

Harmines Isolation and Purification by a New Design of Silica Gel Column Chromatography

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Abstract

Harmines is one of an active compound can be found in harmala seed. It has a nutritional and pharmacological value, harmine has antimicrobial, antiplasmodial, antifungal, antioxidative, antitumor, antimutagenic, cytotoxic and hallucinogenic properties. Other than having the pharmacological properties, it has been used as an ingredient in many herbal formulations, which are used for the treatment of various diseases. In this study, harmine was isolated from harmal seeds using ethyl acetate and sodium bicarbonate method. An isolated harmine has been identified using thin layer chromatography paper and HPTLC. For harmine purification, preparative plate chromatography and designed column chromatography were used. The result of this study revealed that designed column chromatography has several advantages compared to preparative plate Chromatography (PLC) and classical column Chromatography. The amount of purified harmine was two times more, saved time and reduces the amount of solvent. The purity of isolated harmine was confirmed using FTIR spectra and ¹³CNMR.

Keywords: *Harmines; Classical Column Chromatography; New Designed Column Chromatography; Isolation and Purification; C13 NMR*

Abbreviations

IR: Infrared Spectra; RF: The retardation factor; HPLC: High-Performance Liquid Chromatography; UV: Ultraviolet

Introduction

Harmane is a B-carboline alkaloid. It is widely distributed in plants, well-cooked foods, tobacco smoke, mammals as well as in human tissues and body fluids [1,2]. The use of harmine as a multipurpose product has been transferred to several commercial applications. Harmines is an active plant component with a high nutritional value and healthy. It has many traditional medicinal uses and pharmacological activity such as antimicrobial, antitumor and anti-parasitic properties [3-7]. Harmines isolation and purification can be done using several techniques such as Thin Layer Chromatography (TLC), preparative chromatography (PLC), Liquid-solid chromatography (LSC) and high- liquid chromatography (HPLC). Column chromatography, PLC and TLC Chromatography are the ideal method of purification and separation. The disadvantages of using both classical column and TLC chromatography are that: time consuming, requiring the use of large amount of organic solvents, laborious [8]. The disadvantages of TLC compared to classical column chromatography are that using specific reagents to immediately reveal the nature of the separated compound, overloading (volume or mass), need for a larger sample size and its lower sensitivity [9]. PLC can be used for both process isolation and purification of plant extracts rich in non-polar or polar ballast [10]. The advantage of PLC, it can be used as a pilot technique for preparative column chromatography, for a selective system optimization and determination of the effects of overloading as maintained by Głowniak, *et al* [11]. The disadvantage of PLC in order to purify compound using PLC, it needs to use techniques such as TLC, GC or HPLC for isolation of fractions before [12].

In this study, a new design of column chromatography suggested in order to isolate and purify harmine from the extract of *Peganum harmala* seeds. The designed column has unparalleled advantages, in increasing productivity and purity, saving time and reducing the amount of organic solvents.

