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Green extraction of silymarin from milk thistle seeds and its encapsulation using the spray drying

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Abstract

he milk thistle seed, Silybum marianum (L.) Gaernt, is rich in silymarin, which has been found to have a number of pharmacological effects, including antioxidant, anti-inflammatory, anticancer, and cardioprotective activities. A comparison study of extract yield, silymarin content and antioxidant activity of various extraction methods was evaluated. Green extraction was performed using two different polarities of food-grade solvents (ethanol and ethyl acetate) using maceration extraction (ME) and the ultrasound-assisted extraction (UAE) method assays. The antioxidant activities of the resultant extracts were measured using the DPPH and ABTS scavenging radical assays.. The highest silymarin content (88.90 and 95.87.%) and antioxidant activities (306.40 and 137.014 µM Trolox Eq./mg for DPPH and ABTS, respectively) were found using ethanol solvent and UAE method compared to ethyl acetate and ME methods. During the encapsulation process, the size of the particles decreased as the percentage of gum Arabic (GA) increased, but the encapsulation efficiency improved, reaching a high of 84.45% at 20:80 MD: GA. The results also showed that adding MD and GA enhanced the encapsulation process, which is consistent with the efficiency of encapsulation. It can be said that silymarin can be extracted effectively using ethanol and the UAE. The addition of MD and GA improved the silymarin extract encapsulation process for functional foods.

Keywords: Silymarin; maceration; ultrasound-assisted extraction; encapsulation; spray drying; maltodextrin; gum arabic.

1. Introduction

In many developing countries, the use of herbal products and dietary supplements has significantly increased [1]. Milk thistle (*Silybum marianum L. Gaertn*) is one of the most ancient herbal medicines. It is an annual or biennial plant that belongs to the family Asteraceae (*Compositae*). It is native to the Mediterranean and has been cultivated in other warm and dry regions [2]. The plant is usually found and widespread in Egypt on roadsides, waste grounds, and cereal crops.

Silymarin is gaining an increasingly widespread clinical consensus as a natural remedy in the treatment of numerous chronic liver diseases such as cirrhosis and hepatocellular carcinoma due to its antioxidant, anti-inflammatory, anticancer, and antiviral activities. It is also able to increase the stability of the cell membrane. Silymarin is one of the basic substances used as an element of adjunctive therapy in treating liver diseases [3]. It is active in the regeneration of tissues and prevents cardiac diseases through numerous mechanisms that include the action of chelating promoters of metals and scavenging free radicals [4]. Silymarin extracted from milk thistle (*Silybum marianum*) seeds contains many flavonolignans such as silybin (50%-60%), silychristin (20%), silydianin (10%), and isosilibine (5%) as well as a flavonoid (taxifolin) [5, 6]. In the food industry, silymarin is considered a healthy nutritional supplement. Although clinical trials have shown silymarin is safe at high doses (>1500 mg/day) for humans, the pharmacokinetic studies over the past

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three decades proved that silymarin have revealed poor absorption, rapid metabolism, and ultimately poor oral bioavailability. Therefore, the encapsulation of silymarin is necessary for a suitable dosage form that can sufficiently enhance bioavailability [7]. Conventional methods of extraction like soxhlet, maceration, reflux, and hydro distillation have been used over decades [8]. Among these conventional methods is maceration, which is a technique where the plant materials are soaked in a solvent and placed in a closed container for at least three days [9]. UAE is a technology that can be used both on a small and large scale in the industry to extract natural products [10]. Many reports on the beneficial effects of UAE concerning natural products have been published, with significant improvements over conventional extraction methods offering much lower extraction times and enhanced efficiency [11, 12]. Encapsulation provides an effective method to cover an active compound with protective wall material and thus offers numerous advantages. Therefore, encapsulating silymarin into a suitable dosage form that can sufficiently enhance bioavailability is necessary [7]. Encapsulation mechanisms can provide the following benefits: targeted and maintained release of the active ingredient; improved adhesion, penetration, and release of the active principles under physiological changes in pH and temperature; safe handling of toxic compounds; separation of incompatible materials; masking of organoleptic such as color, taste, and odor of substances; reduced potency of toxicities; and reduction of drug dosage [13, 14].

The solubility of an active pharmaceutical entity is the most important parameter for developing a successful formulation. Actually, the active ingredient in silymarin has poor aqueous solubility, so it needs to be made water-soluble before utilization [15]. Gum Arabic is one of the most commonly used wall materials for microencapsulating hydrophobic compounds by spray drying because of its good emulsifying properties, high solubility and low viscosity [16]. Also, maltodextrins have been widely used for the microencapsulation of food due to their low cost, high solubility and low viscosity at high concentrations. However, the greatest disadvantage of this encapsulating agent is its lack of emulsifying properties due to its limited affinity for hydrophobic components [17]. Thus, using gum Arabic combined with maltodextrin could offer a good compromise between cost and effectiveness via the spray drying method. Spray drying is a low-cost and large-scale technique for encapsulating bioactive ingredients due to its efficiency, cost-effectiveness, and ability to produce tolerably good quality particles [18,19]. Therefore, the objectives of this study were to: (i) evaluate silymarin extraction as affected by extraction methods (maceration and ultrasonic-assisted extraction) and solvents (ethyl acetate, ethanol); (ii) encapsulate the extracted silymarin using safe edible materials (combination of gum Arabic and maltodextrin to increase its solubility and bioavailability using spray drying); and (iii) evaluate the physical and chemical characteristics of the resulting capsules.

