

Methods of analysis of the plant *Silibum Marianum* Gaertn. L.

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Abstract: *Silibum marianum* Gaertn. L., commonly known as milk thistle, is a medicinal plant renowned for its hepatoprotective and antioxidant properties. Various analytical methods are employed to assess its chemical composition, biological activities, and pharmacological properties. This article explores different techniques used in the analysis of *Silibum marianum*, including phytochemical screening, chromatographic methods, spectroscopic techniques, and biological assays. These methods provide valuable insights into the plant's bioactive potential, contributing to its application in pharmaceutical and herbal medicine.

Keywords: *Silibum marianum*, milk thistle, phytochemical screening, chromatography, spectroscopy, biological assays.

Introduction:

Silibum marianum Gaertn. L., commonly known as milk thistle, is a medicinal plant widely used for its hepatoprotective and antioxidant properties. The plant has been traditionally employed for liver disorders, including cirrhosis, jaundice, and hepatitis. Modern pharmacological studies have confirmed its potential in treating liver diseases and other conditions such as diabetes, cardiovascular diseases, and cancer [5, 36-40]. Consequently, various methods of analysis have been developed to assess its chemical composition, biological activities, and pharmacological properties. Understanding the different analytical techniques is crucial for researchers and pharmaceutical industries aiming to maximize the medicinal potential of this plant. This article explores different techniques employed in the analysis of *Silibum marianum*, including phytochemical screening, chromatographic methods, spectroscopic techniques, and biological assays.

Phytochemical Screening To begin with, phytochemical screening is a fundamental method used to identify the presence of bioactive compounds in *Silibum marianum*. This preliminary analysis involves qualitative tests for flavonoids, alkaloids, tannins, saponins, and other secondary metabolites. For instance, the Ferric Chloride test is used to detect

tannins, whereas the Shinoda test is employed for flavonoids. Additionally, the Dragendorff reagent test is commonly used to identify alkaloids, and the frothing test can indicate the presence of saponins. These tests provide essential information regarding the plant's chemical composition and potential medicinal benefits. Although qualitative in nature, these tests serve as an essential first step before employing more advanced analytical techniques. Furthermore, recent developments have enabled the semi-quantitative estimation of these compounds through colorimetric and spectrophotometric methods, providing a more detailed phytochemical profile.

Chromatographic Methods. In addition to phytochemical screening, chromatographic techniques play a crucial role in the qualitative and quantitative analysis of *Silibum marianum*. High-Performance Liquid Chromatography (HPLC) is widely used to separate and quantify flavonolignans such as silybin, silydianin, and silychristin, which are the primary active compounds in milk thistle. HPLC offers high sensitivity and specificity, making it a preferred method for quality control in pharmaceutical formulations. Moreover, Gas Chromatography-Mass Spectrometry (GC-MS) is employed to analyze essential oils and other volatile components present in the plant. This method is

particularly useful in detecting and identifying fatty acids, sterols, and terpenoids that contribute to the pharmacological properties of *Silibum marianum*. Furthermore, Thin Layer Chromatography (TLC) serves as a rapid and cost-effective method for the preliminary identification of bioactive compounds. By utilizing various solvent systems, TLC allows for the separation of compounds based on their polarity and molecular weight. Advances in chromatographic techniques, such as Ultra-Performance Liquid Chromatography (UPLC), have further improved sensitivity and efficiency, enabling faster and more precise analysis [4, 154-163].

Spectroscopic Techniques. Furthermore, spectroscopic techniques provide detailed structural information about the constituents of *Silibum marianum*. Ultraviolet-Visible (UV-Vis) spectroscopy is commonly utilized to assess the presence of phenolic compounds by measuring their absorbance at specific wavelengths. This method is particularly useful in determining the total phenolic and flavonoid content, which are indicative of the plant's antioxidant capacity. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) is applied to identify functional groups in the plant's chemical compounds. FTIR spectroscopy provides valuable insights into the molecular composition of *Silibum marianum*, allowing researchers to differentiate between various chemical constituents based on their infrared absorption spectra. Likewise, Nuclear Magnetic Resonance (NMR) spectroscopy allows for the detailed elucidation of molecular structures, particularly in flavonolignans. NMR spectroscopy is an essential tool in confirming the purity and structural integrity of the plant's bioactive compounds, thus ensuring their effectiveness in pharmaceutical applications. The use of Raman spectroscopy and Mass Spectrometry (MS) has further expanded the capabilities of structural analysis, offering high-resolution data for compound identification and quantification.

Biological Assays. Apart from chemical analysis, biological assays are conducted to evaluate the pharmacological properties of *Silibum marianum*. Antioxidant assays, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test, measure the plant's ability to neutralize free radicals. The results of such assays help determine the extent to which *Silibum marianum* can protect against oxidative stress-related diseases, such as liver damage and cardiovascular conditions. Furthermore, antimicrobial assays determine its effectiveness against bacterial and fungal strains, making it an important candidate for the development of herbal antibiotics. The disc diffusion method and minimum inhibitory concentration (MIC) tests are commonly employed to assess the

antimicrobial potential of milk thistle extracts. Additionally, cytotoxicity tests assess its potential in anticancer therapy, often using cell viability assays such as the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to evaluate the impact of *Silibum marianum* on cancer cell lines. Recent studies have also explored its role in anti-inflammatory, antidiabetic, and neuroprotective applications, broadening its therapeutic scope.

Challenges in the Analysis of *Silibum Marianum*.

Despite the advancements in analytical techniques, the analysis of *Silibum marianum* presents several challenges. One major issue is the variability in chemical composition due to environmental factors such as soil conditions, climate, and cultivation practices. These variations can affect the concentration of active compounds and, consequently, the plant's medicinal efficacy. Another challenge lies in the standardization of extraction methods. Different solvents and extraction techniques can yield varying results, making it necessary to establish standardized protocols to ensure consistency in phytochemical analysis. Additionally, the complexity of the plant's chemical matrix requires the use of sophisticated instruments and advanced methodologies to obtain accurate and reproducible results.

CONCLUSION

In conclusion, the analysis of *Silibum marianum* involves a combination of phytochemical screening, chromatographic methods, spectroscopic techniques, and biological assays. Each method provides unique and complementary information regarding the plant's chemical composition and medicinal properties. By utilizing these analytical approaches, researchers can gain a comprehensive understanding of *Silibum marianum*'s potential benefits, thereby supporting its application in pharmaceutical and herbal medicine. However, challenges such as variability in chemical composition and extraction standardization must be addressed to enhance the reliability of analytical results. Future studies should focus on improving analytical techniques, exploring novel methods, and developing standardized protocols to further unlock the therapeutic potential of *Silibum marianum*.

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