Materials and Methods

- Isolation of *P. harmala* crude alkaloid:** Seeds of *P. harmala* were collected from local markets in Misurata city, Libya and taxonomically identified by the botanists Dr. M. Elgaroshii, in Botany Department, College of Science, University of Misurata. The dried and powdered seeds (20g) were boiled in 100ml 30% acetic acid for two hours then the extract left to settle overnight, filtered and the murky yellow water was transferred into a large pot to make sure that all insoluble materials were removed. Filtration process was repeated up to five times. The extracted fraction which contains alkaloids were detected, using Myer's reagent (Potassium mercuric iodide solution [13] and Dragendorff's reagent (Potassium bismuth solution) [14] and TLC chromatography [15]. For basification, a substantial amount of sodium carbonate was slowly added and while string until the solution became cloudy. The free alkaloids were then precipitated after about 24 hours of the addition of sodium carbonate (pH 8). The residues were dissolved in water and hot acetic acid (1:3 v/v) to yield harmala acetates. The resulting harmala acetate was filtered and NaCl (10g) was added to the hot solution (100 cm³). The solution was then cooled (left in the refrigerator or monitored in a freezer) during which the harmala precipitated out as flakes [16]. The obtained harmala flakes were filtered and air-dried.
- Isolation of harmine:** The obtained harmala alkaloid crystals were dissolved in a mixture of hot acetic acid and water (3:1 v/v) then sodium carbonate was added until the pH reached 8.75. The solution was kept 2 hours then filtered. Harmine (~ 1g) was isolated in unpurified form (the purity was determined by TLC). The resulting harmine was recrystallized from hot 75% ethanol to give yellowish crystals. The determination of harmine was done using TLC and HPTLC technique.
- Harmine purification:** Harmine was purified using two chromatography techniques (Plc and column chromatography). Preparative-plates Chromatography: Chromatography was performed on 50 mm × 100 mm glass plates percolated 2 mm layers of silica gel Si 60. The fraction was purified by preparative TLC to give 11.1 mg pure harmine. Designed column chromatography: harmine was also purified by using a new design of column chromatography. The column was prepared as follows: Aluminum foil was formed as a funnel (10.5 cm lengths, 3 cm diameter). A funnel was packed with a dough contains 47.5g of silica gel Si60, 2.5g of calcium sulfate and water. Packed column was subjected into the oven at 105°C for 24h. Aluminum foil was slowly removed.
- Harmine purification by designed column chromatography:** Isolated harmala alkaloid which contains unpurified harmine was dissolved in acetone. Harmine extract was applied to the column by use of the mobile phase distributor of the DS chamber [17]. the column was developed face-down, to a distance of 9 cm, in a horizontal chamber, after conditioning for 10 min with mobile phase vapor (9 methanol: 1 chloroform). After development, the mobile phase was evaporated to dryness. The dried column was subjected to UV lamp 365 - 254 nm. Harmine was identified by comparing its R_f value and IR spectra data with published literature [18-20].

Results and Discussion

The evaluation of the alkaloid of *Peganum harmala* seeds revealed the existence of at least seven distinct spots, which are present in large amounts (Figure 1). According to R_f value spots [2-5] are harmaline, harmine, harmol and harmalol. Date published by Elgubbi, *et al.* [21] was in agreement with the result of this study.

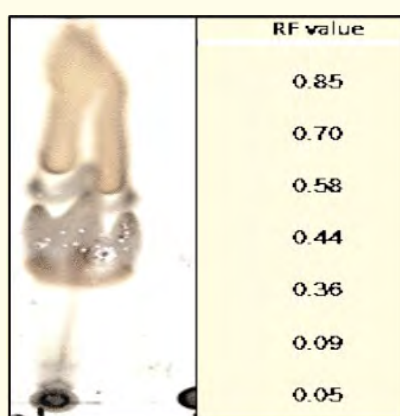


Figure 1: Thin layer chromatography of alkaloids seeds of *Peganum harmala*. Mobile phase: toluene /ethyl acetate / 9: 1 (v/v). detection of alkaloids was performed by use of sulfuric acid.

The presence of alkaloid was further investigated by Mayer's and modified Dragendorff's tests. The reaction resulted as cream colored precipitate and Orange colouration respectively.

An extract rich in harmala alkaloid was prepared as mentioned by [22] using ethyl acetate and sodium carbonate method. The result of the study showed that several compounds seen on the TLC plate and HPTLC result. The preliminary HPTLC studies revealed that the solvent system, toluene-methanol-formic acid (7.0: 2.0: 0.3 v/v/v), was ideal and gave well-resolved sample peaks of isolated compound harmine. The spots on the chromatogram were visualized at 270 nm (Figure 2,3) with a 200k filter at R_f values of 0.11, 0.15, 0.25 and 0.56 respectively. The peak at R_f 0.56 was identified as the isolated compound harmine.

