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Physicochemical Evaluation and Development of HPTLC Fingerprint for *Barringtonia acutangula* Linn. Leaf Extract

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Abstract

Barringtonia acutangula L. is a plant traditionally used for the cure and treatment of many ailments. The aim of the current study is to standardize the leaves of *Barringtonia acutangula* for physicochemical parameters, TLC photo documentation along with development of HPTLC fingerprint profiles. The current attempt physico-chemical standardisation, TLC finger print profiles will help to check the quality of the drug prior to its use in traditional medicine.

Keywords
TLC, HPTLC, *Barringtonia acutangula,* Finger print.

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INTRODUCTION

Plants are invaluable sources of pharmaceutical products and plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [1]. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. There is an ever increasing need to limit toxic clinical drugs [2]. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines [3]. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry [4].

Barringtonia acutangula Linn. *(*Family-*Lecythidaceae)* popularly known as *Samudraphal* (Indian Oak in English) in an important medicinal plant of India. It is an evergreen tree of 9-12 m in height common in the sub-Himalayan tracts from the Ganges eastwards to Assam, and in Madhya Pradesh, extending into peninsular India [5]. *Barringtonia acutangula* L. is a plant traditionally used for the cure and treatment of many ailments. In Ayurveda, its root, leaves and fruits are used in the treatment of jaundice, liver disorders, stomach disorders, leprosy and spleenic disorders since many centuries [6]. It is used in the folklore in vitiated conditions of kapha and pitta, leprosy, arthralgia, dysmenorrhea, plumbago, skin disease, diarrhea, inflammation,

flatulence, haemorrhoids as an anthelmintic [7]. Various parts of *Barringtonia acutangula* have been used as a medicine for curing various ailments like hemiplegia, pain in joints, eye diseases, stomach disorders, cough, dyspnoea, leprosy, intermittent fever, spleen disorders and poisoning [8]. In this review, we have attempted to summarize the details available on potency of *Barringtonia acutangula* to explore its therapeutic prospective.

High-Performance Thin-Layer Chromatography (HPTLC) is one type of planar chromatography and most advanced form of instrumental TLC. Now a day, HPTLC is more useful than TLC and HPLC. Because HPTLC is independent of sample application, chromatogram development, detection, etc. it is not only instrumental TLC but entire concept that include widely standardize methodology based on validated method. It is instrument controlled by software. Applications of HPTLC for phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis, and HPTLC future to combinatorial approach, HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser made HPTLC a power analytical tool in the field of analysis. It is noteworthy that utilization of instrumental HPTLC toward the analysis of drug formulations, Bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future. The present study is aimed at standardization of the *Barringtonia acutangula* (BC) leaf extract and development of HPTLC finger profile for quality maintenance.

MATERIALS AND METHODS

Physico-chemical analysis

All the physico-chemical parameters were performed according to the method mentioned in standard books [9, 10].

Sample preparation for HPTLC

Exactly 2 g of coarse leaf powder was taken in a Soxhlet apparatus and extracted with 100 ml each of n-hexane, chloroform and ethanol successively. These extracts were filtered, concentrated over water bath and made up to 10 ml with the corresponding solvents in standard flasks. The extracts were filled in separate sample vials of ATS 4 (CAMAG, Switzerland) for application.

Chemicals, solvents and materials

AR grade solvents n-hexane, chloroform, ethyl acetate, ethanol and formic acid were purchased from Merck. For visualizing purpose vanillin (1 g) sulphuric acid in ethanol (5%) solution (VSA) was used. For HPTLC, automatic sampler 4, twin trough chamber 10 × 10 cm, TLC visualizer, TLC scanner 4, TLC plate heater (all from CAMAG, Switzerland) were used 15 and 20 µl of each extract were spotted using ATS 4 on silica gel 60F_{254} coated aluminium plates (6 × 10 cm) as 8 mm band width with 15 mm inter band distance. First application position was 15 mm and distance from the bottom of the plate was 10 mm. Plates were developed using solvent systems with n-hexane - ethyl acetate - formic acid (7.5:2.5:0.5, v/v/v), toluene - ethyl acetate - formic acid (6.5:4.0:0.5, v/v/v) and chloroform - methanol - formic acid (9:1: 0.8, v/v/v) for n-hexane, chloroform and ethanol extract respectively. The twin trough chamber was previously saturated for 15 min with the above mobile phases prior to development. The developed plates were dried, and photographs were taken by the visualizer under short UV and long UV. Scanning were performed by TLC Scanner 4 (Scanner_210441) under λ 254 and λ 366 nm in absorbance mode (D_2 lamp) and fluorescence mode (Hg lamp) respectively with a slit dimension 6×0.45 mm with scanning speed 20 mm/s. The scanned plates were dipped in VSA and heated over TLC plate heater at 105º C until the appearance of the coloured spots. Photographs were taken immediately at white light mode followed by scanning at λ 520 nm at absorption mode (W lamp).

