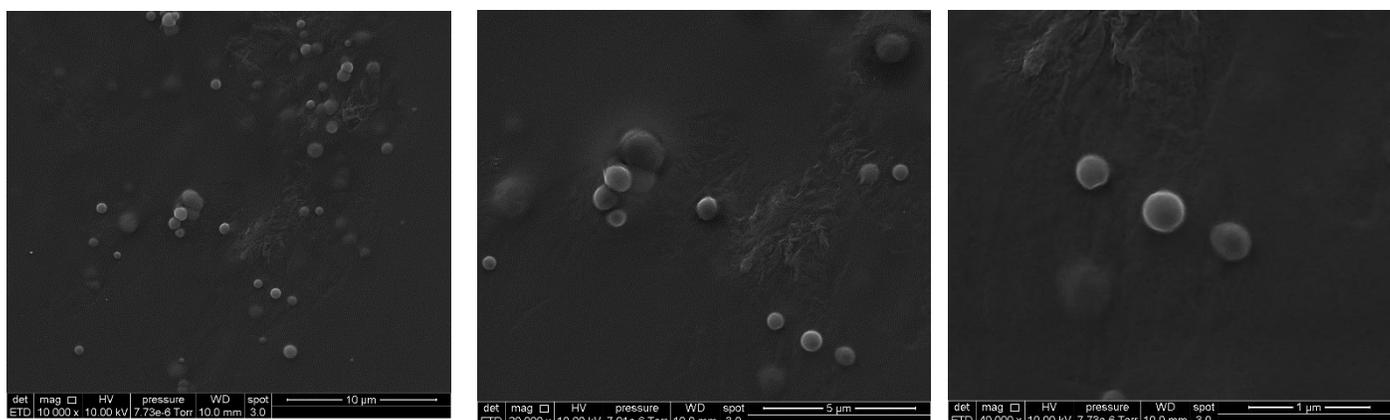
**Fig. 2.** Correlation between PMMA amount (A), Sesame seed oil amount (B) and the cubic radius of nanoparticles. Dots represent the cube radius values of the nanoparticles.

**Table 2.** Encapsulation efficiency and oil loading of sesame seeds oil-loaded PMMA based nanoparticles.

Sesame seed oil amount (mg)	EE (%)	Oil loading ( $\mu\text{g}/\text{mg}$ polymer)
0	/	/
15	$99 \pm 1$	$62 \pm 0$
30	$98 \pm 2$	$123 \pm 3$
60	$100 \pm 1$	$249 \pm 3$
120	$99 \pm 1$	$497 \pm 6$
240	$96 \pm 1$	$961 \pm 6$
300	$86 \pm 1$	$1078 \pm 14$

### 3.2.2. Nanoparticles morphology

The sesame seeds oil loaded NP were examined by SEM (**Fig. 3**) and TEM (**Fig. 4**) and results demonstrated a spherical and regular form.

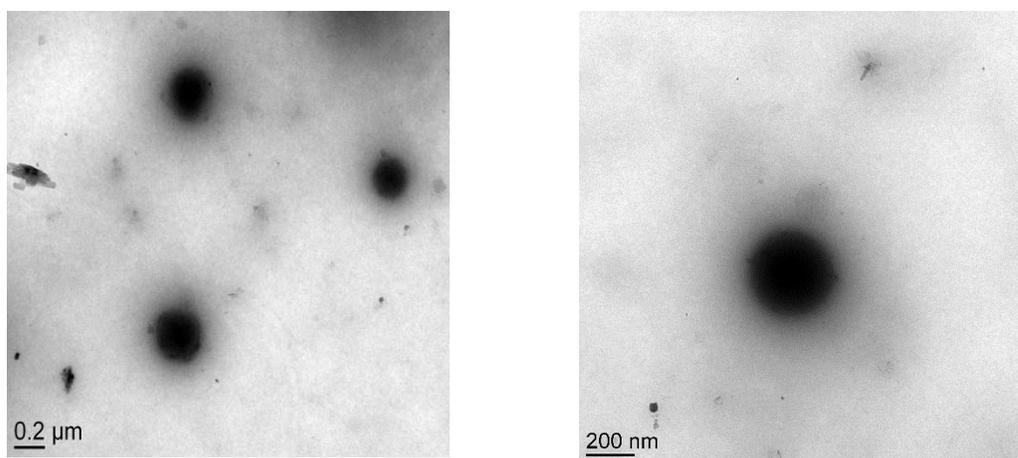


a) 10 µm scale bar

b) 5 µm scale bar

c) 1 µm scale bar

**Fig. 3.** Scanning Electron Microscopy images of sesame oil loaded PMMA nanoparticles, (a) 10 µm scale bar, and (b) 5 µm scale bar and (c) 1 µm scale bar.



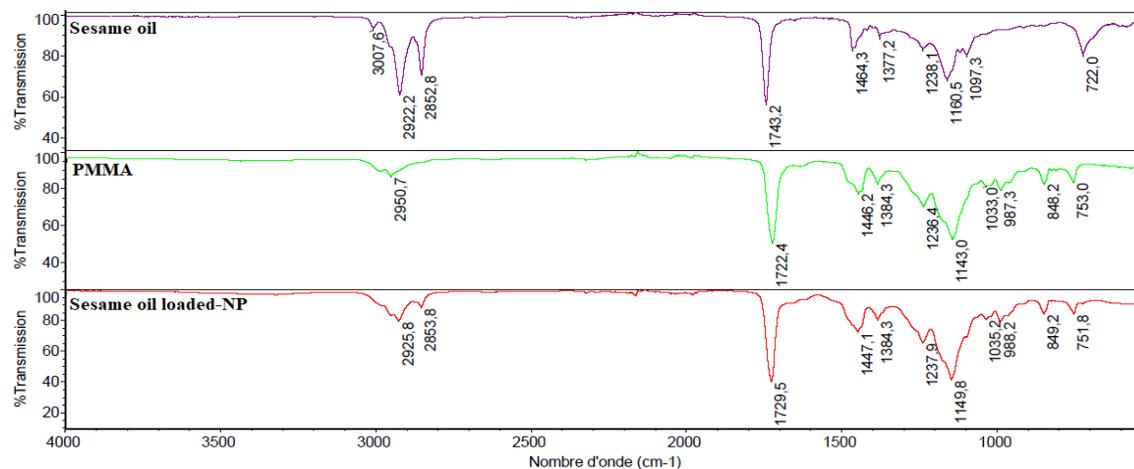
**Fig. 4.** Transmission Electron Microscopy images of sesame oil loaded PMMA nanoparticles.

### 3.2.3. Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy analysis was performed to determine the eventual interaction between PMMA polymer and sesame seeds oil. The FTIR spectra of PMMA, un-encapsulated sesame seeds oil and sesame oil loaded-NP are illustrated in **Fig. 5**. In sesame oil spectrum, the peaks at  $3007\text{ cm}^{-1}$  was attributed to stretching vibration of cis C=CH, whereas peaks at  $2922$  and  $2853\text{ cm}^{-1}$  were assigned to the stretching vibrations of aliphatic C–H in terminal  $\text{CH}_2$  and  $\text{CH}_3$  groups, respectively. The

peaks at 1160 and 1097 $\text{cm}^{-1}$  were due to C-O vibrations. While, that at 722  $\text{cm}^{-1}$  was related to  $\text{CH}_2$  rocking vibration. The peaks at 1743 and 1160  $\text{cm}^{-1}$  correspond to stretching vibration of the C=O and C-O groups of the ester, respectively. These findings were in agreement with those reported in [48–52].

In PMMA spectrum, the peaks at 2950  $\text{cm}^{-1}$  was assigned to C-H stretching of the aliphatic groups. Whereas, peaks at 1143 and 1722  $\text{cm}^{-1}$  were due to the stretching vibrations of C-O and C=O ester groups, respectively. Similar results were reported earlier in [53,54]. Matching up FTIR spectrum of un-encapsulated sesame oil, PMMA with oil loaded-NP demonstrated the appearance of new peaks at 2925 and 2853  $\text{cm}^{-1}$  characterizing sesame seeds oil as well as shifting of other peaks in 1729 and 1149  $\text{cm}^{-1}$ . This finding might be explained by the interaction between sesame seeds oil and PMMA polymer. Thus, sesame seeds oil was efficiently entrapped within PMMA-NP.



