RESEARCH PAPER

Unveiling the Antioxidant and Antimicrobial Potential of Silybum marianum Seed Extract through Phytochemical and Spectroscopic Analysis

Short Title: Functional Properties of Silybum marianum Seed Extract

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ABSTRACT:

Silybum marianum (milk thistle) is a medicinal plant well known for its hepatoprotective and antioxidant properties, because of its rich flavonolignan content. Regardless of its traditional use, comprehensive phytochemical profiling and bioactivity validation remain essential for therapeutic standardization. This study aimed to depict the phytochemical constituents of S. marianum seed extract and estimate its antioxidant and antimicrobial activities using spectroscopic and bioassay-based approaches. Methanolic extracts were prepared through the maceration and undergo qualitative screening, UV–Vis, FTIR, and GC–MS analyses for compound identification. The DPPH radical scavenging assay was used to assess antioxidant potential, while the agar well diffusion method was used to assess antimicrobial activity against specific bacterial and fungus species.IC_{50} values and inhibition zones were statistically evaluated using OriginPro (version 2023). The extract found abundant flavonoids, terpenoids, glycosides, and phenolics. Spectral analyses confirmed the presence of silybin, silychristin, and silydianin. The extract shows dose-dependent antioxidant activity with an IC_{50} value comparable to ascorbic acid. There were moderate antimicrobial effects were observed against E. coli and E. faecalis, while P. aeruginosa and S. aureus were resistance. The findings prove the therapeutic potential of S. marianum seed extract, highlighting its antioxidant efficacy and selective antimicrobial action. These results indicate its use in herbal formulations to combat oxidative stress and infections induced by bacteria.

KEYWORDS: Silybum marianum, pphytochemical analysis, antimicrobial activityy, DPPH assay, GC–MS

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INTRODUCTION :

Numerous phytochemicals found in herbs and shrubs make them a valuable source for the treatment of a variety of illnesses. Among these naturally occurring plant species, milk thistle, or Silybum marianum L., is regarded as a significant and traditional medicinal plant[1.] Silybum marianum is a significant member of the Asteraceae (Compositae), the largest blooming family, which is also known as the sunflower, daisy, or aster family. The family is divided into thirteen subfamilies, which include over two thousand genera[2]. Native to southern Europe, Northern Africa, South and North America, and Australia, it is also used to treat liver problems and helps nursing mothers in some regions of Asia[1.] Other names for S. marianum include pig leaves, royal thistle, Marian thistle, lady's thistle, Christ's crown, snake milk, Venus thistle, Heal thistle, variegated thistle, sow thistle, and wild artichoke. In folk medicine, Silybum marianum L., commonly known as milk thistle, has been used for over 2000 years to treat a variety of illnesses, including rheumatism, liver and kidney issues, gastronomic disorders, cardiac issues, and gall bladder-related conditions like cirrhosis, hepatitis, and jaundice[3.] Due to its antioxidants, flavonoids, and total phenolics, Silybum marianum is used

medicinally to treat a variety of illnesses. . The dehydration-condensation process of dihydroflavonols and phenylpropanoid derivatives yields silymarin, a class of flavonolignans that includes silybin, isosilybin, silydianin, and silychristin[4] .Hepatoprotective, anti-inflammatory, anti-cancer, antioxidant, hypoglycemic, neuroprotective, cardioprotective, and immunomodulatory properties are all exhibited by silymarin[5,6]. Although the entire plant is used medicinally, the seeds have the highest concentration of silymarin[7].In primary research, silymarin has demonstrated efficacy as an antibacterial agent against both Gram-negative (Escherichia coli, Pseudomonas aeruginosa) and Gram-positive (Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus) bacteria[8].It was established that silybin had increased synergistic activity against Methicillin-resistant Staphylococcus aureus (MRSA) [9]when paired with either ampicillin or oxacillin. In Gram-positive bacteria, silibinin decreased the confusion of macromolecules, such as proteins and RNA[10]. Since most research has focused on milk thistle seeds, the purpose of this study was to examine and contrast the phytochemical content, antioxidant activity, and antibacterial properties of the seeds[11].

