Comparative Analysis of Selected Plant Extraction Methods for Antibacterial Activity Against *Pectobacterium carotovorum*

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Abstract

The extraction process itself significantly contributes to the yield and content of phytochemicals, with solvent selection and extraction method varying in their effectiveness in dissolving bioactive compounds, such as flavonoids, phenolics, tannins, and alkaloids. This research explores the antibacterial efficacy of two locally available medicinal herbs, *Silybum marianum* and *Malva parviflora*, against the most destructive phytopathogenic bacterium *Pectobacterium carotovorum* subsp. *carotovorum* (Gene Bank Accession Number PV653278). Different extraction methods including aqueous, methanol, hexane, ethyl acetate, pH-adjusted solutions, and microwave-assisted techniques were employed to evaluate their effects on both antibacterial activity and extraction yield. Microwave extracts of *S. marianum* at 10 and 15 minutes recorded the highest yields, at 30.5% and 27.5%, respectively. These results confirm the efficiency of microwave-assisted extraction in enhancing the recovery of bioactive constituents. The aqueous extract 10% (w:v) of *S. marianum* exhibited the strongest antibacterial activity, with a 34.66 mm inhibition zone, followed by the microwave-assisted extract (32 mm). However, pH 8 and pH 5 extracts were weakly active, producing inhibition zones of only 1 mm and 2 mm, respectively. The Time-Kill Assay demonstrated that the aqueous extract of *S. marianum* exhibited strong and sustained antibacterial activity against *P. carotovorum*, with a significant reduction in bacterial count over time. The GC-MS analysis identified key bioactive compounds, including 2,4-di-tert-butylphenol and cyclohexasiloxane, dodecamethyl, in *S. marianum*, which are likely responsible for its antimicrobial properties. These findings provide valuable insights into the chemical composition and efficacy of the extracts. Altogether, the study highlights the critical role of extraction method and solvent choice in unlocking the antibacterial power of medicinal plants, with *S. marianum* emerging as a strong candidate for natural antimicrobial development.

***Keywords: Silybum marianum*, *Malva parviflora*, Antibacterial activity, Silver nanoparticles.**

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Introduction

Pectobacterium carotovorum is the most destructive bacterial pathogen affecting potato crops globally [30, 28]. These bacteria cause soft rot and blackleg, leading to significant yield losses, reduced tuber quality, and elevated production costs [14, 26]. Management is complicated by the pathogen's ability to spread quickly in both field and storage conditions and the limited efficacy of conventional bactericides. Plant-derived antimicrobial agents have gained attention as sustainable alternatives to synthetic chemicals [5]. These natural products offer bioactivity with lower risks of toxicity and environmental damage [5]. Compounds such as flavonoids, phenolics, and essential oils are well-documented for their antibacterial properties [36]. The effectiveness of plant extracts is, however, influenced by the method of extraction, including solvent type, pH, and processing conditions [13, 17]. Numerous studies have investigated how extraction parameters affect the quality and antimicrobial efficacy of plant extracts. For instance, microwave-assisted extraction and pH modification techniques have shown increased yield and potency by targeting specific bioactive compounds [24]. Salah demonstrated the relevance of solvent polarity and its impact on bioavailability and antibacterial activity in plant-based systems [23]. Moreover, recent global trends emphasize the role of green chemistry in refining plant extract production for agriculture [11, 10]. This study builds on such foundations, focusing on *Silybum marianum* and *Malva parviflora* extracts evaluated against *Pectobacterium carotovorum* isolated from infected potato tissues. The objective was to compare multiple extraction methods and assess both antibacterial activity and extract yield.

2. Materials and Methods

2.1. Pathogen Isolation, Pathogenicity and Molecular Identification

Soft-rot symptomatic potato tubers were obtained from the local market and transported to the research institute's laboratory. The tubers were surface sterilized, rinsed with sterile distilled water, and aseptically cut into small pieces. The pieces were air-dried on sterile filter paper and then plated onto Nutrient Agar (NA) plates. Afterward, they were incubated at 28°C for 48 hours. Probing bacterial colonies was subcultured repeatedly to obtain pure isolates.

Three bacterial isolates were harvested and examined for pathogenicity. Healthy potato tubers were surface-sterilized and washed with sterile distilled water and air-dried. One pore was made in each tuber with a sterile cork borer, and a 24-hour-old plug of bacterial culture was pushed into every pore. Tubers were incubated at room temperature (28–30 °C) in a laminar flow hood for three days. For the control treatment, the pores were not inoculated with bacteria. All treatments comprised five replicates with five tubers in each replicate.

Pure bacterial colonies were subcultured and characterized by molecular characterization using universal 16S rRNA gene primers. The sequence was deposited in the NCBI GenBank database, and an accession number was given [22].

