

## RESEARCH ARTICLE

HPTLC Studies of Ethanolic Extract of *Dioscorea bulbifera*Asha Jyothi V<sup>\*1</sup>, Satyavati D<sup>2</sup><sup>1</sup>Dept. of Pharmacology Shadan Womens College of Pharmacy, Khairatbad, Hyderabad, Telangana, India<sup>2</sup>Brilliant college of Pharmacy, Koheda, Near Ramoji film city, Hyderabad, Telangana, India

Received 10 Sep 2015; Revised 05 Dec 2015; Accepted 17 Dec 2015

## ABSTRACT

To establish phytoestrogenic profile for the medicinally important plant *Dioscorea bulbifera* using high performance thin layer chromatography (HPTLC). Preliminary phytochemical screening was carried out to identify the presence of chemical constituents of ethanolic extract of *Dioscorea bulbifera* bulbil. Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed as mobile phase for flavonoids. TLC Plates dried at 100°C in hot air oven for 3 min. The plate was photo-documented at UV 254 nm. Carbohydrates, proteins, fats, steroids, volatile oils, glycosides, flavonoids and phenolic compounds are present in the ethanolic extract of *Dioscorea bulbifera* bulbil. The ethanolic extracts of *Dioscorea bulbifera* bulbil displayed the presence of mainly 3 types of flavanoids and related compounds with major 3 different Rf values ranging from 0.22 to 0.85. The ethanolic extract of *Dioscorea bulbifera* bulbil illustrated the presence of 3 different types of flavonoids with 3 different Rf values of the peaks at 0.22, 0.50 and 0.85.

**Key words:** *Dioscorea bulbifera*, HPTLC, Chromatography, Ethanolic extract, Rf value and Phytoestrogens.

## INTRODUCTION

Plants generally contain primary and secondary metabolites namely alkaloids, terpenoids, flavonoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, amino acids, sugars, proteins etc. These phyto-constituents impart the specific characteristics and properties of plants. Therefore, it is obligatory to resolve all of the phytochemical constituents present in the plants in order to ensure the consistency and repeatability of pharmacological, antimicrobial and clinical research, to understand their bioactivities, identify the active principles (components) and possible side effects of active compounds and to enhance product quality control [1]. These phyto-constituents are estimated quantitatively and qualitatively by a variety of techniques such as spectroscopy and chromatography. Chromatography techniques are the most useful and popular tools used for the qualitative and separation studies. High performance thin layer chromatography (HPTLC) chromatographic fingerprints can be applied for this kind of certification. Finger print analysis by HPTLC has developed into an effective and powerful tool for

linking the chemical constituents' profile of the plants with botanical identity and for estimation of chemical and biochemical markers. [2-10]

*Dioscorea bulbifera*, the Air potato, belongs to yam species. It is also known as Varahi in Sanskrit, Kaachil in Malayalam and Dukkar Kandin Marathi. The Air potato plant is native to Africa and Asia. It is an invasive species in many tropical areas, including Florida.

*Dioscorea bulbifera* is a perennial vine with broad leaves and has two types of storage organs. The plant forms bulbils in the leaf axils of the twining stems, and tubers beneath the ground. These tubers are like small, oblong potatoes, family belong to Solanaceae or Dioscoreaceae, they are edible and cultivated as a food crop, especially in West Africa. It typically climbs to the tops of trees and has a tendency to take over native plants. New plants develop from bulbils that form on the plant, and these bulbils serve as a means of dispersal. The aerial stems of air potato die back in winter season, but resprouting occurs from bulbils and underground tubers. The primary

means of reproduction through bulbils. The fruits are in capsular form. Air potato has been used as a folk remedy to treat conjunctivitis, diarrhoea and dysentery, among other ailments<sup>[12]</sup>.

## MATERIALS AND METHODS

Dried *Dioscorea bulbifera* bulbil was procured from the authorized botanist Dr. Madhukar Reddy of Heritage bionaturals, Habsiguda, Hyderabad. Shade dried samples were grounded to fine powder using pulverizer. The powdered samples were then stored in a refrigerator for further use.

