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Antioxidant Profiling of *Silybum marianum* Flower crude Aqueous Extract and Its Antimicrobial Efficiency Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Silybum marianum L. is a widely recognized medicinal plant for its bioactive phytochemicals and therapeutic potential. The study assessed the crude aquatic extract antioxidant activity, antimicrobial effectivity, and synergetic effect combined with ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. The agar well diffusion method utilized to evaluate the antimicrobial activity, while antioxidant potential was identified by the DPPH radical scavenging assay. The extract putative major compound was also evaluated for physicochemical and ADME properties. The antimicrobial activity revealed that the aquatic extract of the seed–ciprofloxacin mixture exhibited the highest inhibition zone against *E. coli* (40 mm), followed by the extract of the flower–ciprofloxacin mixture (37 mm), compared with ciprofloxacin alone (33 mm). The flower extract–ciprofloxacin exhibited a 31 mm inhibition zone against *S. aureus,* whereas the seed extract–ciprofloxacin and ciprofloxacin alone showed 30 mm. The flower extract demonstrated strong antioxidant activity using the DPPH assay, with 75% radical scavenging activity at 100 mg/mL and a predicted IC_{50} value of 14 µg/mL, which was close to ascorbic acid (13.9 µg/mL). The investigated extract was further investigated using ADME analysis, which showed that the extract associated compound has high water-solubility, non-BBB permeability, free from PAINS and Brenk alerts, and estimated low gastrointestinal absorption. Finally, these results suggest that *S. marianum* flower extract has promising antioxidant activity and might increas ciprofloxacin activity, particularly against *E. coli*. Further MIC, MBC, synergy, and phytochemical validation studies are required to confirm its antimicrobial potential.

Keywords: *Silybum marianum*; Aqueous flower extract; Antioxidant activity; DPPH assay; Antimicrobial activity; Ciprofloxacin potentiation; *Escherichia coli*; *Staphylococcus aureus*

1. Introduction

The continuous emergence of antimicrobial resistance has become one of the most serious challenges in modern medicine, particularly because common bacterial pathogens are increasingly developing reduced susceptibility to clinically used antibiotics. Among these pathogens, *Staphylococcus aureus* and *Escherichia coli* are highly important due to their involvement in skin, wound, urinary tract, gastrointestinal, and systemic infections [1].

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Figure 1 Morphological appearance of *Silybum marianum* L. flower

The image shows the characteristic purple flower head of *Silybum marianum* L. surrounded by spiny green bracts, representing the plant material used for aqueous crude extract preparation in the present study.

