https://doi.org/10.48047/AFJBS.6.15.2024.4087-4094

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Quantitative Estimation of Sylibin in *Anastatica hierochuntica* L. Extract Using High Performance Thin Layer Chromatography

Nilofer Sayyed[*1], Kiran Bhise[1]

Department of Pharmaceutics, M.C.E. Society's Allana College of Pharmacy, Pune, Maharashtra, India.

Corresponding Author

Nilofer Sayyed, Allana College of Pharmacy, 2390, K.B. Hidatyatullah Road, Azam Campus, Camp, Pune 411001

E-mail ID: sayyednilofer.24@gmail.com

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.4087-4094

Abstract

The present study focuses on the High-Performance Thin-Layer Chromatography (HPTLC) analysis of *Anastatica hierochuntica* L., a medicinal plant known for its traditional use in treating fertility problems, menstrual ailments, etc. The aim was to develop a precise, reproducible, and robust HPTLC method for the qualitative and quantitative analysis of the main bioactive compound Silybin present in the flower. Methanolic extracts of AH were prepared and subjected to HPTLC analysis on silica gel plates using Methanol: Ethyl acetate: Toluene: Formic acid (2.0: 3.0: 5.0: 0.2 v/v/ v/v) as mobile phase. The plates were scanned and densitometric analysis was performed at 291nm to identify and quantify the major phytoconstituent. Silybin was used as a chemical marker in the TLC fingerprinting of the methanol extracts, which resulted in the discovery of spots under UV light. The R_F of 0.526 in these spots confirmed the presence of silybin in the AH extract at concentration of 1.076 mg in 10.000 mg. The developed HPTLC method provides a reliable and efficient tool for the standardization and quality control of AH extracts, ensuring their efficacy and safety in herbal formulations.

Keywords: *Anastatica hierochuntica* L., Methanolic extracts, Silybin

1. Introduction:

The sole member of the genus Anastatica is *Anastatica hierochuntica* L., which is a member of the Brassicaceae family. Kaff-e-maryam, also called "the true rose of Jericho," is a resurrection herb that grows in desert regions and is primarily found in Saudi Arabia, Egypt, and Iran. It is a tiny, grey plant with rectangular, dentate leaves that can reach a height of 15 cm. It has tiny, white, sessile flowers. It is well-known for its capacity to withstand dry circumstances; when the next rain arrives, it dries into a ball with a diameter of 4–10 cm and shows tiny, delicate leaves

and white flowers [1]. Soils from the desert and let them dry. One of the popularly utilized medicinal herbs in Hijaz, Najd, and Al Rub'Al Kali is *Anastatica hierochuntica*. In traditional medicine, the plant is used to treat difficult labor, uterine hemorrhage, and to help expel deceased fetuses [2]. Traditionally, it is drunk as a herbal tea prior to childbirth to ease the birthing process, lessen uterine hemorrhage, treat respiratory conditions, asthma, diarrhea, colds, fevers, headaches, conjunctivitis, and fight sterility. Antioxidant, antibacterial, anti-melanogenesis, nitric oxide inhibitor, hepatoprotective, gastroprotective, anti-inflammatory, and immunostimulatory qualities have also been shown to be present. Three neolignans, hierochins A, B, and C, as well as two novel benzofurano flavanone compounds, Anastatin A and B, Silybin have been found in the plant [3]. According to the earlier molecular docking research cited by Nilofer Sayyed, Silybin (Silibinin) and the estrogen receptor are shown to be creating a stable complex [4]. High-Performance Thin-Layer Chromatography (HPTLC) of *Anastatica hierochuntica* is a precise and efficient analytical technique used to separate, identify, and quantify bioactive compounds present in this plant. This technique can effectively identify and quantify secondary metabolites such as flavonoids, phenolic acids, and other phytochemicals that contribute to the plant's medicinal properties. HPTLC offers several advantages, including high resolution, sensitivity, and the ability to analyze multiple samples simultaneously. It provides a comprehensive phytochemical profile of *Anastatica hierochuntica*, aiding in the standardization of its extracts for therapeutic applications and ensuring consistency in quality. Additionally, the data obtained from HPTLC analysis can be correlated with pharmacological studies to understand the biological activities of the plant's constituents, further supporting its use in traditional and modern medicine.

