HPTLC Finger Print Profile of Extracts from Dried Aerial Parts of *Bryophyllum Pinnatum* in Different Solvents

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ABSTRACT

Introduction: *Bryophyllum pinnatum* Kurz. is perennial herb growing widely and used in folkoric medicine in tropical Africa, America, India, china and Australia. The divine herb has potent medicinal values and used in traditional medicine for the treatment of variety of ailments and well known for its haemostatic and wound healing properties. **Methods:** Chromatographic techniques were used for separation of components from different extracts of plant parts. This study was planned to develop a HPTLC fingerprint profile of drug extracts from aerial parts of *Bryophyllum pinnatum* in different solvents such as petroleum ether, benzene, chloroform, acetone and methanol. **Results:** A High Performance Thin Layer Chromatography (HPTLC) method for the separation of the active constituents in *Bryophyllum pinnatum* extracts has been developed and TLC of these extracts on silica gel precoated aluminum plates of Merck by automatic TLC applicator and using solvent system Chloroform: Ethanol (9.8:0.2) was performed. In the present study, HPTLC finger print of various extracts of dried aerial parts of *Bryophyllum pinnatum* have been carried out and the results provide referential information for standardization. **Conclusion:** The HPTLC method for routine quality control of present species can be carried out using this method for different extracts of plant parts and serve in qualitative, quantitative and was appropriate for standardization of the drug. The HPTLC fingerprint is also suitable for rapid and simple authentication and comparision of subtle differences among samples of identical plant resource.

Key words: Authentication, bioactive molecules, HPTLC analysis, standardization.

INTRODUCTION

Bryophyllum pinnatum Kurz (syn. *B. calycium* and *Kalanchoe pinnata*) commonly known as parnbija, Zakhm-e-hyat (Hindi), life plant, love plant, air plant (Mexican), Good luck or resurrection plant.[1] It is a glabrous, ornamental, crassulescent herb, cultivated in houses and gardens. It is a perennial medicinal herb popularly used as folkloric medicine in tropical Africa, India, China, Australia and tropical America and other parts of the world to treat various inflammatory diseases. The leaves of the plant have great medicinal value and possess various properties like haemostatic, refrigerant, emollient, mucilaginous, vulnerary, depurative, anti-inflammatory, disinfectant and tonic. It is also employed for kidney stones, gastric ulcers, skin disorders and edema of the legs. It contains triterpenoids, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids.[2-4]

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It is a succulent perennial plant that grows 1-1.5 m in height and the stem is hollow four-angled and usually branched. Leaves are opposite, decussate, succulent, 10-20 cm long, distributed all over India. In traditional medicine, the leaves of this plant have been used for antimicrobial, antifungal, antiulcer, anti-inflammatory, analgesic, antihypertensive, potent anti-histamine and anti-allergic activity.[5, 6] In the recent year advancement in of chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines.[7] Chemical finger prints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and its products and therefore be used for authentication and identification of herbal plant.[8] HPTLC is more efficient, faster method and the results are more reliable and reproducible. In combination with digital scanning profiling, HPTLC also provides accurate and precise R_f values and quantitative analysis of sample by *in situ* scanning densitometry aided by formation of easily detected derivatives by post-chromatographic chemical reactions as required, as

well as a record of the separation in the form of a chromatogram with fractions represented as peaks with defined parameters including absorbance (intensity), Rf, height and area.[9] Furthermore, the feature of a pictorial fluorescence image of HPTLC coupled with a digital scanning profile is more and more attractive to herbal analysts for constructing an herbal chromatographic fingerprint by means of HPTLC. The main objective of this study was to evaluate, develop and to optimize the HPTLC fingerprint method in standardization of *Bryophyllum pinnatum* to provide beneficial information in regarding the separation, identification and standardization of drug according to WHO guidelines. These HPTLC fluorescence images coupled with scanning profiles provided adequate information and parameters for comprehensive identification, assessment and comparision of major active constituent fingerprints in the samples studied to serve as a basis for their use in medicinal preparations.[9-11]

MATERIAL AND METHODS

Collection and Identification

The plant of *Bryophyllum pinnatum* Kurz were collected from Tau Devilal Herbal garden, Churpur and positively identified. The specimen was submitted to the A. R College of pharmacy, Vallabh Vidya Nagar, Anand. The collected plant material was made thoroughly free from any foreign organic matter. The aerial parts of the plant were separated, cut into small pieces, shade dried and powdered with mixer and sieved.

