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Phenolic composition and biological Activities of *Silybum marianum (L.)* extracts.

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Abstract :
This study consists to analyze the (Methanol extract **(MeOH)**, Chloroform extract **(CH)**, Ethyl acetate extract **(Ac)** and Aqueous extract **(Aq)**) from *Silybum marianum* seeds. The yields of extracts are ranging from 14.30% to 2.06%.
The total polyphenol content of this plant is 24,52±2,78 mg EAG/g and flavonoids are of the order of 15.14 ± 2.28 mg EQ/g.
Appalling antibacterial activity, all extracts reacted positively at least one of the bacterial strains tested, Ac and Aq extracts of *S. marianum* have witnessed a strong antibacterial activity against the most of the strains studied.
Evaluation of antioxidant made by following the method of trapping of free radical DPPH and that of β-carotene has shown that MeOH and Ac extracts showed good antioxidant activity greater than 80%. On the other hand, the β-carotene test revealed that the MeOH and Ac extracts have a higher reducing power than the Ch and Aq extracts but relatively lower than that of ascorbic acid (98.04%).
The results of separation by high-performance liquid chromatography (HPLC) revealed variability in the phenol and flavonoid content among the plant samples, which may be responsible for biological *Silybum marianum* activities.
Keywords: *Silybum marianum*, polyphenols, flavonoides, antibacterial activity, antioxidant activity, bioactive compounds.

Introduction :

In Africa, The use of medicinal plants is as old as human civilization, utilizing local plants, herbs and their infusions to treat and prevent diseases and their symptoms (Bettaieb et al., 2016) According to the World Health Organization (WHO), nearly 80% of population depend on traditional medicine to cure diseases. However, the secondary metabolites contained in these plants were not known (Bettaieb et al., 2016). The discovery of several classes of molecules, with phenolic compounds being the most studied and recognized for their excellent antimicrobial and antioxidant properties (Bouayed, 2007).

Bioactive molecules from these plants still little explored. Indeed, the scientific community has carried out several scientific works to study medicinal plants for the research and the valorization of new molecules in order to develop and support folks medicine (Bremness, 2011). The role of these plants as antioxidants is due to their redox properties, which allow them to act either as reducing agents or metal chelators (Macheix, 2013). Due to their beneficial effects on health, research on polyphenols is gaining increasing importance (Macheix, 2013). Indeed, they play a role in the prevention and treatment of different diseases, including cancer, cataracts, atherosclerosis, diabetes, hypertension, neurodegenerative diseases, and infectious diseases (Quy et al., 2014).

In Algeria, as in other developing countries, infectious diseases, notably bacterial diseases pose a public health problem due to their frequency and severity, owing to the complexity of bacteria and the rapid development of resistance against the used antibiotics. Thus, the conception of new antibacterial agents is crucial to saving public health (Bendahou et al., 2007).

Regarding the immerging concerns raised about the current abusive utilization of antibiotics and the dangerous side effects related to their administration, a great deal of attention has been given to crude plant extracts (phytomedicine) and exploiting their antioxidant and antibacterial properties to combat public health problems.

S. marianum is an annual biennial plant belonging to the family of Asteraceae, grows throughout Europe and North America, India, China, South America, Africa, Australia, and also in Algeria. It is a species particularly representative of uncultivated fields, ditches, and debris, commonly found everywhere. Its flowering period is from May to July (Belouahem, 2009). This plant is of medicinal and pharmaceutical interest due to its richness in bioactive substances such us ; lipids, albumins, essential oils, flavonoids, silibinin, and silymarin (Polyak, 2010**)**. But remains little studied in Algeria. Thats why this research focused on this medicinal

plant, extracting and analyzing compounds from its seeds to assess their antioxidant and antibacterial activities.

Material and methods

Materials :

Biological material :

1- Plant material:

The plant material used comprises seeds of *Silybum marianum* (milk thistle) harvested during February 2013 from various regions (Oued fodda, Harchoune, Bir safsaf, El Karimia and Chettia) of Chlef- Algeria. After determining the moisture content, the seeds were dried at room temperature and away from sunlight to preserve the integrity of the molecules as much as possible (figure 01). The plant material was then coarsely ground in an electric mill, and the resulting ground material constituted the dry matter used for the extraction of polyphenols.

Figure 01. The dried seeds of *S. marianum*

2- The pathogenic bacteria studied :

For antibacterial activity tests, a range of 6 pathogenic bacteria responsible for gastrointestinal infections was selected. Three international collection strains from ATCC (American Type Culture Collection): *Staphylococcus aureus ATCC 25223*, *E. coli ATCC 25922*, and *Enterobacter aerogenes ATCC 13048*. These were provided by the microbiology laboratory at the Pasteur Institute in Algiers.

The other pathogenic bacteria listed below were isolated from the stools of patients suffering from digestive disorders :

- *Proteus mirabilis,*
- *Proteus vulgaris,*
- *Klebsiella oxytoca.*

Methods :

Determination of plant moisture content :

The method used is desiccation by evaporation. The plant material (fine powder of *S. marianum* seeds) is desiccated at a temperature of 103°C ± 2°C in a ventilated oven until a constant weight is achieved (Audigie, 2018). According to the following formula :

Moisture content (H%) = (initial mass (IM) - final mass (FM) / test sample mass (p)) × 100

The dry matter (DM) is obtained as follows:

Dry matter (DM%) = 100 - H%

Determination of total ash content :

The method used is the mineralization of plant material by incineration in a furnace at a temperature of 550°C until the complete destruction of all carbonaceous particles (Laurent, 1991). The organic matter content is calculated using the following formula :

Organic matter content (OM%) = (initial mass (IM) - final mass (FM) / test sample mass (p)) × 100

The ash content is calculated as follows:

Ash content (A%) = 100 - OM%

Extraction methods :

The extraction is performed according to the method of Markham (1989), with modifications inspired by the method of Bruneton (1993). It is based on the solubility of polyphenols in organic solvents. This method includes two major steps : the first extraction phase was done with methanol to solubilize the polyphenols, then the second phase was carried out with solvents of increasing polarity (from petroleum ether, chloroform, and ethyl acetate) (Mahmoudi et al., 2013 ; Kebièche et al., 2011).