2. Materials and Methods

2.1 Materials

The milk thistle plant was obtained from the widespread plant grown on roadsides in Egypt. It was identified by the taxonomist of the Botany Department, Faculty of Agriculture, Al-Azhar University. The seeds were separated from the plant. Ethyl acetate, ethanol, and n-hexane were purchased from Sigma chemicals company. Gum Arabic (GA) and maltodextrin (MD) were obtained from Alfasol Co., Turkey. All chemicals and reagents used in this work were of analytical grade.

2.2 *Methods*

2.2.1. Preparation of milk thistle seed powder

The seeds of the milk thistles plant were washed with tap water, then distilled water and dried in an oven dryer at 50ºC for 6 h. Then it was kept at room temperature (20±5ºC) for 4 h. Brown Multiquick blender (Germany) was used to mill the seeds, and the powder was stored in polyethylene bags and frozen at -18°C until use.

2.2.2. Extraction of Silymarin

Silymarin was processed using a two-step process; extraction of oil using the ultrasound assistant extraction (UAE) method (defatting by n-hexane) and then extraction of silymarin using solvent maceration and the UAE methods.

2.2.2.1 Oil extraction using the UAE

Oil of milk thistle seed powder was extracted using the UAE according to the method of (20) with some modifications: Typically, 500 g of powdered seeds were extracted using n-hexane for 30 min at

room temperature. A thermocouple inside the extraction mixture monitored the temperature by using an ice cooling bath around the extraction vessel to keep the temperature constant at 25.5 °C). Extraction was performed using an Ultrasonic Processor UP4200S (200 watts, 24 kHz, Hielscher). An ultrasonic probe with a tip diameter of 20 mm was employed for direct sonication (the horn tip position inside the extraction vessel was 1 cm below the solvent level). After extraction, the extract was centrifuged at 4000 rpm. The supernatant was evaporated under vacuum at 40°C to a constant weight, and yellow-colored residual oil was dried, then weighed and stored at −18 °C for analysis.

2.2.2.2 Silymarin extraction using maceration method

The defatted seeds were soaked in ethyl acetate and ethanol for 3 days. The collected extract was filtered through a Fisher brand QL100, 150-mm filter paper. The filtrated extract was evaporated till dryness under reduced pressure at 45°C and then weighed and stored at −18 °C for analysis. The obtained yield was calculated by the following Equation:

$$\textbf{\textit{Yield}}\ (\%) = \frac{\textbf{Weight of the extract (g)}}{\textbf{Weight of the dried sample (g)}}\ \text{X}\ 100$$

2.2.2.3 Silymarin extraction using the USE method

Silymarin was extracted according to Sherif et al. [20] with some modifications as follows: Briefly, the marc left after defatting was thoroughly extracted using ethyl acetate and ethanol for 30 min at room temperature. After extraction, the collected extract was filtered throughout a Fisher brand QL100, 150 mm filter paper. The filtrated extract was evaporated till dry under reduced pressure at 45°C, weighed and stored at −18 °C for high-performance liquid chromatography (HPLC) analysis. The obtained yield was calculated by the previous Equation 1.

2.2.3 Determination of total silymarin concentration in extracts

The concentration of silymarin in various samples was estimated using a JENWAY 6305 spectrophotometer. The reference standard of silymarin was dissolved in methanol to obtain a final concentration of 8 µg/ml. The absorption of silymarin standard and test solutions were measured at 288 nm. The silymarin concentration in extracts was expressed as % according to the following equation [21]:

$$\textbf{\textit{Silymarin}}\ (\%) = \frac{\textbf{A sample} \times \mathbf{m_0}}{\mathbf{A_0} \times \textbf{m sample}}\ \text{x}\ 100$$

Where: A sample is the absorption value of the sample, A_0 is the absorption value of the standard, m_0 is the mass of the standard, and m sample is the mass of the sample.

2.2.4 Identification of silymarin

The HPLC (Agilent 1100 series) was used to determine the chemical composition of each extract as well as the standard silymarin, equipped with G1315 B diode array detector (DAD), G1313A Autosampler, G1311A Quaternary Pump, G1322A Vacuum Degasser, G1321A Fluorescent Detector, G1316A Column Comp. The control and acquiring data systems were installed with the Agilent Chemo station for the LC system. Milk thistle seed (*S. marianum*) extract and standard silymarin were injected separately into semi-prep HPLC using different proportional of H_2O and methanol as mobile phase. The mobile phase was 90:10:1 methanol: H_2O: formic acid [Solvent A] and H_2O (containing 0.1% formic acid) [Solvent B]. At a flow rate of 0.5 ml/min, the oven was set at ~25 °C, and the injection volume was 5 µl. Detection was carried out by monitoring the absorbance signals at 288 nm.

