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Effect of Particle Size on Total Extraction Yield and Silymarin Content of *Silybum marianum* L. Seeds.

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ABSTRACT

The effect of particle size on the composition and mass fraction of silymarin in *Silybum marianum* L. has been investigated. The seeds particles were produced by two methods: first one using ball mill at moderate conditions of the following milling parameters: 3 h of milling time, Grinding mode: dry, Ball powder ratio (BPR) maintained at 10:1, the morphology of milled powders was analyzed using a field emission scanning electron microscope to get particles size of 30-200 nm. In the second method, the seeds were ground using a blender and sieved to obtain the particle size varied from 300 µm until 700 µm. Both types of ground *Silybum marianum* seeds, obtained either by ball mill or by normal blender, were extracted by 80% methanol using Ultrasound-Assisted Extraction under the same conditions. The mass fraction of *Silybum marianum* L. was determined by total methanolic extraction yield where the yield was about 8.5 % for normally ground seeds and about 10% for nano sized particles. On the other hand, concerning silymarin components, some variations have been observed. The extract of nano ground seeds showed highest percentage yield of Silychristin, Silydianin, Silybin A, Silybin B and Iso-Silybin B while Taxifolin and Iso-Silybin A have been decreased, compared to that of normal ground seeds. Optimization of milling parameters was found to be a crucial step in determining the extracted silymarin contents. As expected, a smaller particle size has led to higher extraction yield.

Keywords: *Silybum marianum*, Silymarin, silybin, Planetary ball mill, Particle Size, HPLC, Ultrasound-Assisted Extraction.

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INTRODUCTION

Natural products have been important sources of drugsand will continue to play an important role as a major source of new drugs in the years to come [1]. As bioactive components exist in a low concentration; many methods has introduced and developed to retrieve the analyte from their cell matrix; among them particle size reduction. Ball milling is a common method in the size reduction of material. The milling process produces nano powders through the impact forces generated by action of centrifugal forces. Milling parameters such as milling time, mass concentration, and bead amount are important parameters that need to be considered in producing superfine powders [2,3].

Plant material can undergo grinding or milling before extraction to reduce the particle size. The smaller the particle size of the material, the shorter the path that the solvent has to travel, which decreases the time for maximum phytochemical content to be extracted [4]. Also, grinding or milling the plant material to reduce the particle size damages the plant cells which can also lead to increased extraction of phytochemical compounds [5]. The disadvantage of grinding or milling the plant before extraction is that plant material of small particle size may block filters quicker than bigger particles and this could possibly result in wastage of the extract and extended extraction times [6].

Silymarin is one of the oldest traditional herbal medicines used to combat different organ disorders. It is predominantly composed of five flavonolignan isomers; silybin, Taxifolin, isosilybin, silychristine and silydianin amongst which, silybin (Sb), is the key biologically active compound and constitutes 34% by mass of Sm [7,8]. Hepatoprotective effects of Sb have been demonstrated repeatedly in humans and the most remarkable use of Sb was in the treatment of acute mushroom (*Amanita phalloides)* poisoning [9]. The compound is lowly toxic and exerts significant anti-carcinogenic and anti-inflammatory effects and its biomolecular mechanisms in different disease modifications have also been established [10,11].

HPLC currently represents the most popular and reliable technique for analysis of phenolic compounds. Various supports and mobile phases are available for the analysis of phenolics including anthocyanins, proanthocyanidins, hydrolysable tannins, flavonols, flavan-3-ols, flavanones, flavones, and phenolic acids in different plant extract and food samples [12-21]. Moreover, HPLC techniques offer a unique chance to analyze simultaneously all components of interest together with their possible derivatives or degradation products [22, 23].

MATERIALS AND METHODS

Plant Material

Silybum marianum Seeds family Asteraceae (Compositae) was collected from Cairo- Alexandria Desert Road during April 2013. The plant was identified by Prof. Dr. Kamal M. Zayed and Dr. Ibrahim Elgarf, Taxonomists, Cairo Univ., Faculty of Science, Botany Dept., Cairo, Egypt, to whom the authors are deeply indebted. The seeds of the plant were ground into fine powder using blender. A voucher specimen was kept in the herbarium of Cairo University.