Firstly, the dry material was finely ground with 85% methanol. The macerate was filtered under reduced pressure using a Büchner funnel, then subjected to low-pressure evaporation at 35°C (Rota Vapor, Büchi 461, Germany) (figure 02). The resulting aqueous phase was stored for 48

hours at 4°C to inhibit the action of polyphenol oxidases and to accelerate the diffusion of molecules in the solvents, then filtered (figure 03). The filtrate is then freed from waxes, lipids, and chlorophyll by three successive washes with petroleum ether (V/V) to yield an aqueous phase. This phase was considered the crude extract of the plant, where 50 ml of this extract was frozen to determine its dry matter content and extraction yield (Kebièche et al., 2011).

Figure 02. Solvent separation

Figure 03. Salting out

Calculation of extraction yield

Yield percentage of the various extracts obtained was defined as being the ratio between the mass of the extract and that of the dry vegetable matter, calculated by the following formula :

$$R\ (\%)\ =\ \frac{\text{weight of the extracted residue}}{\text{weight of the plant powder}}\ X\ 100$$

Quantitative analysis of phenolic compounds :

A phytochemical study was conducted to characterize the extracts prepared from *Silybum marianum* seeds.

1- Determination of total polyphenol contents :

The total polyphenol content of the various extracts was achieved colorimetrically with a UV-Vis spectrophotometer using the Folin-Ciocalteu reagent (Waterhouse, 2017).

1 ml of Folin reagent was added to 200 µl of each extract or standard (prepared in methanol) and incubated for 3 minutes. After that, 800 µl of a sodium carbonate (Na2CO3) solution (75 mg/ml) was added to the reaction mixture. After 1 hour of incubation at room temperature, the absorbance of all extracts was measured by a spectrophotometer at 765 nm against a blank without extract (Waterhouse, 2017 ; Chavan and Singhal, 2013). The calibration curve was obtained under the same conditions using a range of concentrations of gallic acid solution prepared in methanol.

Total polyphenols concentration was calculated from the regression equation of the calibration curve, established with the gallic acid standard (5-200 µg/ml) and expressed in milligrams gallic acid equivalent per gram of extract (mg GAE/g DW) (George et al., 2005).

2- Determination of total flavonoids

The determination of total flavonoids was carried out according to the aluminum chloride (AlCl3) method (Aktumsek et al.,2013 ; Djeridane et al., 2006)

1 ml of each extract and the standard (dissolved in methanol) was added to an equal volume of an AlCl3 solution (2% in methanol). The mixture was vigorously shaken and the absorbance at 430 nm was read after 10 minutes of incubation.

A linear calibration curve was obtained using the quercetin standard at different concentrations and the results were expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g).

Biological activity tests :

1- Antioxidant activity :

The *in vitro* antioxidant activity was evaluated using two methods : the DPPH test and the β-carotene bleaching technique.

The DPPH test :

The antiradical activity of the different plant extracts studied was evaluated by measuring the scavenging of DPPH (1,1-Diphenyl-2-picryl-hydrazyl) as a relatively stable free radical, according to the protocol described by Fernandez et al., (2011).

10 µL of reference antioxidants, such as ascorbic acid, BHT, and the tested extracts, were added to 1 ml of the DPPH solution (0.002%) at different concentrations. The mixture was left in the dark for 30 minutes at 25°C. A negative control was also prepared, in parallel.

Absorbance was read at 517 nm after 30 min incubation. Ascorbic acid was used as a positive control.

The antioxidant activity, was estimated by the degree of discoloration of DPPH, the results were expressed as inhibition percentage, calculated according to the equation below:

$$IP\ \% = ((A_{blank} - A_{sample}) / A_{blank}) \times 100$$

A_{blank}: Absorbance of the control reaction

A_{sample} : absorbance of extracts measured after 30 minutes

The absorbance graph variation as a function of concentration allowed for the determination of IC50 (concentration corresponding to 50% inhibition). To better characterize the antioxidant activity, two additional parameters were calculated (El youbi et al., 2012). :

- Effective concentration at 50% (EC50) [$EC_{50} = IC_{50}$/mass of DPPH in mg];
- Antiradical power (ARP) [ARP = 1/EC50].

β-carotene bleaching test :

The antioxidant capacity was determined by measuring the inhibition of β-carotene oxidative degradation (discoloration) caused by linoleic acid oxidation products, resulting in the bleaching of β-carotene and the disappearance of its orange color (Kartal et al., 2007).

The β-carotene/linoleic acid emulsion was prepared by dissolving 2 mg of β-carotene in 1 ml of chloroform. 2 mg of linoleic acid and 200 mg of Tween 40 were added. The chloroform was completely evaporated using a rotary evaporator. Then, 100 ml of distilled water was added, the mixture was vigorously stirred. 350 µL of each extract or reference antioxidants (ascorbic acid and BHT) dissolved in methanol were added to 2.5 ml of the previous emulsion. The absorbance for BHT was measured at 490 nm. Additional readings were taken at different time intervals (2h, 4h, 6h, 12h, and 48h) (Tieppo et al., 2007).

The relative antioxidant activity of the extracts (RAA) after 48 hours was calculated according to the following equation :

$$RAA\% = [Abs_{48h\ (sample)} / Abs_{48h\ (BHT)}] \times 100$$

High-performance liquid chromatography HPLC :

A qualitative analysis of flavonoids was conducted using high performance liquid chromatography (HPLC) using the following standards : catechin, naringin, silybin, rutine, and quercetin. Chromatographic separation was achieved on a C18 column stationary phase (5 µm, 250 × 4.6 mm), bonded to silica particles, with a mobile phase consisting of a mixture of phosphoric acid, methanol, and ultrapure water (0.5 / 35 / 65 ml : V / V / V) (Radjabian et al., 2008 ; Kvasnicka et al., 2003). All samples were filtered through a millipore membrane (0.45 microns), and volume of the standard solution was 20 ml. A UV-visible detector was set at a wavelength of 280 nm. The experiment was carried out at ambient temperature for 30 minutes, with a flow rate set at 1 ml/min. The components of the mixture were subsequently collected and determined based on their rates of adsorption and absorption. Comparing the retention times of the standards with those recorded in the chromatograms enabled the probable identification of flavonoids in our extracts.

