biology and life sciences forum

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Proceeding Paper

Bioactive Potential of Milk Thistle (*Sylibum marianum*) Seeds and Applicability of Its Edible Oil in Food Processing †

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† Presented at the 9th International Seminar (MGIBR) Management and Genetic Improvement of Biological Ressources, Tlemcen, Algeria, 20–22 April 2024.

Abstract: This study aims to explore the potential of Algerian milk thistle (*Silybum marianum*) seeds for food application. Solid–liquid extraction and Soxhlet extraction methods were employed to obtain both aqueous and fatty fractions using different solvents. Spectrophotometry was used to assess the content of bioactive compounds and pigments, while gas chromatography–mass spectrometry (GC-MS) analyzed the fatty acid composition. Additionally, the oxidative stability of the plant oil was evaluated using the Rancimat test. The results indicated a moisture content of 0.779% and a plant oil extraction yield of 0.278%. The polyphenol content in the oil was measured at 142.66 mg/100 g. The choice of solvent significantly impacted the content of bioactive compounds, with the highest values observed in the 80% methanol extract for total polyphenols, the aqueous extract for total flavonoids, and the ethanol extract for reducing power. Furthermore, the present study quantified pigments including chlorophyll, carotenoids, anthocyanins, and carotenoids. GC-MS analysis revealed a diverse range of fatty acids typical of edible oils, including essential fatty acids from the ω3, ω6, and ω9 series. The Rancimat test indicated an oxidation resistance of 14.65 h. Overall, the findings suggest that *Silybum marianum* holds promise as an edible oil source rich in antioxidants, micronutrients, and essential fatty acids.

Keywords: milk thistle; extraction; antioxidants; oxidative stability

Citation: Chikhoune, A.; Ghazi, A.; Adjadj, F. Bioactive Potential of Milk Thistle (*Sylibum marianum*) Seeds and Applicability of Its Edible Oil in Food Processing. *Biol. Life Sci. Forum* **2024**, *36*, 2. https://doi.org/10.3390/blsf2024036002

Academic Editors: Semir Bechir Suheil Gaouar, Rachid Azzi, Tarik Mohammed Chaouch, Mohammed Sidhoum, Ikram Mkedder, Mohammed Rida Mediouni, Sarra Selka, Djalel Eddine Gherissi, Amel Djaout, Hind Houssou and Hakim Tefiel

Published: 26 July 2024

1. Introduction

Oxidation is a major cause of food degradation during manufacturing and storage. This oxidation leads to rancidity, which has sensory, nutritional, hygienic, and therefore economic disadvantages. It is therefore a major problem for the food industry which is faced with the preservation of food and human health. Combating this oxidation is therefore a considerable challenge for food manufacturers [1–3].

Milk thistle is considered a weed. Weeds are plants that generally belong to spontaneous vegetation and are part of the flora. The earliest reported use of milk thistle is that described by Discorides, who recommended it against snake bites. Pliny the Elder (23–79 AD) reported that the juice of the plant mixed with honey was indicated to "train bile". Milk thistle was first revered as an antidote for liver toxins in the Middle Ages and was later used by British herbalist Culpeper to relieve liver obstructions [4]. Thus, the fruit of the milk thistle plant has been a remedy for 2000 years. The active constituents of silymarin, the lipophilic antioxidant fraction of milk thistle, consist primarily of silybin, followed by iso-silybin, silychristine, and silydianine [5]. It has been reported that milk thistle vegetable oil has therapeutic properties as well as high nutritional value due to its

richness in polyunsaturated fatty acids (PUFAs), which are indispensable food components for the human body. It has also been proven to be used as a food-grade lubricant [6,7] and can be considered an edible oil and an interesting source of vitamin E [8].

The fats sector in Algeria suffers from the weight of imports of various vegetable oils including palm, soybean, sunflower, and copra. To date, there is no oilseed crop capable of counterbalancing this import trend, as olive oil is far from satisfying the national market and the growing demand of the Algerian consumer. In light of this, the aim of this work is to analyze the phytochemical arsenal of this plant omnipresent in Algeria and to present it as an oilseed source likely to be of interest to the industry of fats in our country.

2. Materials and Methods

2.1. Plant Material

Milk thistle seeds (*Sylibum marianum*) were harvested in July 2015 in the local area of N'Gaous, Batna, Algeria. The plant material part used for oil extraction consisted of the seeds. The seeds were dried naturally at summer room temperature. Subsequently, they were ground into a fine powder using a grinder (Kinematica, Lucerne, Switzerland) and passed through a sieve (with mesh openings > 500 µm). The powdered seeds were then stored in glass flasks covered with aluminum foil, labeled, and protected from light and moisture until analysis.