2.2. Plant Material Collection

Fresh samples of *Silybum marianum* and *Malva parviflora* were collected during the spring season from the Erbil region of

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Kurdistan, Iraq. The plant material was cleaned, cut into 5 cm segments, and dried under shade to prevent photodegradation. Dried leaves were ground into powder using a laboratory grinder and stored at 4 °C until further analysis [24].

2.3. Preparation of Plant Extracts

2.3.1 Solvent Extraction

Ten grams of powdered plant material were mixed with 100 ml of different solvents: methanol (polar), water (polar), ethyl acetate (semi-polar), and hexane (non-polar). Extracts were left to macerate for 48 hours, filtered, and concentrated by rotary evaporation.

2.3.2 Microwave-Assisted Extraction

Ten grams of the plant powder were mixed with 100 ml of selected solvents and subjected to microwave radiation at 15 and 30 minutes using a domestic microwave oven. The extract was subjected to microwave irradiation in a domestic microwave oven (800W) for 10,15, and minutes, the temperature exceeding 80 °C. The extract was allowed to cool to room temperature and filtered through sterile Whatman No. 1 filter paper.

2.3.3 pH-Modified Aqueous Extraction

Plant powders were mixed with water adjusted to pH 5 and 8 using HCl or NaOH solutions. Extracts were incubated at room temperature for 48 hours before filtration.

2.4. Yield Calculation

All the extracts were first filtered with Whatman No. 1 filter paper to decant solid residues. The filtrates were then evaporated to dryness either reduced pressure using a rotary evaporator until constant weight was achieved. The resulting dried extracts were weighed, and the percentage yield was determined using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of dried plant material}} \times 100$$

Subsequently, the lyophilized extracts were dissolved in sterile distilled water and DMSO to achieve a final concentration of 100 mg/mL and were used for antibacterial assays.

2.5 Antibacterial Assay

The antibacterial activity was determined using the agar well diffusion method [29]. The antibacterial activity of the extracts was determined by using the agar well diffusion assay. The bacterial suspension was standardized to an optical density (OD) of 0.1 at 660 nm, which corresponds to about 10^6 CFU/mL. The suspension was evenly inoculated onto Nutrient Agar plates with 150 µL. Wells of 6 mm in diameter were made in the agar and loaded with 30 µL of each plant extract. The positive control was ampicillin (10 µg/mL) while the negative control was sterile distilled water. The plates were incubated for 24 hours at 30 °C. All treatments were carried out in triplicate, and the experiment was repeated twice to test for reproducibility.

2.6. Time Killing Assay

A time-kill assay was conducted to evaluate the antibacterial activity of *S. marianum* extracts obtained via microwave and water extraction. A standardized bacterial suspension (OD_{660} = 0.1, 1×10^6

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CFU/mL) was treated with 30 µL of each extract in 150 µL of bacterial culture. Ampicillin (30 µg/mL) and sterile distilled water were used as positive and negative controls, respectively. Samples were incubated at 30 °C, and at 2, 4, 6, and 24 hours, 10 µL aliquots were plated on NA to count colony-forming units (CFUs) after 24 hours of incubation. All tests were performed in triplicate and repeated twice to ensure reliability [22].

2.7. GCMS Analysis

A microwave-assisted extraction was conducted by irradiating *S. marianum* in distilled water for 15 minutes at 700 W. The extract was filtered, concentrated under reduced pressure, and stored at 4 °C until analysis. GC-MS was performed using an Agilent 7890 gas chromatograph with a mass selective detector and HP-5MS column (30 m × 0.25 mm, 0.25 µm). A 1 µL methanol-diluted sample (1:10) was injected in splitless mode at 250 °C. The oven started at 60 °C (held 2 min), ramped at 10 °C/min to 300 °C, and held for 10 min. Helium was used as the carrier gas at 1.0 mL/min. The MS operated at 70 eV with an m/z scan range of 50–600. Ion source and transfer line were set at 230 °C and 280 °C, respectively.

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Compounds were identified by comparing spectra to the NIST library, accepting matches ≥80% [32].

2.8. Statistical Analysis

The experiment followed a Completely Randomized Design (CRD) with three replicates per treatment. Data were expressed as mean ± SD. One-way ANOVA followed by Tukey's HSD test was used to compare group means at $p < 0.05$. Treatments not sharing the same letter were considered significantly different. All statistical analyses were performed using SPSS version 26.

3. Results

3.1. Pathogenicity and Identification of the pathogen:

All three isolates were isolated, and pathogenicity tests that were carried out indicated that they were all pathogenic, causing visible and damaging effects after three days. The 16S rRNA gene sequence of isolate A was 100% identical to *Pectobacterium carotovorum subsp. Carotovorum* and it was deposited in GenBank with accession number PV653278

Figure 1. Pathogenicity test of *P. carotovorum* isolates showed visible symptoms after 3 days

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3.2. The extraction yield (%):

The amount of extract obtained varied noticeably depending on the plant used, the solvent, and the extraction method. The highest yield came from *Silybum marianum* using microwave extraction for 10 minutes, producing an impressive 30.5%. A slightly shorter microwave time of 15 minutes also gave a strong result at 27.1%, showing that microwave methods work especially well with this plant. The mixed plant extract with methanol also performed well, yielding 20.0%, which suggests that combining *Malva parviflora* and *S. marianum* may boost extraction efficiency in polar solvents like methanol.