Antibacterial activity :

Two different methods were used to evaluate the antibacterial effect of various extracts obtained from *Silybum marianum* seeds : the agar diffusion method mentioned by (Sacchetti et al., 2005 ; Celiktas et al., 2007), which allows the demonstration of the antibacterial activity of various extracts and employs the microdilution method to determine the minimum inhibitory concentrations (MICs) across a range of product concentrations in the culture medium (Billerbeck et al., 2002).

1- Plant Extract Sensitivity Test : Antibioaromatogram

The organic extracts were dissolved in dimethyl sulfoxide (DMSO), and the aqueous extract was dissolved in sterile distilled water. Muller Hinton agar was poured into 90 mm diameter petri dishes and inoculated with a freshly prepared pure microbial suspension containing 10^6 CFU/ml. Three dishes were used for each strain. A sterile Whatman paper disc of 6 mm in diameter, was soaked with 3 µl of extract (reconstituted to the desired concentration) and then placed on the surface of the inoculated agar. The plates were refrigerated for 1 to 2 hours, then incubated for 24 hours at 37°C (Basli et al., 2012).

The classification of bacterial strains in the antibiogram, based on their response to polyphenolic extracts, is as follows :

- **Sensitive strains** : Ø ≤ 12 mm ≤ D
- **Intermediate strains** : ± (d ≤ Ø < 12 mm)
- **Resistant strains** : Ø < d

Where :

- **D** : The maximum critical diameter (the largest diameter observed for the strain).
- **D** : The minimum critical diameter (the smallest diameter observed for the strain).
- **Ø** : The diameter of the inhibition zone.

2- Minimum inhibitory concentration (MIC) :

This method allows for the determination of the minimum inhibitory concentration (MIC) from a range of extract concentrations in the culture medium. It is the smallest concentration of the product for which no growth is visible after 18 to 24 hours compared to the control without product (Billerbeck et al., 2002).

A mother solution of the extracts at 0.2% was prepared. Dilutions were then made from this solution to obtain the following concentrations : 1/10, 1/20, 1/50, 1/100, 1/200, 1/300, 1/500, 1/1000.Then, 13.5 ml of Muller Hinton and 1.5 ml of each dilution were added to test tubes and properly mixed before pouring them into petri dishes, which were then flooded with 1 ml of the pre-culture of the pathogenic strains. Incubation is done at 37°C for 24 hours (Bouterfas et al., 2014). In parallel, controls containing the culture medium and the 0.2% mother solution without the polyphenolic extracts were prepared and incubated at 37°C for 48 hours.

Results :

Plant water and ash content :

The results obtained from the desiccation of milk thistle powder showed that the water content and dry matter content are approximately 54.75% and 45.25%, respectively, while the calcination results showed an organic matter content of 93.47% and an ash content of 6.53% (figure 04).

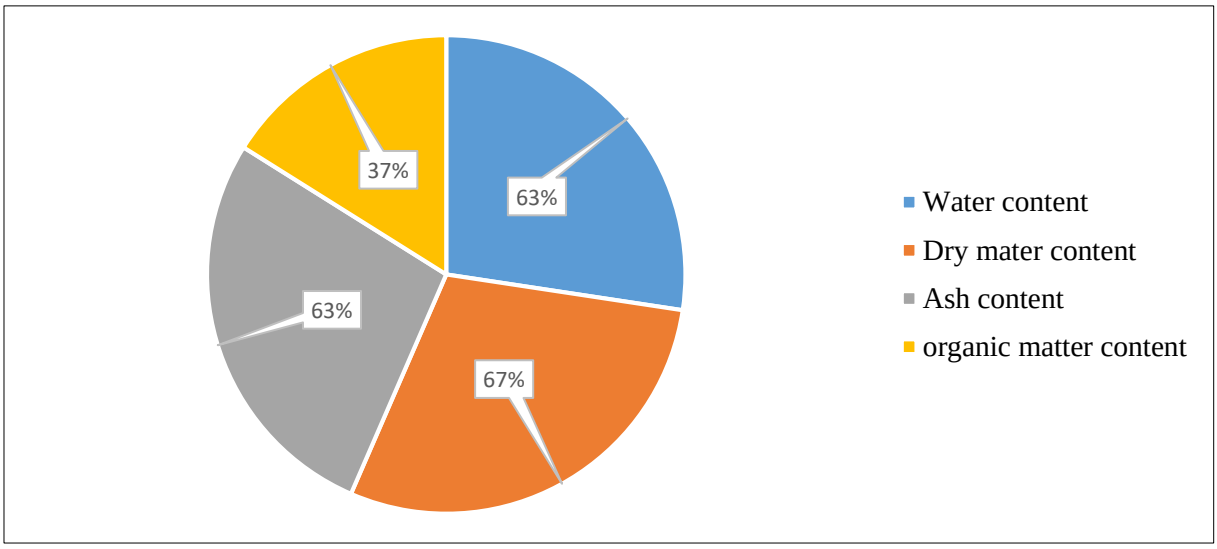

Figure 04. Water and ash content of *S. marianum*

Extraction yield :

The yield was determined relative to the weight of the dried plant material ground into powder, and the results were expressed as a percentage (%). According to these results, the methanolic extract had the highest yield at 14.30%, followed by the chloroform and aqueous extracts with values of 3.33% and 2.06%, respectively, while the lowest yield, at 0.69%, was observed for the acetate extract (figure 05).