2. Materials and Methods

The *Anastatica hierochuntica* L. flower was bought from the local market in Medina, Saudi Arabia. At the Agarkar Research Institute in Pune, India, the authenticity of the AH flower was verified (Ref: AUTH 20-135). Silibinin or Silybin (98%) characterizing compound was purchased from Kurmak traders, Pune and characterized by Ultra-violet (UV) to confirm its identity and purity.

2.1 Instrumentation and Software

Software: Server DESKTOP-5IHGUM1, version 3.2.23095.1
Instruments Used:
Linomat 5 (S/N: 150721)
TLC Scanner 3 (S/N: 151015)
TLC Visualizer 2 (S/N: 290326)

2.2 Extraction procedure

An oven set to 50°C was used to dry two AH blossoms, each weighing roughly twenty to twenty-five grams. Using a mixer grinder, the oven-dried flowers of AH were ground into a coarse powder. After precisely weighing twenty grams of AH power, 250 milliliters of distilled water were added, and the mixture was progressively heated to 80°C for an hour on a heating mantel (LABLINE). Following room temperature cooling, the suspension was filtered via

Whatman filter paper. Using a microwave at 50–60°C, drying was done. At room temperature, the dried extract was scraped off and packed in an airtight container.

2.3 Chromatography plate development

HPTLC was performed on Merck HPTLC Silica gel 60 F_{254}, 100 × 100 mm plates. The plate was pre washed with methanol and heated to 105°C. Methanol: Ethyl acetate made up the mobile phase composition. Formic acid: Toluene (2.0: 3.0: 5.0: 0.2 v/v/v/v) at 20 minutes of saturation time.

2.4 Preparation of standard stock solution

10 mg of Silybin STD was placed in a 10 ml volumetric flask. 5 ml of acetone was added and sonicated for 10 minutes at 25 °C. The solution was diluted with additional acetone to achieve a concentration of 1000 ppm. 1ml of this solution was taken and diluted with 10ml of acetone to achieve a concentration of 100 ppm.

2.5 Preparation of Sample solution

10mg of (AH) extract was taken in 10ml volumetric flask. 5ml of acetone was added and sonicated for 10min at 25°C. The solution was diluted with acetone (1000ppm). The sample solution was filtered through syringe filter.

2.6 Application of sample

A bandwidth of 8 mm, spacing of 8 mm, and flow rate of 150 nL/sec were maintained when the various solvent extract samples were applied using the Linomat 5 (S/N: 150721). Between 5 and 10 microliters of the sample was used.

2.7 HPTLC development

The mobile phase used in the experiment was chosen through empirical selection as a mixture of Methanol: Ethyl acetate: Toluene: Formic acid (2.0: 3.0: 5.0: 0.2 v/v/v/v). The plates were saturated in a tank containing the mobile phase for 20min. The plates were dried at room temperature.

2.8 Spectral Analysis of Silybin Standard from (AH) Extract

The spectra were obtained by doing additional dilutions using methanol from the standard stock solution and scanning it over the 200–800 nm range. Both the medication and the marker were found to have significant absorbance at 291 nm. (as shown in fig. 1)