RESULTS AND DISCUSSION

Physicochemical analysis

The total ash, water soluble ash, acid insoluble ash and sulphated ash of *Barringtonia acutangula* leaves were determined using standard procedures and were found to be 7%, 6.25%, 0.082% and 7.56% w/w respectively. Ash values of a drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values [**Table 1**] of the leaf powder of *B. acutangula* revealed a high concentration of sulphated ash. The extractive values are primarily useful for the determination of exhausted or adulterated drug [11].

Sample preparation for photo documentation:

2 g plant sample was extracted with 20 ml of ethanol, concentrated and made up to 10 ml. 10 and 15µl of extract was applied on TLC plate and developed to a distance of 8 cm using Toluene: Ethyl acetate: Formic acid (7:3:0.5) as mobile phase. After development, the plate was photo documented under UV 254 nm and UV 366 nm under a Visualizer. Then scanned at UV 254 nm (Absorption mode and Deuterium lamp) and UV 366 nm (Absorption mode and Mercury lamp). After dipping the plate in vanillin–sulphuric acid reagent followed by heating at 105°C till development of colour, the plate was first photo

documented in white light under the Visualizer and then scanned at 520 nm (Absorption mode and Tungsten lamp). TLC Photo documentation of ethanol extract of *B. acutangula* shown in **Fig.1**.

HPTLC of ethanol extract of *Barringtonia acutangula*:

The results from HPTLC finger print scanned at wavelength 254 nm (Track 1) for ethanol extract of *Barringtonia acutangula* leaf extract showed the presence of 6 components with their R_f value: R_f – 0.07, 0.12, 0.16, 0.31, 0.51, 0.90. Component number 6 at R_f 0.90 showed maximum concentration of 38.64% (**Fig.2a**); scanned at wavelength 254 nm (Track 2) showed the presence of 7 components with their R_f value: R_f – 0.07, 0.12, 0.17, 0.31, 0.51, 0.85, 0.90. Component number 1 at R_f 0.07 showed maximum concentration of 38.13% (**Fig.2b**). Overall 3D Chromatogram under 254 nm (Track 1 and Track 2) HPTLC fingerprint profile of BC extract shown in **Fig.3**.

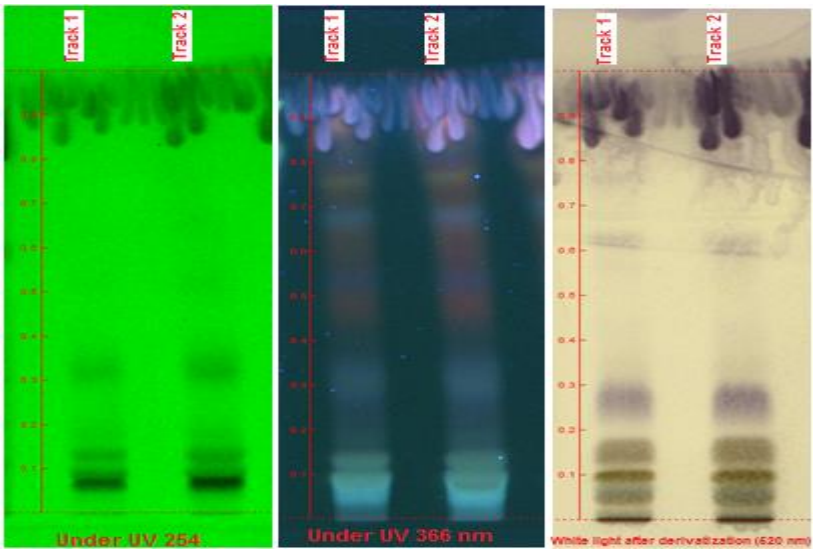
The results from HPTLC finger print scanned at wavelength 366 nm (Track 1) for ethanol extract of *Barringtonia acutangula* leaf extract showed the presence of 7 components with their R_f value: R_f – 0.05, 0.28, 0.66, 0.78, 0.85, 0.88, 0.90. Component number 7 at R_f 0.90 showed maximum concentration of 25.88% (**Fig.4a**); scanned at wavelength 366 nm (Track 2) showed the presence of 9 components with their R_f value: R_f – 0.04, 0.08, 0.28, 0.48, 0.63, 0.65, 0.75, 0.85, 0.90. Component number 9 at R_f 0.90 showed maximum concentration of 36.08% (**Fig.4b**). Overall 3D Chromatogram under 366 nm (Track 1 and Track 2) HPTLC fingerprint profile of BC extract shown in **Fig.5**.