Silybum marianum L (Figure 1), commonly known as milk thistle, is a medicinal plant belonging to the family Asteraceae. It has a long history of use in traditional medicine, especially for liver-related disorders, and its pharmacological importance is mainly associated with silymarin, a mixture of flavonolignans including silybin, isosilybin, silychristin, and silydianin [2]. In addition to its hepatoprotective activity, *S. marianum* has been reported to possess antioxidant, anti-inflammatory, anticancer, and antimicrobial properties. Recent phytochemical investigations have shown that the biological activity of *S. marianum* is strongly influenced by the plant part used and the extraction solvent [3]. Flowers, leaves, stems, and seeds may differ substantially in their chemical composition and extract yield. A recent comparative study demonstrated that *S. marianum* extracts contain several classes of secondary metabolites, including alkaloids, tannins, flavonoids, glycosides, steroids, quinones, phenols, anthraquinones, cardiac glycosides, and terpenoids [4]. The same study also reported that the methanolic flower extract contained particularly high levels of phenolic, flavonoid, and tannin contents, while antimicrobial activity was observed against both Gram-positive and Gram-negative bacteria, as well as *Candida albicans* [5]. These findings suggest that flower-derived extracts may be especially promising for further antimicrobial and antioxidant evaluation. Chemical profiling studies also support the pharmacological potential of *S. marianum*. A study reported that several bioactive constituents, including phytosterols, catechin, terpenes, fatty acids, fatty alcohols, and monosaccharide derivatives, with variable distribution among different plant parts was identified in Iraqi *S. marianum* flowers using GC-analysis [6]. These detected compounds may possess a main role in the plant antioxidant and antimicrobial activity of either individually or through synergistic interactions. However, careful interpretation of the GC-MS-derived compounds is recommended, particularly when derivatization procedures are utilized, because some identified molecules may represent derivatized forms rather than the original native phytochemicals. In addition, several biological studies also support the therapeutic effectiveness of *S. marianum* [7]. Makrane et al (2018) reported that the *S. marianum* aqueous crude extracts demonstrated cytotoxic effects against several cancer cell lines, including Hep-2, AMN-3, and RD cells, while no apparent toxic effect was shown against normal REF cells under the tested environment [8]. Although this finding represents anticancer activity rather than antimicrobial activity, it indicates that the biological activity of *S. marianum* compounds is capable of affecting abnormal cells compared with lower toxicity against normal cells [9]. Therefore, all these findings provide additional justification for investigating *S. marianum* bioactive potential in other biomedical applications, including antibiofilm and antimicrobial studies. However, the main limitation of these studies is that they used agar well diffusion or disk diffusion assays as preliminary antimicrobial screening [10]. Although these implemented experiments are beneficial for preliminary estimation, they do not provide sufficient information about other properties, such as minimum inhibitory concentration, antibiotic interaction, bactericidal activity, and antibiofilm potential. Moreover, insufficient characterization related to the mechanism by which *S. marianum* extracts may increase antibiotic activity [11]. This gap is important because the weak direct antibacterial activity of plant extracts is highly valuable if it enhances the effectivity

of conventional antibiotics, such as ciprofloxacin, ampicillin, or oxacillin. Therefore, for stronger mechanistic evidence generation, several essays such as checkerboard synergy, fractional inhibitory concentration index analysis, antibiofilm, phytochemical profiling, and computational molecular interaction studies are demanded [12].

This study aims to estimate the antioxidant, antimicrobial, antibiotic-potentiating, and antibiofilm activities of *Silybum marianum* flower and seed aquatic extracts against *Staphylococcus aureus* and *Escherichia coli*. In addition, this study aims to relate phytochemical composition to biological activity through GC-MS or LC-MS-based profiling and in silico analysis of selected bioactive compounds. It is hypothesized that flower-derived fractions of *S. marianum* extracts, which are rich in phenolic and flavonoid compounds, may strengthen antibiotic activity and reduce the formation of bacterial cells through multi-target mechanisms. This combined experimental and computational methodology may provide a stronger scientific basis for developing natural antimicrobial adjuvants from *S. marianum*-derived compounds or fractions as.

2. Materials and Methods

2.1. The plant extract preparation

Silybum marianum L fresh seeds and flowers were collected from Tikrit city in January and February 2026, thoroughly washed with distilled water (D.W), and preserved in laboratory conditions (in a dark, clean, and well-ventilated area) away from direct light, heat, and moisture until ready for extraction.

The flower and seed extracts were processed for cold water extraction. For cold water, 10 g dried powder of each part was soaked in 100 ml distilled water and rotated on a shaker at 150 rpm for 24 h. The supernatant was collected and then filtered. Centrifugation and filtration processes were used and the final extract was filtered using Millipore 0.**22** µ m pore size to ensure extract sterility [1]