Among the individual plants, *S. marianum* consistently gave higher yields. Its methanolic extract reached 19.1%, and the water extract produced 17.5%. In contrast, *M. parviflora* showed more modest results13.7% with water and 8.8% with methanol. When adjusting the pH, *S. marianum* still came out on top, yielding 8.2% at pH 5 and 7.4% at pH 8, compared to just 1.9% and 1.1% for *M. parviflora*. Interestingly, *Malva*'s extract using microwave for 15 minutes gave a very low yield of only 0.2%, reflecting how extraction efficiency can drop off depending on time and plant type. In general, S. marianum clearly outperformed *M. parviflora* in almost every method tested, making it a more efficient choice for extracting bioactive compounds under these conditions.

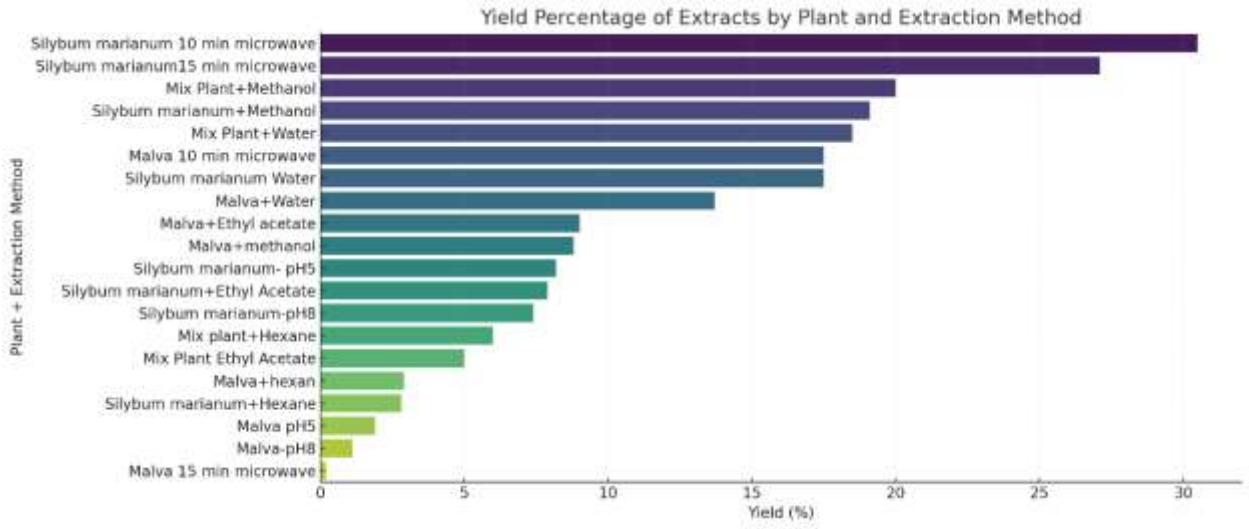

Figure 2. Yield percentage of *M. parviflora* and *S. marianum* extracts obtained using various extraction methods.

3.3. Antibacterial Activity of *Malva parviflora*

The antibacterial activity of *M. parviflora* leaf extracts varied noticeably depending on

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the extraction method used. The methanol and microwave extracts stood out, both producing comparable zones of inhibition averaging 27.67 ± 2.52 mm, and were statistically grouped together, indicating similar levels of effectiveness (Figure 3). Ethyl acetate also showed respectable antibacterial activity (24.33 ± 2.08 mm), though slightly lower. In contrast, the water and hexane extracts failed to show any inhibition at all, and extracts prepared under altered pH conditions (pH 5 and pH 8) exhibited only minimal effects, with inhibition zones no greater than 2 mm. These results suggest that polar solvents and microwave-assisted extraction are more effective in releasing antimicrobial compounds from *M. parviflora* leaves.

