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Original Research Article

High performance thin layer chromatography analysis of seed extracts of Tudri Surkh (*Cheiranthus cheiri*)

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ABSTRACT

Background: To develop the high-performance thin layer chromatography (HPTLC) finger print profile of hydroalcoholic and ethylalcohol extracts of seeds of Tudri Surkh (*Cheiranthus cheiri*).
Methods: Chromatographic technique was used for separation of components from hydroalcoholic and ethylalcohol extracts of seeds and HPTLC was carried out using CAMAG HPTLC system equipped with Linomat V applicator, and WinCATS software.
Results: HPTLC profiling of the extract confirm the presence of various phytochemicals. At 640 nm HPTLC finger print of hydroalcoholic extract revealed 16 components with Rf=0.05-0.94 while ethylalcohol extracts revealed 19 components peaks with Rf=0.07 to 0.96. Well separated and compact greenish, yellow and pink bands were visualized using anisaldehyde sulphuric acid reagent.
Conclusions: We conclude that HPTLC fingerprint profiling of seed extracts of Tudri Surkh (*Cheiranthus cheiri*) revels presence of various compounds and can be utilized as a marker for standardization and proper identification of the plant material to be used for preparation of traditional drugs.

Keywords: Unani, Tudri Surkh, *Cheiranthus cheiri*, HPTLC, Standardization

INTRODUCTION

Traditional systems of medicine including Unani and Ayurvedic systems of medicine have been known for centuries and have been employed for treatment of diseases according to their principles of treatment. All these systems of medicine use herbs as a major source of drugs besides animal products or minerals. Herbal drugs symbolise plants or parts of plants that have been converted into phytopharmaceuticals by the method of various procedures like harvesting, drying and storage.[1] Other variation causing factors are geographical location and time of harvesting.[2] These herbal medicines are used either singularly or in combination with other herbs to achieve the therapeutic effects. According to an estimate about 80% of world's population still rely on medicinal herbs as a source of their primary health care and the global use of herbs is increasing with time.[1] Besides the use of herbs in traditional systems of medicine currently around 40% of the drugs used worldwide originate directly or indirectly from natural products including plants, microorganisms and animals.[3] Whatever the source, all medicines should fulfil the basic requirements of being safe and effective and the quality assessment of herbal products is of utmost importance. The presence of multitude of compounds in single as well as compound herbal formulations, in which no single constituent is responsible for its efficacy, makes quality control of these drugs a challenging task.[4]

The term "quality control" encompasses the processes involved in maintaining the quality and validity of a manufactured product.[5] Standardization is a system that ensures predefined amount of quantity, quality and

therapeutic effect of ingredients in each dose. Standardisation should be done on bioactive extract on the basis of active principles or major compounds along with the chromatographic fingerprints like TLC, HPTLC, GC and HPLC.[6] HPTLC is widely used technique for the standardization and detecting adulterants of herbal products.[7]

A chromatographic fingerprint of an herbal drug or extract is a chromatographic pattern of pharmacologically active or chemically characteristics components. This process offers very powerful separation ability in which complex chemical components in herbal extracts can be separated into many relatively simple sub-fractions.[8] The major advantage of HPTLC is its ability to analyse several samples simultaneously using a small quantity of mobile phase. HPTLC expedites repeated detection of chromatogram with same or different parameters.[9]

Tudri surkh (*Cheiranthus cheiri*) is a perennial herb belonging to family Brassicaceae. Originally a native of European countries it has been introduced in other geographical regions of the world where it is grown primarily as an ornamental garden plant. It is used widely in Unani system of medicine for various ailments and is a constituent of many compound pharmacopeial Unani formulations including Jawarish Atai, Majoone-Regmahi, Majoon Alkula.[10,11] It has also been mentioned as one of the drugs in for the management of renal insufficiency.[10-11] Seeds of *Cheiranthus cheiri* are aphrodisiac and also helps to expel black bile (Sauda) from the body.[12-14] The present study was therefore, designed with the aim to analyze the HPTLC finger printing profile of steroids, glycosides and terpenoids for hydroalcoholic and ethanolic extracts of seeds of Tudri surkh (*Cheiranthus cheiri*).

METHODS

Collection of plant material

Seeds of Tudri surkh (*Cheiranthus cheiri*) used for the investigation was procured from the Khari Baoli, New Delhi and taxonomically identified by taxonomist at national institute of science communication and information resources (NISCAIR), New Delhi.