The antibacterial qualities of distinct plant components will be examined in this study, along with any possible synergistic effects. It offers fresh perspectives that broaden our knowledge of the characteristics of milk thistle extracts and their possible uses. In addition, several phytoconstituents in milk thistle seeds have been identified through investigation using Gas Chromatography–Mass Spectrometry (GC–MS), Fourier Transform Infrared Spectroscopy (FTIR), and UV Spectroscopy.

MATERIALS AND METHODS:

Collection of seeds

Silybum marianum (Milk Thistle) seeds are bought from the authetic Supplier through Amazon.The seeds were air-dried in a well-ventilated area to reduce moisture content for one week. Spread on a tray in a thin layer and let them dry for Seven days. When seeds are air-dried the seeds are grind in a fine powder using a coffee grinder or similar device and store it in tight and sealed jar at cool place until needed for analysis.

Preparation of extracts

Whole seeds powder will be macerated at room temperature with 99.9 % methanol using a plant material to solvent ratio of 1:1,1:2 and 1:3 for one week. 2gm of air-dried powder (seeds) were soaked in 1:1 (2gm+ 100ml), 1:2 (2gm+ 200ml) and 1:3 (2gm + 300ml) of organic solvents like methanol for one week in glass flask. Using Whatman filter paper No. 1, the extracts were filtered, and the filtrate was left to evaporate at room temperature. Consequently, methanol extracts were produced.

Preliminary phytochemical screening

Preliminary phytochemical screening of the methanolic extract of Silybum marianum seeds showed the presence of flavonoids, tannins, terpenoids, sterols, steroids, alkaloids, and glycosides using standard qualitative methods [12-18]

Quantitative Analysis

UV–Visible Spectrophotometric Analysis

The powder from dried seeds of Silybum marainum seeds was dissolved in laboratory grade methanol and methanolic extract of Silybum marianum seeds was prepared by using a double - beam UV-Visbile spectrophotometer (Shimadzu, Japan) was examine at 200 - 400 nm wavelength with methanol as the reference blank. Selection of the wavelength for analysis was based on the previously used certified spectrophotometric methods[19].

Fourier Transform Infrared Spectroscopy (FTIR)

In order to identify which functional groups were present in the Silybum marianum extract, Fourier Transform Infrared Spectroscopy (FTIR) was used**.** PerkinElmer FTIR Spectrophotometer (PE IR SUBTECH, Materials Research Centre) is used for spectral analysis. Homogenization of 10 mg dried extract with spectroscopic grade potassium bromide (KBr) (100 mg) in a ratio of 1:10 (w/w) is made and by using a hydraulic press compressed in to a translucent pellet. Then the pellet was firmly attached in the sample holder, and spectra were recorded in the mid - infrared region of 4000- 400 $cm^{-1,}$ with a spectral resolution of 4 cm^{-1} and averaged over 32 scans.The absorption peaks obtained were evaluated and delegated to the corresponding functional groups based on standard infrared absorption ranges and previously published studies on S. marianum extracts[20].

Gas Chromatography–Mass Spectrometry (GC-MS)

At the Amity Cheminnova Research Facility, Amity University Gurugram (Pachgaon), GC-MS analysis of Silybum marianum seed extract is carried out using a Shimadzu GC-MS system that has a robotic auto-injector. To prepare the samples for GC-MS, the

extracts were derivatized, specifically acetylated, before to examination.

Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is most common and easy assay used for measuring antioxidant activity.This method is well known because its simple, quick, and cost-effective.

DPPH free-radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is used to evaluate the antioxidant potential of the methanolic extract following the protocol of [21].A 0.04 mg/mL DPPH solution (101.5 µM) in methanol was prepared. 2 mL of DPPH solution was mixed with 1 mL of extract at varying concentrations (0–1,000 µg/mL) for each test.A control sample containing DPPH and methanol, as well as ascorbic acid, were also prepared ,used as the positive standard. Following a 30 minutes incubation period in dark at room temprature, the absorbance of the sample was measured at 517 nm using a UV–Vis spectrophotometer (UV-2005, Selecta, Barcelona, Spain).