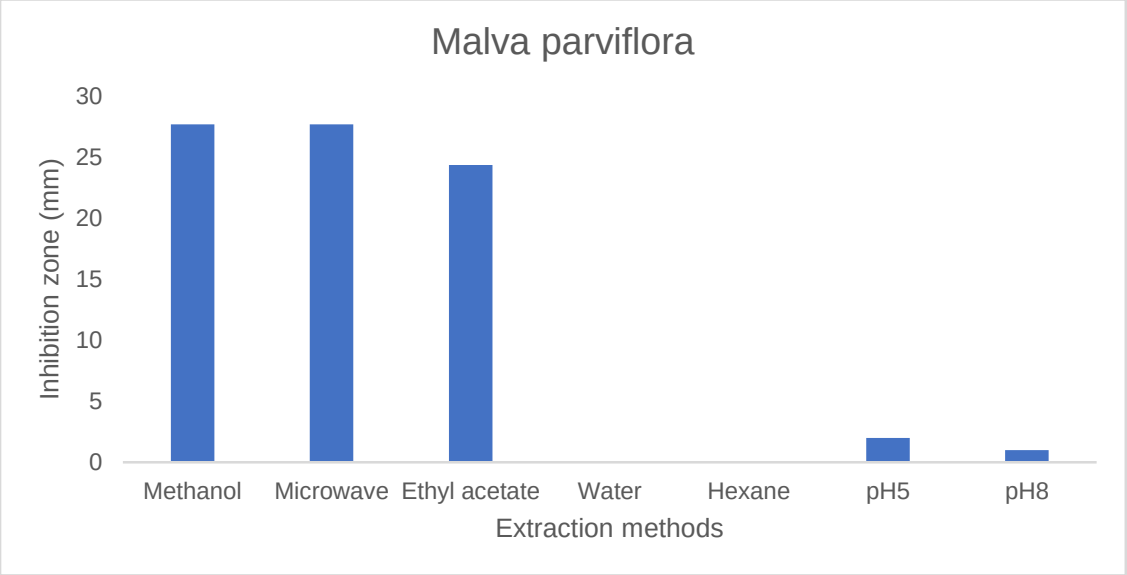

Figure 3. The antibacterial effects of *M. parviflora* leaf extracts prepared using different extraction methods, against *E. carotovora*. Different letters above the bars indicate treatments that were significantly different from each other based on Tukey's test ($p < 0.05$).

3.4.

Antibacterial Activity of *Silybum marianum*

Among all the tested samples, from the Figure 4, *S. marianum* extracts showed the strongest antibacterial activity. The water extract performed best, producing a mean inhibition zone of 34.67 ± 1.53 mm, closely followed by the microwave (32.00 ± 2.00 mm) and ethyl acetate (30.33 ± 2.52 mm) extracts they were statistically similar in effectiveness. The methanol extracts also showed good activity (23.67 ± 1.53 mm), though it was significantly less effective than the top three. Hexane extract produced a much smaller inhibition zone (10.33 ±

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3.51 mm), while pH-adjusted extracts (pH 5 and pH 8) were only minimally active, with inhibition zones under 2 mm. Overall, *S. marianum* responded well to aqueous and microwave-based extraction, making it a strong candidate for antibacterial applications.

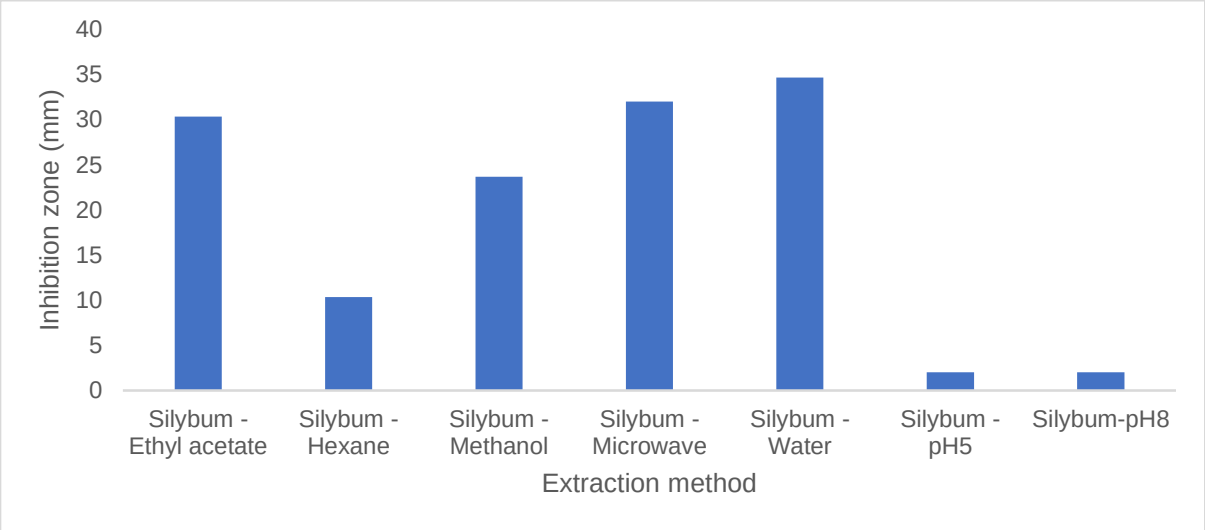

Figure 4. The antibacterial effects of *S. marianum* leaf extracts prepared using different extraction methods, against *E. carotovora*. Different letters above the bars indicate treatments that were significantly different from each other based on Tukey's test ($p < 0.05$).