2.2. Moisture Determination

The water content of milk thistle seeds was determined using the drying method in an oven at 105 °C according to [9] Lako et al. (2007).

2.3. Oil Extraction by Soxhlet

The extraction of plant oil from milk thistle seeds was carried out using a Soxhlet apparatus (VELP Scientifica, Lombardy, Italy). A total of 5 g of seed powder was placed in cartridges and placed in containers of 60 mL hexane. The Soxhlet apparatus was set at a temperature of 130 °C, with immersion, washing, and recovery times of 40 min, 20 min, and 40 min, respectively. The obtained oil was stored in a tightly sealed glass bottle covered with aluminum foil and put in a refrigerator at 4 to 6 °C.

2.4. Extraction and Assessment of Antioxidants

2.4.1. Extraction of Antioxidants from the Seeds

A total of 0.25 g of powdered milk thistle seeds were extracted in 10 mL of water, 80% methanol, 80% ethanol, and 80% acetone. They were then agitated in a water bath at 40 °C for 1 h, then centrifuged at 3500 rpm. The recovered supernatants from each extract were filtered through Whatman paper and stored in a refrigerator at 4 to 6 °C until analysis.

2.4.2. Extraction of Antioxidants from Oil

The oil was diluted in hexane prior to extraction by mixing 1 mL of the oil with 9 mL of hexane. A total of 6 mL from this mixture was mixed with 5 mL of methanol/water (60/40). This new mixture was vigorously agitated for 1 min using a vortex mixer. A volume of 5 mL of hexane was then added and the mixture was agitated again using the vortex mixer. The organic fraction was discarded, leaving only the hydro-alcoholic fraction for analysis.

2.4.3. Assessment of Antioxidants and Antioxidant Activity (Reducing Power) by Spectrophotometry

Total phenolics (TPC), total flavonoids (TFC), anthocyanins, and antioxidant activity via the reducing power test were determined according to [10].

2.4.4. Assessment of Pigments in Extracts and Oil of *Sylibum marianum*

Carotenoids in *Sylibum marianum*'s extracts were determined according to the protocol of [11], whereas carotenoids and chrolophylls of the oil were determined according to the protocol of [12].

2.5. Fatty Acid Composition of Sylibum marianum's Oil by GC-MS

The analysis of fatty acids was carried out on a chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flow modulator. The detector used was a flame ionization detector (FID) at 250 °C. The oven temperature program was 100 °C for 0 min, then 6 °C/min until 230 °C. The inlet temperature was 250 °C. The column consists of a customized ionic liquid column (4MPyC6, 20 m × 0.25 mm × 0.2 µm). High-purity helium was used as the carrier gas. The sample was injected using a 1:100 split mode. The flow rate was 1 mL.min^{-1} with a modulation time of 1.4 s.

2.6. Oxidative Stability by Rancimat Test

The resistant of the oil toward oxidation was determined on a Rancimat (Metrohm, Herisau, Switzerland) according to [13].

2.7. Statistical Analysis

The reported parameters were evaluated in triplicate. Statistical analysis of the data was conducted using analysis of variance (ANOVA) and the Least Significant Difference (LSD) test (STATISTICA 5.5) to identify measures that could be considered statistically different at significance levels of $p < 0.05$.

3. Results

3.1. Moisture Content

The moisture content calculated was 5.47%, which is higher than the one reported by [14] (0.37–0.61)%, but lower than that reported by [15] (7.79%).

3.2. Oil Extraction Yield

The calculated oil extraction yield (27.8%) was higher than that reported by [16] (21.87%) and by [17] (17%).

3.3. Antioxidant Contents in Extracts and Oil of Sylibum marianum

3.3.1. Total Phenolic Content (TPC)

The methanolic extract depicted the highest TPC (15 mg GAE/100 g DW) compared to aqueous and acetonic extracts (10 and 7.9 mg EAG/100 g DW, respectively) ($p \leq 0.05$). The ethanolic extract depicted the lowest TPC (6.4 mgEAG/100 g DW) when compared to other extracts ($p \leq 0.05$).

The TPC calculated in the oil of *S. marianum* (1.42 mg GAE/g oil) was lower than that reported by [18] (3.07 mg EAG/g oil).