2.2.5. Determination of antioxidant activity (AOA)

2.2.5.1. DPPH• radical scavenging activity

Silymarin sample extracts were prepared at 0.2mg/mL concentration in methanol. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to the method of Boly et al [22] Briefly; 100 µL of freshly prepared DPPH reagent (0.1% in methanol) were added to 100 µL of the sample in 96 wells plate (n=6), the reaction was incubated at room temp for 30 min in dark. At the end of incubation time the resulting reduction in DPPH color intensity was measured at 540 nm. Data are represented as means ± SD according to the following equation [23].

$$\%\ \textbf{\textit{inhiption}} = \frac{\textbf{average absorpance of blank} - \textit{average absobance of the test}}{\textit{average absorbance of blank}} \times 100$$

The standard curve was linear between 10 and 800 uM Trolox. Additional dilution was needed if the measured DPPH value was over the standard curve's linear range. Silymarin extract samples were prepared

at the following final concentrations: 25, 50, 100, 200and 400 µg/ml. IC_{50} represents the level where test samples scavenged 50% of the radicals.

2.2.5.2. ABTS• radical scavenging activity

Silymarin sample extracts were prepared at 0.2mg/mL concentration in methanol. The assay was carried out according to the method of Arnao et al. [24], with minor modifications to be carried out in microplates, briefly; 192 mg of ABTS was dissolved in distilled water and transferred to 50 mL volumetric flask, then the volume was completed with distilled water. 1mL of the previous solution was added to 17 µL of 140 mM potassium persulphate, and the mixture was left in the dark for 24 hours. After that, 1mL of the reaction mixture was completed to 50 mL with methanol to obtain the final ABTS dilution used in the assay. 190 µL of The freshly prepared ABTS reagent was mixed with 10 µL of the sample/ compound in 96 wells plate (n=6), and the reaction was incubated at room temperature for 30 min in the dark. At the end of incubation time, the decrease in ABTS color intensity was measured at 734 nm Data are represented as means ± SD according to the following equation:

$$\%\ \boldsymbol{inhiption} = \frac{\textbf{average absorpance of blank} - \boldsymbol{average\ absobance\ of\ the\ test}}{\boldsymbol{average\ absorbance\ of\ blank}} \times 100$$

The standard curve was linear between 10 and 800 uM Trolox. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

Silymarin extract samples were prepared at the following final concentrations: 1.25, 2.5, 5, 7.5 and 10 µg/ml, IC_{50} represents 50% of the radicals that were scavenged by test samples.

2.2.6 Encapsulation of silymarin

Four silymarin formulations were encapsulated using different carrier agents (maltodextrin, MD and gum Arabic, GA) as described in Table 1. Both MD (10%) and GA (10 %) were dissolved in distilled water using gentle magnetic stirring at 50-600C and 40 0C, respectively, until completely dissolved. GA (10%) was dissolved in distilled water by gentle magnetic stirring at 40 ^{0}C. The wall materials, MD and GA were mixed in ratios of 8:2, 6: 4, 4:6 and 2:8 using gentle magnetic stirring for 1 h. Silymarin was dissolved in ethanol and then added to the wall material at a ratio 1:10. The solution capsules were formed using a magnetic stirrer for 15 min and then further homogenized using an Ultra-Turrax homogenizer at 18000 rpm/5 min. Following that, the mixtures were ultrasonically treated at 200 E power, 20 kHz frequency, and a 50% pulse. All formulations were dried using a spray dryer (Mini spray dryer B-290, BÜCHI Labor Technik, Switzerland) as described by El-Messery et al. [25]. The formulations were fed at a flow rate controlled by pump rotation speed. The flow rate of drying air was adjusted at 2.5 m^3/min, and the compressor air pressure of 0.06 MPa. Inlet and outlet air temperatures were adjusted at 180-195 and 71-75^{0}C, respectively. The feed flow rate was adjusted to 5 cm^3/min to prepare the powder form. The spray-dried powder was stored at −18 ^{0}C for further analysis.

Table 1. Encapsulated Silymarin formulations encapsulation using different carrier agents (10% MD and 10% GA).

Formulation code	Carrier agents (MD:GA)*
M1	80:20
M2	60:40
M3	40:60
M4	20:80

* Core: Carrier agents= 1:10

2.2.7. Encapsulation efficiency

The encapsulation efficiency (EE) of silymarin microcapsules was calculated according to the following equation as described by Ades et al. [26].

$$\boldsymbol{EE} = \frac{\textbf{TPC} - \textbf{SPC}}{\textbf{TPC}} \times \mathbf{100}$$

Where, TPC is the total phenolic content and SPC is the surface phenolic compound.