3.5. Antibacterial Activity of Mixed Extracts

Figure 5 shows the extracts prepared from a combination of *M. parviflora* and *S. marianum* generally which showed lower antibacterial activity compared to extracts from each plant alone. The methanol extract of the mixed sample was the most effective, producing a mean inhibition zone of 16.67 ± 2.89 mm. Other solvents, including hexane, ethyl acetate, and water, yielded modest and nearly identical inhibition zones of 10 mm, with no statistical difference among them. These results suggest that combining the two plants did not produce a synergistic effect and may have even diluted the antibacterial potency observed in their individual extracts.

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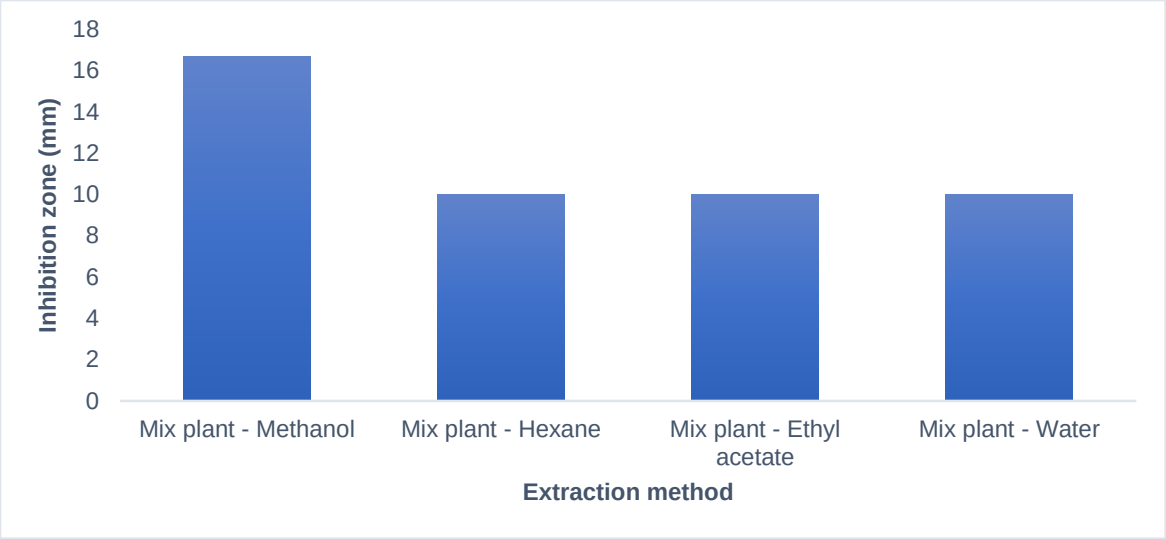

Figure 5. The antibacterial effects of mixed plants *M. parviflora* and *S. marianum* leaf extracts prepared using different extraction methods, against *P. carotovorum*.

3.6. Focused Comparison of Microwave and Aqueous Extraction Methods

In this follow-up experiment (Figure 6), the antibacterial activity of *S. marianum* and *M. parviflora* extracts prepared using microwave-assisted and water-based methods which was evaluated against *P. carotovorum*. The results revealed notable differences in efficacy between plant species and extraction techniques. The water extract of *S. marianum* demonstrated the highest antibacterial activity, with a mean inhibition zone of 36.67 ± 2.89 mm, followed closely by its microwave counterpart (32.33 ± 2.52 mm); both were statistically grouped in letter 'a', indicating no significant difference. Similarly, *M. parviflora* extracts prepared using microwave showed a comparable level of inhibition (28.33 ± 2.89 mm) and also fell within group 'a', suggesting that microwave extraction enhances its antimicrobial potency. In contrast, the water extract of *M. parviflora* despite yielding the same numerical mean as the microwave extract was categorized in group 'b', signifying a statistically lower effect compared to *Silybum* extracts. These findings support the superior efficacy of *S. marianum* in both extraction approaches and highlight the importance of method selection when optimizing the antibacterial potential of plant-based treatments.

