Elucidation of β-sitosterol from *Benincasa hispida* Seeds, *Carissa congesta* Roots and *Polyalthia longifolia* Leaves by High Performance Thin Layer Chromatography

Gaurav Mahesh Doshi[1,2*], Pratip Kashinath Chaskar[1] and Hemant Devidas Une[3]

[1]*Department of Pharmacology and Pharmaceutical Chemistry, Vivekanand Education Society's College of Pharmacy, Mumbai, India*

[2]*Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India*

[3]*Department of Pharmacology, Y. B. Chavan College of Pharmacy, Rouzabagh, Aurangabad, Maharashtra, India.*

ABSTRACT

Background: Fruits of *Benincasa hispida* (BH) is regarded as Valliphala due to its vast plethora of medicinal properties, *Carissa congesta* (CC) is an imperative local plant particularly in rural communities and *Polyalthia longifolia* (PL) is an ornamentally significant traditionally relevance plant in India system. β-sitosterol, an active constituent identified from enormous plants has been reported to possess excellent amount of pharmacotherapeutic potential by number of researchers. **Objective:** In the recent studies, the research team focuses on determining the percentage of the β-sitosterol present in the BH seeds, CC roots petroleum ether extracts as well as PL leaves ethanolic extract by chromatographic technique in harmony with High Performance Thin Layer Chromatography. **Materials and Methods:** Respective parts of BH, CC and PL plants were shade-dried and extracted by appropriate extraction methods followed by identification of β-sitosterol from the extracts by High Performance Thin Layer Chromatography after preliminary phytochemical screening extracts for the constituents. **Results:** The amount of β-sitosterol present in the BH seeds, CC roots and PL leaves extracts was found to be 23.00, 5.94 and 1.81 % w/w respectively. Research studies elucidated a peak that coincided with standard peak of β-sitosterol suggesting the presence of constituent in the extracts. **Conclusion:** Thus, extracts contains important constituent of β-sitosterol in BH, CC and PL.

Key words: *Benincasa hispida*, *Carissa congesta*, HPTLC, *Polyalthia longifolia*, β-sitosterol.

INTRODUCTION

Plant kingdom is regarded as an enormous reservoir of biologically active molecules but least fraction of plants with medicinal importance and activity are explored until date. Phytochemistry is one of the burgeoning fields of research, which has providing immense tool development in correlating data of the plant extracts. The progress has aided immensely by the development of rapid and accurate methods of screening plants for particular constituents present in the extracts.[1] Isolated and characterized phytoconstituents present in the plants have paved the way in contributing to healthcare and income to the consumer as well as government in terms of well- being and cheap price respectively.[2]

Benincasa hispida (Thunb.) Cogn. has been reported as Kushmanda Avaleha, Vasakhanda, Khanda and Rasayana in classical medicine in India.[3,4] Rasayana properties of this plant species is attributed in offering immune protection and is advised during the later years of life at forty-five in

***Corresponding author:**
Prof. Gaurav Mahesh Doshi
Assistant Professor,
Department of Pharmacology and Pharmaceutical Chemistry,
Vivekanand Education Society's College of Pharmacy,
Mumbai, India.
Phone No : +91-9819771515
E-mail: gaurav.pharmacology@gmail.com

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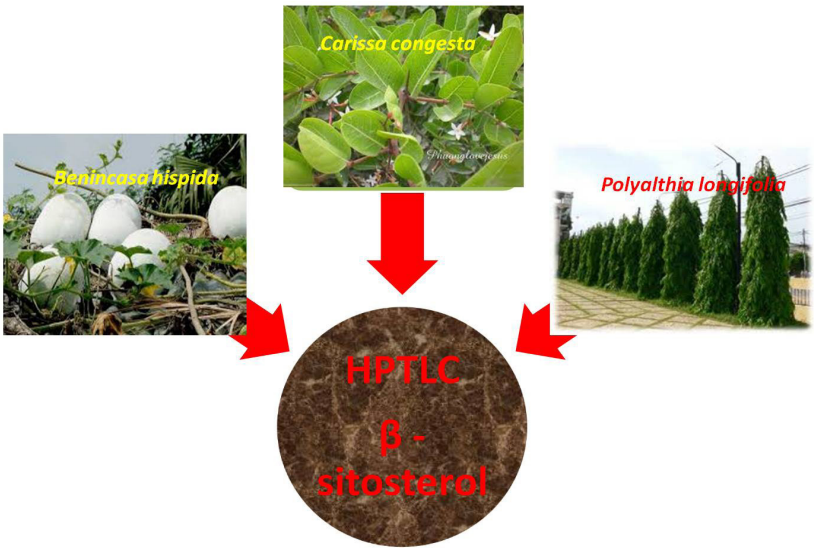