Preparation of Different Size Particles of *Silybum marianum* :

1. The seeds were ground into powder using a blender. Particle size distribution was determined using Vibrator Sieve Shaker to obtain the particle size varied from 300 µm until 700 µm.
2. Ball milling is a common mechanical process to produce superfine powders. In this research, a planetary ball mill was chosen as a grinding tool due to its simplicity. This process was done at CMRDI using the following conditions:

- Vertical planetary ball mill (RETSCH- floor model)
- Grinding jar (500 ml) from agate
- Silicon nitride balls of 20mm diameter
- Ball powder ratio (BPR) maintained at 10:1
- Rotational speed maintained at 100 rpm

- Grinding time 3 hrs
- Grinding mode: dry

The obtained particle size was measured using Transmission Electron Microscope (T.E.M.), model Jeol- JEM 2100-H.R., Made in Japan, under the following conditions: Take one sample drop (suspended in distilled water) on Cupper grid coated with Carbon. Leave to dry then put it in (T. E. M.)

Extraction of different Size Particles of *Silybum marianum* seeds using Ultrasound-Assisted Extraction

43 g of nano size particles of *Silybum marianum* L. seeds were mixed with 800 ml of 80% methonal for 10 min using an Ultrasonic Processor UP400S (400 watts, 24kHz, Hielscher) direct sonication, at power 400 W (amplitude 0.5 and rotation 70 cycles) with an ultrasonic probe with a tip diameter of 20 mm fitted into the flask and the tip was inserted at the half height of the extraction solvent to give extract (A). The same conditions were used to extract normally ground seeds (by blender) to give extract (B). Extracts (A and B) were centrifuged at 4000 rpm and their supernatants were concentrated under reduced pressure and evaporated at 40^{o}C till dryness using a rotary evaporator to yield 4.36 g and 3.64 g; respectively. The extracts were washed twice with 100 ml n-hexane and then finally dissolved in 100 ml 80 % methanol in volumetric flask. The extraction yields as well as its components were compared by HPLC.

HPLC Device Specifications

High performance liquid chromatography (Agilent 1100 series) was used to determine the chemical composition of each extract as well as the standard one, equipped with: G1315 B diode array detector (DAD), G1313A Auto sampler, G1311A Quaternary Pump, G1322A Vacuum Degasser, G1321A Fluorescent Detector, G1316A Column Comp.

The control system and data acquiring system was installed with Agilent Chemo station for LC system.

HPLC Analysis

Extracts A and B and standard silymarin (Segma Co.) were injected separately to Semi-prep HPLC for further analysis using different proportional of H_2O and methanol

Mobile phase: Solvent B: H2O (containing 0.1% formic acid).
Solvent A: MeOH : H2O : formic acid
90 : 10 : 1

Flow rate: 0.5 ml/min

UV detector: 288 nm.

Column temperature: ~25 °C

Stop time: 30 min

Injection volume: 5 µl

RESULTS

Effect of particle size on extraction efficiency

Particle size reduction of plant parts has become a vital aspect that has to be considered and also has a significant effect in the extraction of active compounds. Generally, the efficiency of extraction processes is mainly affected by some extraction parameters such as temperature, time, types of solvent and particle size. Moreover, the sample particle size of the plant part and the extraction temperature significantly affect the efficiency of the extraction process. Particularly, the sample particle size can influence and control the mass

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transfer kinetics and the access of solvent (water) into the soluble components. It is also one of the important studied criteria as it can affect the extraction rate, diffusion rate and extractability.