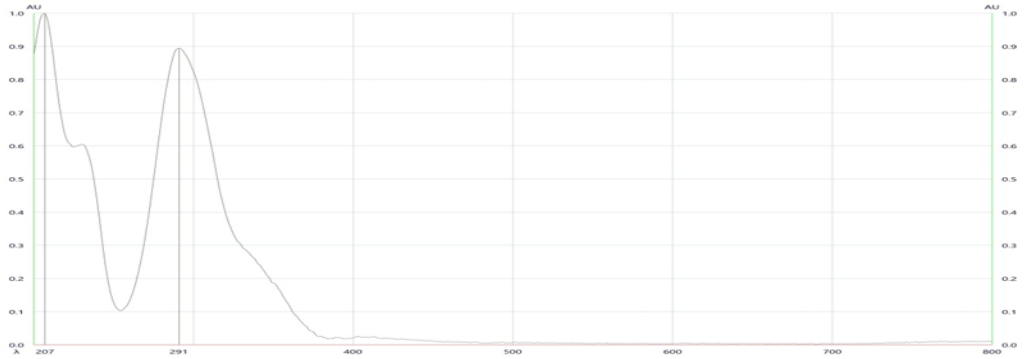
3. Result and Discussion

3.1 Spectral Analysis of Silybin Standard from (AH) Extract

The provided image is a UV-Visible absorption spectrum of a silybin standard from an (AH) extract. This type of spectral analysis is essential for identifying and characterizing chemical compounds based on their absorbance at different wavelengths. The UV-Visible spectrum in Figure 1 displays the absorbance of silybin across a range of wavelengths. The characteristic peaks and their respective absorbance values provide critical information about the concentration and purity of silybin in the extract. The wavelength values range from 206 nm to 800 nm, representing the ultraviolet (UV) to visible light spectrum. The peak appears at approximately 290 nm with a significant absorbance close to 0.9 AU. The first peak at 210 nm indicates the

primary absorption of silybin in the UV range, suggesting the presence of conjugated double bonds and aromatic systems typical of silybin's molecular structure. The second peak at 290 nm i.e secondary peak further confirms the presence of silybin, as it matches known absorption characteristics of this compound.

Figure 1: Spectra of Silybin in AH extract



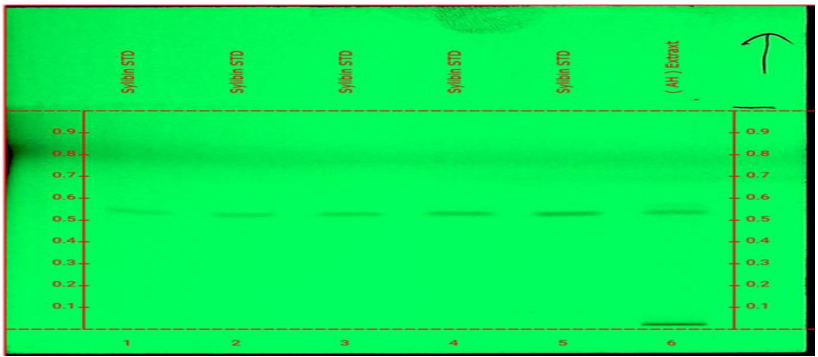
3.2. Thin Layer Chromatography (TLC) Analysis of Silybin Standard and (AH) Extract

A Thin Layer Chromatography (TLC) plate seen at 254 nm under UV light is shown in Figure 2. The picture illustrates the separation and identification of components inside the samples with six tracks: Five for the silybin standard (STD) and one for the AH extract. The presence and purity of silybin in the extract are shown by comparing the migratory patterns of the silybin standard with the (AH) extract in Figure 2 and Table 1. Track 1 to 5 shows a discrete band that corresponds to the silybin standard, with an R_F value of about 0.5. A comparable band that shows up in track 6 at the same R_F value suggests that silybin is present in the (AH) extract. By comparing, this TLC technique successfully proves that silybin is present in the (AH) extract.