2.2. Antibacterial activity assay

The agar well diffusion method was used to evaluate antibacterial activity. Agar plate of Mueller–Hinton were inoculated with standardized bacterial suspension using sterile cotton swabs. Agar wells were made using a sterile cork borer, and different concentrations of the extract were added to the wells. The antibiotic Ciprofloxacin was used as the positive control, and distal water (D.W) was used as the negative control. After incubation at 37 °C for 24 h, inhibition zones were measured in millimeters. All experiments were conducted in triplicate. The minimum inhibitory concentration was detected utilizing the broth microdilution method. The active extract serial dilutions were prepared in 96-well plates, followed by the addition of bacterial inoculum. After incubation at 37 °C for 24 h, the MIC was recorded as the lowest concentration demonstrating no bacterial growth. Furthermore, for the determination of MBC, aliquots from wells without growth were subcultured onto agar plates. The minimum concentration revealed that no colony growth was recorded as the MBC. To determine the synergetic effect of S. *marianum* extract as enhancing antibiotic activity, the active extract was combined with ciprofloxacin. A comparison was made by using the inhibition zones of only extract, only ciprofloxacin, and extract–ciprofloxacin combination. Finally, the increased inhibition zone in the combination group was considered as possible antibiotic-potentiating activity [2; 3].

2.3. Antioxidant activity assay

The antioxidant activity will be evaluated using the DPPH radical scavenging assay. Different concentrations of the extract will be mixed with DPPH solution and incubated in the dark for 30 min at room temperature. Absorbance will be measured at 517 nm using a spectrophotometer. Ascorbic acid will be used as the standard antioxidant. Radical scavenging activity will be calculated as: RSA (%) = [(Acontrol − Asample) / Acontrol] × 100. The IC_{50} value will be calculated from the concentration–response curve[15].

2.4. ADME and drug-likeness analysis

The best-docked compounds was evaluated using SwissADME[16]. Parameters including molecular weight, hydrogen bond donors and acceptors, LogP, topological polar surface area, gastrointestinal absorption, BBB permeability, Lipinski's rule, PAINS alerts, and synthetic accessibility were recorded. Compounds with acceptable docking score and favorable ADME properties will be considered the most promising candidates. All experimental results will be expressed as mean ± standard deviation from three independent replicates.

3. Results and Discussion

3.1. Antibacterial activity of Silybum marianum extract

The *Silybum marianum* flower and seed antibacterial activity extracts were evaluated against the utilized bacteria in this study, which included both *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method. The results revealed that the seed extracts alone exhibited no antibacterial activity compared with the flower extract alone, which exhibited weak activity. However, the extract of flower-ciprofloxacin, and seed- ciprofloxacin combinations demonstrated considerably wide inhibition zones against both investigated bacterial strains **(Table 1)**. The seed extract–ciprofloxacin combination showed large inhibition zones of approximately 30 mm against *S. aureus* and approximately 40 mm against *E. coli*, whereas the flower extract–ciprofloxacin combination exhibited inhibition zones of approximately 31 mm and ~37 mm, respectively. However, when compared with the investigated drug, which was used as a control (ciprofloxacin alone), it showed inhibition zones of ~30 mm against *S. aureus* and ~33 mm against *E. coli.*

Table 1 The *Silybum marianum* extracts and ciprofloxacin combinations antibacterial activity

Treatment	*S. aureus* inhibition zone (mm)	*E. coli* inhibition zone (mm)
Seed extract alone	weak activity	No/weak activity
Flower extract alone	weak activity	No/weak activity
Seed extract + ciprofloxacin	~30	~40
Flower extract + ciprofloxacin	~31	~37
Ciprofloxacin	~30	~33

**Values should be presented as mean ± SD from three independent replicates after final measurement.

The wide range antibacterial activity revealed by the extract–ciprofloxacin combinations suggests that the phytochemical content in *S. marianum* both flowers and seeds may have synergetic effect that might enhance ciprofloxacin. This exhibited effect was evident against *E. coli*, where the seed extract–ciprofloxacin combination exhibited the largest inhibition zone. Recent study was introduced by Mahmoud and Selim (2025), who reported that *S. marianum* extracts contain various phytochemical content, including flavonoids, glycosides, steroids, alkaloids, tannins, quinones, phenols, anthraquinones, cardiac glycosides, and terpenoids, which demonstrated a considered antimicrobial activity against Gram-positive and Gram-negative microorganisms [3]. In addition, another study reported that *S. marianum* aquatic extract possesses a potent in vitro anticancer activity [4]. These findings indicate that *S. marianum* might be considered a potent antibacterial candidate and may contribute to the enhancement of antibiotic activity.