The results from HPTLC finger print scanned at wavelength 520 nm (Track 1) for ethanol extract of *Barringtonia acutangula leaf* extract showed the presence of 13 components with their R_f value: R_f – 0.04, 0.09, 0.15, 0.26, 0.41, 0.54, 0.59, 0.65, 0.73, 0.75, 0.81, 0.88, 0.90. Component number 4 at R_f 0.26 showed maximum concentration of 21.15% (**Fig.6a**); scanned at wavelength 520 nm (Track 2) for showed the presence of 16 components with their R_f value: R_f – 0.04, 0.09, 0.16, 0.26, 0.41, 0.51, 0.54, 0.58, 0.60, 0.65, 0.67, 0.69, 0.72, 0.76, 0.84, 0.90. Component number 4 at R_f 0.26 showed maximum concentration of 26.41% (**Fig.6b**). Overall 3D Chromatogram under 520 nm (Track 1 and Track 2) HPTLC fingerprint profile of BC extract shown in **Fig.7**.

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. These methods were also employed to analyze commercial samples to illustrate their application in qualitative (fingerprint) and quantitative determination, demonstrating their feasibility in the quality control of phytoconstituents from mentioned Herbal drugs and formulations. This will help induced to come out uniform standard products, which will restore faith of product and alternative herbal medicine therapy.

Table 1: Physicochemical constants of leaf powder of *B. acutangula*

Sl.No.	Parameters	% w/w
1	Total ash	7
2	Water soluble ash	6.25
3	Acid insoluble ash	0.082
4	Sulphated ash	7.56



Solvent system: Chloroform: Methanol: Formic acid (8:2:0.5)
Track 1- B.*acu* ethanol extract- 10µl,
Track 2- B.*acu* ethanol extract- 15µl
Fig.1: TLC Photo documentation of ethanol extract of BC