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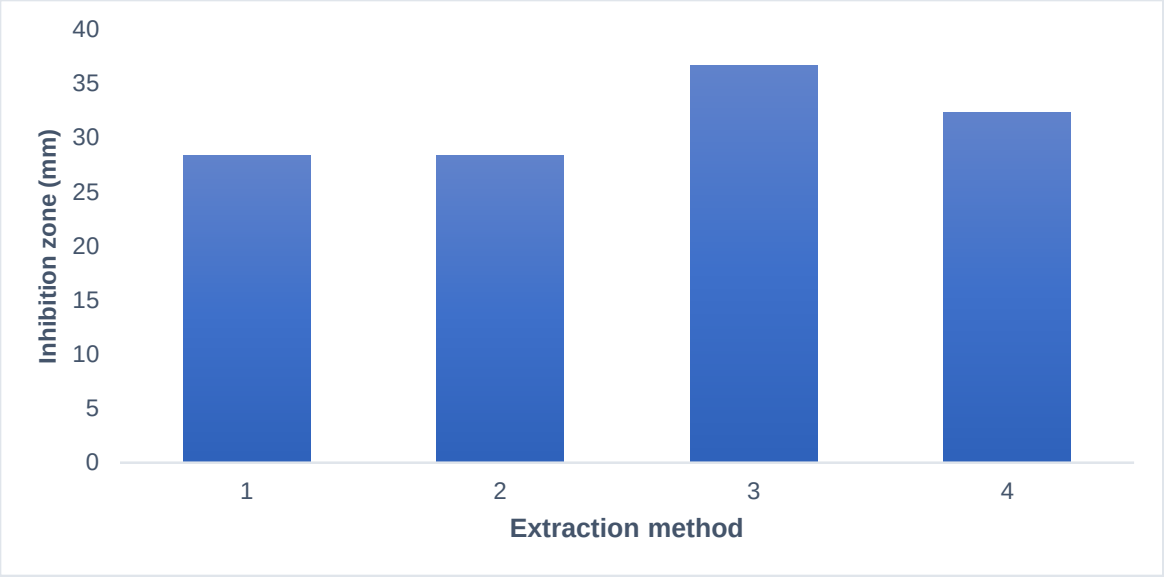

Figure 6. Focused comparison of aqueous and microwave extraction methods for *M. parviflora* and *S. marianum* against *P. carotovorum*. Statistically significant differences in inhibition zones are indicated by different letters ($p < 0.05$).

3.7. Time Killing Assay

Figure 7 indicates that both water and microwave-assisted extracts of *S. marianum* leaves exhibited time and concentration dependent antibacterial activity against *P. carotovorum*. At the highest concentration (100%), both extracts showed a substantial reduction in bacterial viability, with the microwave extract achieving near complete inhibition by 4 hours and maintaining this effect through 24 hours. The water extract, while effective, exhibited a slower and less sustained reduction in bacterial count.

At lower concentrations (50%, 25%, and 1%), the microwave extract still displayed significant antibacterial activity, outperforming the water extract at all concentrations, particularly between 4 and 6 hours. Statistical analysis confirmed highly significant differences across extract type, concentration, and time ($p<0.001$ for all main effects and interactions), with the microwave-assisted extract consistently demonstrating stronger bactericidal effects.

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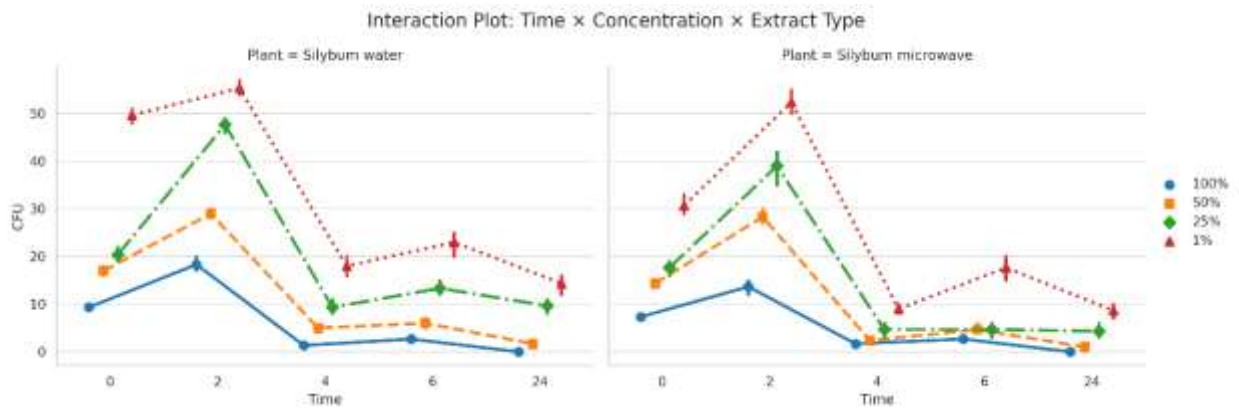

Figure 7. Time Killing Assay of *S. marianum* leaf extracts against *P. carotovorum*. Mean colony-forming unit (CFU) counts measured over 24 hours for water and microwave-assisted extracts at varying concentrations (100%, 50%, 25%, 1%). Error bars represent standard deviations (n = 3).

3.8. GC-MS analysis

The gas chromatography-mass spectrometry (GC-MS) analysis of the sample revealed a diverse array of organic compounds, including siloxanes, hydrocarbons, and ester derivatives. The compounds exhibited a wide range of retention times, from 6.3 to 19.4 minutes, reflecting the structural diversity of the components (Table 1).

A total of 38 compounds were identified, with Tetracontane and Cyclononasiloxane, octadecamethyl being the most abundant, contributing significantly to the area percentages (35.22% and 35.82%, respectively). These compounds, along with others such as Cyclohexasiloxane, dodecamethyl- and Dodecane, 2,6,11-trimethyl-, displayed strong peaks in the chromatogram, suggesting their prominent presence in the sample.