**Fig. 5.** Fourier transformed infrared spectroscopy (FTIR) spectrum noted between 650  $\text{cm}^{-1}$  and 4000  $\text{cm}^{-1}$  for unencapsulated sesame seeds oil, PMMA and sesame oil loaded-nanoparticles.

### 3.3. Stability study

In order to investigate the colloidal stability of the prepared nanoparticles formulation, particle size,  $\zeta$ -potential and EE% were measured during one month as a function of

temperature (4, 25 and 40°C), as demonstrated in **Table 3**. Independently of the temperature and time, the average diameter,  $\zeta$ -potential and EE% remain almost constant during all the storage period.

**Table 3.** Stability of PMMA based nanoparticles containing sesame seeds oil in terms of size,  $\zeta$ -potential and encapsulation efficiency. Measurements from separate assays were combined (n = 3) and represented as mean  $\pm$  SD.

	4°C				25°C				40°C			
	Day 1	Day 10	Day 20	Day 30	Day 1	Day 10	Day 20	Day 30	Day 1	Day 10	Day 20	Day 30
<b>Average diameter (nm)</b>	259 $\pm$ 2	260 $\pm$ 3	258 $\pm$ 4	257 $\pm$ 3	258 $\pm$ 2	261 $\pm$ 1	260 $\pm$ 1	260 $\pm$ 3	259 $\pm$ 3	260 $\pm$ 1	260 $\pm$ 2	259 $\pm$ 3
<b><math>\zeta</math>-potential (mV)</b>	78.2 $\pm$ 5	77.0 $\pm$ 3	78.2 $\pm$ 4	77.1 $\pm$ 3	78.1 $\pm$ 5	79.2 $\pm$ 4	79.2 $\pm$ 3	77.3 $\pm$ 4	78.1 $\pm$ 3	77.6 $\pm$ 4	76.2 $\pm$ 5	77.1 $\pm$ 4
<b>EE %</b>	99 $\pm$ 1	98 $\pm$ 2	97 $\pm$ 2	98 $\pm$ 2	98 $\pm$ 1	98 $\pm$ 2	99 $\pm$ 2	97 $\pm$ 3	100 $\pm$ 1	99 $\pm$ 2	99 $\pm$ 3	98 $\pm$ 2

### 3.4. Antioxidant activity

The human body is subjected to a wide variety of chemical (combustion fumes, smoking, transition metals, toxins), physical (ionizing radiation, ultraviolet, trauma), and metabolic aggressions (exposure to xenobiotics, deprivation of growth or hormonal factors) [55]. Most of these aggressions lead to the oxidative stress, which is defined as a disturbance of the endogenous balance between free radicals (reactive oxygen species) and antioxidant agents in favor of the former, resulting in fats, DNA and protein damages [56]. Free radicals have an unpaired valence electron in the outer orbital; they can be derivatives of nitrogen such as nitric oxide NO•, or of oxygen such as the hydroxyl radical OH• or the superoxide anion O<sub>2</sub>•<sup>-</sup> [56]. In the presence of antioxidants, the balance is restored either by reduction (gain of another electron) or by oxidation (loss of the free electron) [57].

There is no universal method by which the antioxidant profile of samples is quantitatively determined in a very precise manner given the complexity of the oxidation process and the diverse nature of antioxidants. As a result, various methods and analytical tools are developed for the evaluation of scavenging activity [58]. In the present study, four methods were used to evaluate the antioxidant activity: DPPH, ABTS, phenanthroline and reducing power. The activity of different samples was deduced using a spectrophotometer following the scavenging of free radicals which was accompanied by the color change from violet to yellow for DPPH and from blue to colorless for ABTS; or following the reduction of ferric iron which was reflected by the color change from orange to red-orange for phenanthroline and from yellow to blue for reducing power. The results were compared to those of the standard: BHA, and are summarized in **Table 5** in terms of percentage inhibition for each concentration.

**Table 5.** DPPH, ABTS, phenanthroline and reducing power results of sesame oil, loaded nanoparticles and BHA.

<b>C(<math>\mu\text{g/mL}</math>)</b>	<b>12.5</b>	<b>25</b>	<b>50</b>	<b>100</b>	<b>200</b>	<b>400</b>	<b>800</b>
<b>DPPH (% inhibition)</b>							
Oil	-	6.5 $\pm$ 0.4	14.2 $\pm$ 0.2	19.2 $\pm$ 0.1	25.5 $\pm$ 3.1	29.6 $\pm$ 0.1	32.0 $\pm$ 0.4
Loaded NP	15.3 $\pm$ 1.3	28.0 $\pm$ 1.0	30.3 $\pm$ 0.9	33.3 $\pm$ 0.01	35.4 $\pm$ 1.1	40.7 $\pm$ 0.3	51.7 $\pm$ 1.2
BHA	48.2 $\pm$ 2.6	62.4 $\pm$ 2.8	81.1 $\pm$ 1.4	84.2 $\pm$ 0.1	87.2 $\pm$ 1.2	88.9 $\pm$ 2.3	90.2 $\pm$ 2.5
<b>ABTS (% inhibition)</b>							
Oil	-	-	9.5 $\pm$ 2.4	17.5 $\pm$ 0.4	25.6 $\pm$ 1.5	32.6 $\pm$ 2.1	30.5 $\pm$ 1.3
Loaded NP	-	-	-	7.1 $\pm$ 7.5	21.9 $\pm$ 0.4	31.4 $\pm$ 1.9	38.6 $\pm$ 0.7
BHA	64.7 $\pm$ 0.6	78.5 $\pm$ 2.7	88.4 $\pm$ 1.3	95.3 $\pm$ 0.3	95.6 $\pm$ 0.5	95.8 $\pm$ 0.2	95.9 $\pm$ 0.1
<b>C(<math>\mu\text{g/mL}</math>)</b>	<b>3.125</b>	<b>6.25</b>	<b>12.5</b>	<b>25</b>	<b>50</b>	<b>100</b>	<b>200</b>
<b>Phenanthroline (Absorbance)</b>							
Oil	0.10 $\pm$ 0.01	0.19 $\pm$ 0.02	0.25 $\pm$ 0.01	0.26 $\pm$ 0.02	0.29 $\pm$ 0.00	0.32 $\pm$ 0.01	0.38 $\pm$ 0.03
Loaded NP	0.15 $\pm$ 0.01	0.25 $\pm$ 0.02	0.32 $\pm$ 0.01	0.34 $\pm$ 0.01	0.39 $\pm$ 0.01	0.41 $\pm$ 0.01	0.47 $\pm$ 0.01
BHA	0.73 $\pm$ 0.02	0.93 $\pm$ 0.01	1.25 $\pm$ 0.04	2.10 $\pm$ 0.05	4.89 $\pm$ 0.06	4.90 $\pm$ 0.01	4.92 $\pm$ 0.01
<b>Reducing power (Absorbance)</b>							

Oil	0.07±0.01	0.08±0.01	0.10±0.01	0.14±0.01	0.18±0.01	0.25±0.09	0.25±0.10
Loaded NP	0.06±0.01	0.09±0.01	0.11±0.01	0.14±0.01	0.18±0.00	0.24±0.03	0.26±0.03
BHA	0.09±0.01	0.12±0.01	0.19±0.02	0.38±0.02	0.77±0.02	1.72±0.03	3.52±0.02

(-): No absorbance.

Mean values are expressed as mean ± SD of three parallel measurements.

Oil: sesame oil; loaded NP: nanoparticles containing sesame oil.

**Table 6.** Results of the measurement of IC<sub>50</sub> and A<sub>0.5</sub> of sesame oil, loaded nanoparticles and BHA for the various tests (DPPH, ABTS, phenanthroline and reducing power).