The percentage inhibition of DPPH radicals was calculated using the formula:

$$\% \text{ Inhibition} = n = \frac{\text{Absorbance of Blank - Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100 \quad (2)$$

IC_{50} Determination

To determine the IC_{50} value, the concentration is required to inhibit 50% of the DPPH radical. The percentage of inhibition was plotted against extract concentration using OriginPro's nonlinear regression. IC_{50} values that are lower suggest greater antioxidant activity.

Antimicrobial Activity

The agar well diffusion assay, was utilized to investigate the antimicrobial activity of Silybum marianum seed extracts. The study included a representative selection of both Gram-positive and Gram-negative bacterial strains specifically, Staphylococcus auxus, Enterococcus faecalis, Pseudomonas aeruginosa, and Escherichia coli as well as the fungal species Aspergillus niger. Microbial strains were first cultivated on nutrient media and incubated for 24 hours at 37 °C prior to use. Nutrient agar was put onto sterile Petri-dishes after being melted and chilled to about 40°C. After solidification, wells were created 24 mm apart using a sterile metal cork borer with a diameter of 6 mm. By using sterile cotton swabs, bacterial suspension, aged 4-8 hours were evenly dispersed over the agar surface. The streaking process is repeated three times per plate and rotation of plates is done to ensure the uniform coverage .Each well is filled with 2 mL of Silybum marianum seed extract that has been dissolved in dimethyl sulfoxide [DMSO] at a concentration of 1 mg/mL. Control wells received either standard antibiotics (positive control) or DMSO alone (negative control).Following preparation, For a whole day, the plates were incubated at 37°C. A digital Vernier caliper was used to measure the zones of inhibition in millimeters. Every assay was performed in triplicate, and the findings are presented as mean ± standard deviation. To identify significant variations in microbial responses, three repetitions of the experiments were conducted, {triplicate (n = 3);' and statistical analysis was done using one-way ANOVA and Tukey's post hoc test. A p value less than 0.05 indicates significance ($p < 0.05$).

Statistical Analysis

The results are shown as mean ± standard deviation (SD), and each experiment was conducted in triplicate.By using the DPPH radical scavenging assay, Antioxidants activity is quantified while IC_{50} values were calculated by nonlinear regression analysis using a four-parameter logistic model in OriginPro (version 2023). Antimicrobial activity was estimated by measuring zones of inhibition (mm), and statistical comparisons between treated and control groups were conducting using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test to determine pairwise differences. A p-value < 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Qualitative Phytochemical Analysis of Silybum marianum Seed extract

Figure. 1. Qualitative phytochemical screening of Silybum marianum seed extract

The findings from the phytochemical analysis of S. marianum methanolic extracts can be seen in Table 1,(+) and (-) sign indicating presence and absence compounds.

Table 1: Phytochemical Analysis of Silybum marianum Methanolic seed extract

Sr. No	Compound Test	Result
1.	Flavonoid	+
2.	Tanin	+
3.	Saponin	-
4.	Terpenoid	+
5.	Sterol and Steroids	+
6.	Alkaloid	-
7.	Glycoside	+

When a methanolic extract of the seeds was subjected to phytochemical analysis, flavonoids, tannins, glycosides, terpenoid, sterol, and steroids were found. Saponins and alkaloids were likewise absent from a methanolic extract of the seeds, according to phytochemical study[15].

Quantitative Analysis of Silybum marianum methanolic seed extract

Their quantities in the extract were determined using conventional colorimetric and spectrophotometric techniques. According to the results, the extract had a high concentration of flavonoids and phenolics, which supported its usage as a natural antioxidant and demonstrated its potent ability to scavenge free radicals

UV - Spectrophotometer Analysis

The UV - Vis spectroscopic analysis of the methanolic extract of Silybum marianum showed two clear absorption peak at 245nm and 295nm. The absorbance value for these two peaks were 4.7180 a.u and 4.5118 a.u. The high absorbance values, nearing 5.0 and 4.0 absorbance units (a.u.) , indicate a significant concentration of UV-active phytochemicals in the extract, as shown in Figure 2.