2.2.8. Characterization of encapsulated silymarin

Photon correlation spectroscopy was used to determine the particle size of encapsulated silymarin in terms of average intensity diameters and

polydispersity index (PDI) using a particle size analyzer, Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom) at a fixed angle of 173° at 25°C. The same equipment was used to determine the zeta potential and samples were analyzed in triplicate. The surface morphology of encapsulated silymarin was evaluated by Scanning Electron Microscopy (Quanta FEG 250 SEM) (Thermo Fisher Scientific, Oregon, USA).

2.2.9. Fourier transforms infrared spectroscopy (FTIR):

The function groups for capsule powder, silymarin, gum arabic, and maltodextrin were checked by FTIR (JASCOFT/IR 6100 using KBr wafer technique) in the region of 400-4000/cm. Each spectrum was obtained at a resolution of 1 /cm.

2.2.10 Statistical Analyses

SPSS (IBMR SPSSR, 2017) was used to analyze data (means ±standard division). Differences at 5% significance were assessed using ANOVA, Student's t-test and Duncan's multiple range tests ($P \leq 0.05$;). All experiments were repeated three times, and the analysis was performed in triplicate.

3. Results and Dissection

3.1. Silymarin yield in milk thistle seeds extract

Table 2 shows that the yield of silymarin was markedly affected by the type of solvent, ethanol and ethyl acetate, and the extraction methods. The extraction yield ranged from 5.02 to 4.08% when the maceration method was used and ranged from 4.62 to 3.14% when using ultrasonic-assisted extraction in ethanol and ethyl acetate, respectively. This means that the maceration method was more efficient in silymarin extraction than the UAE method, But the ME takes 3 days compared to the UAE, which only needs 30 min. These results were consistent with those found by Khalil-ur-Rahman et al. [27], who found that the silymarin content of milk thistle seed was 4.6% (46 mg/g). Also, Halbach et al. [28] mentioned that the silymarin content of the same varieties of MTS ranged between 4 and 6% (based on dry weight). Our result was slightly lower than that as reported by Abd Raboh [29], who found that the silymarin content of MTS exhibited 70 mg/g. This variation of silymarin content may be related to the varieties, geographic and climatic conditions, determination method and other agents. Ramawat ey al. [30], reported several concentrations of silymarin ranging from 1 to 6 % in the ripe fruit of MTS. From the previous results, it could be concluded that the yield of silymarin increased using ethanol as an extraction solvent compared to ethyl acetate. Moreover, using the USE extraction method for 30 min showed the best results compared with the maceration method. Hamouda and El-Adawi [31] demonstrated that extraction of defatted seeds for 3 h with ethanol (95%) at 50°C recorded the highest yield of phenolics-rich silymarin extract compared to methanol, acetone, acetonitrile and ethyl acetate. The solvent is one of many parameters that could affect the extraction of polyphenols [32]. The extraction may be carried out by solvents such as water, methanol, ethanol and acetone. However, aqueous solvents give better yields of extraction than absolute solvents [33].

Table 2. The yield of *Silybum marianum* L. seeds extracts as affected by the extraction methods and solvents.

Extraction method	Yield extract (g/100 g)	
	Ethanol	Ethyl acetate
Maceration (3 days)	5.02±0.11*	4.08±0.72
Ultrasonic (45 min)	4.62±0.22*	3.14±0.46

Data are the mean values of triplicate samples ± SD * significant at $P \leq 0.05$ using Student's t test

3.2 Silymarin concentration in milk thistle seeds extract

The concentration of silymarin extract obtained by using maceration varied from 88.90 to 83.27 g/100 g using ethanol or ethyl acetate solvent, respectively. Meanwhile, ultrasonic-assisted extraction achieved higher values that ranged from 95.87 to 90.54 g/100 g for ethanol or ethyl acetate solvent, respectively, as shown in Table 3. These results are confirmed by Saleh et al. [34], who reported that ultrasonic-assisted extraction was evaluated as a more effective alternative method than the conventional maceration extraction method for the extraction of silymarin from *Silybum marianum* L. seeds. They found the % increase of silymarin contents obtained using UAE. This increase could be attributed to the UAE mechanism of extraction. When a liquid is ultrasonically irradiated, acoustic cavitation bubbles occur. Bubbles form, grow and suddenly collapse, producing tremendous amounts of localized energy. In a system containing solid particles, the bubbles collapse, releasing high temperatures and pressures [~5000 °K and ~2000 atm] but also creating a high-speed jet directed towards the solid surface, in our case the herb particles [35,36]. The generated ultrasonic jets hit the herb particles at an extremely high speed, allowing better solvent penetration into the plant particles. The jets could also contribute to cells' pore enlargement, acting like a micro-pump forcing the solvent into the cell, where it can dissolve the

compounds and transport them into the bulk solvent [37]. The quantitative analysis by UV-visible spectrophotometry showed that the silymarin concentration varies from one extract to another. The highest content of silymarin was found in the ethanol extract compared to the ethyl acetate extract.