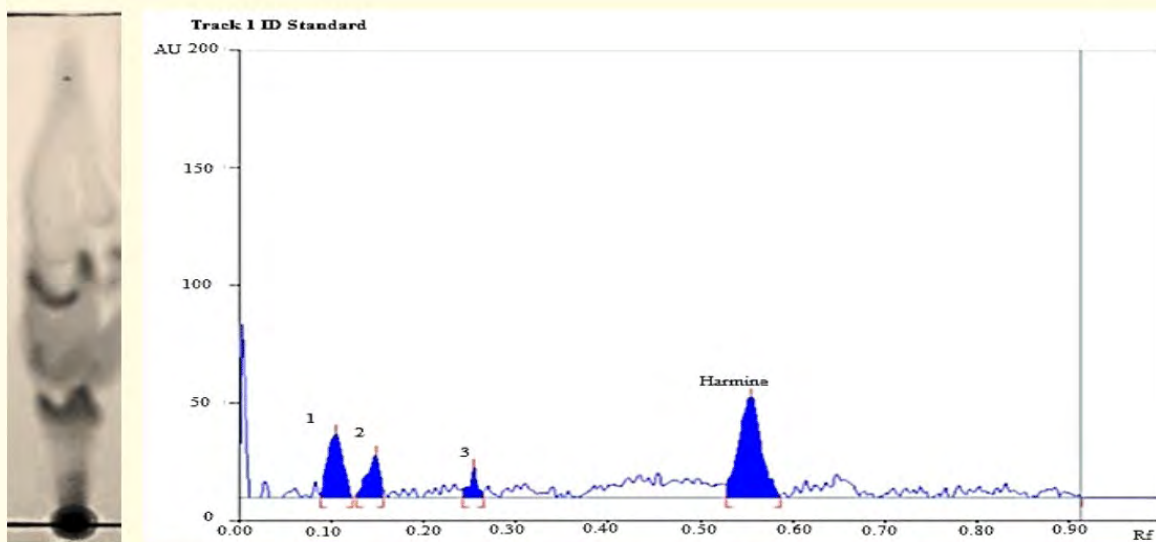


Figure 2: Thin layer chromatography and HPTLC chromatogram of methanolic extract scanned at 366 nm. of alkaloids seeds of Peganum harmala.

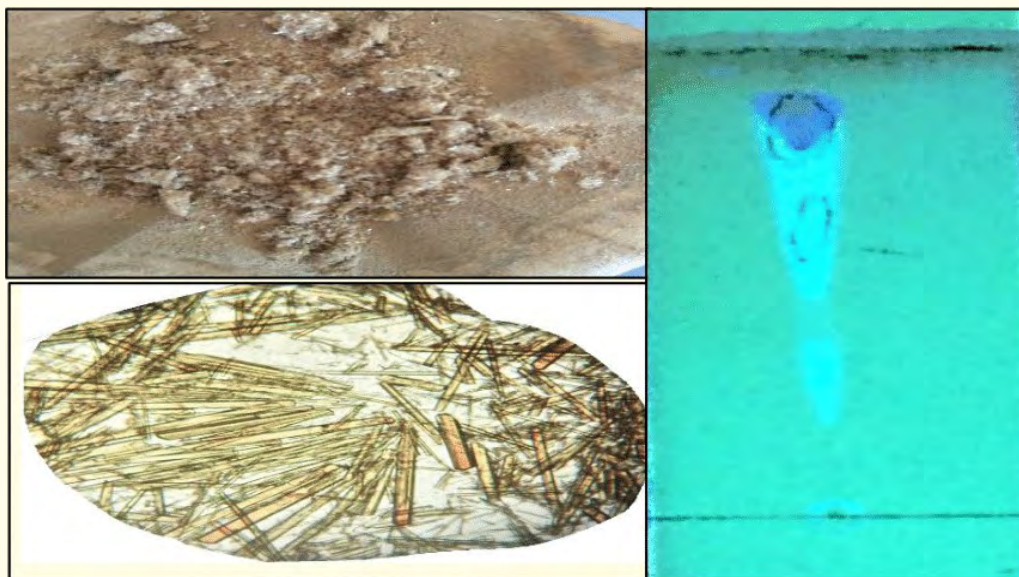


Figure 3: Needle crystal shapes of harmine under microscopic (40X). Thin layer chromatogram of the alkaline extract (UV lamp 365 - 254 nm).

To isolate harmine from an alkaloids extract that will involve justifying pH value up to 8.75 by adding sodium carbonate. The alkaline extract was filtered, dried then dissolved in ethyl acetate (20%) and analyzed by TLC. The result of TLC illustrated that the alkaline extract was reached in unpurified harmine (Figure 4). The result of the microscopic test of the dried powder emphasized that harmine with needle crystals shape presented in huge amount but also there was a few amount of a plate shape crystals refers to the presence of harmaline.