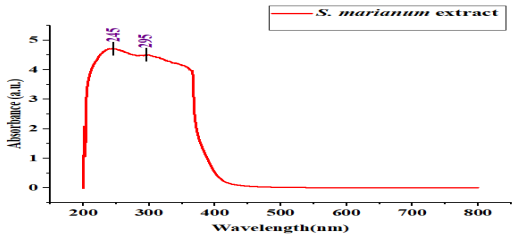

Figure. 2..UV–Vis absorption spectrum of Silybum marianum methanolic extract showing two major peaks at 245 nm and 295 nm with absorbance values of 4.7180 and 4.5118 a.u., respectively. These peaks correspond to π→π* and n→π* transitions, indicating the presence of phenolic acids and flavonolignans. The spectrum confirms strong UV activity and supports the extract's polyphenolic composition.

The peak at 245 nm is due to π→π* transitions, typically linked with aromatic rings and conjugated double bonds. This suggests the presence of phenolic acids, simple flavonoids, and other aromatic compounds. The second peak at 295 nm is associated to n→π* transitions. This is characteristic of sturucture with carbonyl groups like flavonolignans—namely silybin, silydianin, and silychristin which is key constituents of the silymarin complex[16,17]. A sharp drop in absorbance occur beyond 295 nm, with almost near-zero absorbance above 400 nm. This validate the absence of visible-range chromophores and fortifies the extract's UV-active nature. These spectral features are uniform with previously reported profiles of S. marianum extracts and support the presence of bioactive polyphenolic compounds[18] . Strong UV absorbance indicates the extract is rich in antioxidant constituents, which might contribute to its reported hepatoprotective, anti-inflammatory, and antimicrobial properties. These findings accompany the antimicrobial assay results and provide a biochemical basis for the observed bioactivity.

FTIR Spectral Characterization of Silybum marianum Methanolic Extract

The FTIR spectrum analysis of the methanolic extract of Silybum marianum showed distinct absorption bands. These bands correspond to specific functional groups of phytoconstituents, confirming the presence of various bioactive compounds in the methanloic seed extract.

key peaks include O–H stretching at 3469.75 cm^{-1}, C–H stretching at 2946.00 and 2833.33 cm^{-1}, and C≡C or C≡N stretching near 2226.19 cm^{-1}. The strong band at 1650.80 cm^{-1} suggests C=O stretching, while peaks in the fingerprint region (1402.63–418.33 cm^{-1}) shows complex vibrational modes of aromatic rings and C–O functionalities. These spectral features indicates the presence of flavonolignans and polyphenolic compounds in the extract. A broad, intense absorption band at 3470 cm^{-1} is linked to O–H stretching vibrations, charcterstics of hydroxyl groups commonly found in polyphenolic compounds such as flavonoids and flavonolignans This matches with the antioxidant-rich profile of S. marianum, particularly its silymarin complex, which includes silybin, silychristin, and silydianin (Figure.3).

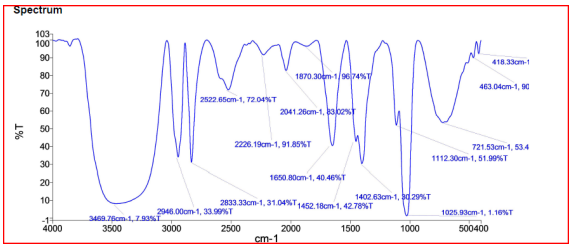

Figure 3. Shows the FTIR transmission spectrum of the Silybum marianum extract, It highlights the characteristic absorption bands that correspond to specific functional groups.