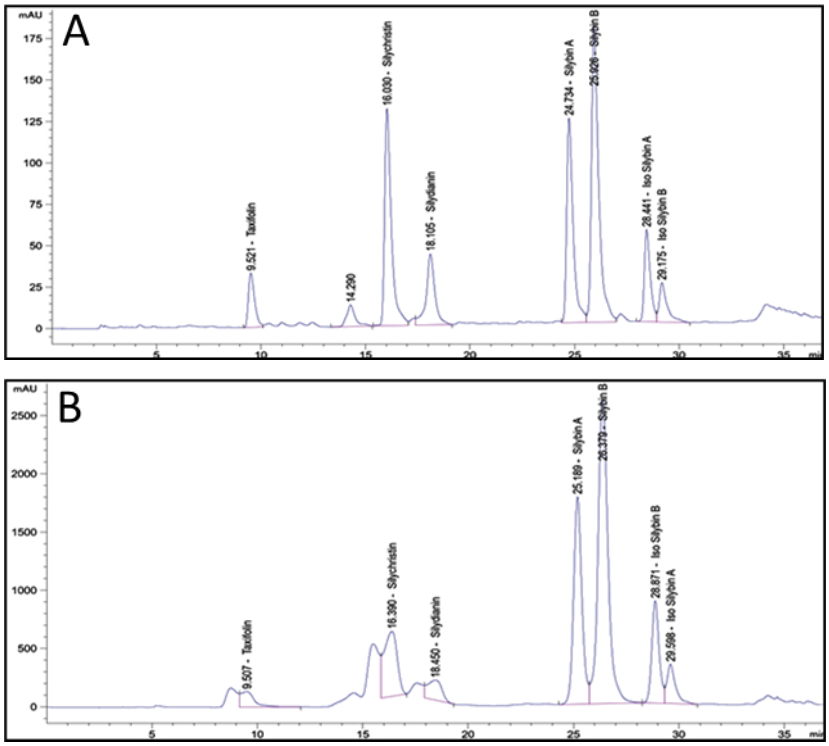
Table 3. Silymarin concentration in *Silybum marianum L.* seeds extract as affected by the extraction methods and solvents by spectrophotometer method

Extraction method	Silymarin concentration (g/100 g)	
	Ethanol	Ethyl acetate
Maceration (3 days)	88.90±0.97*	83.27±1.30
Ultrasonic (45 min)	95.87±1.69*	90.54±1.49

Data are the mean values of triplicate samples ± SD * significant at P ≤ 0.05 using Student's t test

3.3 Identification of silymarin components in Silybum marianum L. seeds using HPLC

Figs 1c and 1b illustrate the HPLC chromatograms of ethanol and ethyl acetate extracts of *Silybum marianum* seeds samples compared to the silymarin standard Fig. 1a. As seen in chromatograms, eight principal peaks were observed; each peak was identified as one of the flavonolignans constituents of the silymarin samples. The peaks were identified as: (1) Taxifolin, (2) Silychristin, (3) Silydianin, (4) Silybin A, (5) Silybin B, (6) Iso silybin A, and (7) Iso silybin B, respectively.Silymarin components of MTS were fractionated and determined by HPLC, and the results are shown in Table (4). Silybin B recorded the highest concentration in the ethanol extract of *Silybum marianum* seeds ethanol extract (353.16 mg/g), followed by Silybin A (195.61mg/g), Silychristin (135.64 mg/g), Iso silybin A (85.34 mg/g), Silydianin (85.01 mg/g), Iso silybin B (41.31 mg/g) and Taxifolin (m27.40 mg/gm). Also, Silybin B was the highest concentration in the ethyl acetate extract of Silybum marianum seeds, which was recorded (390.85 mg/g), followed by Silybin A, Silychristin, Iso silybin A, Iso silybin B, silydianin, and taxifolin respectively. The Silybin B constituent had the highest concentration in the ethanol extracts or ethyl acetate extract, while taxifolin was the lowest. The main component of *S. marianum* fruit extract (silymarin) is a flavonolignan called silybin, which is not only the major silymarin element but is also the most active ingredient of this extract, which has been confirmed in various studies [38].