Several compounds, including Cyclooctasiloxane, hexadecamethyl- and Cyclononasiloxane, octadecamethyl-, exhibited highly consistent signal intensities (SI values in the range of 80–94), indicating their stability and potential significance in the sample matrix.

The chemical structures of the identified compounds were mapped based on their retention times, area percentages, and CAS numbers, with formulas assigned to each structure for clarity. This comprehensive analysis provides valuable insight into the chemical composition of the sample and highlights key compounds that could be of interest for further investigation, particularly in applications related to material science, siloxane chemistry, and environmental analysis.

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Table 1. GC-MS Analysis of Phytochemicals in *Silybum marianum* Microwave extract.

Formula	Height %	Area %	Re Time	.Name
C6H18O6Si6	3.6	1.57	6.301	Cyclohexasiloxane, dodecamethyl-
C18H54O12Si6	4.38	1.43	7.304	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
C15H32	0.44	0.14	7.522	Dodecane, 2,6,11-trimethyl-
C15H22O	4.28	1.5	7.689	2,4-Di-tert-butylphenol
C15H32	0.73	0.29	7.817	Dodecane, 2,6,11-trimethyl-
C18H38O	0.36	0.15	7.895	Dodecyl octyl ether
C14H28O4	2.79	1.12	8.234	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
C16H50O8Si8	3.35	1.06	8.39	Cyclooctasiloxane, hexadecamethyl-
C14H22O2	1.76	0.8	8.88	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-
C16H34	0.69	0.18	9.325	Hexadecane
C6H18O6Si6	3.28	1.08	9.492	Cyclohexasiloxane, dodecamethyl-
C16H50O8Si8	3.05	0.95	10.56	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
C17H34O2	3.18	1.3	10.633	Hexadecanoic acid, methyl ester
C26H53I	0.52	0.15	10.865	Hexacosane, 1-iodo-
C26H28Cl2N2O2	0.7	0.4	10.911	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene
C15H32	0.38	0.32	10.992	Tetradecane, 4-methyl-
C12H22O2	0.93	0.4	11.265	Cyclopropaneoctanoic acid, 2-hexyl-, methyl

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				ester
C16H50O8Si8	2.57	0.73	11.569	Cyclooctasiloxane, hexadecamethyl-
C18H38O	0.34	0.15	11.782	1-Octadecanol
C20H34O2	0.71	0.23	11.847	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
C20H32O2	1.65	0.64	11.89	11,14,17-Eicosatrienoic acid, methyl ester
C19H38O2	1.63	0.51	12.047	Methyl stearate
C18H54O9Si9	2.6	0.78	12.484	Cyclononasiloxane, octadecamethyl-
C14H22O4	1.45	0.53	12.696	2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester
C18H54O9Si9	2.39	0.7	13.325	Cyclononasiloxane, octadecamethyl-
C18H35NO	2.56	1.36	13.632	9-Octadecenamide, (Z)-
C40H82	14.88	35.22	14.098	Tetracontane
C18H18	0.68	0.25	14.34	Cyclohexane, 1,3,5-triphenyl-
C18H36O3	0.43	0.38	14.665	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
C24H38O4	1.47	0.61	14.812	Bis(2-ethylhexyl) phthalate
C18H54O9Si9	1.67	0.72	15.177	Cyclononasiloxane, octadecamethyl-
C18H54O9Si9	1.31	0.77	16.375	Cyclononasiloxane, octadecamethyl-
C24H38O4	12.54	7.29	16.744	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
C18H54O9Si9	0.66	0.47	17.883	Cyclononasiloxane, octadecamethyl-
C40H82	16.05	35.82	19.369	Tetracontane

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4. Discussion

Various extraction methods were evaluated to determine the antibacterial activities of *Silybum marianum* and *Malva parviflora* against *Pectobacterium carotovorum*. Among these, aqueous and microwave-assisted extractions emerged as the most effective. In the case of *M. parviflora*, methanol and ethyl acetate extracts were particularly potent, likely due to the ability of these solvents to extract a broad range of polar phytochemicals such as flavonoids and phenolic acids, which are well-known for their antimicrobial properties [27, 12, 13]. These findings are consistent with previous studies where methanol and ethyl acetate extracts of *M. parviflora* exhibited inhibitory effects against *Escherichia coli* and *Staphylococcus aureus* [33, 38].