Bands at 2946 cm^{-1} and 2833 cm^{-1} show C–H stretching of aliphatic chains, suggesting the presence of terpenoids and fatty acids. These compounds helps in membrane stabilization and anti-inflammatory activity. A sharp peak at 1651 cm^{-1} indicates C=O stretching and aromatic C=C vibrations, validate the presence of conjugated carbonyl systems like those in flavonoids and phenolic acids. These compounds have antioxidants (free radical scavenging) and metal-chelating properties. The absorption bands at 1452 cm^{-1} and 1403 cm^{-1} showst CH_2/CH_3 bending and aromatic skeletal vibrations, confirming the presence of lignans and aromatic flavonoid rings. Peaks at 1112 cm^{-1} and 1026 cm^{-1} are charctestics of C–O stretching, indicates alcohols, ethers, and glycosidic linkages matching silymarin glycosides known for hepatoprotective effects. The 721 cm is $^{-1}$ band is aromatic C–H bending, Peaks at 463 cm^{-1} and 418 cm^{-1} indicates complex skeletal vibrations involving aromatic and heteroatom-rich structures. These features shows the structural diversity of the extract and support its potential in pharmacology.

Overall, the FTIR spectral data supports previous reports on the phytochemical makeup of S. marianum, confirming the presence of flavonolignans, flavonoids, phenolic acids, and fatty acids as major constituents in Silybum marainum seeds[19-21]. The abundance of hydroxyl and carbonyl functional groups in the extracts further supports its antioxidant potential and therapeutic significance in reducing oxidative stress and heavy metal toxicity[22,23].

GC - MS Analysis

Gas chromatography–mass spectrometry (GC-MS) analysis was used to identify the volatile and semi-volatile phytoconstituents present in the methanolic extract of Silybum marianum. The chromatogram showed a complex mixture of bioactive compounds spread across three distinct elution phases: initial (3 -6 min), mid (6-24 min), and late (24- 29 min) (Figure.4).

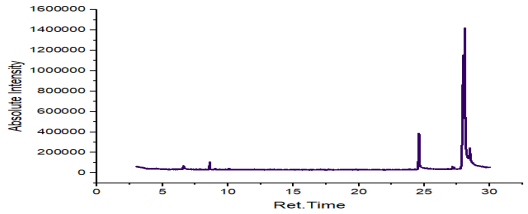

Figure 4. shows the GC–MS chromatogram of the methanolic extract of Silybum marianum.

The major peaks are labeled with their retention times.The peak with highest abundance is 28.11 min and corresponds to flavolignans. In the initial phase, low-intensity peaks showed up at retention times of 3.02 and 3.09 min. These corresponded to small, volatile molecules like short-chain alcohols or esters. Though they are minor, they might contribute to the extract's solubility and initial bioactivity. During the mid-phase (6.-24 min) ,peak intensity gradually increasing, showing mid-polarity compounds such as terpenoids, small flavonoids, and phenolic acids are present in the ex These constituents are reowned for their anti-inflammatory and antioxidant properties. Their elution pattern matches previous reports on S. marianum seed extracts[24.] The late phase demonstrated a more significant peak at 28.110 min, with a maximum intensity of 1,419,649 units.This suggest the presence of high molecular weight, less volatile compounds. This distinctive retention behavior is displayed by the flavonolignans, which include silybin, silychristin, and silydianin, the main bioactive components of silymarin[25,26]. These substances are known to have hepatoprotective, antioxidant, and metal-chelating properties, making them therapeutically significant in relation to heavy metal toxicity and oxidative stress[27,28].

S. marianum's phytochemical richness is confirmed by the GC-MS profile, which shows a wide range of constituents from complex polyphenolics to tiny volatiles. In addition to confirming the FTIR results, which also showed a substantial presence of hydroxyl and carbonyl functional groups, the preponderance of late-eluting flavonolignans confirms the extract's pharmacological potential (Table 4). Based on both radical scavenging and chelation mechanisms, these findings support the use of S. marianum as a strong source of antioxidant and hepatoprotective substances and support its function in reducing heavy metal-induced toxicity.

Antioxidants Analysis(DPPH Radical Scavenging Activity)

Using UV-Vis spectrophotometry to measure absorbance, the DPPH free radical scavenging test

was used to evaluate the antioxidant effectiveness of the Silybum marianum methanolic extract (Figure 5).