	Oil	Loaded NP	BHA
IC <sub>50</sub> DPPH (µg.mL <sup>-1</sup> )	> 800	736.6±6.8	12.7 ± 3.8
IC <sub>50</sub> ABTS (µg.mL <sup>-1</sup> )	> 800	> 800	6.7 ± 0.5
A <sub>0.5</sub> Phenanthroline (µg.mL <sup>-1</sup> )	> 200	> 200	0.9 ± 0.1
A <sub>0.5</sub> Reducing power (µg.mL <sup>-1</sup> )	> 200	> 200	32.9 ± 0.3

By comparing the results obtained from all the tests with those of BHA, it can be deduced that sesame oil had a very low antioxidant activity. No color change was detected on the microplates from the different tests. Regarding DPPH test, the IC<sub>50</sub> values were very high (> 0.8mg.mL<sup>-1</sup>) (**Table 6**). The same was for the values of IC<sub>50</sub> for ABTS test and A<sub>0.5</sub> for phenanthroline and reducing power methods. The IC<sub>50</sub> (DPPH) found presently did not match with those obtained in previous studies. Ruslan et al. revealed that the IC<sub>50</sub> (DPPH) of ethanolic extracts of the two varieties of sesame seeds was in the range of 8.88–44.21µg.mL<sup>-1</sup> [59]. Bopitiya and Madhujith demonstrated that the methanolic extract of sesame oil had an IC<sub>50</sub> of 26 µg.mL<sup>-1</sup> [60].

According to the literature, the natural phenolic compounds have received increasing interest in the last decade due to their antioxidant power, among other plant components [61]. In order to explain the low antioxidant activity of sesame oil, the content of total

polyphenols was quantified and the results revealed a low quantity ( $0.20 \pm 0.02$  mg Gallic acid equivalent/mg oil). The principle of the used method is inserted in **Supporting Information**. These results are attributed to the polarity of the solvent used in the extraction process. Different studies have revealed a proportional relationship between the polarity of the used solvent, the content of total polyphenols and the antioxidant potential [62–65]. In the present study, since CO<sub>2</sub> is non-polar, it had a low ability to dissolve polar compounds such as polyphenols; resulting in low antioxidant activity. Adding a co-solvent such as methanol to the supercritical CO<sub>2</sub> extraction process is reported to improve the level of extracted polyphenols [66,67]. A similar study conducted by Djenidi et al. demonstrated considerable extracted amounts of polyphenols ( $56.7\mu\text{g}\cdot\text{mg}^{-1}$ ) by adding methanol [68].

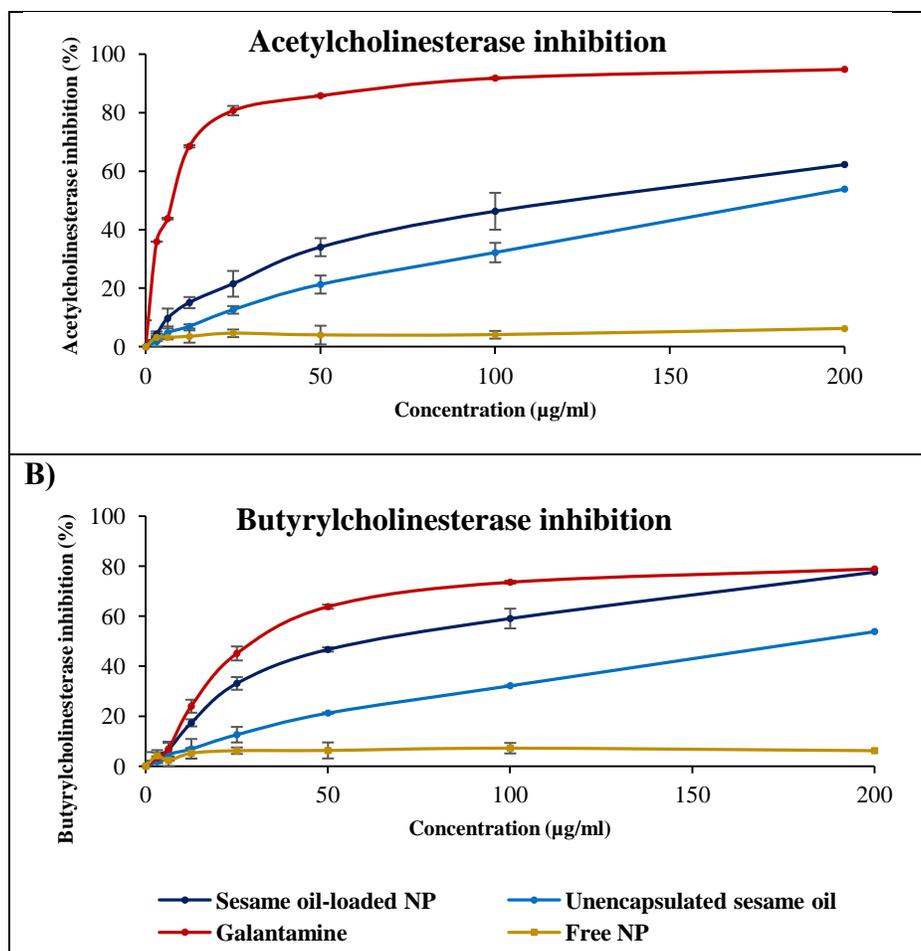
### **3.5. Enzyme inhibition activities**

#### **3.5.1. Cholinesterase inhibition activity**

It is well established that memory deficit and cognitive problems are signs of Alzheimer's disease mainly due to destructive neurodegeneration.

It results from a significant decline in acetylcholine amount following its hydrolysis by both acetylcholinesterase and butyrylcholinesterase enzymes [69]. Therefore, the inhibition of these two enzymes can boost acetylcholine levels and symptoms associated with the progressive loss of cholinergic function in Alzheimer's disease [70,71]. The anticholinesterase activity was investigated and compared to that of galantamine. The results of enzymes inhibition are illustrated in **Fig. 6**.

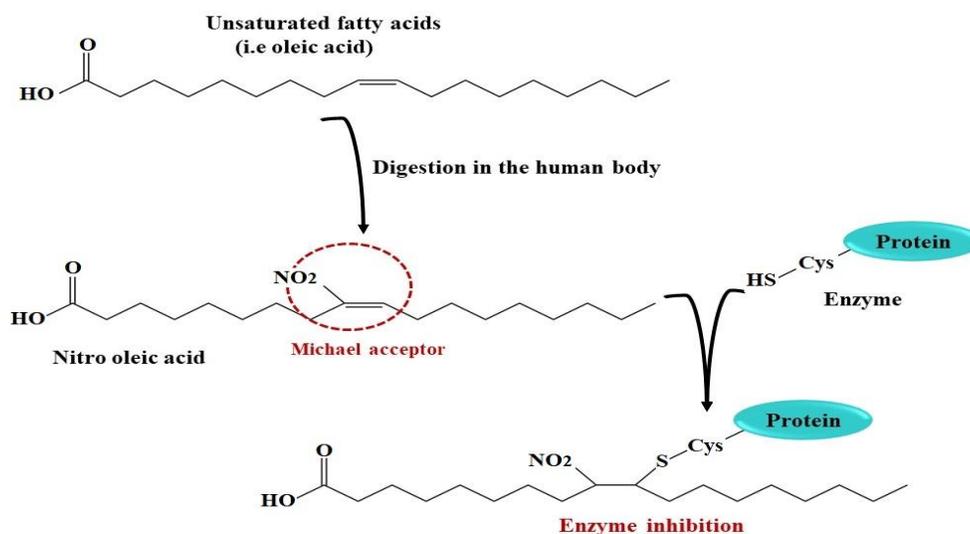
A)



**Fig. 6.** Inhibition by sesame oil, loaded-nanoparticles and galantamine of acetylcholinesterase (A) and butyrylcholinesterase (B).

The inhibition of the two enzymes is dose dependent. Sesame oil had cholinesterase inhibitory activity since the free particles did not exhibit any enzyme inhibition. From **Fig. 6**, it can be seen also a weak activity of sesame oil on acetylcholinesterase. Galantamine showed the highest inhibitory effect in terms of percentage (94.8%) for a concentration of  $200\mu\text{g.mL}^{-1}$  followed by sesame oil loaded-NP and un-encapsulated sesame oil with inhibition rates of 62.3 and 53.8%, respectively. In respect to butyrylcholinesterase, a moderate activity of the oil is noticed; the IC<sub>50</sub> were equal to 90.1, 62.2 and  $34.7\mu\text{g.mL}^{-1}$  for the un-encapsulated oil, the loaded NP and galantamine, respectively (**Table 7**). The cholinesterase used in this study were horse

butyrylcholinesterase and *Electrophorus electricus* acetylcholinesterase. It was reported in our previous work elaborated on *Saussurea lappa* essential oil [30] that horse derived butyrylcholinesterase is similar to the human serum butyrylcholinesterase at 90.1% and it contained sulfhydryl groups which interact with electrophilic groups by Michael-type reactions. Frasco et al. noticed an irreversible human cholinesterase inhibition in the micromolar range as a consequence of inhibiting the free sulfhydryl groups [72]. According to the literature, during digestion in the human body, mono and polyunsaturated fatty acids like linoleic and oleic acids, the main fatty acids of sesame oil, transform into nitrated fatty acids having electrophilic characteristics and promoting with histidine and cysteine residues Michael-type addition reactions, as illustrated in **Fig. 7** [73–75]. This may explain the chemical interaction between sesame oil and butyrylcholinesterase.