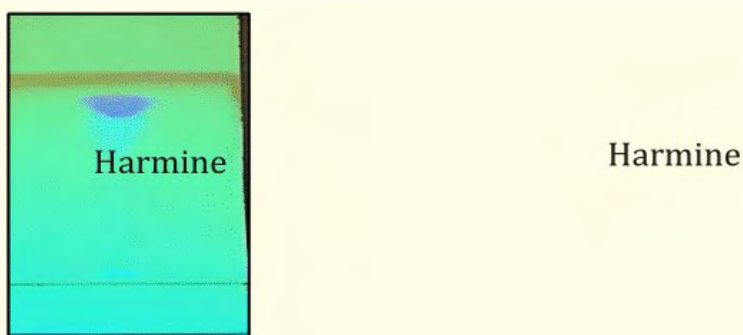


Figure 4(a): TLC and PLC of purified harmine.



Figure 4(b): Designed column chromatography for harmine purification.

Harmine was purified, using both techniques preparative layer chromatography (PLC) and newly designed column chromatography Figures 4 (A and B), then analyzed by TLC, IR, C13NMR. Thus, the result of analysis emphasized the purity of tested harmine (Figures 5 and 6).

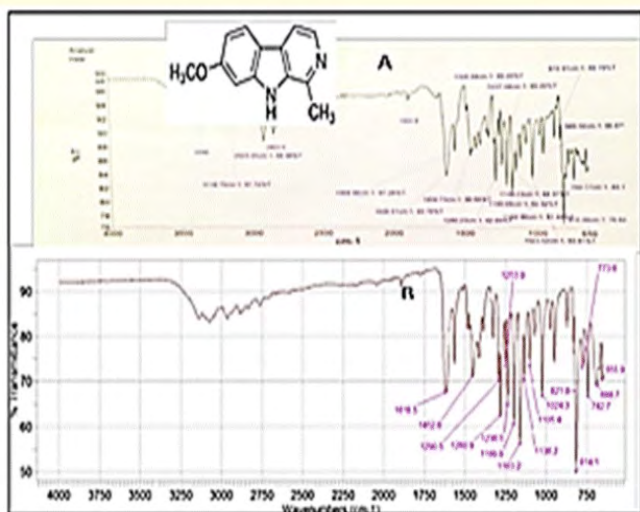


Figure 5: IR for purified harmine (A) and harmine standard.

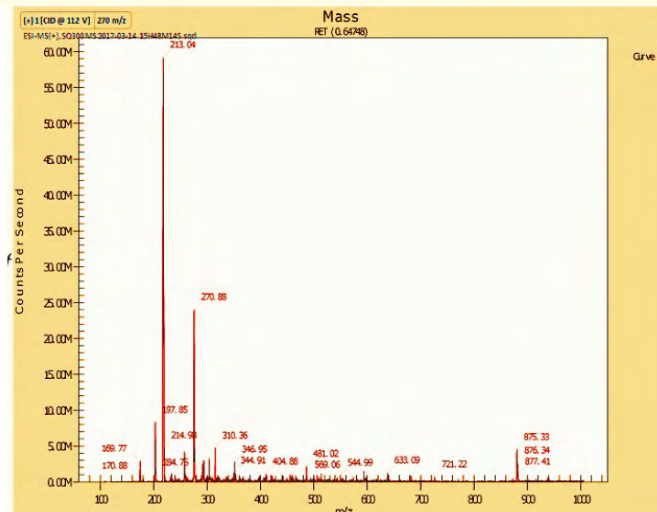


Figure 6: C¹³ NMR spectrum of harmine.

Designed Column chromatography and preparative TLC of total alkaloids of seeds of *P. harmala* led to the isolation of harmine, with total amount 35 mg and 11 mg respectively, whose structures were identified by comparing their spectral data with published literature [23,24].

Conclusions

A new silica gel column chromatography was designed in this work. Unlike classical column chromatography, the new design column require an aluminum foil, dough contains Silica gel Si 60 and calcium carbonate. From data obtained in this study, designed silica gel column chromatography is a more favorable mode of separation and purification compare to classical silica gel column and preparative plate chromatography. The advantage of the new design column over classical silica gel column chromatography and preparative plate chromatography is that it is significantly reduces the amount of solvent and time required to achieve the same degree of separation. Also, Amount of pure harmine isolated was two times more compared to the preparative plate chromatography.