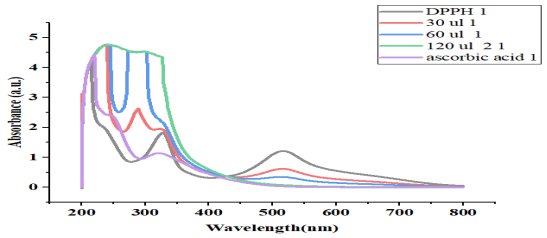

Figure 5. UV–Vis absorption spectra showing DPPH radical scavenging by methanolic extract of Silybum marianum at different concentrations (30, 60, and 120 µL) compared with DPPH control and ascorbic acid standard. A dose-dependent reduction in absorbance confirms the antioxidant potential of the extract.

The DPPH control showed a significant absorbance peak in the 200–400 nm range, with a noticeable maximum close to 517 nm. This is indicative of the stable DPPH radical's purple chromophore. There was a concentration-dependent decrease in absorbance after the plant extract treatment. With increasing extract volume, the 30 µL sample produced a slight drop, while the 60 µL and 120 µL concentrations produced increasingly larger declines, suggesting increased radical scavenging action.

This pattern implies that S. marianum's bioactive components, particularly its polyphenols and flavonolignans, efficiently donate electrons or hydrogen atoms to neutralize DPPH radicals, reducing the absorption band's strength. At the maximum concentration tested (120 µL), the extract's scavenging activity was very similar to that of the positive control, ascorbic acid. This resemblance highlights S. marianum's strong antioxidant potential, which is probably due to its diverse phytochemical composition, which includes substances like silybin, silychristin, and silydianin[29,30] .The ability of these flavonolignans to donate electrons and neutralize free radicals is well-established.The extract's capacity to neutralize free radicals in a dose-responsive fashion is confirmed by the observed lowering of absorbance across increasing doses. These results are consistent with earlier studies that showed milk thistle extracts have strong antioxidant activity in the DPPH and ABTS tests[31.] All of the evidence points to S. marianum's medicinal value as a natural antioxidant that may be used to reduce oxidative stress and toxicity brought on by heavy metals.

Using the absorbance suppression across various extract volumes, the IC_{50} value the dose needed to inhibit 50% of DPPH radicals was calculated in order to further evaluate the antioxidant activity. The extract showed a steep dose-response curve, and the IC_{50} was substantially below this threshold at 120 µL concentration, which was almost completely quenching radicals. The quick decrease in absorbance within minutes after extract addition suggests fast electron transfer kinetics, which is a feature of high-reactivity polyphenols, even though detailed kinetic modeling was outside the purview of this experiment. These results not only confirm the extract's strength but also point to its potential for use in therapeutic settings where quick antioxidant action is needed, like acute oxidative stress or cellular damage brought on by heavy metals.

Antimicrobial Activity of Silybum marianum Seed Extracts

Four pathogenic strains Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, and Staphylococcus aureus were used to test the antibacterial activity of Silybum marianum seed extracts. The selected bacterial strains, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, and Staphylococcus aureus, include both Gram-negative and Gram-positive pathogens. This allows us to assess the extract's broad-spectrum antibacterial activity. These strains are important in clinical settings. E. coli and P. aeruginosa are common Gram-negative pathogens linked to gastrointestinal and hospital acquired infections. In contrast, E. faecalis and S. aureus are Gram-positive bacteria often related to wound, urinary tract, and systemic infections. Testing the extract against this group helps us evaluate its effectiveness across various bacterial cell wall structures and resistance mechanisms, offering valuable insights into its therapeutic potential.

Agar well diffusion assay was used to quantify the zones of inhibition, which were then compared to DMSO (negative control) and conventional antibiotics (positive control). Table 2 presents a summary of the findings.