Track	Vial ID	Description	Volume	R_F value	Type
1	1	Silybin STD	5.0 µL	0.583	Reference
2	1	Silybin STD	7.5 µL	0.561	Reference
3	1	Silybin STD	10.0 µL	0.575	Reference
4	1	Silybin STD	12.5 µL	0.556	Reference
5	1	Silybin STD	15.0 µL	0.585	Reference
6	2	AH Extract	12.0 µL	0.592	Sample

Table 1: Quantification of Silybin from the AH extract by TLC

Figure 2: Thin Layer Chromatography (TLC) Analysis of Silybin Standard and (AH) Extract



3.3 Description and Analysis of the Overlay Densitogram and 3D Densitogram

The central peak in Figure 3, which is about at R_F 0.5, is Silybin. The chromatographic profiles of the (AH) extract and the Silybin standard are contrasted in Figure 3's overlay densitogram. The absorbance units (AU) are shown by the Y-axis, while the retention factor (R_F), which ranges from 0.0 to 1.0, is represented by the X-axis. Silybin can be found at roughly RF 0.5, which is the center peak. The presence of silybin in the (AH) extract is shown by the near alignment of the peaks for the standard and the extract. The precision and repeatability of the chromatographic technique used are confirmed by the yellow dashed lines, which show the predicted range for silybin detection. The 3D densitogram from Figure 4 illustrates the chromatographic profile of silybin standard across five different concentrations. The X-axis represents the Retention Factor (R_F), ranging from 0.0 to 1.0. The Y-axis denotes the sample number or concentration, while the Z-axis shows absorbance units (AU). Each vertical peak in Figure 4 corresponds to a specific concentration of silybin, consistently positioned around an R_F value of approximately 0.5. The uniform height and sharpness of the peaks indicate a high degree of linearity and reproducibility in the detection of silybin. This confirms the reliability of the chromatographic method for quantitative analysis of silybin.

Figure 3: Overlay Densitogram of silybin STD and extract

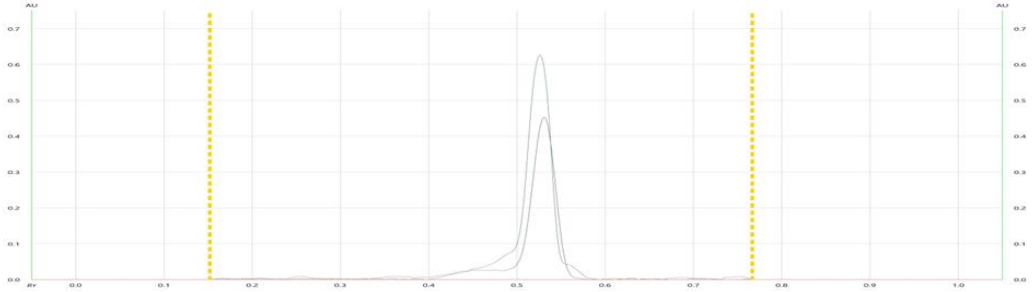
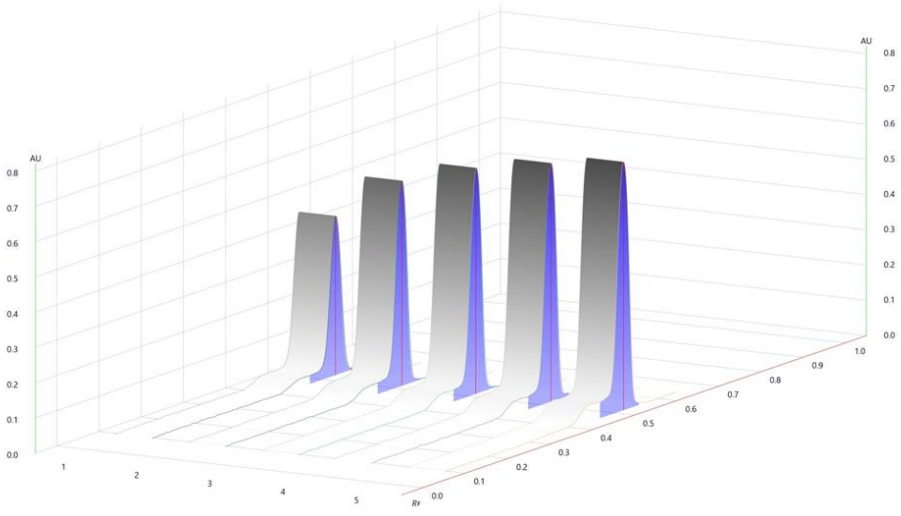