Graphical Abstract

Uses: *Benincasa hispida*, *Carissa congesta* (CC) and *Polyalthia longifolia* are significantly important plant in India system. These plants are well known among trial communities. The plants were shade-dried and extracted by appropriate extraction methods followed by identification of β-sitosterol from the extracts by High Performance Thin Layer Chromatography.

both the sexes.[5] The fruits has been found to contain ample quantity of proteins, enzymes, vitamin B_1 and C, flavonoid C-glycoside, terpenes, phenolic acids and free sugars as glucose, rhamnose, mannitol, uronic acid, trace metals, peptic polysaccharides (sequential extraction)[6,7] whereas phenolic compounds as astilbin, catechin and naringenin (high-speed counter current chromatography).[8-10]

Carissa congesta is well known in local people in India among the tribal communities due to its multiple properties as well as whole plant is powdered, mixed with cow's milk and given in diabetes.[11,12] The plant has been known to produce pentacyclic triterpenoids and glycosides yielding oderside H, digitoxigenin and sugars like D-glucose and D-digitalose.[13] The roots have yielded a various volatile principles like 2-acetylphenol, lignans as carinol, and a mixture of sesquiterpenes as carissone and carindone. Des-n-methylnoracronycine has been identified with constituents such as lupeol.[14-16]

Polyalthia longifolia is has common flowering plant in India with leaves used mostly in traditional medicine as an aromatic and essential oils due to its usefulness in various disorders.[17-19] Numbers of chemical constituents has been identified from the leaves such as azafluorene alkaloid and three new Aporphine N-oxide alkaloids.[20] Activity guided fractionation has elucidated presence of quercetin, quercetin-3-o-β-glucopyranoside, kaemperfol-3-o-α-rhamnopyranosyl-β-glucopyranoside,kaempferol-3-o-α-rhamnopyranosyl-(1-6)-β-gluco-pyranoside, rutin, β-sitosterol and allantoin from butanol fraction. Ethanolic extract has been found to contain bulbocapnin and steroids like β-sitosterol, stigmasterol and campesterol constituents.[21]

However, literature reports several methods for scrutinaztion of β-sitosterol from various plants species, no studies on HPTLC guided fraction for β-sitosterol from these plant extracts has been noticed. Selection of BH, CC and PL species for present research studies was based on its easily availability in local market and enormous pharmacological potential. Minute doses of β-sitosterol have been reported to increase the in vitro proliferative activity of T-lymphocytes as well as acting as chemopreventive agent in colon and breast cancer.[22,23] Taking pictograph of above viewpoints, our research article focuses on β-sitosterol percentage estimation in the BH seeds, CC roots petroleum ether, and further on PL leaves ethanolic extracts.

EXPERIMENTAL

Part A: Collection, authentication and extraction

All the studies undertaken in Part A have been previous reported by us in identification of lupeol from these extracts.[24-26]

Part B: Reagents and Biomarker

Standard biomarker used for identification purpose in chromatographic studies was procured from Sigma-Aldrich Private Ltd., Bangalore, India, and solvents from Merck Ltd., Mumbai, India.

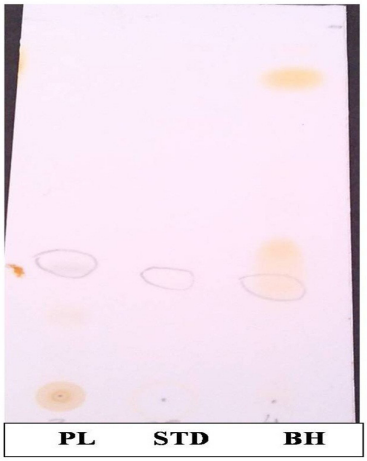


Figure 1: TLC of *Benincasa hispida* seeds and *Polyalthia longifolia* leaves extracts

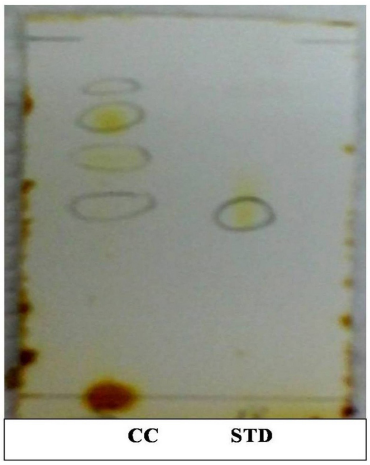


Figure 2: TLC of *Carissa congesta* for β-sitosterol root extract

Part C: Instrumentation

- *Instrument*: CAMAG Linomat 5
- Linomat 5 application parameters
  - *Spray gas*: Inert gas
  - *Sample solvent type*: Ethanol
  - *Dosage speed*: 100 nl/s
  - *Predosage volume*: 0.2 µl
- Sequence
  - *Syringe size*: 100 µl
  - *Number of tracks*: 10
  - *Application position Y*: 12.0 mm
  - *Band length*: 8.0 mm
- Calibration parameters
  - *Calibration mode*: Single level
  - *Statistics mode*: CV
  - *Evaluation mode*: Area and peak height