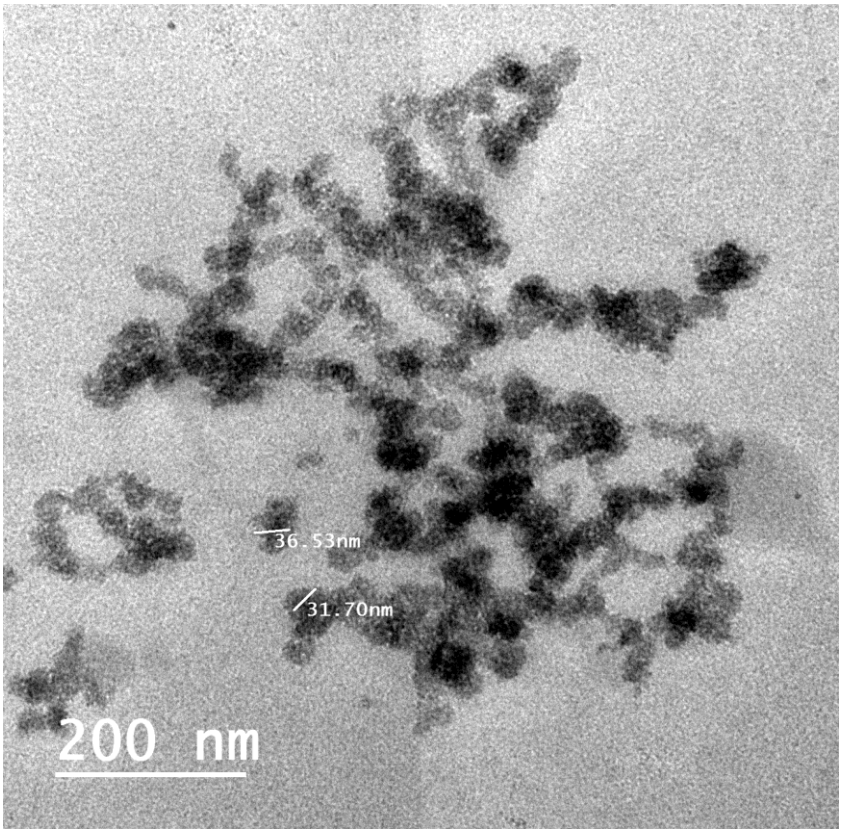

Figure 1: *Silybum marianum* seeds obtained using ball mill with particle size distribution 30-200 nm

HPLC Analysis:

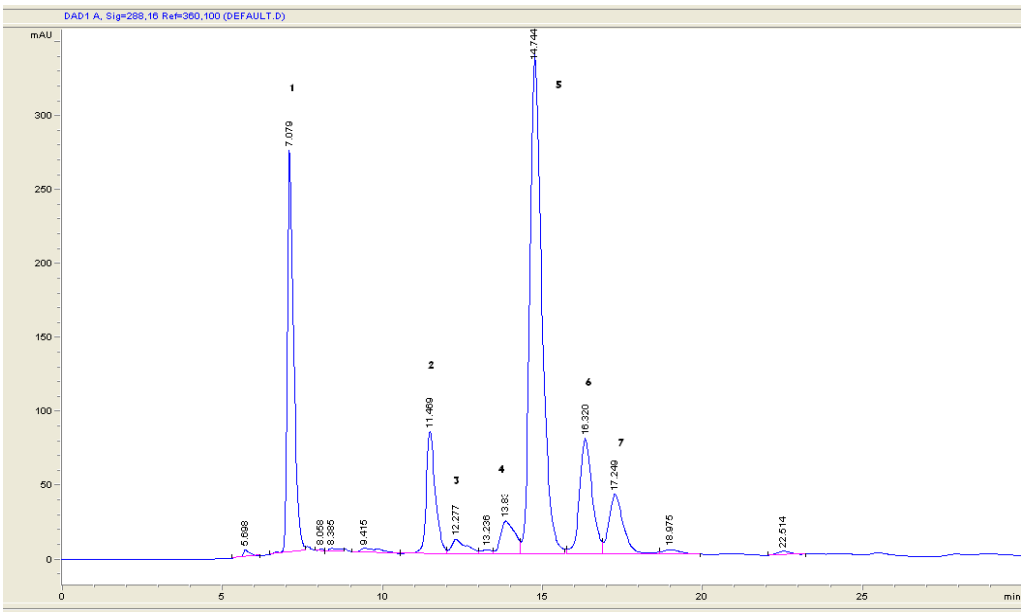

Figure 2: HPLC Chromatogram of Standard Silymarin of *Silybum marianum* Seeds (Sigma Co.).

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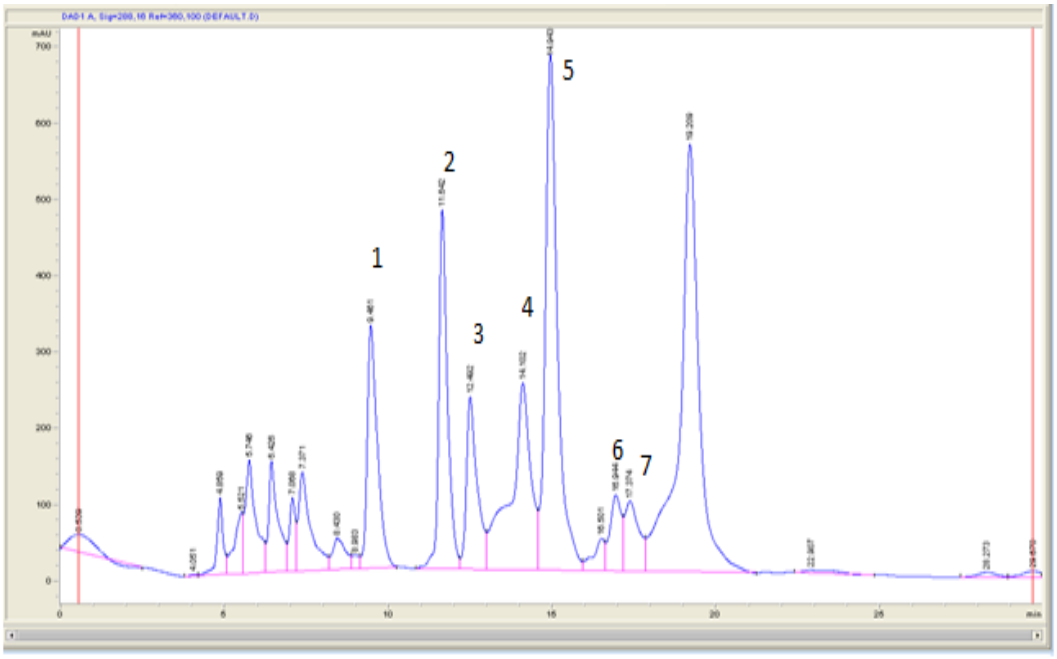