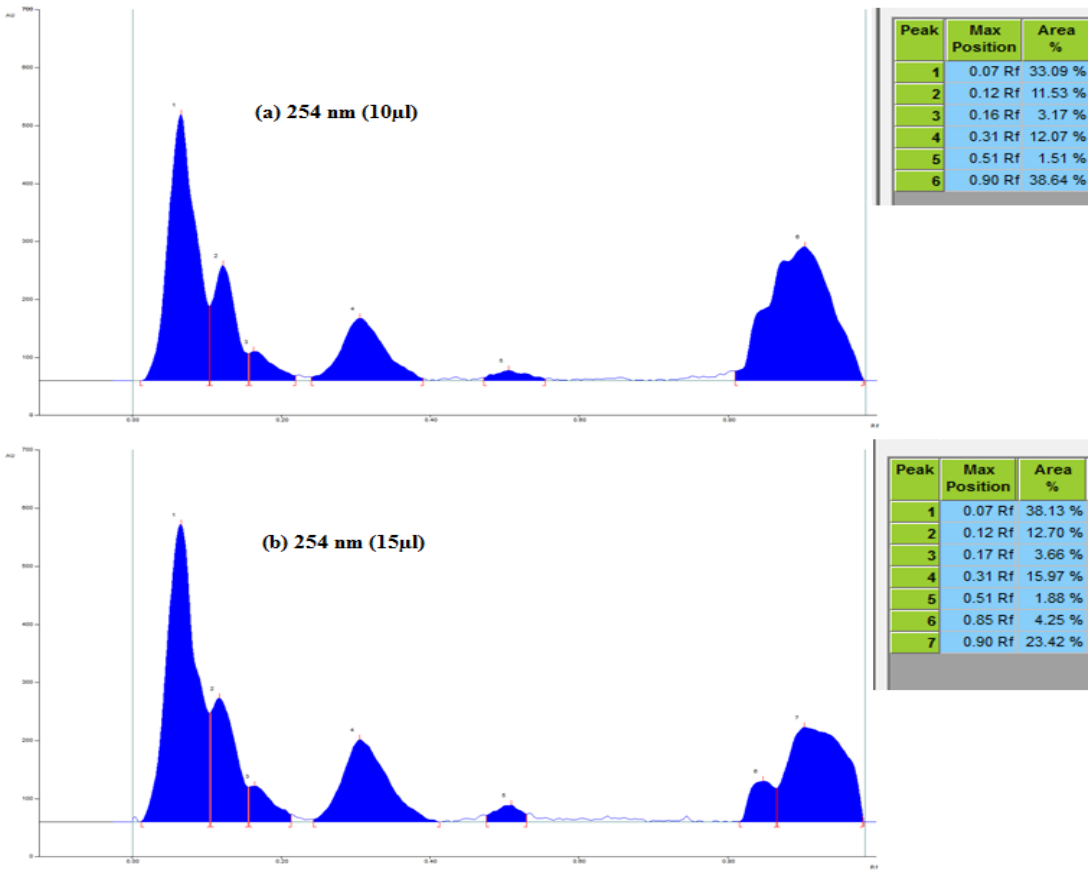


Fig.2: HPTLC fingerprint profile of BC extract scanning at 254 nm (a) Track 1 (10 µl) (b) Track 2 (15 µl)