3.2. The flower extract of *S. marianum* DPPH radical scavenging activity

Table 2 DPPH radical scavenging activity of *Silybum marianum* flower extract and ascorbic acid

Sample	Concentration	Absorbance at 517 nm	RSA (%)	IC_{50}	Activity level
Flower extract	100 mg/mL	0.20	75.0	14 µg/mL	Strong
Flower extract	80 mg/mL	0.22	72.5	—	Strong
Flower extract	40 mg/mL	0.26	67.5	—	Moderate
Flower extract	20 mg/mL	0.30	62.5	—	Moderate
Flower extract	10 mg/mL	0.80	0.0	—	No RSA
Ascorbic acid	100 mg/mL	0.10	87.5	13.9 µg/mL	Very strong
Ascorbic acid	80 mg/mL	0.20	75.0	—	Strong
Ascorbic acid	40 mg/mL	0.26	67.5	—	Moderate
Ascorbic acid	20 mg/mL	0.27	66.25	—	Moderate
Ascorbic acid	10 mg/mL	0.80	0.0	—	No RSA

The antioxidant activity of *S. marianum* flower aqueous extract was evaluated using the DPPH radical scavenging assay. As demonstrated in **Table 2**, the extract exhibited a different range of antioxidant activity depending on the assessed extract concentration, with the upgrading of the assessed concentrations, the demonstrated activity had a stronger effect of radical scavenging. The highest concentration initiated as 100 mg/mL, which exhibited the highest radical scavenging activity, achieving 75% RSA. The different concentrations of 80, 40, and 20 mg/mL demonstrated different values of RSA of 72.5%, 67.5%, and 62.5% were observed at, respectively, however, no detectable radical scavenging activity was reported at 10 mg/mL. Thus, the flower extract predicted IC_{50} value was 14 µg/mL.

In this experiment, the antioxidant reference used was ascorbic acid, which exhibited stronger activity at the highest concentration, resulting in 87.5% RSA and an IC_{50} value of 13.9 µg/mL. Nevertheless, the flower extract exhibited a comparable antioxidant activity to that demonstrated by ascorbic acid at several concentrations. It's worth mentioning, both experimented samples revealed identical RSA values (67.5%) at 40 mg/mL. The close range between the IC_{50} values of the assessed extract and the standard antioxidant indicates substantial free radical scavenging capacity.

The observed antioxidant activity in the assessed flower extract may be attributed to the presence of flavonoids, phenolic compounds, and other hydroxyl-containing phytochemicals. Previous studies have consistently reported significant antioxidant activity in S. marianum and associated these effects with silymarin, silybin, and related flavonolignans [2]. Furthermore, Lekmine et al. [18] reported that S. marianum contains numerous bioactive metabolites with antioxidant potential. Therefore, the present findings support the potential application of S. marianum flower extract as a natural antioxidant source.

3.3. Putative bioactive compound and ADME profile

The main chemical compound, which hypothesized to have antimicrobial and antioxidant activity was retrieved from GC–MS analysis of previous studies literature as putative compound associated with the *S. marianum* flower extract as Myo-inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-[5; 6]. The chemical structure of the identified compound is presented in **Fig 2**.

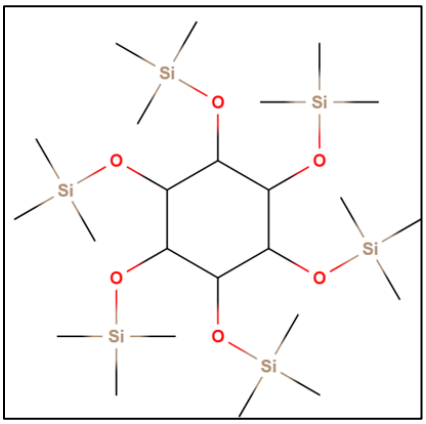

Figure 2 Chemical structure of the putative GC-MS-detected [6] compound from *Silybum marianum* flower extract

The structure represents Myo-inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, which was considered as a putative phytochemical constituent associated with the extract profile.