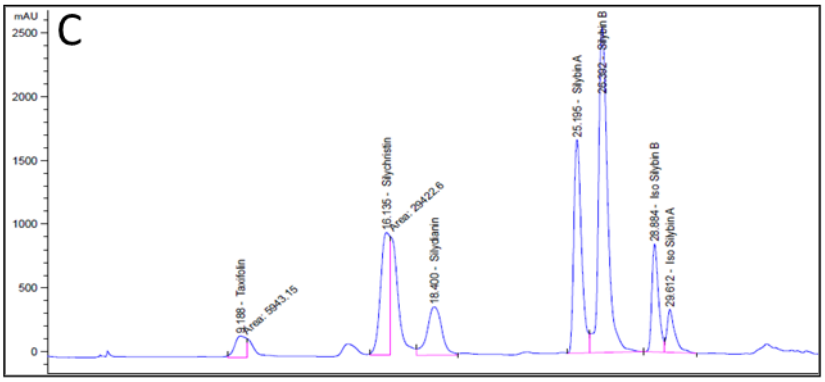


Fig. 1. HPLC chromatogram of A. standard silymarin, B. ethyl acetate extract, and C. ethanol extract.

Table 4. HPLC analysis of silymarin compounds in milk thistle seed extract by UAE as affected by the solvents

Compounds	Ethanol extract		Ethyl acetate extract	
	(mg/g)	(%)	(mg/g)	(%)
Taxifolin	27.40	2.97	27.24	2.97
Silychristin	135.64	14.69	107.98	11.76
Silydianin	85.01	9.21	35.93	3.91
Silybin A	195.61	21.18	217.74	23.71
Silybin B	353.16	38.24	390.85	42.56
Iso-Silybin A	85.34	9.24	95.20	10.37
Iso-Silybin B	41.31	4.47	43.38	4.72
Total	923.5	100.00	918.3	100.00

3.4 The antiradical activity of extracted silymarin

Milk thistle seeds are known to be a source of antioxidants [39]. DPPH and ABTS are the most popular and commonly used due to their ease, speed, sensitivity and stabile radicals [40,41]. As shown in Table 5, the AOA of silymarin extracts in ethanol and ethyl acetate was determined by using two assays (DPPH and ABTS methods). The antioxidant activity of silymarin was higher in ethanol extract (306.40 ±1.03) compared to ethyl acetate extract (260.03 ±18.25 µM Trolox eq/mg). This means that ethanol extract was the most effective in improving the antioxidant activity compared with ethyl acetate extract. However, for ethanol and ethyl acetate, the IC_{50} values for the DPPH assay significantly dropped to 94.59 and 196.40 g/ml, respectively. The AOA of silymarin extracts was evaluated using the ABTS method. The ethanol extract (137.014) showed the highest effect (137.014 µM Trolox eq/100) compared to the ethyl acetate extract (98.637 µM Trolox eq/100 mg). In addition, IC_{50} values for the ABTS assay were duplicated from 2.926 to 5.80 µg/mL for ethanol and ethyl acetate, respectively. The lower IC_{50} value of the used plant extract had a higher free radical scavenging activity. In our study, the antioxidant capacity of silymarin, measured using the DPPH and ABTS methods, is highest in the case of ethanol compared with ethyl acetate extract.

Table 5. The antiradical activity and IC_{50} of silymarin extracts using DPPH and ABTS methods

Assay	Antioxidant activity (µM Trolox eq/mg)		IC_{50} (µg/mL)	
	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate
DPPH	306.40±1.03*	260.03±18.25	94.59 ±1.02	196.40 ±1.03*
ABTS	137.01±519.45*	98.64±80.79	2.93 ±0.087	5.81±0.171 *

Data are the mean values of triplicate samples ± SD * significant at $P \leq 0.05$ using Student's t test

3.5 Properties of encapsulated silymarin

The particle size of the droplets determines the diameter of the treatments produced with different wall material ratios. Particle size measurements with the DLS analyzer revealed that the mean diameter decreased from 981.100 to 376.866 nm for wall materials with an 80:20 and a 20:80 ratio (MD: GA) (Table 6). GA is known to have stabilizing and emulsifying effects on encapsulation [42]. From these results, we note that by increasing the percentage of GA, the size of the particles decreases. As shown in Table 7, the zeta potential of M1, M2, M3 and M4 had negative values within -9.03, -7.43, -8.87 and -18.63 mV, respectively. These formulations also exhibited good stability without significantly affecting the MD: GA ratios. The negative zeta potential was initiated with the increment of dispersion of the formulation's droplet, which explained the good formulation stability [43,44]. The zeta potential results of this study were in good agreement with the formulation stability values and fit the previous results on the behavior of MD: GA, which occurs on the emulsion surface [44]. In this range of zeta potential, MD and GA are covalently cross-linked and repulsion between droplets occurs [44]. The particle distribution index (PDI) defines the particle distribution curve. The obtained results were interesting in that the PDI of (M1 80:20Md: GA) was higher than that of other treatments, which was attributed to the aggregation of particles during spray drying.