**Fig. 7.** Michael addition of nitro fatty acids with thiol groups of enzymes.

Regarding acetylcholinesterase, it was reported that *Electrophorus electricus* acetylcholinesterase does not contain free sulfhydryl groups and the enzyme inhibition happened in the millimolar range [72]. At the same time, several reports have illustrated

the major effect of polyphenols on the inhibition of cholinesterase enzymes and more particularly on acetylcholinesterase [76,77]. Lan et al. showed that cinnamic acid had an acetylcholinesterase inhibitory potential three times higher than the standard drug (donepezil) [78]. Additionally, Balkis et al. noticed that the position, the substitution, the number of hydroxyl groups, and the oxidation of flavonoids affect acetylcholinesterase inhibition [79]. Taking into account the results of this work, the low activity of sesame oil on acetylcholinesterase can be attributed to its very low content of total polyphenols. This explained the difference between the IC50 values sesame oil against the two enzymes.

**Fig. 6** illustrates also that the encapsulation of sesame oil enhances its anti-cholinesterase potential. Lower IC50 values were shown for both enzymes (**Table 7**). This was in agreement with other studies where they noticed improved biological activity upon the encapsulation of plant oils [80].

Overall, the developed sesame oil NPs can be considered as a reliable way to manage Alzheimer's disease.

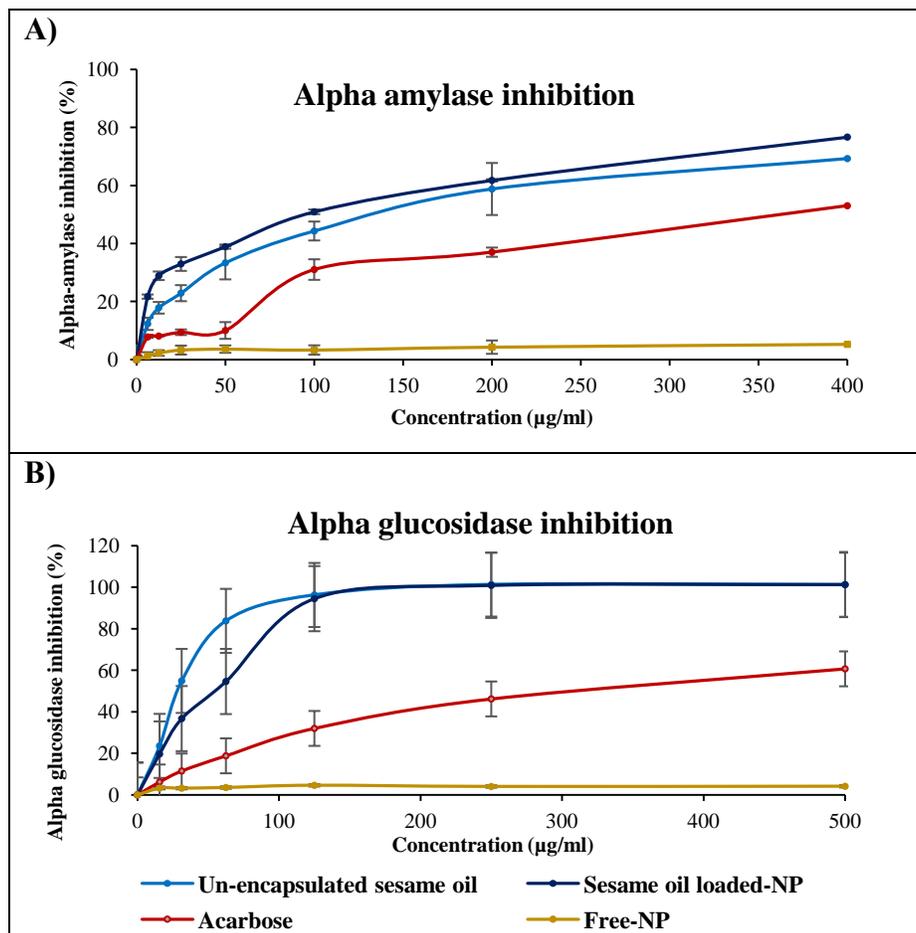
**Table 7.** Concentrations of 50% inhibition of enzymes for sesame oil, loaded-nanoparticles and standard drugs.

IC50 ( $\mu\text{g.mL}^{-1}$ )	AchE	BchE	$\alpha$ -amylase	$\alpha$ -glucosidase	Urease
Sesame oil	182.60 $\pm$ 8.66	90.12 $\pm$ 8.19	107.77 $\pm$ 7.39	33. 3 $\pm$ 4	182.29 $\pm$ 4.31
Oil loaded-NP	130.74 $\pm$ 3.54	62.21 $\pm$ 8.69	77.72 $\pm$ 3.62	53.6 $\pm$ 2	77.73 $\pm$ 8.25
Galantamine	6.3 $\pm$ 1.2	34.7 $\pm$ 1.9	-	-	-
Acarbose	-	-	365.3 $\pm$ 10.7	271.7 $\pm$ 7.1	-
Thiourea	-	-	-	-	11.57 $\pm$ 0.68

AchE: Acetylcholinesterase; BchE : Butyrylcholinesterase.

### 3.5.2. Inhibition activities of Alpha amylase and $\alpha$ -glucosidase

Diabetes mellitus, a complex chronic disease characterized by high glucose level in blood attributable to insulin deficiency, is considered an important cause of death worldwide [81]. Correcting the postprandial hyperglycemia may form part of the strategy for the management of diabetes. Currently, providing diabetic people with healthy diet is challenging [82]. Many researchers revealed the link between  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition and the management of diabetes [83]. The inhibition of these two enzymes was evaluated and compared to that of acarbose and the results are demonstrated in **Fig. 8** and **Table 7**.



**Fig. 8.**  $\alpha$ -amylase (A) and  $\alpha$ -glucosidase (B) inhibition by sesame oil, loaded-NPs and Acarbose

By comparing the activities of empty and loaded NP, it can be deduced that the antidiabetic effect was due to the presence of sesame oil and more specifically to unsaturated fatty acids. Indeed, several studies have demonstrated the effectiveness of unsaturated fatty acids in the management of type 2 diabetes [84–86]. The GC-FID analysis revealed that sesame oil consisted mainly of unsaturated fatty acids (81.858%) including 40.784% of oleic acid and 39.840% of linoleic acid.

**Table 7** demonstrates that sesame oil in its free or encapsulated form exhibited better inhibition than acarbose against both enzymes. The IC<sub>50</sub> values were 107.8, 77.7 and 365.3  $\mu\text{g}\cdot\text{mL}^{-1}$  against  $\alpha$ -amylase; and 33.3, 53.6 and 271.7  $\mu\text{g}\cdot\text{mL}^{-1}$  against  $\alpha$ -glucosidase for, free oil, oil loaded-NP and acarbose, respectively.