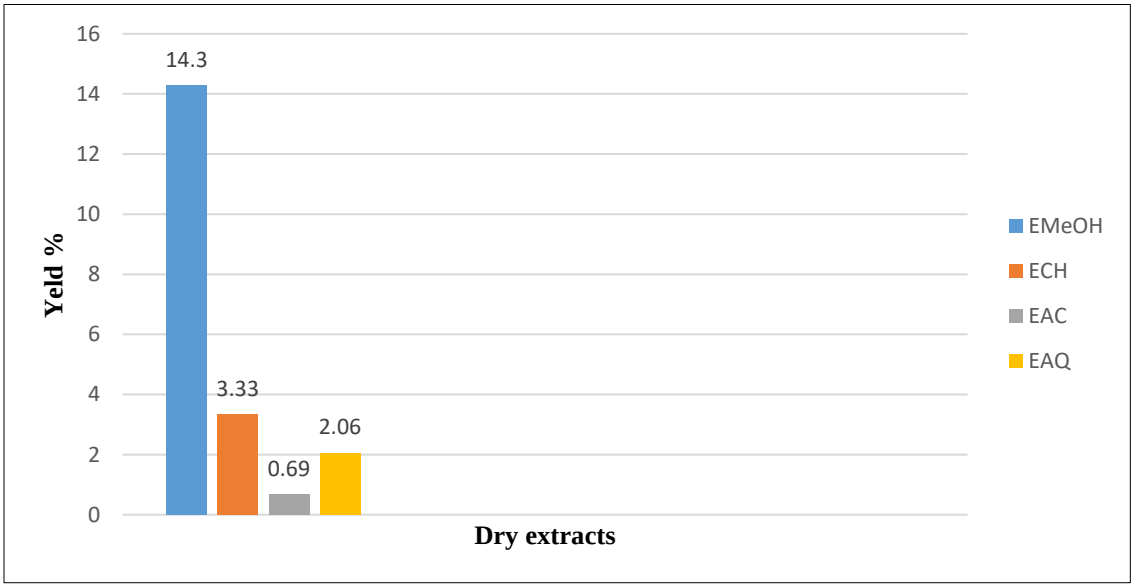

Figure 05. Extraction yields of *S. marianum* seeds.

Quantitative analysis of phenolic compounds :

Total polyphenol assays :

The results reveal that the highest levels of polyphenols were recorded in the methanolic extracts with a value of 24.52 ± 2.78 mg GAE/g of DM, followed by the chloroform extract (ECh) with 20.94 ± 3.27 mg GAE/g of DM, the acetate extract (EAc) with 15.66 ± 2.71 mg GAE/g of DM, while the aqueous extract (EAq) contains only 11.32 ± 2.51 mg GAE/g of DM (figure 06).

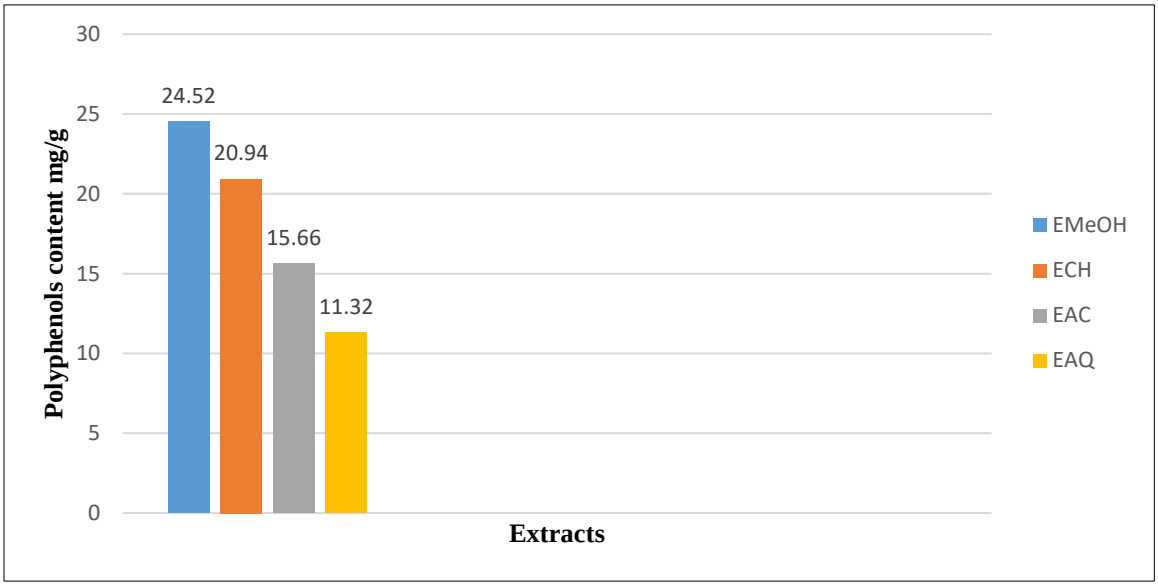

Figure 06. Polyphenol content of *S. marianum* extracts in mg GAE/g of DM.

Total flavonoids content :

The quantitative determination of total flavonoids in milk thistle seeds using the aluminum chloride method reveals that the ethyl acetate extract and the methanol extract are the richest in flavonoids, with values of 10.32 ± 2.62 mg QE/g ; 15.14 ± 2.28 mg QE/g of DM, respectively. Following these, the aqueous extract has 08.61 ± 2.05 mg QE/g of DM. However, the lowest content 05.67 ± 3.47 mg QE/g of DM was found in the chloroform extract (figure 07).