Extraction of Plant Material

The powder was extracted with different solvents ranging from non-polar to polar solvents. About 10 g of the crude drug powder was subjected for extraction (Soxhlet extraction) in round bottom flask, first with petroleum ether (60-80°C) for 2-3 hours. The extract was concentrated under reduced pressure at 50-60°C. The dried marc of *Bryophyllum pinnatum was* once again subjected to successive extraction with different solvents viz. benzene, Chloroform, acetone, methanol.

Extracts were concentrated under vacuum and finally made up to 10 ml with HPLC grade methanol and ready for HPTLC analysis.

Chromatography

A highly sensitive and accurate HPTLC method was developed and used for *Bryophyllum pinnatum* extracts. 5 µl aliquots of each of the extracts were separately applied on aluminium plates precoated with Silica gel 60 F_{254} HPTLC plates, 10 × 10 cm (Merck, Darmstadt, Germany) with the help of Camag Linomat-V applicator and eluted the plate to a distance of 7 cm at room temperature (25°) in solvent system Chloroform: Ethanol (9.8:0.2). Sample solution was applied on 6 mm wide band using Camag Linomat-V automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nL/sec from syringe.

Development, Detection and Quantitation: After sample application, plates were developed in a Camag twin through glass tank pre-saturated with the mobile phase Chloroform: Ethanol (9.8: 0.2) for 20 min., the plate was developed horizontally in Camag horizontal developing chamber (10 × 10 cm) at the room temperature. After heating the plate at 100°C for 5 min., derivatization of the chromatogram was performed by Camag glass reagent spray by spraying still hot plate with 5% methanolic- sulphuric acid system. The plate was observed after 30 min. under UV-366 nm light in Camag UV cabinet and the HPTLC fluorescence image documented. The corresponding digital scanning profiling was carried out with a Camag TLC scanner III fitted with winCATS- V1.2.3 software at a single wavelength 490 nm. Documentation of chromatograms was carried out with digital camera. The components get separated by the principle of adsorption, having differential migration rates of individual component towards the phases.

RESULTS AND DISCUSSION

The various extracts of *Bryophyllum pinnatum* were subjected to HPTLC analysis by specific solvent system Chloroform: Ethanol (9.8:0.2) and detected under UV at 366 nm and 490 nm. The HPTLC images shown in Figure 1, 2 and 3 indicate that all sample constituents were clearly separated without any tailing and diffuseness. The R_f value of the corresponding component as obtained through the software system attached with the instrument. Area corresponds to each peak for the corresponding spot or component determines the concentration of the component in the solution. It is evident from Table 1 that in the Petroleum Ether extract of aerial parts of *Bryophyllum pinnatum* there are 10 spots at the following R_f 0.10, 0.18, 0.22, 0.26, 0.30, 0.38, 0.50, 0.58, 0.65, 0.76 as shown in Figure 4, indicating the occurrence of atleast 10 different components in Petroleum Ether extract. It is also clear from Table 1 and the chromatogram as shown Figure 4 that out of 10 components, the component with R_f values 0.38 (light blue, violet), 0.22 (light blue, violet), 0.76 (pinkish blue, purple), 0.18 (reddish brown, reddish brown) and 0.26 (reddish brown, light purple) at 366 nm and visible 490 nm were found to be more predominant as the percentage area is more with 30.31%, 14.73%, 12.94%, 10.63% and 8.92% respectively. And remaining components were found to be very less in quantity as the percentage area for all the spots was less than 7.5%.

It is evident from Table 2 that in the Benzene extract of aerial parts of *Bryophyllum pinnatum*, there are 7 spots at the following