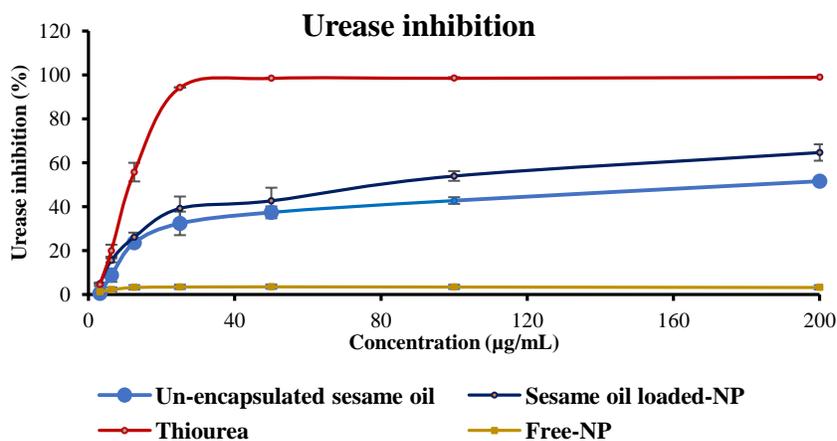
**Table 7** revealed also that sesame oil was more effective in inhibiting  $\alpha$  glucosidase compared to  $\alpha$  amylase. As mentioned previously concerning the chemical structure of diabetes key enzymes, almost all  $\alpha$ -glucosidases are extremely inhibited by sulfhydryl groups-binding reagents [87,88]. Additionally, Su et al. reported that oleic acid was a competitive inhibitor of  $\alpha$ -glucosidase [89]. Miyazawa et al. stated that increasing the number of fatty acid double bonds enhanced  $\alpha$ -glucosidase inhibition [90]. This explains the high IC<sub>50</sub> for sesame oil comparing with date seeds oil (IC<sub>50</sub> = 88.9  $\mu\text{g}\cdot\text{mL}^{-1}$ ), investigated in a previous work [30] where the oil contained only 58.027% of unsaturated fatty acids. With respect to  $\alpha$ -amylase, Schramm et al. pointed out that the complete oxidation of sulfhydryl groups concurred with 80% inactivation of  $\alpha$ -amylase [91], thus these groups were not absolutely required in enzyme inhibition. Moreover, it was reported that polyphenols can inhibit  $\alpha$ -amylase in a competitive, non-competitive or

mixed way [92]. Therefore, the low content of polyphenols present in the oil explained the moderate activity on this enzyme.

In overall, sesame oil in its free or encapsulated form represent a promising formulation system for the inhibition of diabetes-associated enzymes and can be used as an alternative to oral hypoglycemic drugs, the intake of which is often associated with adverse reactions like diarrhea, gastrointestinal discomfort, nausea, cramps, flatulence and weight gain [93].

### 3.5.3. Urease activity

Urease is generally found in different bacteria like *Helicobacter pylori*, an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate, which decomposes rapidly and spontaneously, yielding a second molecule of ammonia. These reactions may increase the pH and cause negative effects in human health like gastric and duodenal ulcers [94]. Targeting urease for treating diseases caused by urease-producing bacteria has gained tremendous attention over the years [95,96]. In this context, the urease activity of sesame oil and its corresponding NP has been tested and compared to that of a standard drug: Thiourea and the results are demonstrated in **Fig. 9**.



**Fig. 9.** Inhibition by sesame oil, loaded-nanoparticles and thiourea of urease.

**Fig. 9** revealed that sesame oil exhibited a moderate urease activity which was enhanced upon its encapsulation. The ranked order of inhibition at the higher concentration ( $200 \mu\text{g.mL}^{-1}$ ) was as follow: thiourea (98.9%) > sesame oil loaded NP (64.6%) > un-encapsulated sesame oil (51.5%). These results were further confirmed by the values of IC50, demonstrated in **Table 7**, where thiourea showed the best enzyme inhibition. The effect of sesame oil on urease enzyme was related to its composition in fatty acids. For instance,

According to Thompson et al. unsaturated fatty acids have shown inhibitory effects on *Helicobacter pylori* urease. The higher is the unsaturation degree of the fatty acids, the greater are these effects, following, for instance, the typical order: (eicosapentaenoic acid [C20:5] > arachidonic acid [C20:4] > linolenic acid [C18:3] > linoleic acid [C18:2] > oleic acid [C18:1]) [97].

To elucidate the reaction mechanism between sesame oil and the enzyme, it has been reported that Jack bean urease reactivity, the enzyme used in this study, is linked to its thiol groups. Riddles et al. related urease activity to the unique active-site cysteine residue which was inhibited by adding  $\beta$ -mercaptoethanol [98]. Sakaguchi et al. illustrated that diazonium-1H-tetrazole modified specifically cysteine residues resulting in inhibition of urease enzyme and loss of polymerization ability [99]. Thus, it may be assumed that oleic and linoleic acids of the studied oil interacted with urease cysteine residues via Michael type reactions.

#### **4. Conclusion**

The current research explored a novel approach of polymeric NP for the encapsulation of sesame oil produced by a green, an innovative, a rapid and a reproducible technique. The

physicochemical characterization revealed that the vegetable oil consisting of oleic and linoleic as the major constituents, was efficiently encapsulated in PMMA based NP of 259 nm diameter, a  $\zeta$ -potential value of +78 mV and an encapsulation efficiency equal to 99%. Furthermore, *in vitro* enzymatic and antioxidant activities were for the first time carried out and compared to that of standard drugs. Results revealed that the developed NP were more effective in inhibiting diabetes key controlling enzymes. The IC<sub>50</sub> against  $\alpha$ -glucosidase and  $\alpha$ -amylase were equal to 53.6 and 77.72  $\mu\text{g}\cdot\text{mL}^{-1}$ , respectively. Moderate antiurease and anti-Alzheimer activities were also observed with IC<sub>50</sub> around 77.73, 62.21 and 130.74  $\mu\text{g}\cdot\text{mL}^{-1}$  against urease, butyrylcholinesterase and acetylcholinesterase, respectively. Finally, a very low antioxidant property was noticed due to the low content of total polyphenols. Further *in vivo* clinical studies would be warranted for a more complete characterization of the systems developed in this study.

#### **Authors' contributions**

Narimane Lammari: Investigation, Methodology, Writing original draft; Mehdi Louaer and Ahmed Zermane: Investigation; Ouahida Louaer: Conceptualization, Supervision; Chawki Bensouici: Formal analysis; Abdelhamid Elaissari: Conceptualization, Supervision; Abdeslam Hassan Meniai: Writing - review & editing.

#### **Competing interests**

All authors declare no conflict of interests.

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## References

- [1] C. Veeresham, Natural products derived from plants as a source of drugs, *J. Adv. Pharm. Technol. Res.* 3 (2012) 200. <https://doi.org/10.4103/2231-4040.104709>.
- [2] D.A. Dias, S. Urban, U. Roessner, A historical overview of natural products in drug discovery, *Metabolites.* 2 (2012) 303–336.
- [3] M.M. Robinson, Z. Xiaorui, The world medicines situation (Traditional medicines: global situation, issues and challenges), *World Heal. Organ.* (2011).
- [4] S. Wachtel-Galor, I.F.F. Benzie, Herbal medicine: An introduction to its history, usage, regulation, current trends, and research needs, in: I.F.F. Benzie, S. Wachtel-Galor (Eds.), *Herbal Medicine: Biomolecular and Clinical Aspects*, CRC Press, 2011, pp. 1–10.
- [5] G.M. Cragg, D.J. Newman, Biodiversity: A continuing source of novel drug leads, *Pure Appl. Chem.* 77 (2005) 7–24.
- [6] K.R. Anilakumar, Nutritional, medicinal and industrial uses of sesame (*Sesamum indicum* L.) seeds - an overview, *Agric. Conspec. Sci.* 75 (2010) 4.
- [7] S. Abbas, M.K. Sharif, M. Sibte-Abbas, T.F. Teferra, M.T. Sultan, M.J. Anwar, Nutritional and therapeutic potential of sesame seeds, *Biol. Potential Chem. Compos. Funct. Food.* 2022 (2022) 6163753.
- [8] C. Muthulakshmi, R. Sivaranjani, S. Selvi, Modification of sesame (*Sesamum indicum* L.) for Triacylglycerol accumulation in plant biomass for biofuel applications, *Biotechnol. Reports.* 32 (2021) e00668.
- [9] M.G. Agidew, A.A. Dubale, M. Atlabachew, W. Abebe, Fatty acid composition, total phenolic contents and antioxidant activity of white and black sesame seed

varieties from different localities of Ethiopia, *Chem. Biol. Technol. Agric.* 8 (2021) 14.