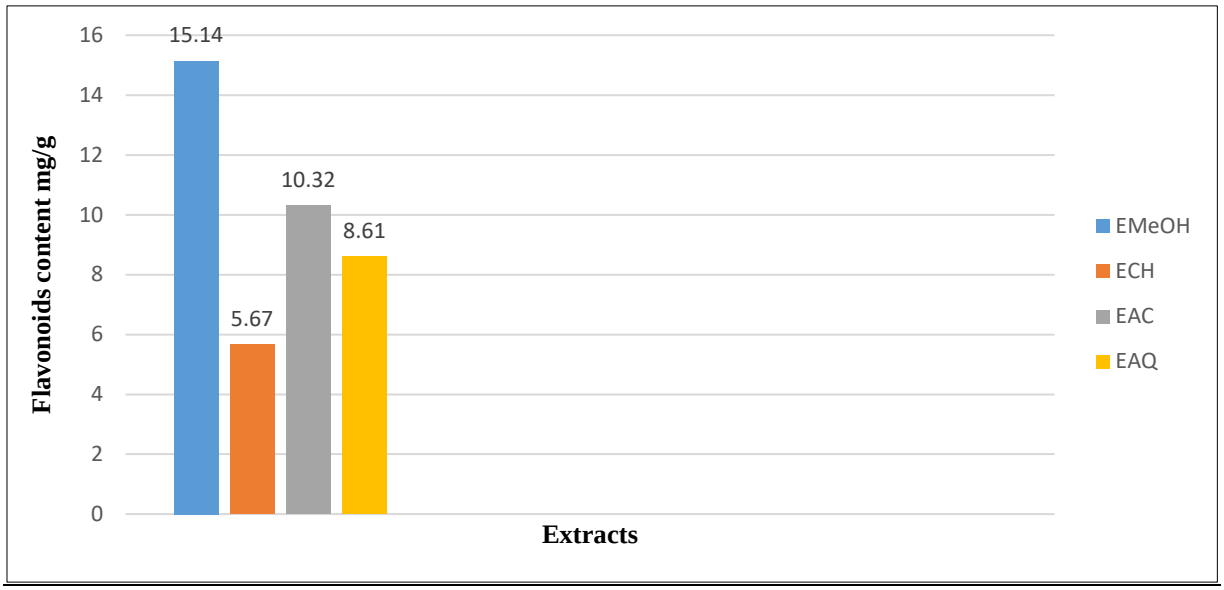

Figure 07. Flavonoid content of *S. marianum* extracts in mg QE/g of DM.

Determination of antioxidant activity :

DPPH radical test :

The inhibition profiles obtained revealed that the different extracts of S. marianum exhibit dose-dependent activity. Their comparison confirmed that the EMeOH was the most active, with an IC50 of 0.88 mg/ml and an estimated antiradical power (ARP) of 2.27, followed by the EAc with an IC50 of approximately 1.28 mg/ml and an ARP of 1.56, then ECh with an IC50 of 1.51 mg/ml and an estimated APR of 1.32. The EAq showed the weakest antiradical activity (IC50 = 2.09 mg/ml, ARP twice less active than MeOH) (table 01).

Table 01. Antiradical activity of *S. marianum* extracts and ascorbic acid.

	IC50 (mg/ml)	EC50 (mg/ml)	ARP
Ascorbic acid	0,53±0.018	0,26±0.04	3,77±1.9
EMeOH	0,88±0.47	0,44±1.47	2,27±0.002
ECh	1,51±1.25	0,75±0.45	1,32±0.003
EAc	1,28±2.08	0,64±0.07	1,56±0.073
EAq	2,09±3.79	1,04±0.14	0,95±0.002

β-carotene bleaching test

The results from (figure 08) indicated that extracts of milk thistle as well as BHT, inhibited the coupled oxidation of linoleic acid and β-carotene compared to the negative control. According to these results, it was observed that the standard (BHT) and all tested extracts effectively inhibited the coupled oxidation of linoleic acid and β-carotene compared to the negative control (where the extract was replaced by methanol), which represents 98% of peroxidation. The highest inhibition was provided by EMeOH (91.73%), followed by EAc (56.25%), then ECh (50.15%), finally EAq (22.39%).

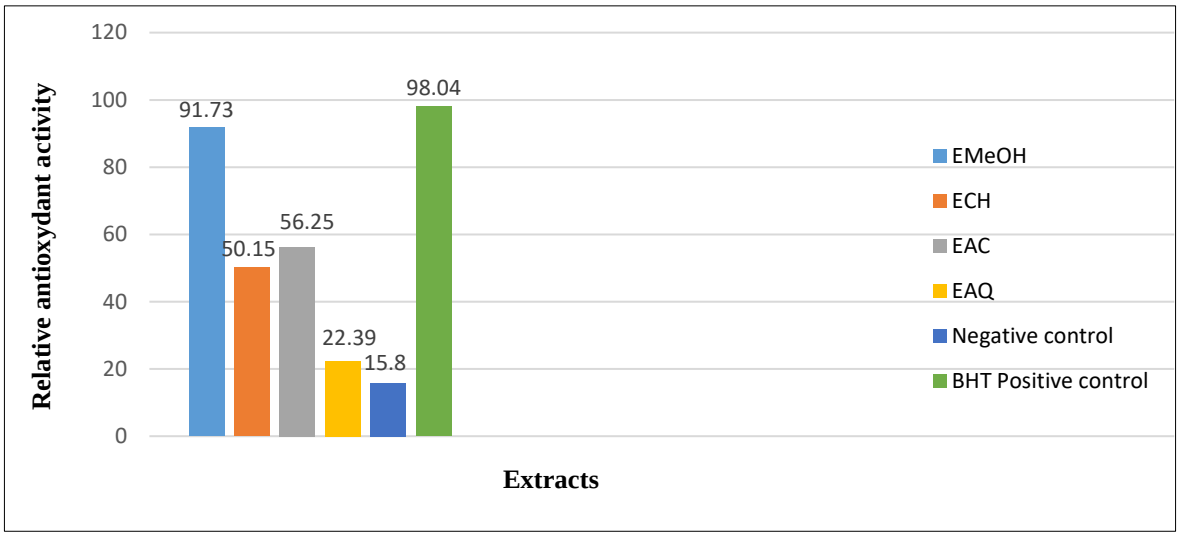

Figure 08. Relative antioxidant activity of *S. marianum* extracts, BHT, and the negative control.

Phenolic profiling by HPLC :

The separation of the various plant extracts using high-performance liquid chromatography (HPLC) is shown in (Figure 09) and detailed in (Table 04). Comparing the values in the (Table 03) with those recorded in the chromatograms of the analyzed samples (Table 04) allowed for the identification of the following flavonoids : Silybin in EAc with a Rt of 8.43 minutes, Naringin in AC and Aq extracts with an Rt of 6.90 minutes, catechin in MeOH

and AQ extracts with an Rt of 7.60 minutes, rutin in the MeOH extract with an Rt of 5.41 minutes and quercetin in the ECH extract with an Rt of 8.53 minutes.