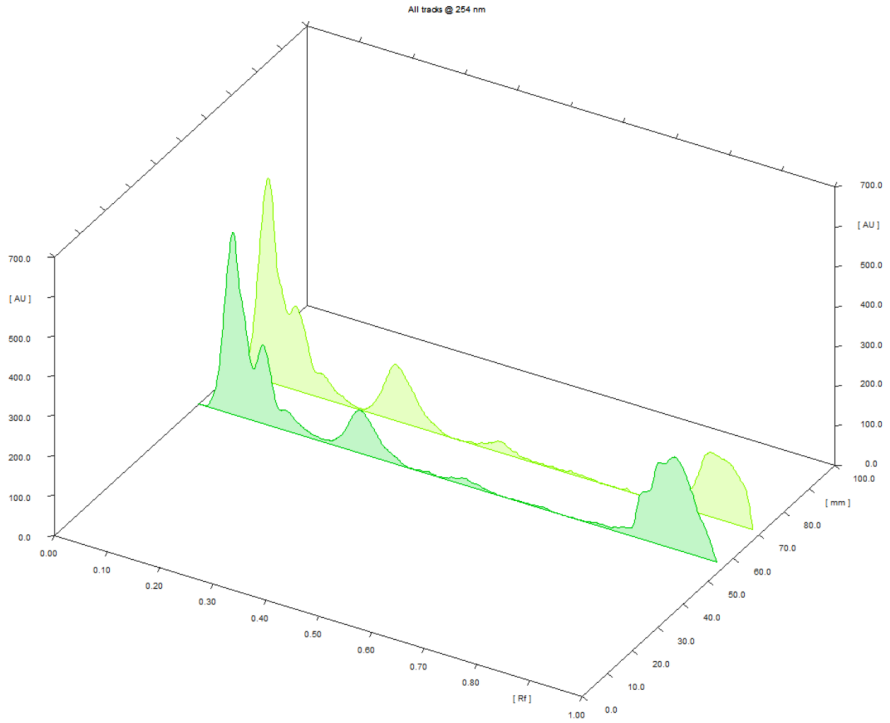
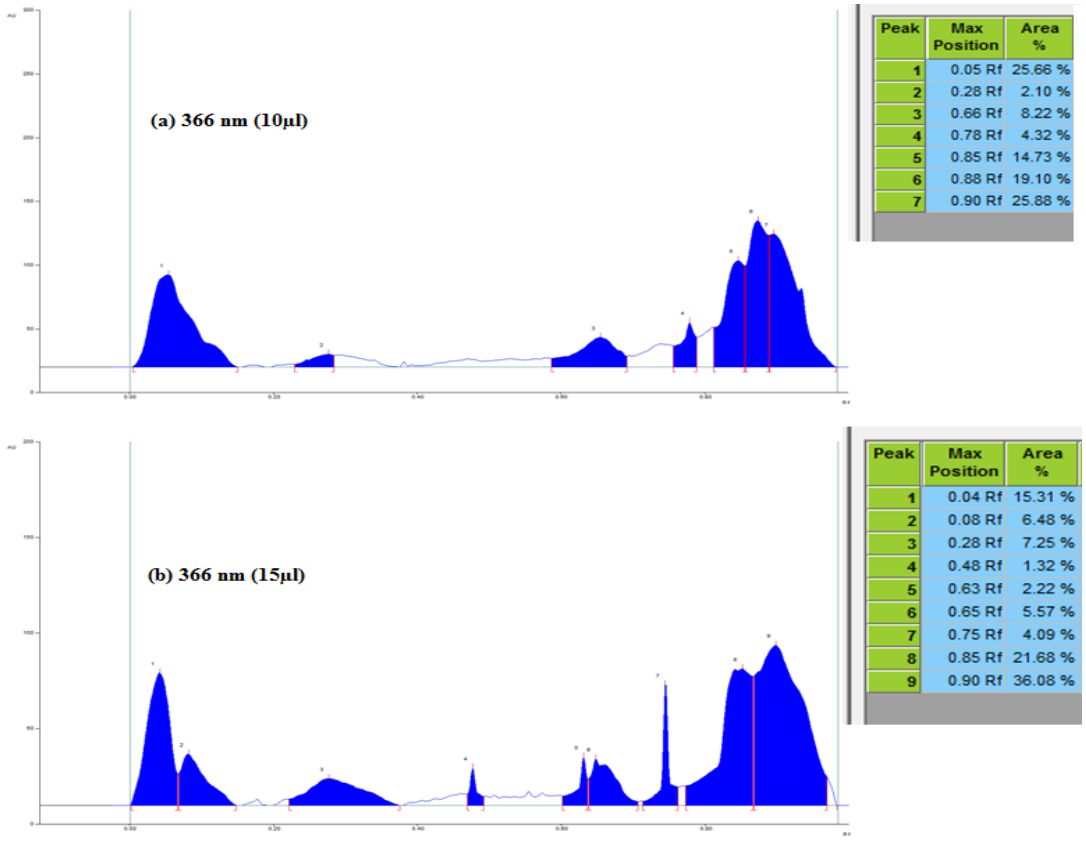


Fig.3: 3D Chromatogram under 254 nm (BC extract)



Peak	Max Position	Area %
1	0.05 Rf	25.66 %
2	0.28 Rf	2.10 %
3	0.66 Rf	8.22 %
4	0.78 Rf	4.32 %
5	0.85 Rf	14.73 %
6	0.88 Rf	19.10 %
7	0.90 Rf	25.88 %

Peak	Max Position	Area %
1	0.04 Rf	15.31 %
2	0.08 Rf	6.48 %
3	0.28 Rf	7.25 %
4	0.48 Rf	1.32 %
5	0.63 Rf	2.22 %
6	0.65 Rf	5.57 %
7	0.75 Rf	4.09 %
8	0.85 Rf	21.68 %
9	0.90 Rf	36.08 %

Fig.4: HPTLC fingerprint profile of BC extract scanning at 366 nm (a) Track 1 (10 µl) (b) Track 2 (15 µl)