- [10] K.I. Sallam, S.M. Abd-Elghany, K. Imre, A. Morar, V. Herman, M.A. Hussein, M.A. Mahros, Ensuring safety and improving keeping quality of meatballs by addition of sesame oil and sesamol as natural antimicrobial and antioxidant agent, *Food Microbiol.* 99 (2021) 103834. <https://doi.org/10.1016/j.fm.2021.103834>.
- [11] M. Arshad, M. Ashfaq, B. Rizwan, R. Zanib, A. Fatima, R. Pervaiz, Z. Gulzar, A. Fatima, Hypolipidemic effect of sesame seed oil: A review, *Biomed. J.* 5 (2022) 3–8.
- [12] H.F. Elbakry, H.A.R.A. Salam, S.S. Abdelgayed, D.A. Mohamed, Hepatorenal protective effects of sesame seeds oil, flaxseed oil and their mixture against methotrexate toxicity in rats, *Iran. J. Toxicol.* 16 (2022) 51–62.
- [13] C. Wekpa, U.A. Obisike, H. Brown, E.O. Nwachuku, Evaluation of the effect of diet containing sesame seed (*Sesamum indicum*) on some biochemical variables of diabetic albino wistar rats, *Int. Res. J. Gastroenterol. Hepatol.* 5 (2022) 25–37.
- [14] A.F. Majdalawieh, S.M. Yousef, I.A. Abu-Yousef, G.K. Nasrallah, Immunomodulatory and anti-inflammatory effects of sesamin: mechanisms of action and future directions, *Crit. Rev. Food Sci. Nutr.* 62 (2022) 5081–5112.
- [15] S. Naeem, L. Ali, R. Ikram, S.S. Khan, H. Shareef, Y. Shafiq, N. Alam, A. Adil, Comparative antioxidant and analgesic effect of sesame oil, fish oil and their combination in experimental animal model, *Pak. J. Pharm. Sci.* 34 (2022) 499–506.
- [16] A.E. Allam, Y. Amen, A. Ashour, H.K. Assaf, H.A. Hassan, I.M. Abdel-Rahman,

- A.M. Sayed, K. Shimizu, In silico study of natural compounds from sesame against COVID-19 by targeting Mpro, PLpro and RdRp, *RSC Adv.* 11 (2021) 22398–22408.
- [17] M.A. Mujtaba, H.M. Choc, H.H. Masjukiad, M.A. Kalama, H.C. Onga, M. Gulae, M.H. Haritha, M. Yusoffa, Critical review on sesame seed oil and its methyl ester on cold flow and oxidation stability, *Energy Reports.* 6 (2020) 40–54.
- [18] J.B. Morris, Food, industrial, nutraceutical, and pharmaceutical uses of sesame genetic resources, in: J. Janick, A. Whipkey (Eds.), *Trends New Crop. New Uses*, ASHS Press, Alexandria, VA, 2002, pp. 153–156.
- [19] U.M. Omar, H.M. Alsufiani, A.A. Almalki, A.S. Alsolami, L.W. Kuddah, N.M. Alghashmari, T.S. Aldayel, R.A. Mansouri, Enhancing the stability of polyunsaturated sesame oil against oxidation via addition of natural antioxidant during storage, *Food Public Heal.* 10 (2020) 72–80.
- [20] A. Rustan, C. Drevon, Fatty acids: structures and properties, in: I.E.A. Board (Ed.), *Encycl. Life Sci.*, Nature Publishing Group, London, 2005, pp. 1–7.
- [21] L. Tao, Oxidation of polyunsaturated fatty acids and its impact on food quality and human health, *Adv. Food Technol. Nutr. Sci.* 1 (2015) 135–142.
- [22] S. Jurić, M. Jurić, M.A. Siddique, M. Fathi, Vegetable oils rich in polyunsaturated fatty acids: nanoencapsulation methods and stability enhancement, *Food Rev. Int.* 38 (2020) 1–38.
- [23] A. Fadda, D. Sanna, E.H. Sakar, S. Gharby, M. Mulas, S. Medda, N.S. Yesilcubuk, A.C. Karaca, C.K. Gozukirmizi, M. Lucarini, G. Lombardi-Boccia, Z. Diaconeasa, A. Durazzo, Innovative and sustainable technologies to enhance the oxidative

stability of vegetable oils, *Sustainability*. 14 (2022) 849.

- [24] J. Rodríguez, M.J. Martín, M.A. Ruiz, B. Clares, Current encapsulation strategies for bioactive oils : From alimentary to pharmaceutical perspectives, *Food Research Int.* 83 (2016) 41–59. <https://doi.org/10.1016/j.foodres.2016.01.032>.
- [25] N. Lammari, O. Louaer, A. Hassen, H. Fessi, A. Elaissari, Plant oils : From chemical composition to encapsulated form use, *Int. J. Pharm.* 601 (2021) 120538. <https://doi.org/10.1016/j.ijpharm.2021.120538>.
- [26] M. Elgegren, S. Kim, D. Cordova, C. Silva, J. Noro, A. Cavaco-Paulo, J. Nakamatsu, Ultrasound-assisted encapsulation of Sacha Inchi (*Plukenetia volubilis* Linneo.) oil in alginate-chitosan nanoparticles, *Polymers (Basel)*. 11 (2019) 1245.
- [27] P. Ferreira, T. Haddi, F.T. Corrêa, V.L.B. Zapata, T.B. Piau, L.F.N. Souza, Prolonged mosquitocidal activity of *Siparuna guianensis* essential oil encapsulated in chitosan nanoparticles, *PLoS Negl. Trop. Dis.* 13 (2019) e0007624.
- [28] J. Ghitman, G.G. Pircalabioru, A. Zainea, L. Marutescu, H. Lovu, E. Vasile, C. Stavarache, B.S. Vasile, R. Stan, Macrophage-targeted mannose-decorated PLGA-vegetable oil hybrid nanoparticles loaded with anti-inflammatory agents, *Colloids Surfaces B Biointerfaces*. 213 (2022) 112423.
- [29] N. Lammari, F. Froiio, M. Louaer, M.C. Cristiano, C. Bensouici, D. Paolino, W. Louaer, A.H. Meniai, A. Elaissari, Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammoniumethyl methacrylate chloride) (Eudragit RS 100) nanocapsules as nanovector carriers for *Phoenix dactylifera* L. seeds oil: a versatile antidiabetic agent., *Biomacromolecules*. 21 (2020) 4442–4456. <https://doi.org/10.1021/acs.biomac.0c00255>.

- [30] N. Lammari, T. Demautis, O. Louaer, A. Hassen, A. Bentaher, A. Elaissari, Nanocapsules containing *Saussurea lappa* essential oil: Formulation, characterization, antidiabetic, anti-cholinesterase and anti-inflammatory potentials, *Int. J. Pharm.* 593 (2021) 120138. <https://doi.org/10.1016/j.ijpharm.2020.120138>.
- [31] H. Fessi, F. Puisieux, J.P. Devissaguet, N. Ammoury, S. Benita, Nanocapsule formation by interfacial polymer deposition following solvent displacement, *Int. J. Pharm.* 55 (1989) R1–R4.
- [32] M.. Blois, Antioxidant determinations by the use of a stable free radical, *Nature*. 4617 (1958) 1119–1200.
- [33] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 26 (1999) 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- [34] M. Oyaizu, Studies on product of browning reaction prepared from glucose amine, *Japanese J. Nutr. Diet.* 44 (1986) 307–315.
- [35] A. Zydowska-Czerniaka, C. Dianoczeki, K. Recseg, G. Karlovits, E. Szlyk, Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods, *Talanta*. 76 (2008) 899–905.
- [36] X. Yang, M. Huang, Y. Jin, L.-N. Sun, Y. Song, H.-S. Chen, Phenolics from *Bidens bipinnata* and their amylase inhibitory properties, *Fitoterapia*. 83 (2012) 1169–1175. <https://doi.org/10.1016/j.fitote.2012.07.005>.
- [37] R. Vadivelan, R.G. Krishnan, R. Kannan, Antidiabetic potential of *Asparagus racemosus* wild leaf extracts through inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase, *J.*

<https://doi.org/10.1016/j.jtcme.2017.10.004>.