METHODS

Our research studies enlist the same flow pattern as we have reported in our previous studies for lupeol identification in these extracts. Here, we have estimated percentage of β-sitosterol present as an active constituent in these extracts by Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC).[24-26]

(a) Thin Layer Chromatography (TLC)

Mobile Phase: Hexane: Ethyl Acetate (8.3:1.7-BH and PL) and (7: 3-CC)

Dilution: The standard and the sample were dissolved in ethanol and was filtered using Whatman Filter paper no. 41 before spotting on TLC plate.

Saturation: Chamber was saturated for 30 mins.

(b) High Performance Thin Layer Chromatography (HPTLC)

The HPTLC was performed at Radiant Research Services Private Limited, Bangalore, India. The HPTLC plates (20x10 cm) coated with silica gel 60 F254 were used and scanning of the developed plates was performed at 336 nm (before derivatization) and 550 nm (after derivatization). The standard and the sample were prepared by dissolving 5.16 mg and 47.5 mg in 10 ml of ethanol each. Spots of 3 µg/l, 6 µg/l, 9 µg/l and 12 µg/l were applied on plates per extract.

Percentage of β-sitosterol = sample area × standard dilution × purity × 100/ standard area × sample dilution × 100

RESULTS

(a) Extraction and Preliminary phytochemical analysis

The results of extraction yield and preliminary analysis of the plant extracts were been previously reported by us. [24-26]

(b) Thin Layer Chromatography (TLC)

In TLC, Chromatographic analysis of extracts revealed that Rf value for standard β-sitosterol and the extracts (BH, CC and PL) were found to be 0.72 to 0.77 which confirms the presence of the constituent in these plants (Figures 1 and 2).

(c) High Performance Thin Layer Chromatography (HPTLC)

HPTLC reports interpreted that BH, CC and PL extracts showed well-resolved spots at tracks 5 and 6 in

Table 1: HPTLC analysis of *Benincasa hispida* and standard β-sitosterol

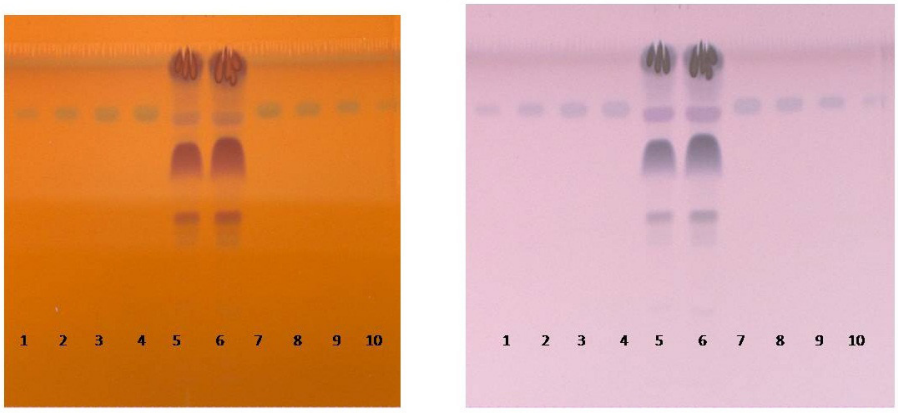
Track No	Details	R_f Value	Area
1	β-sitosterol standard (3 µg/l)	0.74	1297.3
2	β-sitosterol standard (6 µg/l)	0.74	2240.1
3	β-sitosterol standard (9 µg/l)	0.73	2620.9
4	β-sitosterol standard (12 µg/l)	0.73	2784.4
5	*Benincasa hispida* petroleum ether extract (6 µg/l)	0.72	4476.6
6	*Benincasa hispida* petroleum ether extract (9 µg/l)	0.72	4155.6
7	β-sitosterol (12 µg/l)	0.74	2767.4
8	β-sitosterol (9 µg/l)	0.74	2386.8
9	β-sitosterol (6 µg/l)	0.75	1923.0
10	β-sitosterol (3 µg/l)	0.76	891.5

Table 2: HPTLC analysis of *Carissa congesta* roots extract and standard β–sitosterol