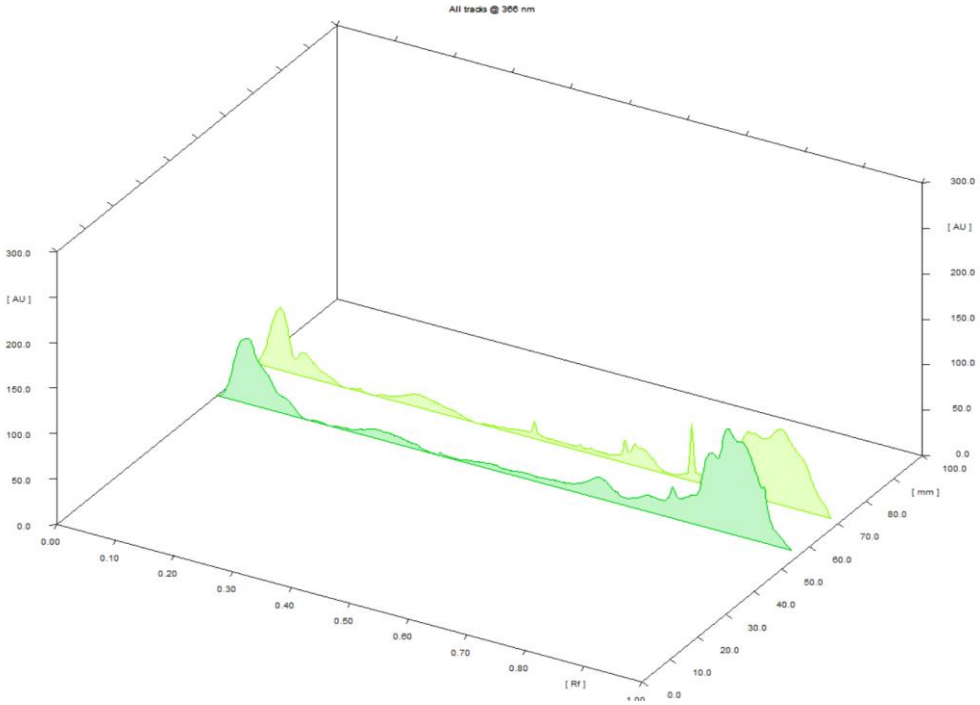
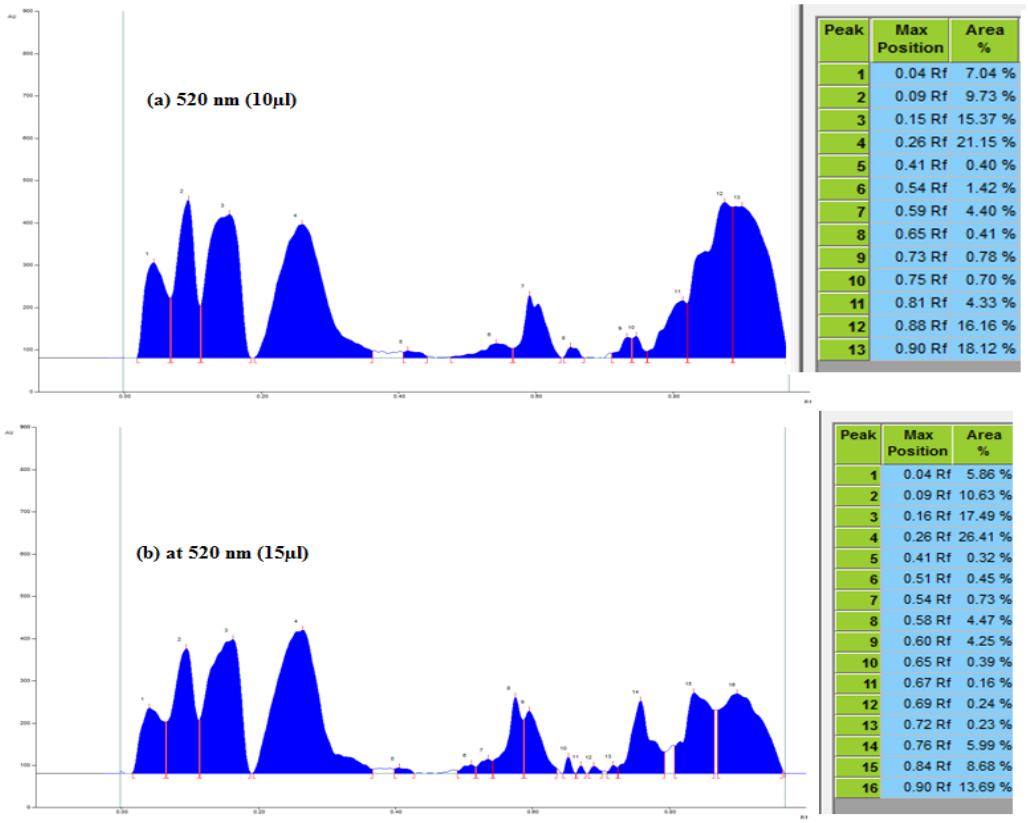