- [38] K. Rhee, M. Van de Meent, K. Ingkaninan, R. Verpoorte, Screening for acetylcholinesterase inhibitors from amaryllidaceae using silica gel thin-layer chromatography in combination With bioactivity staining, *J. Chromatogr. A.* 915 (2001) 217–223.
- [39] G.. Ellman, K.. Courtney, V. Andres, R.. Featherston, A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* 7 (1961) 88–95.
- [40] M.. Weatherburn, Phenol-hypochlorite reaction for determination of ammonia, *Anal. Chem.* 39 (1967) 971–974.
- [41] M. Biglar, Profiling of major fatty acids in different raw and roasted sesame seeds cultivars, *African J. Biotechnol.* 11 (2012) 6619–6623.
- [42] C. Kurt, Variation in oil content and fatty acid composition of sesame accessions from different origins, *Grasas Aceites.* 69 (2018) 1–9.
- [43] B. Were, A. Onkware, S. Gudu, M. Welander, A. Carlsson, Seed oil content and fatty acid composition in East African sesame (*Sesamum indicum* L.) accessions evaluated over 3 years, *F. Crop Res.* 97 (2006) 254–260.
- [44] V. Rosset, N. Ahmed, I. Zaanoun, B. Stella, H. Fessi, A. Elaissari, Elaboration of argan oil nanocapsules containing Naproxen for cosmetic and transdermal local application, *J. Colloid Sci. Biotechnol.* 1 (2012) 218–224.
- [45] W. Badri, K. Miladi, R. Eddabra, H. Fessi, A. Elaissari, Elaboration of nanoparticles containing indomethacin: Argan oil for transdermal local and

- cosmetic application, *J. Nanomater.* 2015 (2015) 1–9.  
<https://doi.org/10.1155/2015/935439>.
- [46] F. Froiio, A. Mosaddik, M.T. Morshed, D. Paolino, H. Fessi, A. Elaissari, Edible polymers for essential oils encapsulation: Application in food preservation, *Ind. Eng. Chem. Res.* 58 (2019) 46.
- [47] S.A. Tayel, M.A. El-Nabarawi, M.I. Tadros, W.H. Abd-Elsalam, Positively charged polymeric nanoparticle reservoirs of Terbinafine hydrochloride: Preclinical implications for controlled drug delivery in the aqueous humor of rabbits, *AAPS PharmSciTech.* 14 (2013) 782–793.
- [48] M. Khudzaifi, S.S. Retno, A. Rohman, The employment of FTIR spectroscopy and chemometrics for authentication of pumpkin seed oil from sesame oil, *Food Res.* 4 (2020) 42–48. [https://doi.org/10.26656/fr.2017.4\(2\).313](https://doi.org/10.26656/fr.2017.4(2).313).
- [49] I. Tarhan, A.A. Ismail, H. Kara, Quantitative determination of free fatty acids in extra virgin olive oils by multivariate methods and Fourier transform infrared spectroscopy considering different absorption modes, *Int. J. Food Prop.* 20 (2017) S790–S797. <https://doi.org/10.1080/10942912.2017.1312437>.
- [50] H. Jahangirian, M.J. Haron, N.A. Yusof, S. Silong, A. Kassim, R. Rafiee-Moghaddam, M. Peyda, Y. Gharayebi, Enzymatic synthesis of fatty hydroxamic acid derivatives based on palm kernel oil, *Molecules.* 16 (2011) 6634–6644. <https://doi.org/10.3390/molecules16086634>.
- [51] J.A. Olowokere, A.I. Onen, M.C. Odineze, I.D. B’aga, E.G. Kefas, Extraction and characterization of oil from date palm (*Phoenix dactylifera*) seed, *Asian J. Appl. Chem. Res.* 3 (2019) 1–9. <https://doi.org/10.9734/ajacr/2019/v3i430095>.

- [52] M.A. Ali, T.A. Al-hattab, I.A. Al-hydary, Extraction of date palm seed oil (Phoenix Dactylifera) by soxhlet apparatus, *Int. J. Adv. Eng. Technol.* 8 (2015) 261–271.
- [53] S.B. Sibokoza, M.J. Moloto, F. Mtunzi, N. Moloto, Diphenyldiselenide mediated synthesis of copper selenide nanoparticles and their poly(methyl methacrylate) nanofibers, *Asian J. Chem.* 30 (2018) 1455–1459. <https://doi.org/10.14233/ajchem.2018.21166>.
- [54] M.F. Khurshid, T. Hussain, R. Masood, N. Hussain, Development and evaluation of a controlled drug delivery wound dressing based on polymeric porous microspheres, *J. Ind. Text.* 46 (2016) 986–999. <https://doi.org/10.1177/1528083715612231>.
- [55] C. Migdal, M. Serres, Espèces réactives de l’oxygène et stress oxydant, *Médecine/Sciences.* 27 (2011) 405–412. <https://doi.org/10.1051/medsci/2011274017>.
- [56] A. Favier, Le stress oxydant: Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique, *Actual. Chim.* 270 (2003) 108–115.
- [57] S.B. Nimse, D. Pal, Free radicals, natural antioxidants, and their reaction mechanisms, *RSC Adv.* 5 (2015) 27986–28006. <https://doi.org/10.1039/C4RA13315C>.
- [58] I. Gülçin, Antioxidant activity of food constituents: An overview, *Arch. Toxicol.* 86 (2012) 345–391. <https://doi.org/10.1007/s00204-011-0774-2>.
- [59] K. Ruslan, S. Happyniar, I. Fidrianny, Antioxidant potential of two varieties of

Sesamum indicum L. collected from Indonesia, J. Taibah Univ. Med. Sci. 13 (2018) 211–218.

- [60] D. Bopitiya, T. Madhujith, Antioxidant activity and total phenolic content of sesame (*Sesamum indicum* L.) seed oil, Trop. Agric. Res. 24 (2014) 296–302.
- [61] N. Pourreza, Phenolic compounds as potential antioxidant, Jundishapur J. Nat. Pharm. Prod. 8 (2013) 149–150.
- [62] M.R.S. Ardekani, M. Khanavi, M. Hajimahmoodi, M. Jahangiri, A. Hadjiakhoondi, Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran, Iran. J. Pharm. Res. 9 (2010) 141–146. <https://doi.org/10.22037/ijpr.2010.849>.
- [63] A. Daoud, D. Malika, S. Bakari, N. Hfaiedh, K. Mnafigui, A. Kadri, N. Gharsallah, Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars, Arab. J. Chem. 12 (2019) 3075–3086. <https://doi.org/10.1016/j.arabjc.2015.07.014>.
- [64] W. Kchaou, F. Abbès, C. Blecker, H. Attia, S. Besbes, Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (*Phoenix dactylifera* L.), Ind. Crops Prod. 45 (2013) 262–269. <https://doi.org/10.1016/j.indcrop.2012.12.028>.
- [65] A. Siahpoosh, A.M. Taleb, H. Almasi, In vitro evaluation of antioxidant activity and total phenol contents of some extracts from ripe fruits of *Phoenix dactylifera* var Berhi, Int. J. Pharmacogn. Phytochem. Res. 8 (2016) 1855–1862.
- [66] A.M. Fariás-Campomanes, M.A. Rostagno, J.J. Coaquira-Quispe, M.A.A. Meireles, Supercritical fluid extraction of polyphenols from lees: overall extraction

- curve, kinetic data and composition of the extracts, *Bioresour. Bioprocess.* 2 (2015) 45.
- [67] K. Tyśkiewicz, M. Konkol, E. Rój, The application of supercritical fluid extraction in phenolic compounds isolation from natural plant materials, *Molecules.* 23 (2018) 2625. <https://doi.org/10.3390/molecules23102625>.
- [68] H. Djenidi, S. Khennouf, A. Bouaziz, Antioxidant activity and phenolic content of commonly consumed fruits and vegetables in Algeria, *Prog. Nutr.* 22 (2020) 224–235. <https://doi.org/10.23751/pn.v22i1.7701>.
- [69] G.T. Grossberg, Cholinesterase inhibitors for the treatment of Alzheimer's disease, *Curr. Ther. Res.* 64 (2003) 216–235. [https://doi.org/10.1016/S0011-393X\(03\)00059-6](https://doi.org/10.1016/S0011-393X(03)00059-6).
- [70] M. Mesulam, A. Guillozet, P. Shaw, B. Quinn, Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and alzheimer brain, *Neurobiol. Dis.* 9 (2002) 88–93. <https://doi.org/10.1006/nbdi.2001.0462>.
- [71] R.M. Lane, S.G. Potkin, A. Enz, Targeting acetylcholinesterase and butyrylcholinesterase in dementia, *Int. J. Neuropsychopharmacol.* 9 (2005) 101. <https://doi.org/10.1017/S1461145705005833>.
- [72] M.F. Frasco, J.P. Colletier, M. Weik, F. Carvalho, L. Guilhermino, J. Stojan, D. Fournier, Mechanisms of cholinesterase inhibition by inorganic mercury, *FEBS J.* 274 (2007) 1849–1861. <https://doi.org/10.1111/j.1742-4658.2007.05732.x>.
- [73] F.J. Chopfer, C. Cipollina, B.A. Freeman, Formation and signaling actions of electrophilic lipids, *Chem. Rev.* 111 (2011) 5997–6021.
- [74] C. Batthyany, F.J. Schopfer, P.R.S. Baker, R. Durán, L.M.S. Bake, Y. Huang, C.