3.3.2. Total Flavonoid Content (TFC)

The aqueous extract depicted the highest TFC (5.7 mg QE/100 g DW) compared to ethanolic and ethanolic extracts (2.3 mg QE/100 g DW and 2.2 mg QE/100 g DW) ($p \leq 0.05$). The TFC of the acetonic extract was reported to be the lowest (0.79 mg QE/100 g DW) ($p \leq 0.05$).

3.3.3. Pigment Content

The content of pigments (anthocyanins, carotenoids, and chlorophylls) in the seeds and oil of *S. marianum* is reported in Table 1.

Table 1. Pigments in seeds and oil of *S. marianum*.

Sample	Anthocyanins [1]	Carotenoids [2]	Chlorophyll [3]
Seed extract	$(6.067 \pm 1.604) \times 10^{-5}$	$(7.623^{b} \pm 0.226) \times 10^{-3}$	/
Oil	/	$0.188^{a} \pm 0.018$	0.281 ± 0.051

[1] g Cy-3-Glu E/100 g DW; [2] g β-carotene E/100 g DW; [3] g Pheophytine E/100 g DW. Results are reported as means ± SD of three replicates. Means not sharing the same letters (a,b) in column are significantly different ($p < 0.05$).

Table 1 reveals notable differences in carotenoid content between the seeds and the oil, with higher concentrations found in the latter. Moreover, the carotenoid contents in the seeds and oil exceeded those of other pigments. The presence of anthocyanins in milk thistle seeds can be attributed to the flavonoid content obtained from solvent extraction. In particular, the aqueous extract yielded the highest flavonoid content ($p \leq 0.05$), indicating the polar nature of the flavonoids present in the sample. Anthocyanins, being glycosylated flavonoids, are well known for their solubility in polar solvents.

The presence of carotenoids in both the seeds and oil from *S. marianum*, with higher concentrations observed in the latter, signifies the richness of milk thistle seeds in natural antioxidants. Indeed, [18] reported the presence of carotenoids in the oil of milk thistle seeds (2.30 µmole/kg).

3.3.4. Reducing Power of Extracts from Seeds of *S. marianum*

In this study, the antioxidant capacity of *S. marianum* extracts was assessed by measuring their ability to reduce ferric iron to ferrous iron. The values (expressed in g AAE/100 g of powder) were determined from the calibration curves of ascorbic acid. The results of reducing activity expressed in AAE clearly indicate that the ethanolic extract exhibits the most significant ability to reduce Fe^{3+} ions (the strongest antioxidant potential at 4.3 mg AAE/100 g DW compared to other solvents) ($p \leq 0.05$). In contrast, the 80% methanolic extract showed the lowest reducing power (1.7 mg AAE/100 g DW) ($p \leq 0.05$).

3.3.5. Fatty Acid Composition (FAC)

The identified fatty acids in this case range from 14 carbon atoms (myristic acid C14:0) to 24 carbon atoms (lignoceric acid C24:0). Only one trans fatty acid was detected, represented by linolelaidic acid (all-trans-Δ9,15), according to the results. Certain fatty acids are predominant over others. Two fatty acids are notably predominant: linoleic acid (all-cis-Δ9,12) and oleic acid (cis-Δ9), with respective contents of 57.43% and 25.52%. Saturated fatty acids are also present, mainly represented by palmitic acid (C16:0) and stearic acid (C18:0), with respective contents of 7.54% and 3.95%. Myristic acid (C14:0) and lignoceric acid (C24:0) have the lowest contents, at 0.32% and 0.18%, respectively. These fatty acids represent the three families of fatty acids:

- Omega-3 (n-3) family: α-linolenic acid (all-cis-Δ9,12,15);
- Omega-6 (n-6) family: linoleic acid (all-cis-Δ9,12);
- Omega-9 (n-9) family: oleic acid (cis-Δ9).

3.3.6. Oxidative Stability

The oil in the present study demonstrates greater stability against oxidation compared to that reported by [18] (13.30 h). This confirms its potential for use as a food-grade oil, given its favorable fatty acid profile and its resistance to oxidation.

4. Discussion

4.1. Moisture Content

According to [19], water contents below 4% protect seeds from insect infestation and mold growth during storage. Additionally, oil-rich plant seeds tend to have lower

moisture contents compared to starch-rich plant seeds, even under similar environmental humidity conditions.