Fig.5: 3D Chromatogram under 366 nm (BC extract)



Peak	Max Position	Area %
1	0.04 Rf	7.04 %
2	0.09 Rf	9.73 %
3	0.15 Rf	15.37 %
4	0.26 Rf	21.15 %
5	0.41 Rf	0.40 %
6	0.54 Rf	1.42 %
7	0.59 Rf	4.40 %
8	0.65 Rf	0.41 %
9	0.73 Rf	0.78 %
10	0.75 Rf	0.70 %
11	0.81 Rf	4.33 %
12	0.88 Rf	16.16 %
13	0.90 Rf	18.12 %

Peak	Max Position	Area %
1	0.04 Rf	5.86 %
2	0.09 Rf	10.63 %
3	0.16 Rf	17.49 %
4	0.26 Rf	26.41 %
5	0.41 Rf	0.32 %
6	0.51 Rf	0.45 %
7	0.54 Rf	0.73 %
8	0.58 Rf	4.47 %
9	0.60 Rf	4.25 %
10	0.65 Rf	0.39 %
11	0.67 Rf	0.16 %
12	0.69 Rf	0.24 %
13	0.72 Rf	0.23 %
14	0.76 Rf	5.99 %
15	0.84 Rf	8.68 %
16	0.90 Rf	13.69 %

Fig.6: HPTLC fingerprint profile of BC extract scanning at 520 nm (a) Track 1 (10 µl) (b) Track 2 (15 µl)

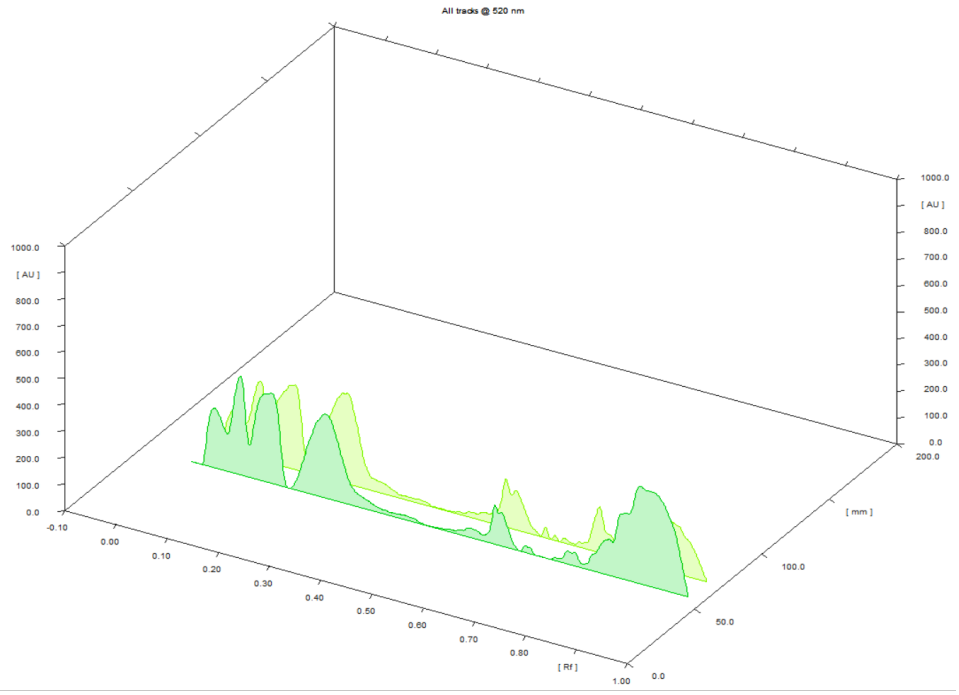


Fig.7: 3D Chromatogram under 520 nm (BC extract)

CONCLUSION

The current attempt of physico-chemical standardisation, HPTLC finger print profiles will help to check the quality of the drug prior to its use in traditional medicine. HPTLC chromatogram of ethanolic extract results showed that there are many compounds in *Barringtonia acutangula* leaf extract. From the HPTLC studies, it has been found that ethanol extracts containing mixture of compounds and so it is established that the pharmacological activity shown by them are due to the cumulative effect of all the compounds in composite.

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