The physicochemical and ADME analyses revealed that the compound possessed a molecular formula of $C_6H_{12}O_6$ and a molecular weight of 180.16 g/mol. The molecule contained six hydrogen bond acceptors and six hydrogen bond donors, with a topological polar surface area of 121.38 $Å^2$. The consensus LogP value of −3.41 indicated high hydrophilicity and was consistent with the prediction of high aqueous solubility. However, low gastrointestinal absorption and a lack of blood–brain barrier permeability were revealed by the predicted pharmacokinetic analysis, suggesting restricted systemic distribution and limited membrane permeability following oral administration.

The compound was further assessed by drug-likeness assessment, which indicated that it complied with Lipinski's rule of five, except for one violation related to the count of hydrogen bond donors. The compound also demonstrated high polarity and low lipophilicity, which was compiled using the Veber and Egan criteria but failed the Ghose and Muegge filters. In addition, a favorable structural safety profile was demonstrated as no PAINS or Brenk alert detection. The

compound also exhibited moderate drug-likeness despite limited oral absorption, indicating a score of 0.55 bioavailability.

The parent inositol multiple hydroxyl groups may contribute to antioxidant activity through radical stabilization and hydrogen donation. Furthermore, *S. marianum* had various active biological compounds, including flavonoids, phenolics, terpenoids, and related metabolites, exhibit antimicrobial and antioxidant properties [19]. Therefore, several phytochemicals identified compounds may contribute to the biological activities observed recently. However, further phytochemical validation is required to determine its exact biological significance.

3.4. *S. marianum* extracts antimicrobial activity in combination with ciprofloxacin

The combination of *S. marianum* seed and flower extracts with ciprofloxacin demonstrated antimicrobial activity, which was evaluated against *E. coli* and *S. aureus* using the agar well diffusion method. The results detailed in **Table 3** revealed that the combination of extract–ciprofloxacin produced clear inhibition zones against both investigated bacterial strains. The highest antibacterial activity against *E. coli* was shown by the seed extract–ciprofloxacin combination, as it exhibited of ~40 mm inhibition zone, followed by the flower extract–ciprofloxacin combination with ~37 mm. However, comparing with ciprofloxacin alone produced an inhibition zone of ~33 mm. These comparative results are demonstrated in **Fig 3A**.

Table 3 Antimicrobial activity of *Silybum marianum* extracts combined with ciprofloxacin

Treatment	*S. aureus* inhibition zone (mm)	*E. coli* inhibition zone (mm)
Seed extract + ciprofloxacin	30	40
Flower extract + ciprofloxacin	31	37
Ciprofloxacin	30	33

The combination of flower extract – ciprofloxacin exhibited an inhibition zone of ~ 31 mm against *S. aureus*, whereas both the ciprofloxacin alone and seed extract – ciprofloxacin combination exhibited inhibition zones of ~ 30 mm. **Fig 3B** represents agar well diffusion plates revealing visible inhibition zones. It's evident that the extract potentiating effect was more obvious against *E. coli* than against *S. aureus*.

The increased antibacterial activity shown by the extract – ciprofloxacin combinations suggest a potential synergistic interaction between *S. marianum* phytochemicals and ciprofloxacin. Previous study conducted by Mahmoud and Selim (2025), reported that *S. marianum* contains numerous antimicrobial phytochemicals [3], However, another study conducted by Eldalawy et al. (2020) reported identification of phytosterols, terpenes, catechins, fatty acids, and related compounds in various plant parts [19]. Such compounds may play a pivotal role in enzyme inhibition, membrane disruption, and increased bacterial cell antibiotic susceptibility [20]. Furthermore, the effectiveness of S. marianum extracts against multidrug-resistant bacteria has been reported in recent studies, which demonstrated and highlighted their potential antimicrobial complementary agents [23].