Sample extraction

Hydroalcoholic extract

Hydroalcoholic extract of Tudri surkh (*Cheiranthus cheiri)* (HATS) was prepared by the method of Soxhlation. 100 gm of plant material was air dried and grinded to fine powder and placed in thimble chamber of Soxhlet apparatus. About 250 ml ethanol was mixed with 250ml water and added to a round bottom flask. Extraction solvent was heated on isomantle for 48 hours. After 48 hours solvent was recovered and extract was dried on water bath. The percentage yield was calculated after weighing the extract.

Ethanolic extract

Ethanolic extract of Tudri surkh (*Cheiranthus cheiri)* (EATS) was prepared by extracting plant material with ethanol in Soxhlet apparatus. Plant material (100 gm) was air dried, grinded to fine powder and placed in thimble chamber of Soxhlet apparatus. About 500 ml of 95% ethanol was added to round bottom flask and extracted by heating on isomantle for 48 hours. Then after recovering solvent filtrate was dried on water bath and weighed. The percentage yield was calculated.[15]

HPTLC analysis

HPTLC analysis of Tudri surkh (*Cheiranthus cheiri*) extracts was carried out for their qualitative analysis and simultaneous fingerprinting analysis of extracts was carried out using newly developed HPTLC method following the ICH guidelines.

HPTLC sample preparation and chromatographic conditions

The hydroalcoholic extract and ethanolic extract of Tudri Surkh (*Cheiranthus cheiri*) were reconstituted using methanol (HPLC grade) and 10 mg/mL concentration of the extracts was prepared. The samples were spotted in the form of bands (4.0 mm width), with a CAMAG microlitre syringe on pre-coated HPTLC silica gel aluminium plates ($60F_{254}$; 20×10 cm, Merck KGaA, Germany) using a CAMAG Linomat V (Muttenz, Switzerland) and were controlled by WinCATS software (CAMAG). A constant application rate of 10 µL/s was employed and the space between two bands was 6.0 mm. The slit dimension was maintained at 5.0×0.3 mm, and 20 mm/s scanning speed was employed. The solvent system of the Tudri Surkh (*Cheiranthus cheiri*) seed extracts consisted of choloroform: ethyl acetate: methanol: formic acid (2:8:2:1 v/v/v). Linear ascending development was carried out in a 20×10 cm twin trough glass chamber, saturated with the solvent system. The optimized chamber saturation time for the solvent system was 15 min at room temperature. The length of chromatogram run was 80 mm. Subsequent to development, HPTLC plates were dried in an oven at 60°C for five min. Densitometric scanning was performed on a CAMAG TLC scanner IV (absorbance mode 530 nm) with WinCATS software after spraying the developed plate with anisaldehyde-sulphuric acid reagent and heating it on a hot air oven at 110° C for five minutes at different wavelength of 254, 340, 540 and 640 nm.

RESULTS

The results from HPTLC fingerprint scanned at wavelength 254 nm for HATS (Figure 3) revealed the

presence of 11 polyvalent phytoconstituents. The Rf=0.11 to 1.06. Out of 11 components, the component 10 with Rf. value 0.99 was found to be predominant as the percentage area is 34% and highest concentration of the phytoconstituent 1 was found to be 27.3% and its corresponding Rf value was found to be 0.11. Where as in EATS at 254 nm (Figure 4) revealed the presence of 11 components with Rf=0.12 to 1.09. The component having Rf=0.12 showed higher concentration of 21.03% and higher percentage area of 24.79%.

Both extracts, HATS and EATS at 340 nm (Figure 5 and 6) revealed 11 components with a range of 0.11 to1.06 and 0.12 to 1.09 respectively. In HATS, component 1 with Rf=0.11 revealed higher percentage concentration of 27.3% and component 10 with Rf=0.99 showed maximum percentage area of 34%. EATS scanning at 340 nm further revealed component 1 with Rf=0.12 having maximum percentage concentration of 21.03% and component 1 also had maximum percentage area of 24.79%.

At 540 nm (Figure 7) HATS showed 16 components with a range 0.05 to 0.94 having component 16 (Rf=0.94) with higher percentage concentration of 23.55% and component 14 (Rf=0.89) with maximum percentage area of 47.07%. Whereas EATS (Figure 8) showed 15 components ranging from 0.07 to 0.96 with component 1 (Rf=0.07) having maximum percentage concentration of 27.49% and component 2 (Rf=0.17) having higher percentage area of 32.64%.