Figure 3: HPLC Chromatogram of Nano Particles of *Silybum marianum* Seeds (Extract A).

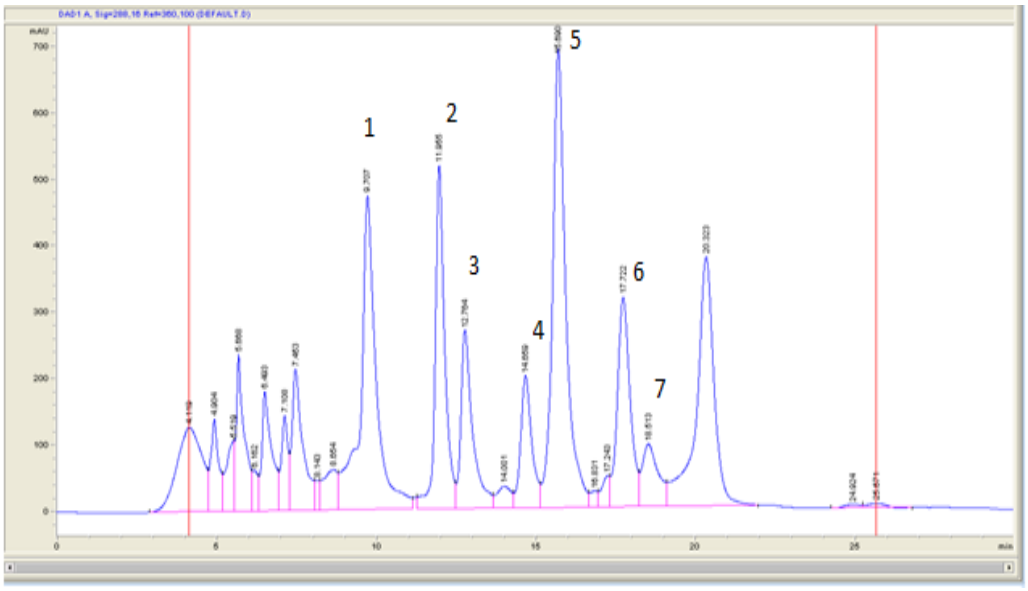

Figure 4: HPLC Chromatogram of Normal Ground Particles of *Silybum marianum* Seeds (Extract B).

Table 1: Silymarin Components Percentage in (Extract A) and (Extract B)

No of peaks	Compound Name	Ret Time (St.)	Ret Time (A)	Ret Time (B)	Area % (St.)	Area % of Extract A (Nano-particles)	Area % of Extract B(normal particles)
1	Taxifolin	7.079	9.461	9.707	19.5982	7.2095	13.8492
2	Silychristin	11.469	11.642	11.955	8.5316	9.2178	9.1926
3	Silydianin	12.277	12.492	12.764	1.6461	5.8462	5.4779
4	Silybin A	13.832	14.102	14.659	3.5891	11.0526	4.1710
5	Silybin B	14.744	14.943	15.690	45.9876	18.2349	16.9934
6	Iso-Silybin A	16.320	16.944	17.722	11.2543	2.4570	7.4678
7	Iso-Silybin B	17.249	17.374	18.513	6.6571	2.9653	2.8278

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CONCLUSION

Particle size had a significant effect in the total methanolic extraction yield as well as silymarin components, where the total methanolic extraction yield was about 8.5 % for normally ground seeds and about 10% for Nano sized particles.

Concerning silymarin components, some variations have been observed. The percent of Silychristin, Silydianin, Silybin A, Silybin B and Iso-Silybin B have been increased while Taxifolin and Iso-Silybin A have been decreased.

This means that particle size reduction has increased the obtained extraction yield. Optimization of milling parameters was found to be a crucial step in determining the extracted silymarin contents.

Anyway, this experiment is a preliminary investigation for the effect of nano size particles on the extraction yield and the on the components concentration. Further investigation is highly recommended as promising results are obtained and need to be continued.

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