In contrast, *S. marianum* generally showed moderate to low antibacterial activity across most solvent systems. However, its aqueous extract stood out with a notably strong inhibition zone, sometimes surpassing those observed with organic solvents. This suggests that water can effectively extract hydrophilic bioactive compounds from *S. marianum*, reinforcing earlier findings that water-soluble flavonolignans such as silybin exhibit antimicrobial effects [36, 8]. The extract yield data supports this observation, with the aqueous extract of *S. marianum* yielding 17.5%, indicating a high efficiency in recovering bioactive components. Literature has consistently highlighted the antimicrobial potential of silymarin and its constituents, particularly silibinin, which has shown inhibitory effects against *E. coli* (MIC = 64 µg/mL), while silymarin itself displayed lower activity (MIC ≈ 512 µg/mL) [18]. Moreover, recent studies confirm that ethanol extracts from *S. marianum* seeds exert strong antibacterial effects against multidrug-resistant bacteria, including *Staphylococcus aureus* and *Stenotrophomonas maltophilia*, with GC–MS analysis identifying several antimicrobial phytochemicals [6].

Time-Kill Assay results further corroborated the superior bactericidal efficacy of the aqueous extract of *S. marianum*, particularly during early exposure periods. Microwave-assisted extraction also showed promising results, especially with a 10-minute extraction, which yielded the highest extract percentage (30.5%) and potent antibacterial activity [4]. This aligns with previous reports demonstrating that microwave-assisted extraction—commonly conducted at temperatures around 60–80 °C enhances efficiency by disrupting plant cell walls and promoting the rapid diffusion of phytochemicals into the solvent [4]. The enhanced performance of this technique highlights its suitability for eco-friendly and scalable applications in agricultural antimicrobial strategies.

Interestingly, mixed extracts of *S. marianum* and *M. parviflora* exhibited higher yields (20% for methanol and 18.5% for water) but reduced antibacterial activity. This suggests potential antagonistic interactions between bioactive constituents, underscoring the importance of phytochemical compatibility in polyherbal formulations. While synergistic effects are often reported in mixed plant extracts [3, 34], these findings caution that combining extracts does not always enhance bioactivity and may sometimes diminish it.

GC–MS analysis provided further insight into the chemical composition of the extracts, revealing significant differences in bioactive constituents based on the extraction method. For instance, the aqueous extract of *S. marianum* contained antimicrobial compounds such as cyclohexasiloxane, dodecamethyl-, which have demonstrated antibacterial and antiseptic activities [25]. In contrast, ethyl acetate extracts tended to contain more volatile and less bioactive compounds. These findings underscore the importance of solvent choice in determining the pharmacological profile of plant extracts [9, 2, 39, 7, 19].

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Among the identified compounds, 2,4-di-tert-butylphenol (2,4-DTBP) emerged as a particularly potent bioactive. Widely reported in the literature, 2,4-DTBP exhibits broad-spectrum antimicrobial activity, including bactericidal, antifungal, and antiviral effects, often through mechanisms such as membrane disruption and quorum sensing inhibition [41]. It also possesses antioxidant and anti-inflammatory properties and has demonstrated cytotoxic activity against cancer cell lines [37, 40]. Furthermore, recent reviews have emphasized the wide-ranging antimicrobial potential of silybin and related flavonolignans, identifying membrane integrity disruption, efflux pump inhibition, and anti-biofilm activity as key modes of action [41]. A study using Moroccan *S. marianum* leaf and stem extracts also recorded antibacterial effects against *S. aureus* and *Klebsiella pneumoniae*, further validating the leaf as a viable source of active compounds [21].

Furthermore, pH modification of extracts selectively improved bioactivity by optimizing solubility and stability of active compounds, although yields were lower at pH 5 and pH 8 [15, 24]. This suggests that pH tuning may be best employed alongside other techniques, such as microwave-assisted extraction, to maximize antibacterial efficacy.

Taken together, these findings highlight the antimicrobial potential of *S. marianum* and *M. parviflora*, particularly when extracted using microwave-assisted or aqueous techniques. These methods offer effective, sustainable alternatives to synthetic bactericides, contributing to environmentally friendly pest management strategies in agriculture. The use of GC–MS as a diagnostic tool for identifying key phytochemicals further strengthens the scientific foundation for harnessing plant-based antimicrobials [1, 25, 16].

5. Conclusion

This study highlights the significant antibacterial potential of *Silybum marianum* against *Pectobacterium carotovorum*, with both aqueous and microwave-assisted extractions demonstrating strong and sustained bactericidal effects. The aqueous extract exhibited notable efficacy, likely due to its ability to solubilize hydrophilic bioactive compounds such as silybin and 2,4-di-tert-butylphenol. Microwave-assisted extraction, yielding 30.5%, proved especially efficient in enhancing both yield and antibacterial activity by improving cell wall disruption and compound diffusion. GC–MS analysis revealed several antimicrobial constituents including 2,4-di-tert-butylphenol and cyclohexasiloxane derivatives supporting the observed biological activity. These findings aqueous and microwave-assisted extraction as eco-friendly, scalable alternatives to synthetic bactericides, offering a promising strategy for sustainable crop protection. Future research should prioritize the isolation, quantification, and field validation of these bioactive compounds to optimize their application in agricultural disease management.

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