HPTLC scanning at 640 nm (Figure 9) revealed 16 components in HATS. Rf=0.05 to 0.94. The component with Rf value 0.89 has highest percentage area of 47.07% and component with Rf=0.94 maximum concentration of 23.55%. Whereas EATS (Figure 10) showed highest number of components i.e., 19 ranging from 0.07 to 0.96 with component 1 (Rf=0.07) having maximum percentage of concentration of 27.49% and component 2 (Rf=0.17) having maximum percentage area of 32.64%.

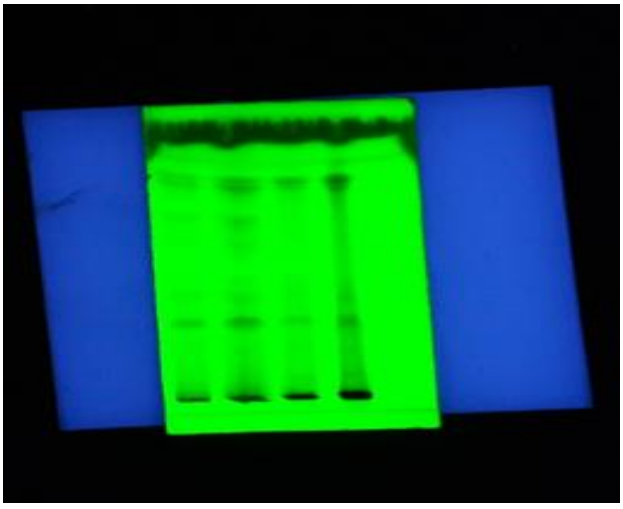

Figure 1: Under UV light of 254 nm.

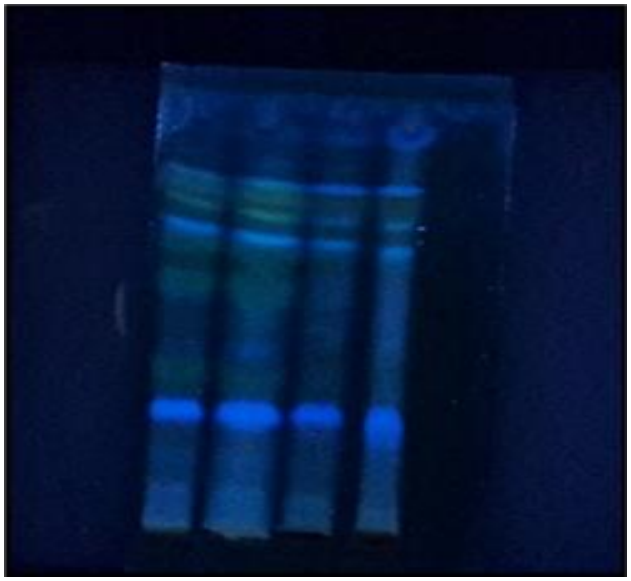

Figure 2: Under UV light of 360 nm.

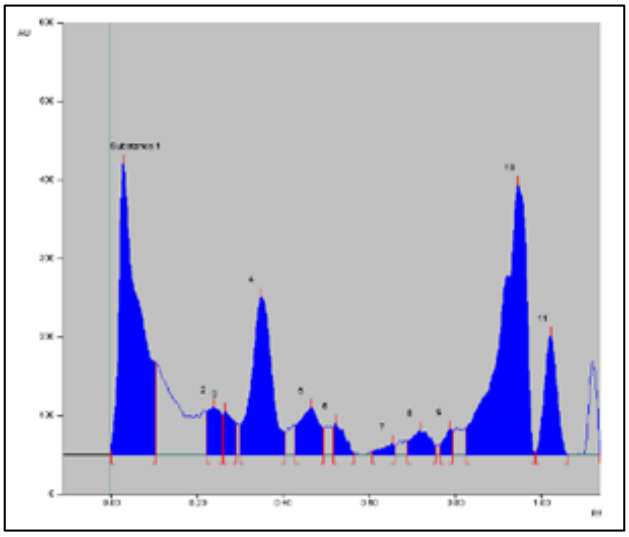

Figure 3: Chromatogram of HATS at 254 nm.