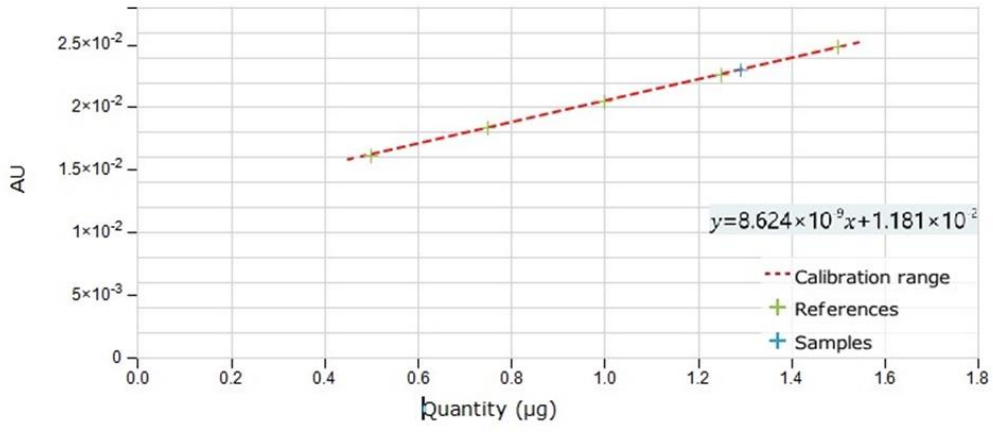
Figure 4: Densitogram of Sylibin Standard across Different Concentrations



3.4 Quantification of silybin from AH extract:

An HPTLC method for determining the concentration of Silybin in an AH extract is described. The high correlation coefficient and calibration data in Figure 5 show that the method is dependable for quantifying Silybin. The presence and concentration of silybin in the AH extract were confirmed by the identification and quantification of the particular peaks for silybin in both the sample and the reference standard. The report provides excellent precision and consistent results, validating the established HPTLC method. It was computed how much silybin was present in the AH Extract. 107.6 µg/mL was the average concentration that was discovered. The sample contained 1.291 µg of silybin.

Figure 5: Area calibration for substance Sylibin STD at 291 nm



4. Conclusion:

Given the results obtained in this study conclude that essential information for silybin identification and quantification in the (AH) extract is provided by UV-visible absorption spectrum. The identified absorption properties of silybin are compatible with the observed peaks at 210 and 291 nm, suggesting that the extraction and isolation procedure was successful. By comparing it to the silybin standard, this TLC examination successfully proves that the (AH) extract contains silybin. The identity of silybin is confirmed by the matching R_F values and fluorescence patterns, indicating that it was extracted successfully and that it may be used in future pharmacological research.

5. References:

1. Nilofer A. Sayyed and Kiran S. Bhise, Rose of Jericho - A Glorious Herb. International Journal of Pharmaceutical Education and Research, 2021; 3(2)
2. Rajaa K.Baker, et al. The Effect of Aqueous Extract of Anastatica hierochuntica on Some Hormones In Mouse Females. Journal for pure and applied science. May 2013, Vol. 26 (2) 2013.
3. R Saranya1 et al, Phytochemical, Fluorescence Screening and GC-MS Analysis of Various Crude Extracts of Anastatica hierochuntica. Journal of Pharmaceutical, Chemical and Biological Sciences, March - May 2019; 7(1):44-56.
4. Nilofer A. Sayyed and Kiran S. Bhise. Estrogen receptor compared with sylibin as a target for postmenopausal symptoms via molecular docking. International Journal for Research Trends and Innovation. 2022, Volume 7, Issue 12, ISSN: 2456-3315.