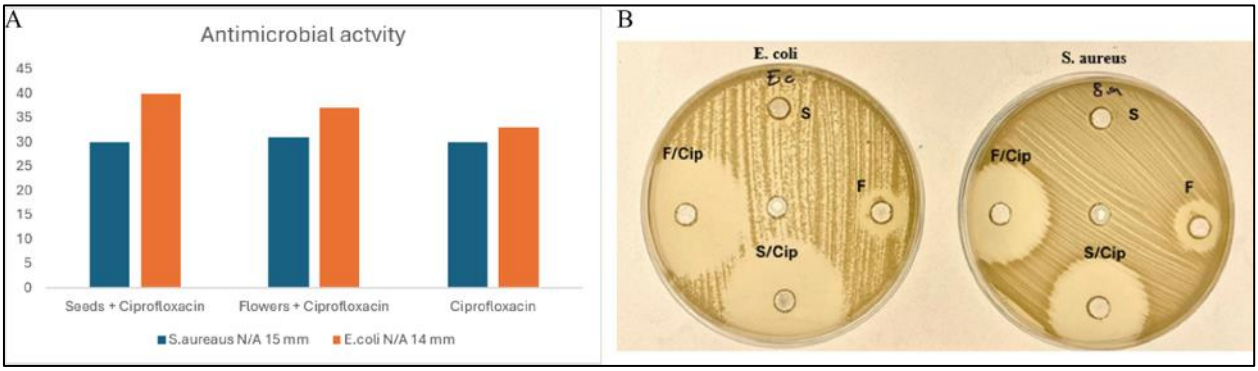

Figure 3 Antimicrobial activity of Silybum marianum extract–ciprofloxacin combinations against *E. coli* and *S. aureus.*

(A) Bar graph showing inhibition zones produced by seed extract + ciprofloxacin, flower extract + ciprofloxacin, and ciprofloxacin alone. (B) Agar well diffusion plates showing visible inhibition zones against *E. coli* and *S. aureus*. S/Cip = seed extract + ciprofloxacin; F/Cip = flower extract + ciprofloxacin; Cip = ciprofloxacin.

All in all, these results indicate that *S. marianum* extracts, particularly flower extracts, may function as antibiotic adjuvants potent candidates capable of increasing ciprofloxacin efficacy against Gram-negative and Gram-positive bacteria. However, to confirm the mechanism and extent of this interaction further investigations involving MIC and MBC determination, checkerboard synergy assays, antibiofilm studies, and time-kill kinetic analyses are required [24]. For further strengthen the biomedical relevance of these findings, future studies involving multidrug-resistant clinical isolates are required, which might support the development of plant-based approaches for combating antimicrobial resistance [25].

4. Conclusion

The crude aqueous of *Silybum marianum* flower extract represented in this study demonstrated that it possesses potent antioxidant activity and may contribute to increased antimicrobial activity when combined with ciprofloxacin. The broadest antibacterial activity was observed against *E. coli*, where seed extract -ciprofloxacin combination introduced a ~ 40 mm inhibition zone, followed by flower extract - ciprofloxacin combination with ~ 37 mm, both boarder than ciprofloxacin alone. Against *S. aureus*, the extract - ciprofloxacin combinations showed only lower activity than ciprofloxacin alone, indicating that the augmenting effect was more evident against Gram-negative bacteria. However, considerable antioxidant activity was validated by the DPPH assay, with radical scavenging activity of flower extract ~ 75% at 100 mg/mL and an IC_{50} value close range to that of ascorbic acid. The ADME profile of the putative detected compound suggested high water solubility, absence of major structural alerts, and no predicted blood–brain barrier permeability, although low gastrointestinal absorption may limit systemic oral activity. Overall, *S. marianum* flower extract may serve as a promising natural antioxidant source and a possible antibiotic-supporting agent. However, further studies using MIC, MBC, checkerboard synergy assay, antibiofilm testing, and LC-MS/HPLC-based compound confirmation are necessary before strong antimicrobial claims can be made.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares no conflicts of interest associated with this manuscript.

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