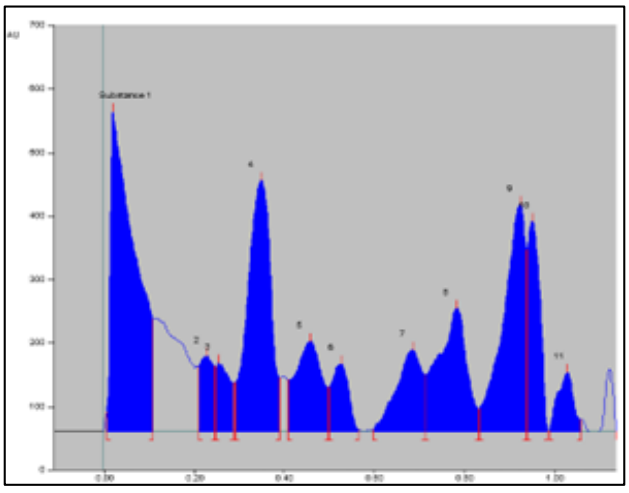

Figure 4: Chromatogram of EATS at 254.

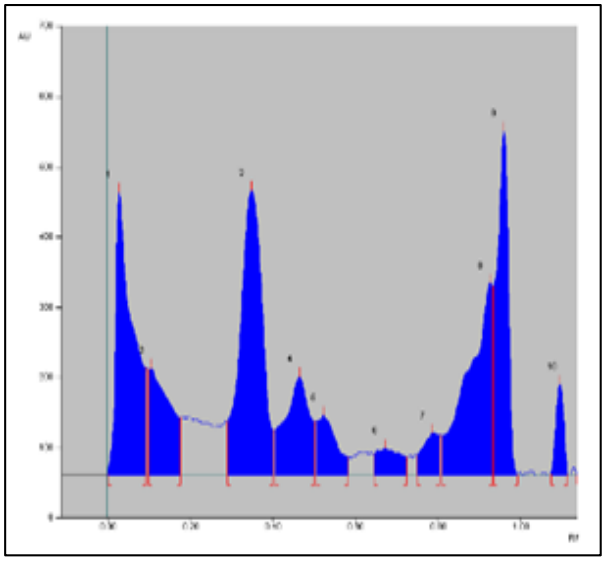

Figure 5: Chromatogram of HATS at 340 nm.

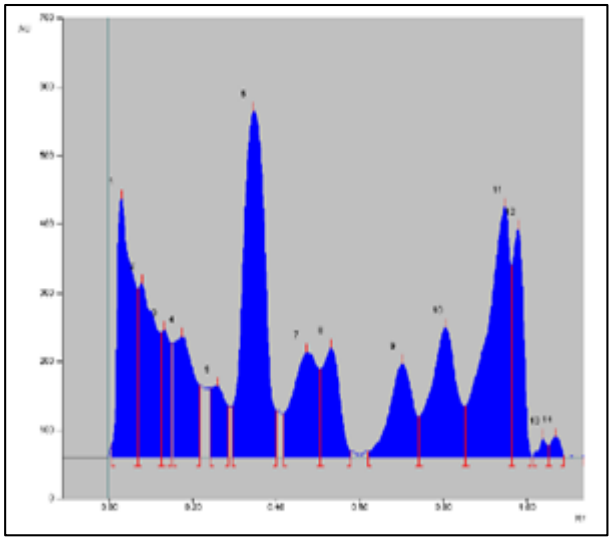

Figure 6: Chromatogram of EATS at 340 nm.

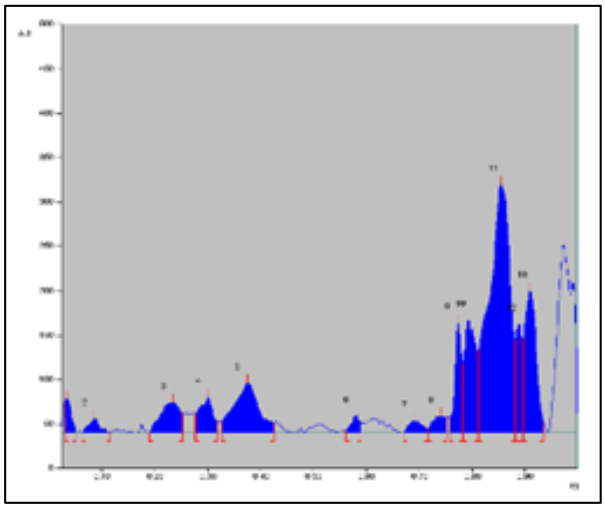

Figure 7: Chromatogram of HATS at 540 nm.