The powdered bulbils of *Dioscorea bulbifera* were extracted using ethanol with gentle stirring for 72 h separately at room temperature. The extracts were then filtered through Whatmann No. 1 filter paper and concentrated using rotaevaporator.

HPTLC studies were performed at ICT, Hyderabad. All the solvents used for HPTLC analysis was obtained from MERCK. The samples (5  $\mu$ L) were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60F-254 (20  $\times$  10 cm with 250  $\mu$ m thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (flavanoids) and the plate was developed in the respective mobile phase up to 90 mm. The Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed as mobile phase for flavanoids. Linear ascending development was carried out in 20 cm  $\times$  10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical constituents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25  $\pm$  2) °C. The developed plate was dried by hot air to evaporate solvents from the plate. After the spray of the flavinoids reagents plates are dried at 100°C in the hot air oven for 3min. The plate was photo-documented at UV 366 nm<sup>[11]</sup>.

## RESULTS

From the preliminary phytochemical evaluation the constituents like Carbohydrates, proteins, fats, steroids, volatile oils, glycosides, flavonoids and

phenolic compounds are present in the ethanolic extract of *Dioscorea bulbifera* bulbil. Various combinations of Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed as mobile phase for flavonoids. TLC Plates dried at 100°C in hot air oven for 3 min. The plate was photo-documented at UV 254 nm. The ethanolic extracts of *Dioscorea bulbifera* bulbil displayed the presence of mainly 3 types of flavanoids and related compounds with major 3 different Rf values ranging from 0.22 to 0.85. The ethanolic extract of *Dioscorea bulbifera* bulbil illustrated the presence of 3 different types of flavonoids with 3 different Rf values of the peaks at 0.22, 0.50 and 0.85.

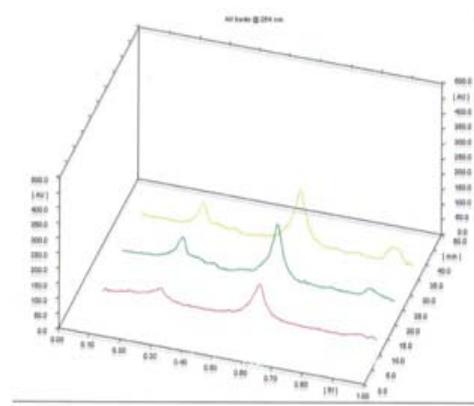


Fig.3. All tracks sample-III  
Absorption/reflectance mode at 254 nm.

Fig 1: 3D display of HPTLC chromatogram of ethanolic extract of *Saraca asoka*

## DISCUSSION

Secondary metabolites are produced by a large variety of organisms, including bacteria, fungi, plants and animals especially by higher plants for their defensive mechanisms to protect themselves from the biotic and abiotic factors. Flavanoids possess lots of pharmacological and pharmaceutical properties and are used as medicines, as recreational drugs, or in entheogenic rituals<sup>13</sup>. The quality and quantity of the alkaloids present in the plants are varied depending on the type of plants and parts or tissue of the plants.

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on the plant, and these bulbils serve as a means of dispersal. The aerial stems of air potato die back in winter season, but resprouting occurs from bulbils and underground tubers. The primary means of reproduction through bulbils. The fruits are in capsular form. Air potato has been used as a folk remedy to treat conjunctivitis, diarrhoea and dysentery, among other ailments<sup>[12]</sup>.

The results of the present study authenticates and confirms the ayurveda usage, traditional practices, ethnobotanical, anti-microbial and pharmacological values of the medicinally important plant *Dioscorea bulbifera* bulbil and suggest that the leaves extracts of *Dioscorea bulbifera* bulbil possess compounds with bioactivity properties that can be used as active principles or agents in new drugs for the therapy of infectious diseases. A recent review proves that the HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology<sup>[13]</sup>. In addition, HPTLC was recommended for identification of the medicinal plants and finds solution for the taxonomical problems<sup>[8-10]</sup>. Similar to the previous observations, in the present study we produced the HPTLC profile for the various organic solvent extracts of in practical use as a pharmacognostical tool to identify this medicinally important plant. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic. Further, the separation and characterization of the bioactive compound (principles) from the plants is to be evaluated and reported in near future.

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