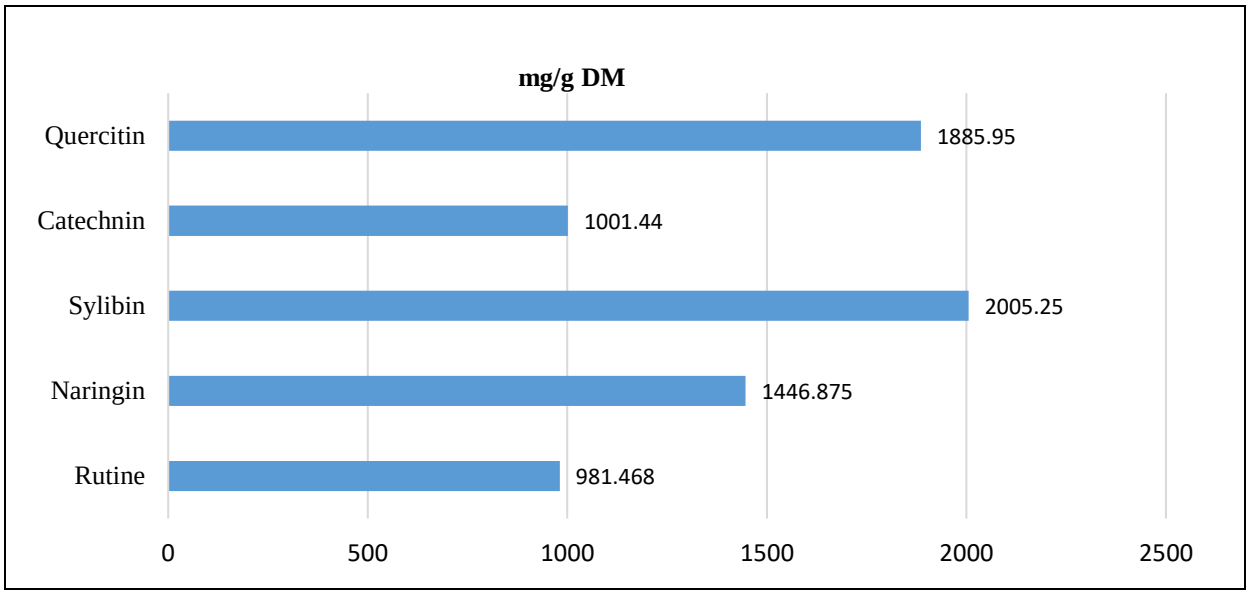

Figure 09. Phenolic compounds of *S. marianum* seeds extracts using HPTLC.

Table 03 : Retention time of standards flavonoids used at λ= 280 nm.

Retention time (min)	**Standards**
8.43 7.60 6.90 5.41 8.53	Silybin Catechin Naringin Rutine Quercetin

Table 04 : Retention times of flavonoids content in the plant extracts at λ= 280 nm.

Retention time (min)				**Flavonoids compounds**
ECH	**EAC**	**EMeOH**	**EAQ**	Silybin in ECH, Naringin in EAC Catechin in EAQ and EMeOH Rutine in EMeOH Quercetin in EAQ - - - -
8.43 8.96 9.54 11.92 11.62	**6.90** 7.94 5.36 10.93 11.78	**7.60** **5.41** 6.98 8.37 10.20	**7.60** **8.53** 10.09 11.06 6.90	

(-): absence of reference flavonoïdes.

Antibacterial activity :

1- Sensitivity to the tested extracts (Aromatogram) :

The antibacterial activity is indicated by the appearance of a halo around the disk impregnated with the tested extracts, demonstrating its inhibitory effect. The results of the inhibition zones from the aromatogram (Table 05) showed that all extracts have antibacterial activity against at least one of the tested strains. However, only inhibition zones more than 12 mm are considered positive (Hassan et al., 2006). This depends on the bacteria and the tested extract. For this reason, there is a wide range in the diameter of the inhibition zones, ranging from 5 to 32 mm. The largest inhibition diameter is observed respectively with *S. aureus ATCC 25223*, *En. aerogenes ATCC 13048*, *E. coli ATCC 25922*, *P. vulgaris*, and *P. mirabilis* against extracts from milk thistle seeds (figure 10).

Table 05: Determination of the inhibition zones of *S. marianum* extracts against the tested strains.

Extracts / Bacteria	EMeOH	ECh	EAc	EAq
P. vulgaris	13±4,04	0,00±0,00	14,33±2,08	0,00±0,00
P. mirabilis	12±1,65	0,00±0,00	07±2,08	0,00±0,00
K. oxytoca	0,00±0,00	0,00±0,00	0,00±0,00	14±2,50
En. aerogenes ATCC 13048.	0,00±00,00	07±2,08	0,00±0,00	32±2,30
E. coli ATCC 25922	10,66±1,75	0,00±0,00	05,33±1,15	18,00±2,84
S. aureus ATCC 25223	00,00±0,00	22,00±1,25	8,00±1.15	29,00±1,08