4.2. Oil Extraction Yield

The authors of [20] successfully employed n-hexane for extracting oil from milk thistle seeds, optimizing an enzymatic pretreatment using response surface methodology. However, ref. [21] reported that petroleum ether is the most effective solvent for extracting vegetable oil from *Silybum marianum* seeds using the Soxhlet technique. In this study, the vegetable oil was extracted using hexane. Hexane is the recommended solvent for extracting vegetable oils from oilseeds due to its availability, low cost, excellent diffusivity through oilseed cell walls, high solubility in edible oils, and low solubility in water.

4.3. TPC

The extraction of phenolic compounds is influenced by their solubility in the solvent used, with the polarity of the solvent playing a significant role in enhancing phenolic solubility. This is why organic solvent/water extraction systems are generally preferred [22]. The seeds of *Silybum marianum* represent an undeniable source of bioactive substances, including polyphenols [23]. Moreover, the polyphenol content in the seeds appears to be light-dependent. In contrast to observations in seeds of other plant species, the polyphenol content decreases in milk thistle seeds following exposure to light [24].

4.4. TFC

Silybum marianum seeds contain a biologically active substance known as silymarin, a mixture of flavonolignans resulting from the biosynthetic condensation of Taxifolin and a coniferyl alcohol, a precursor of lignins and lignans. They also contain flavonoids (such as Taxifolin), fatty acids, and other phenolic compounds, along with a residual fraction (not chemically defined) composed of condensed polyphenols. Silymarin is considered an antioxidant and radical scavenger [25–28].

4.5. Pigment Contents

The antioxidant effect of anthocyanins can be explained by several mechanisms. Flavonoids are primarily recognized for their ability to scavenge peroxyl and alkoxyl radicals [29]. Due to their polyphenolic structure, these molecules possess sites susceptible to oxidation by free radicals. Moreover, the radical generated by the oxidation of a flavonoid can undergo electronic delocalization through the pi system (double bonds), resulting in a more stable radical [30]. The antioxidant action of anthocyanins is largely attributed to their metal chelating property. The *o*-diphenol substitution in the B ring and the conjugated double-bond system are associated with their scavenging property of free radicals by donating a hydrogen atom and subsequent radical stabilization [31]. Carotenoids are the most efficient molecules for trapping singlet oxygen. Like many antioxidants, they exert antioxidant activity through several distinct yet likely complementary mechanisms. Their action involves the deactivation of singlet oxygen ($^1O2$) into triplet oxygen ($^3O2$) [32].

4.6. FAC

The extracted oil contained higher levels of unsaturated fatty acids (86.1%) than saturated ones (13.4%). This is of significant nutritional importance, as polyunsaturated fatty acids (PUFAs) have been reported to influence cellular signaling, membrane structure, gene expression, prostaglandin biosynthesis, and the mediation of nervous, endocrine, and immune systems [33]. It is also noteworthy that the fatty acid composition of milk thistle seed vegetable oil is similar to that of sunflower oil. It has been reported that this oil contains fatty acids such as linoleic, oleic, linolenic, palmitic, and stearic acids. Therefore, it has been suggested as a suitable oil for culinary use [34].

5. Conclusions

Based on these results, we can conclude that milk thistle (*Silybum marianum*) seeds represent a promising alternative to other oilseeds given their bioactive potential revealed in both milk thistle extracts and extracted oil. Due to its spontaneous growth nature, it would be feasible to consider intensive cultivation for large-scale oil production and the valorization of waste resulting from extraction. Further research and consideration of its food application would be worthwhile, aiming to determine the proportions in which the vegetable oil can be formulated and its effect on the specific food matrix. The food valorization of such oil is essential, especially in the current economic context marked by a decline in oil prices. Developing an investment strategy based on the valorization of natural resources is crucial.

Author Contributions: Conceptualization, A.C.; methodology, A.C., A.G. and F.A.; software, A.C.; validation, A.C.; formal analysis, A.C.; investigation, A.C., A.G. and F.A.; resources, A.C., A.G. and F.A.; data curation, A.C.; writing—original draft preparation, A.C., A.G. and F.A.; writing—review and editing, A.C., A.G. and F.A.; visualization, A.C.; supervision, A.C.; project administration, A.C.; funding acquisition, A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors deeply thank INATAA, Universite Constantine 1, for their technical support. The authors also thank Mikhail Shashkov, from Novosibirsk state University and Novosibirsk, Russian Federation, for help with GC-MS analysis.

Conflicts of Interest: The authors declare no conflicts of interest.

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