- Cerveñansk, B.P. Branchaud, B.A. Freeman, Reversible post-translational modification of proteins by nitrated fatty acids in vivo, *J. Biol. Chem.* 281 (2006) 20450–63.
- [75] S.R. Salvatore, D.A. Vitturi, M. Fazzari, D.K. Jorkasky, F.J. Schopfer, Evaluation of 10-nitro oleic acid bio-elimination in rats and humans, *Sci. Rep.* 7 (2017) 39900.
- [76] N.R. Jabir, F.R. Khan, S. Tabrez, Cholinesterase targeting by polyphenols: A therapeutic approach for the treatment of Alzheimer's disease, *CNS Neurosci. Ther.* 24 (2018) 753–762. <https://doi.org/10.1111/cns.12971>.
- [77] M. Jung, M. Park, Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*, *Molecules.* 12 (2007) 2130–2139. <https://doi.org/10.3390/12092130>.
- [78] J.S. Lan, J.W. Hou, Y. Liu, Y. Ding, Y. Zhang, L. Li, T. Zhang, Design, synthesis and evaluation of novel cinnamic acid derivatives bearing N -benzyl pyridinium moiety as multifunctional cholinesterase inhibitors for Alzheimer's disease, *J. Enzyme Inhib. Med. Chem.* 32 (2017) 776–788. <https://doi.org/10.1080/14756366.2016.1256883>.
- [79] A. Balkis, K. Tran, Y.Z. Lee, K. Ng, Screening flavonoids for inhibition of acetylcholinesterase identified Baicalein as the most potent inhibitor, *J. Agric. Sci.* 7 (2015) 26–35. <https://doi.org/10.5539/jas.v7n9p26>.
- [80] F. Froiio, L. Ginot, D. Paolino, N. Lebaz, A. Bentaher, H. Fessi, A. Elaissari, Essential oils-loaded polymer particles: Preparation, characterization and antimicrobial property, *Polymers (Basel).* 11 (2019) 1017. <https://doi.org/10.3390/polym11061017>.

- [81] A.T. Kharroubi, H.M. Darwish, Diabetes mellitus: The epidemic of the century, *World J. Diabetes*. 6 (2015) 850–867. <https://doi.org/10.4239/wjd.v6.i6.850>.
- [82] S. Li, J. Wang, B. Zhang, X. Li, Y. Liu, Diabetes mellitus and cause-specific mortality: a population-based study, *Diabetes Metab. J.* 43 (2019) 319–341. <https://doi.org/10.4093/dmj.2018.0060>.
- [83] A. Duraiswamy, D. Shanmugasundaram, M.C. Sanjay, Changam Sheela Sasikumara, M.C. Kotturathu, Development of an antidiabetic formulation (ADJ6) and its inhibitory activity against  $\alpha$ -amylase and  $\alpha$ -glucosidase, *J. Tradit. Complement. Med.* 6 (2016) 204–208.
- [84] H. Teng, L. Chen,  $\alpha$ -Glucosidase and  $\alpha$ -amylase inhibitors from seed oil: A review of liposoluble substance to treat diabetes, *Crit. Rev. Food Sci. Nutr.* 57 (2017) 3438–3448. <https://doi.org/10.1080/10408398.2015.1129309>.
- [85] E.K. Vassiliou, A. Gonzalez, C. Garcia, J.H. Tadros, G. Chakraborty, J.H. Toney, Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF- both in vitro and in vivo systems, *Lipids Health Dis.* 8 (2009) 1–10. <https://doi.org/10.1186/1476-511X-8-25>.
- [86] T. Nguyen, B. Um, S. Kim, Two unsaturated fatty acids with potent alpha-glucosidase inhibitory activity purified from the body wall of sea cucumber (*Stichopus japonicus*), *J. Food Sci.* 76 (2011) 208–214.
- [87] A. Faridmoayer, C.H. Scaman, Binding residues and catalytic domain of soluble *Saccharomyces cerevisiae* processing alpha-glucosidase I, *Glycobiology.* 15 (2005) 1341–1348. <https://doi.org/10.1093/glycob/cwj009>.
- [88] H.M. El-Shora, M.A. Metwally, S.A. Khlaf, Essential groups and stability of  $\alpha$ -

- glucosidase of *Penicillium notatum*, *Ann. Microbiol.* 59 (2009) 285–291.
- [89] C.H. Su, C.H. Hsu, L.T. Ng, Inhibitory potential of fatty acids on key enzymes related to type 2 diabetes, *BioFactors.* 39 (2013) 415–421. <https://doi.org/10.1002/biof.1082>.
- [90] M. Miyazawa, N. Yagi, K. Taguchi, Inhibitory compounds of alpha-glucosidase activity from *Arctium lappa* L, *J. Oleo Sci.* 54 (2005) 589–594.
- [91] M. Schramm, Unmasking of sulfhydryl groups in pancreatic  $\alpha$ -amylase, *Biochemistry.* 3 (1964) 1231–1234. <https://doi.org/10.1021/bi00897a008>.
- [92] H. Nyambe-Silavwe, J.A. Villa-Rodriguez, I. Ifie, M. Holmes, E. Aydin, J.M. Jensen, G. Williamson, Inhibition of human  $\alpha$ -amylase by dietary polyphenols, *J. Funct. Foods.* 19 (2015) 723–732. <https://doi.org/10.1016/j.jff.2015.10.003>.
- [93] T. Fujisawa, H. Ikegami, K. Inoue, Y. Kawabata, T. Ogihara, Effect of two  $\alpha$ -glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms, *Metabolism.* 54 (2005) 387–390. <https://doi.org/10.1016/j.metabol.2004.10.004>.
- [94] M. Amin, F. Anwar, F. Naz, T. Mehmood, N. Saari, Anti-*Helicobacter pylori* and urease inhibition activities of some traditional medicinal plants, *Molecules.* 18 (2013) 2135–2149.
- [95] Z.P. Xiao, X.D. Wang, Z.Y. Peng, Molecular docking, kinetics study, and structure-activity relationship analysis of quercetin and its analogous as *Helicobacter pylori* urease inhibitors, *J. Agric. Food Chem.* 60 (2012) 10572–10577.
- [96] X. Zhu-Ping, S. Da-Hua, L. Huan-Qiu, Polyphenols based on isoflavones as

inhibitors of *Helicobacter pylori* urease, *Bioorganic Med. Chem.* 15 (2007) 3703–3710.

- [97] L. Thompson, A. Cockayne, R. Spiller, Inhibitory effect of polyunsaturated fatty acids on the growth of *Helicobacter pylori*: a possible explanation of the effect of diet on peptic ulceration, *Gut.* 35 (1994) 1557–1561.
- [98] P.W. Riddles, R.K. Andrews, R.L. Blakeley, B. Zerner, Jack Bean Urease VI. Determination of thiol and disulfide content: Reversible inactivation of the enzyme by the blocking of the unique cysteine residue, *Struct. Mol. Enzymol.* 743 (1983) 115–120.
- [99] K. Sakaguchi, K. Mitsui, J. Hase, K. Kobashi, Modification of thiol groups of Jack bean urease with diazonium-1H-tetrazole, *J. Biochem.* 95 (1984) 535–541.