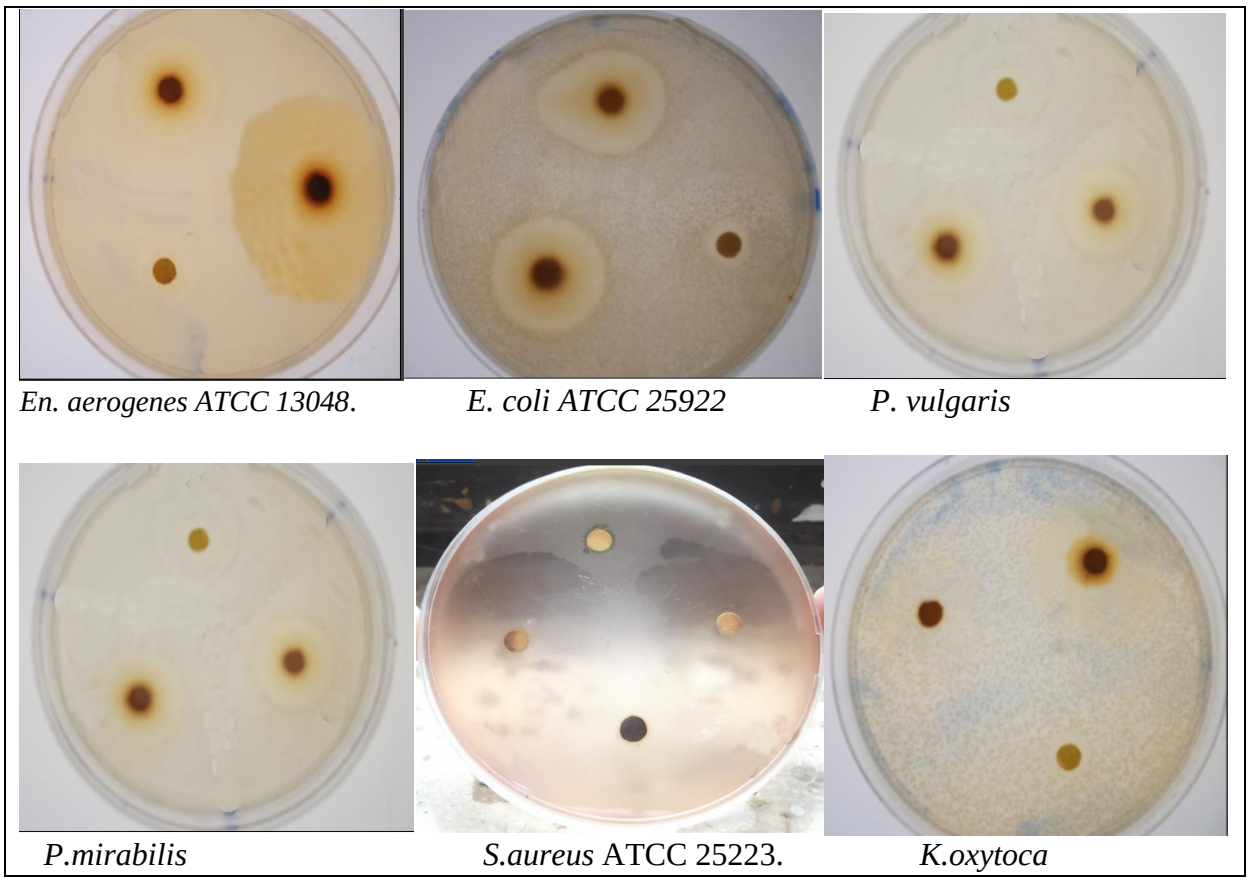

Figure 10. Antibacterial effects of the plant extracts on the tested bacterial strains.

2- The minimum inhibitory concentration of *S. marianum* :

According to the results in table 06, the Aq extract of milk thistle demonstrated a forte inhibition against ; *En. aerogenes ATCC 13048*, *E. coli ATCC 25922*, *S. aureus ATCC 25223*, and *K. oxytoca* at a concentration of 1/100, *En. aerogenes ATCC 13048*, *S. aureus ATCC 25223*, and *K. oxytoca* at a concentration of 1/200, and *K. oxytoca* at a concentration of 1/300. On the other hand, a concentration of 1/100 of EAc was able to inhibit the growth of *P. vulgaris.* For *P. mirabilis* and *P. vulgaris,* an inhibitory concentration of 1/200 was detected for EMeOH. However, 1/100 of ECh inhibited the growth of *S. aureus ATCC 25223.*

Table 06. MIC results of the various milk thistle extracts (where the diameters of the inhibition zones are ≥ 12 mm).

Extracts	EMeOH					ECh					EAc					EAq				
IMC v/v / Bacteria	1/100	1/200	1/300	1/500	1/1000	1/100	1/200	1/300	1/500	1/1000	1/100	1/200	1/300	1/500	1/1000	1/100	1/200	1/300	1/500	1/1000
P.vulgaris	+	-	+	+	+	/	/	/	/	/	-	+	+	+	+	/	/	/	/	/
E.coli ATCC 25922	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	-	+	+	+	+
En.aerogenes	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	-	-	+	+	+
S.aureusATCC 25223	/	/	/	/	/	-	+	+	+	+	/	/	/	/	/	-	-	+	+	+
K.oxytoca	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	-	-	-	+	+
P. mirabilis	-	-	+	+	+	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/

(+) : Growth, (-) : No growth (/) : Not tested, v : The volume of the polyphenolic extract relative to the volume of the mother solution.

Discussion :

According to these results, it was found that *Sylibum marianum* seeds have a high moisture content of 54.75%. This variation is likely due to : Differences in species, exposure to different pedoclimatic conditions, geographical distribution and stages of maturation and harvesting.

Regarding extraction yield, the highest value was obtained by EMoH with a rate of 14.30%, while the lowest yield was from EAc with a value of 0.69%. This variation was related to several parameters, including the plant material studied (particle size), physicochemical characteristics, and the polarity of the solvents used (Majhenic et al., 2007).

Anandharajan et al., (2006) performed a progressive extraction of the dry powder of *Aegle marmelos* and *Syzygium cumini* using solvents of increasing polarity : hexane, dichloromethane, ethyl acetate, and methanol. These researchers obtained yields ranging from 0.1% to 0.15% for hexane and dichloromethane extractions, 10% and 2% for methanol and ethyl acetate extractions. In our case, the extraction with ethyl acetate indeed yielded a low result (0.69%). According to the literature, this can be explained by the fact that methanol (MeOH) is capable of extracting almost all of the constituents from the plant material, which accounts for the high yield, while the low productivity obtained with chloroform and ethyl acetate is primarily due to the selective nature of these two solvents.

Furthermore, the genetic properties of the used parts, geographic origin, storage duration, harvest period, and extraction technique affect the total phenols and flavonoids content, as well as antioxidant and antimicrobial capacities (Lee et al., 2003).

The extraction method must allow for the complete extraction of the target compounds and prevent their chemical modification (Turkmen et al., 2007). This is why we have chosen the Folin-Ciocalteu method, which meets the feasibility and reproducibility criteria and is well standardized (Vuorela, 2005). Monica and her collaborators (2008) determined that the polyphenol content in the ethanolic extract was 6.12 ± 2.02 mg EAG/g. Comparing this with the polyphenol content found in the EMeOH extract of *S. marianum* seeds (24.52 ± 2.78 mg EAG/g) and that of the ethanolic extracts of the same plant studied by Monica and her collaborators, allowed us to deduce that the polyphenol content in our study was four times higher.

However, it is difficult to compare these results with those from the literature because the use of different extraction methods reduces the reliability of comparisons between studies (Falleh et al., 2008).

Flavonoids represent the most important polyphenolic class, with over 5,000 compounds already described (Gomez-Caravaca et al., 2006).