Table 2. Zones of inhibition (mm) produced by Silybum marianum seed extracts against Both Gram positive and Gram negative microbial strains

Microorganism	Zone of Inhibition (mm)	Statistical Significance
E. coli	14.2 ± 0.6	a
E. faecalis	12.8 ± 0.4	a
P. aeruginosa	6.1 ± 0.3	b
S. aureus	5.4 ± 0.2	b

Note: Values are mean ± SD (n = 3). Different superscripts (a, b) indicate statistically significant differences ($p < 0.05$) based on Tukey's post hoc test.

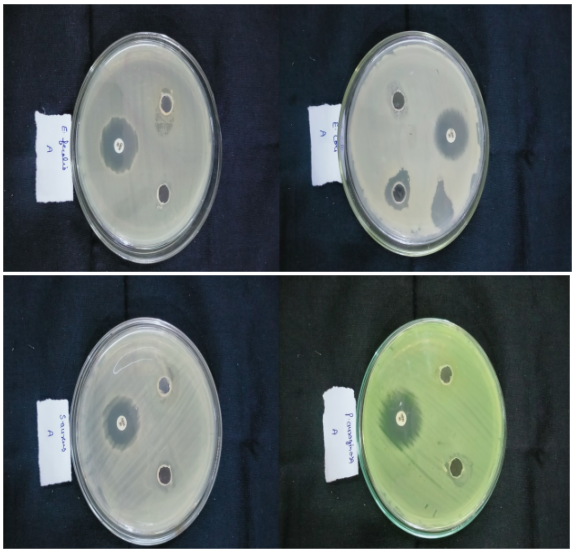

Figure.6. Antimicrobial activity of Silybum marianum seed extracts against selected pathogenic strains using the agar well diffusion method. Series A: inhibition zones observed against Escherichia coli, showing moderate antibacterial activity. Series B: no inhibition observed against Pseudomonas aeruginosa, indicating resistance to the extract. Series C: mild inhibition zones against Enterococcus faecalis, suggesting partial sensitivity. Series D: no inhibition observed against Staphylococcus aureus, demonstrating lack of efficacy under tested conditions.

With inhibition zones varying from 5.6 to 17.9 mm across replicates, the extract demonstrated moderate antimicrobial efficacy against E. coli and E. faecalis. The presence of bioactive chemicals with specific efficiency against Gram-negative bacteria is suggested by the maximum inhibition found against E. coli. This is in line with earlier findings that flavonolignans, including silybin and silychristin, have antibacterial qualities, especially against Salmonella and E. coli[30,32]Under the tested conditions, no inhibition zones were seen against P. aeruginosa and S. aureus, suggesting that the seed extracts had little to no effectiveness against these bacteria. Its strong efflux mechanisms and impermeable outer membrane, which lessen vulnerability to phytochemicals, may be the cause of P. aeruginosa's resistance[33.]With inhibition zones of 5.6 and 10.5 mm, the moderate activity against E. faecalis indicates partial sensitivity. Even though E. faecalis is Gram-positive, it has built-in resistance mechanisms that could reduce the potency of some plant-based medications[34.]The observation that the negative control (DMSO) showed no inhibition indicates that the seed extracts, not the solvent, were the cause of the observed antimicrobial activities. The test conditions were validated and the dependability of the experimental setup was confirmed by the constant production of inhibition zones above 16 mm by the positive control, ciprofloxacin.

CONCLUSION

This investigation highlights that Silybum marianum seeds are notably rich in phytochemicals, especially flavonolignans, which are recognized for their potent antioxidant properties. The presence of significant secondary metabolites, including flavonoids, tannins, phenolics, alkaloids, terpenoids, and glycosides, which are known to support antioxidant and medicinal properties, was discovered using qualitative phytochemical analysis. Spectroscopic and chromatographic analyses clearly identified prominent bioactive compounds such as silybin and silychristin. All of these findings point to S. marianum's pharmacological effectiveness and motivate more study into its potential as a natural remedy for microbial infections and illnesses linked to oxidative stress. To maximize therapeutic efficacy, future research should focus on identifying its mechanisms of action and exploring potential synergistic combinations.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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