5. Natividad Chaves, et al. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. Antioxidants. 2020 Jan; 9(1): 76. doi: 10.3390/antiox9010076
6. Sabine Adeline Fanta Yadang, et al. Quantification of Bioactive Compounds and Evaluation of the Antioxidant Activity of Carissa edulis Valh (Apocynaceae) Leaves. The Scientific World Journal. 15 May 2019. https://doi.org/10.1155/2019/7549620.
7. Marium Ahsan, et al. Bioactive and Medicinal Potential of Edible Flowers, Medicine and Clinical Science. 2024; 6(3):1-7.
8. Maria G. J. Almeida, et al. Validation of an Alternative Analytical Method for the Quantification of Antioxidant Activity in Plant Extracts. January 2013, Latin American Journal of Pharmacy 32(1):90-95.
9. Muzafar A. Macha, et al. Emerging potential of natural products for targeting mucins for therapy against inflammation and cancer. Cancer Treatment Reviews. Volume 41, Issue 3 March 2015, Pages 277-28. https://doi.org/10.1016/j.ctrv.2015.01.001
10. K. Sonia, et al. HPTLC method development and validation: An overview. January 2017, Journal of Pharmaceutical Sciences and Research 9(5):652-657.
11. World Health Organization. (2011). Quality control methods for herbal materials. Geneva: WHO Press.
12. Yoshikawa, M., Xu, F., Morikawa, T., Ninomiya, K., & Matsuda, H. (2003). Anastatins A and B, new skeletal flavonoids with hepatoprotective activities from the desert plant Anastatica hierochuntica. Bioorganic & Medicinal Chemistry, 13, 1045-1049.
13. Zin, S. R. M., Kassim, M. N., Alshawsh, M. A., Hashim, N. E., & Mohamed, Z. (2017). Biological activities of Anastatica hierochuntica L.: A systematic review. Biomedicine & Pharmacotherapy, 91, 611-620.
14. Ghazanfar, S. A. (1994). Handbook of Arabian medicinal plants. Florida: CRC Press.
15. Kim, S. L., & Lean, K. S. (2013). Herbal medicines: Malaysian women's knowledge and practice. Evidence-Based Complementary and Alternative Medicine, 2013, 438139. doi: 10.1155/2013/ 438139.
16. Friedman J, Stein Z. The influence of seed-dispersal mechanisms on the dispersion of Anastatica hierochuntica (Cruciferae) in the Negev Desert, Israel. J Ecol 1980: 43-50.
17. AlGamdi N, Mullen W, Crozier A. Tea prepared from Anastatica hirerochuntica seeds contains a diversity of antioxidant flavonoids, chlorogenic acids and phenolic compounds. Phytochem 2011; 72(2-3): 248-254.
18. Abou-Elella F, Hanafy EA, Gavamukulya Y. Determination of antioxidant and anti-inflammatory activities, as well as in vitro cytotoxic activities of extracts of Anastatica hierochuntica (Kaff Maryam) against HeLa cell lines. J Med Plants Res 2016; 10(7): 77-87.
19. Tayel AA, El-Tras WF. Possibility of fighting food borne bacteria by egyptian folk medicinal herbs and spices extracts. J Egypt Public Health Assoc 2009; 84(1-2): 21-32.

20. Nakashima S, Matsuda H, Oda Y, Nakamura S, Xu F, Yoshikawa M. Melanogenesis inhibitors from the desert plant Anastatica hierochuntica in B16 melanoma cells. Bioorg Med Chemi 2010; 18(6): 2337-2345.
21. Yoshikawa M, Morikawa T, Xu F. (7R, 8S) and (7S, 8R) 8-5'Linked Neolignans from Egyptian herbal medicine Anastatica hierochuntica and inhibitory activities of lignans on nitric oxide production. Heterocycles 2003; 60(8): 1787-1792.
22. Yoshikawa M, Xu F, Morikawa T, Ninomiya K, Matsuda H. Anastatins A and B, new skeletal flavonoids with hepatoprotective activities from the desert plant Anastatica hierochuntica. Bioorg Med Chemi Lett 2003; 13(6): 1045-1049.