Bibliography

1. Allen J., *et al.* "The simple b-carboline alkaloids". *Phytochemistry* 19.8 (1980): 1573-1582.
2. Callaway J., *et al.* "Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca". *Journal of Analytical Toxicology* 20.6 (1996): 492- 497.
3. Patel K., *et al.* "A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid "Harmine". *Asian Pacific Journal of Tropical Biomedicine* 2.8 (2012): 660-664.
4. Miraj S., *et al.* "A review of chemical components and pharmacological effects of *Melissa officinalis* L". *Der Pharmacia Lettre* 8.6 (2016): 229-237.
5. Al-shaibani M., *et al.* "Antitumor and immunomodulatory activities of *Peganum harmala* extracts". *Journal of Biotechnology Research Center* 7.1 (2013): 11-20.
6. Boeira J., *et al.* "Genotoxic effects of the alkaloids harman and harmine assessed by comet assay and chromosome aberration test in mammalian cells in vitro". *Journal of Pharmacology and Toxicology* 89.6 (2001): 287-294.
7. Herraiz T., *et al.* "Beta-carboline alkaloids in *peganum harmala* and inhibition of human monoamine oxidase (MAO)". *Food Chemical Toxicology* 48.3 (2010): 839-845.
8. Lacaze A., *et al.* "Solid-phase extraction and liquid chromatography-mass spectrometry for the determination of free fatty acids in shellfish". *Journal of Chromatograph A* 1145.1-2 (2007): 51-57.
9. Moreda W., *et al.* "Gas and liquid chromatography of hydrocarbons in edible vegetable oils". *Journal of Chromatography* 936.1-2 (2001): 839-845.
10. Waksmundzka-Hajnos M., *et al.* "On-line purification and microprpreative separation of contaminated fruit extract by TLC in equilibrium sandwich chamber". *Journal of Planar Chromatography* 5 (1992): 169-174.
11. Główniak K., *et al.* "The application of zonal thin-layer chromatography to the determination of paclitaxel and deacetylbaecat III in some *Taxus* species". *Journal of Planar Chromatography* 12.5 (1999): 328-335.
12. Hajnos M., *et al.* "Optimization of the isolation of some toxoids from yew tissues". *Journal of Chromatography* 14 (2001): 119-125.
13. Sousek J., *et al.* "Alkaloids and organic acid content of eight *Fumaria* species". *Phytochemical Analysis* 10.1 (2009): 6-11.
14. AL-Shahaat N., *et al.* "Plants and Medicinal Herbs". Dar AL-Bahaar, Beirut (1986): 140-146.
15. Preston RD. "Hormonal regulation of development molecular aspects of plant hormones". *Cell* 25.2 (1981): 573-574.
16. Harrington N. "Harmala alkaloids as bee signaling chemicals". *Journal of Student Research* 1 (2012): 23-32.
17. Waksmundzka-Hajnos M., *et al.* "Strategy for preparative separation of quaternary alkaloids from *Chelidonium majus* L. by Thin-layer chromatography". *Journal Planar Chromatography* 15.4 (2002): 289-293.
18. Shao H., *et al.* "Main Alkaloids of *Peganum harmala* L. and Their Different Effects on Dicot and Monocot Crops". *Molecules* 18.3 (2013): 2623-2634.
19. Kartal M., *et al.* "HPLC methods for the analysis of harmal, harmalol, harmaine and harmaline in the seeds of *Peganum Harmala*". *Journal of Pharmacology Biomedical Analysis* 31.2 (2003): 263-296.

20. Aripova S., *et al.* "Alkaloids of the cultivated species". *Chemistry of Natural Compounds* 33.6 (1997): 698-699.
21. Elgubbi H., *et al.* "Cytotoxicity studies of harmine isolated and purified from *Peganum harmala* l. seeds". *The Third Symposium on Theories and Applications of Basic and Biosciences* 3 (2016): 22-29.
22. Brobst A., *et al.* "The free base extraction of harmaline from *Peganum harmala*". *American Journal of Undergraduate Research* 8.2-3 (2009): 1-4.
23. Duan A., *et al.* "Studies on the chemical constituents of *Peganum multisectum* Maxim II. The alkaloids from seeds and antitumour activity". *Journal of China Pharmaceutical University* 29.1 (1998): 21-23.
24. Wang X. "Separation and purification of harmine and harmaline from *Peganum harmala* using pH-zone-refining counter-current chromatography". *Journal of Separation Science* 31.20 (2008): 3543-354.

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