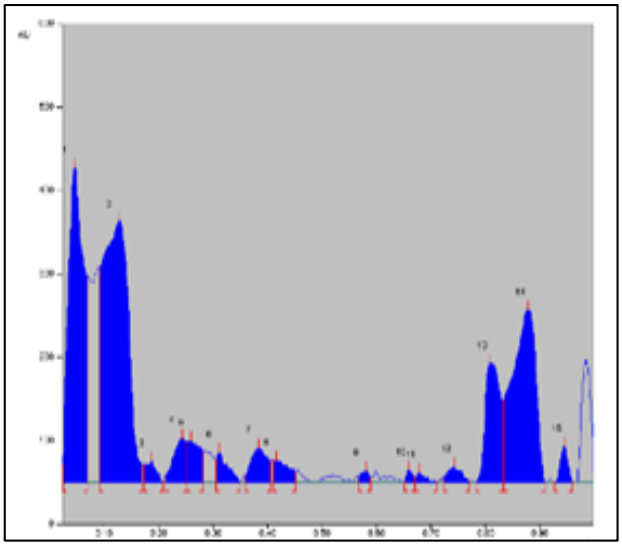

Figure 8: Chromatogram of EATS at 540 nm.

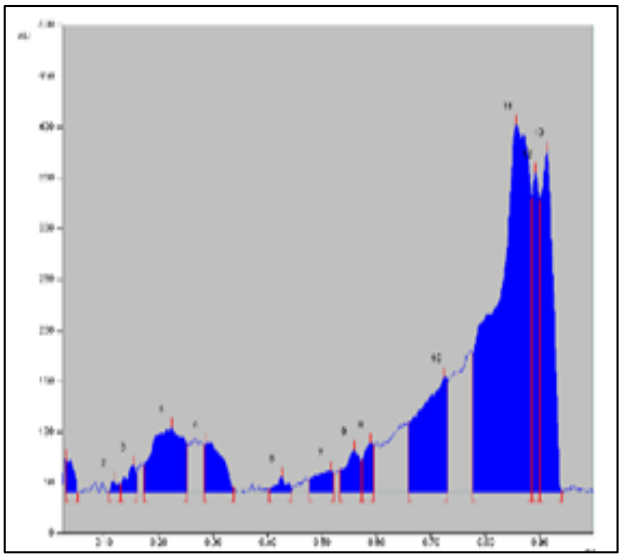

Figure 9: Chromatogram of HATS at 640 nm.

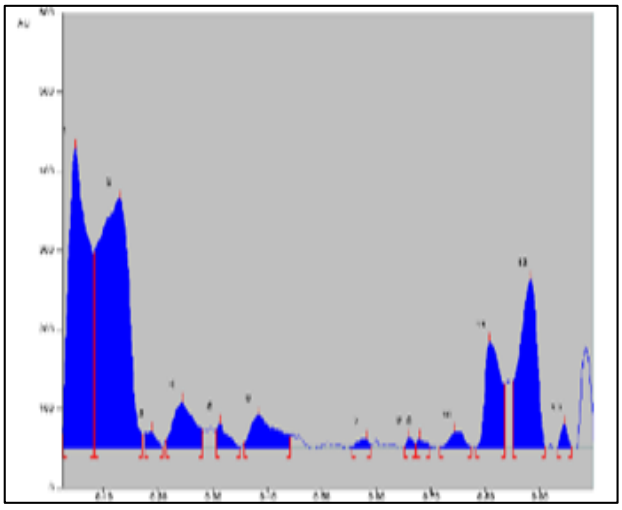

Figure 10: Chromatogram of EATS at 640 nm.

DISCUSSION

Medicinal plants or their products are best source of large number of pharmacologically useful compounds or constituents which alleviate various disease including AIDS, cancer and many degenerative diseases. Extraction and phytochemical analysis help to separate the medicinally active portions of plants by using universal solvent including water and alcohol through standard procedure.[16]

Free radical scavenging molecules such as phenolic acids, flavonoids, tannins and terpenoids are present in Unani herbal medicines. Chromatographic fingerprinting is one of the potent approaches for the standardization and quality control of herbal medicine. It helps to identify and assess the stability of chemical constituents observed by chromatography.[17]

HPTLC fingerprint studies confirmed the results of phytochemical screening by the presence of various colored bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytocompounds. HPTLC fingerprints of all the extracts revealed different peaks confirming the presence of various constituents. Rf values indicate the presence of different chemical constituents like glycosides, tannins, proteins, triterpenes, saponins, steroids, and amino acids.[18]

CONCLUSION

A novel method for HPTLC analysis of extract of Tudri surkh (*Cheiranthus cheiri)* has been presented along with results that show the presence of secondary metabolites such as steroids, terpenoids and glycosides in the ethanolic and hydroalcoholic extract of plant material. The essences of these metabolites are beneficial for maintenance of human health and chronic degenerative diseases.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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