Table 6. The particle size, PDI, zeta potential and encapsulation efficiency of encapsulated silymarin using the spray drying method

Formulas	Size (nm)	Zeta potential (mV)	PDI	EE (%)
M1	981.10±19.30^{a}	-9.03±1.5511^{b}	0.76±0.06^{a}	55.48±3.8^{d}
M2	411.27±19.10^{b}	-7.43±1.193^{b}	0.42±0.05^{c}	65.97±1.68^{c}
M3	434.90±16.382^{b}	-8.87±0.639^{b}	0.39±0.02^{c}	77.11±4.30^{b}
M4	376.87±13.92^{c}	-18.63±1.0507^{a}	0.50±0.07^{b}	84.45±3.25^{a}

Means (±SD) with the same letters in the same column are not significantly different at $p \leq 0.05$; PDI (particle distribution index)EE, (Encapsulation efficiency)

Encapsulation efficiency (EE) is defined as the amount of core material that is encapsulated in the capsules. Silymarin EE was 55.47, 65.97, 77.10 and 84.451% for M1, M2 M3 and M4, respectively, As shown in Table 6. It was noticed that the increase in the GA ratio increased the EE of the silymarin extract. The MD: GA ratio of 20:80 recorded an EE of 84.45%, decreased to 55.47 with a decreasing MD: GA ratio of 80:20. The surface polyphenol content of capsules was needed to calculate the EE. In a highly efficient encapsulation process, a small amount of polyphenol content remains on the surface. There was a significant ($p \leq 0.05$) difference between the surface polyphenol content of the mixture having wall material of 80:20, 60:40, 40:60, and 20:80 MD: GA ratios. These results confirmed that increasing the percentage of GA in the mixture increases the encapsulation efficiency. This can be explained by the stabilizing and emulsifying effects of GA on encapsulation [46]. GA can form a dried matrix around core material, which prevents contact of the core material with air as indicated by reference [46]. The surface-active characteristic of GA has increased its intended use as an encapsulation material to protect chemically reactive and volatile compounds [48].

3.6 Morphology of silymarin microcapsules

The particle surface morphology of silymarin microcapsules was captured using the SEM technique (Fig 2). The particle size distribution of the spray-dried microparticles process provided predominantly crooked spherical particles, without visible cracks, collapses, or porous structures, but also clustered particles which caused few agglomerations of particles. This is in accordance with Alftrén et al. [46], which confirmed that microcapsules produced using gums could provide a degree of agglomeration. GA used in this study as a polysaccharide contained many sugars, which probably had a role as filler or plasticizer, preventing surface wrinkling during the spray drying process. Some collapses in structure and irregular shapes with some hollows are probably the result of the rapid drying process of this type of carrier [49-51]. As it is shown in Fig 2, the usage of GA as a carrier gave well-formed particles with varied size distribution (as appeared from PPSD results) without significant irregularities on the targeted microcapsules. This is very important for industrial applications since particle shape and surface morphology could significantly affect the flow rate of obtained microparticles [50-52].