The study conducted by Akkaya and Yilmaz (2012) on the flavonoid content in methanolic extracts of *S. marianum* showed that this plant had a content of 7.35 ± 0.01 mg EQ/g of DM. Comparing this with our results led us to conclude that the flavonoid content in the studied extract was twice as high (15.14 mg EQ/g of DM).

The results of the antioxidant activity of *S. marianum* align with other studies on the polyphenols of milk thistle in Turkey (IC = 94.94 ± 0.62%) (Akkaya and Yilmaz, 2012) and in Romania (Hadaruga and Hadaruga, 2009). Thus, we have observed that the flavonoid compounds in our plant's seeds exhibit significant antioxidant strength. It is also possible that the radicals associated with flavonoids vary, since antioxidant activity largely depends on the number of hydroxyl groups, their positions, and the radicals attached to the molecular strucutre Ethanolic extracts of *S. marianum* fruits exhibited significant activity with an IC50 of 0.47 mg/ml and 0.43 mg/ml for silymarin. The fruit extract showed *in vitro* antioxidant activity (55% inhibition) comparable to that of ascorbic acid (69.6% inhibition) and silymarin (74.3% inhibition). Based on these results, we can deduce that the antioxidant potential of the EMeOH extract of *S. marianum* from Algeria (Chlef) remained higher than that of the previously mentioned ethanolic extracts (Elwekeel et al., 2012).

Phenolic compounds appear to be good candidates for antioxidant activities due to the presence of numerous hydroxyl groups that can react with free radicals (Klervi, 2005 ; Panovska, 2005 ; Sokol-Letowska, 2007).

To measure the antioxidant activity of substances in oxidizing emulsions, the β-carotene decolorization technique was applied.

The antioxidant behavior of compounds in emulsions has not been fully explained (*Gachkar* et al., 2017). The raw extracts derived from *S. marianum* consist of a variety of compounds : primarily nonpolar in the EAc and ECh extracts, and polar in the MeOH and Aq extracts. The MeOH and Ac fractions, being rich in flavonoids, indicate a possible link between the antioxidant activity of these fractions and their flavonoid content.

We also found that the tested extracts have modest antioxidant properties, which is likely due to the β-carotene bleaching assay's strong preference for lipophilic compounds (Gachkar et al., 2017).

Indeed, achieving effective antioxidant activity relies on various factors, including concentration, isomeric forms, and synergistic interactions with other components (Almela et al., 2006).

Extracts obtained with highly polar solvents proved to be much more effective compared to those obtained with less polar solvents. The polarity of solvents influences their ability to dissolve specific antioxidant compounds, thereby affecting the evaluation of antioxidant activity (Hayouni et al., 2007 ; Turkmen et al., 2007).

Our sensitivity analysis of the plant extracts indicates their effectiveness against almost all strains tested, which is in line with various studies focused on the antibacterial properties of *S. marianum* extracts. These studies, including those by Djeridane et al., (2006), Rajaei et al., (2010), Magiatis et al., (2012), Bessam and Mehdadi, (2014), and Guemari et al., (2020), have all highlighted their significant antibacterial activity.

The Ac and EAq extracts show notable activity against gram-negative bacteria (*P. vulgaris*, *E. coli ATCC 25922*, *E. aerogenes ATCC 13048*, and *K. oxytoca*). The observed antimicrobial effect is probably due to these molecules direct interaction with the cell wall, leading to its dissolution (Bousseboua, 2001).

The sensitivity of *S. aureus* ATCC 25223 to chloroform and aqueous extracts can be ascribed to the flavonoids in the plant, which inhibit bacterial growth through multiple mechanisms, including the inhibition of protein and membrane phospholipid biosynthesis, as well as nucleic acid synthesis, ultimately leading to the complete dissolution of the membrane (Hassan et al., 2006).

The extracts exhibited varying levels of antibacterial activity against the different bacterial species tested. Pathogenic strains showed significant sensitivity to the polyphenolic extracts of milk thistle, particularly *E. coli* ATCC 25952, *S. aureus* ATCC 25223, and *E. aerogenes* ATCC 13048. At a concentration of 1/100, no growth was observed for any of the strains. These results align with findings reported by Newman (2003).

Notably, even bacteria resistant to multiple antibiotics were found to be sensitive to the extracts. These observations are in agreement with studies by Ghosh et al., (2008) and Ullah et al., (2020).

On the other hand, polyphenols such as tannins and flavonoids, including epigallocatechin, catechin, naringin, quercetin, rutin, and luteolin are crucial antibacterial agents, which accounts for the positive effects observed in the various extracts (Shan et al., 2007). This was confirmed by HPLC (high-performance liquid chromatography), which allowed for the separation and

identification of the flavonoid compounds present in the different extracts. By comparing with reference standards, we identified silybin in the chloroform extract (EAc), catechin and rutin in the methanol extract (EMeOH), naringin in the ethyl acetate extract (EAc), and both quercetin and catechin in the aqueous extract.

According to Lee (2003) and Fatima Bessam and Mehdadi (2014), the sensitivity of Gram-positive bacteria is attributed to the inhibitory action of silybin on protein synthesis and RNA, which explains the positive effect of the chloroform extract in our study against this type of pathogen.

Conclusion :

From these results, we deduced that the seeds of *S. marianum* possess a significant bactericidal activity against highly antibiotic-resistant pathogenic germs. The Aq and CH extracts were found to be the most active, with inhibition diameters of 32 ± 2.30 mm and 22 ± 1.25 mm, respectively. The study of the antioxidant effect of the extracts using the DPPH test and the β-carotene bleaching method confirmed that the EMeOH extract of milk thistle was the most effective at scavenging DPPH, a relatively stable free radical, and inhibiting the oxidation of linoleic acid coupled with β-carotene. This confirms that our plant has a very active antioxidant capacity. HPLC analysis of the extracts from *S. marianum* seeds helped complete its phytochemical profile, showing the presence of five classes of flavonoids (silybin, catechin, naringin, rutine, and quercetin) responsible for the antimicrobial potency of our species.

This study confirmes the effectiveness of this plant and rationalizes its use in traditional medicine.

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