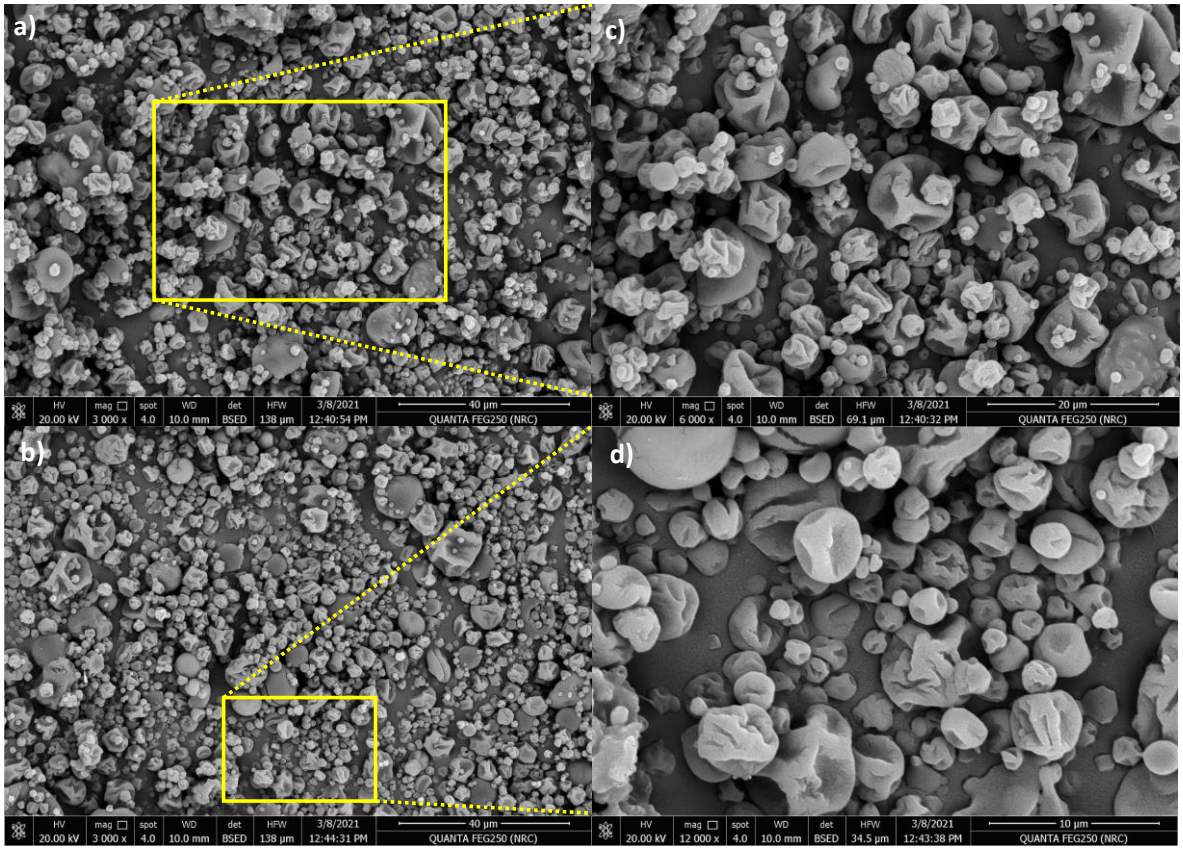


Fig. 2. SEM of the spray-dried microcapsules containing silymarin: a) 3000X, b) 3000X c) 6000X and d) 12000X magnifications

3.7. FTIR spectroscopy of silymarin microcapsules

FTIR spectra were recorded in the region from 4000 to 400 cm^{-1}. The maltodextrin (A), gum Arabic (B), silymarin (C), and silymarin (D) capsules' chemical structures were analyzed by FTIR spectra (Fig. 3), in their solid state in order to be unaffected by the strong water absorption. The main characteristic peak in the FTIR spectrum of pure silymarin was observed at 3400 cm^{-1} for the phenolic OH groups [51]. In the FTIR spectrum of GA, Fig.3, the OH stretching vibration, characteristic of the glucoside ring, participated in the existence of broadband at 3400 cm^{-1}. While at 2975 cm^{-1}, GA exhibited a signal for CH stretching. Besides, the COO-symmetric and asymmetric stretching of GA provided a dominant peak at 1637 and 1454 cm^{-1}, respectively. While at 1200 to 900 cm^{-1}, there is a fingerprint of carbohydrates [54]. The FTIR spectrum for MD exhibited a strong, broad absorption band centered at 3400 cm^{-1} and a weak band at 1635 $cm^{-1,}$ which arise from OH-stretching and OH-bending (in-plane) modes, respectively, Fig.3. Also, the CH-stretching absorption band was found at 2975 cm^{-1}. Compared to the spectrum of crystalline silymarin microcapsules, absorption bands in the region of carbohydrate fingerprint (1500-1800 cm^{-1}) are broader, showing extensive overlapping [55].

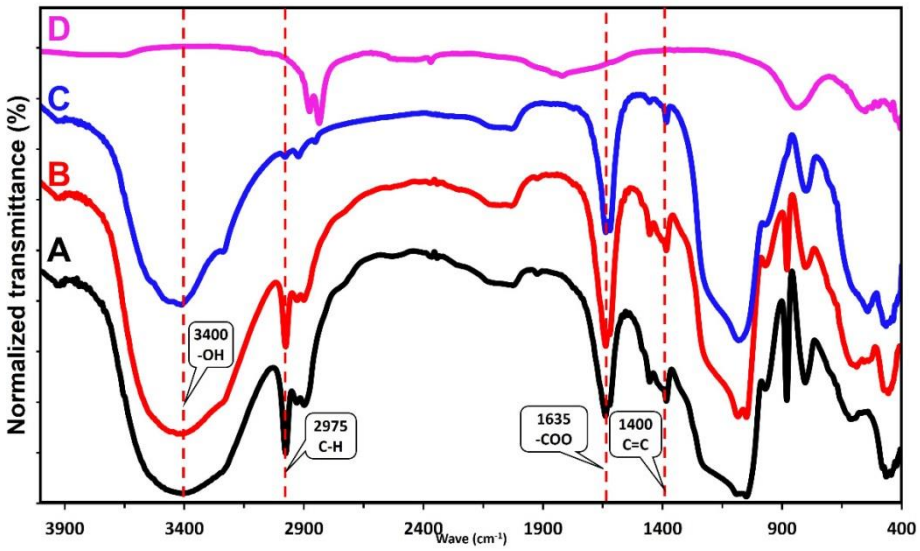


Fig. 3. FTIR Spectra of maltodextrin, A. gum Arabic, B. silymarin, C and D. silymarin capsule.

4. Conclusion

UAE has proven to be a more effective technique to replace conventional maceration. Comparison with conventional extraction methods revealed that UAE could save a lot of time and be more efficient. The highest yield, antioxidant activities and silymarin content were found using ethanol solvent compared to ethyl acetate. Silymarin extract can be encapsulated by different ratios GA and MD utilizing the spray drying method. The highest encapsulation efficiency was observed when using GA: MD at a ratio 80: 20.

5. Conflict of interest

The authors declare that there is no conflict of interest.

6. Funding source

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8. References

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