Track No	Details	R_f Value	Area
1	β–sitosterol Standard (3 µg/l)	0.75	2215.5
2	β–sitosterol Standard (6 µg/l)	0.75	3383.8
3	β–sitosterol Standard (9 µg/l)	0.74	3924.8
4	β–sitosterol Standard (12 µg/l)	0.73	4634.4
5	*Carissa congesta* petroleum ether extract (6 µg/l)	0.73	2123.1
6	*Carissa congesta* petroleum ether extract (9 µg/l)	0.73	2603.9
7	β–sitosterol Standard (12 µg/l)	0.73	4695.7
8	β–sitosterol Standard (9 µg/l)	0.73	4226.0
9	β–sitosterol Standard (6 µg/l)	0.74	3597.7
10	β–sitosterol Standard (3 µg/l)	0.74	2465.7

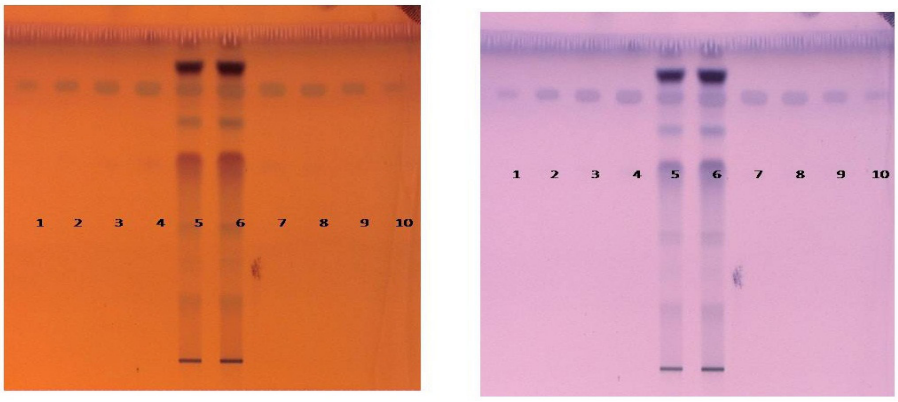
Table 3: HPTLC analysis of *Polyalthia longifolia* leaves extract and standard β–sitosterol

Track No	Details	R_f Value	Area
1	β–sitosterol Standard (3 µg/l)	0.74	2184.2
2	β–sitosterol Standard(6 µg/l)	0.74	2937.1
3	β–sitosterol Standard (9 µg/l)	0.76	3143.2
4	β–sitosterol Standard (12 µg/l)	0.73	3598.5
5	*Polyalthia longifolia* leaves extract (6 µg/l)	0.77	538.9
6	*Polyalthia longifolia* leaves extract (9 µg/l)	0.77	638.7
7	β–sitosterol Standard (12 µg/l)	0.72	3739.8
8	β–sitosterol Standard (9 µg/l)	0.74	3473.7
9	β–sitosterol Standard (6 µg/l)	0.72	2977.6
10	β–sitosterol Standard (3 µg/l)	0.74	2307.4



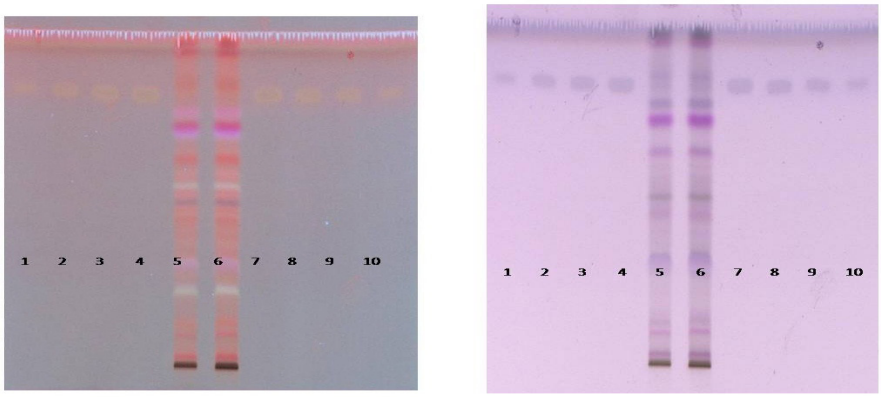
HPTLC Image before derivatization HPTLC Image after derivatization

Figure 3: HPTLC chromatograms of *Benincasa hispida* seeds and standard for β-sitosterol



HPTLC Image before derivatization **HPTLC Image after derivatization**

Figure 4: HPTLC chromatograms of *Carissa congesta* roots and standard for β-sitosterol



HPTLC Image before derivatization **HPTLC Image after derivatization**

Figure 5: HPTLC chromatograms of *Polyalthia longifolia* leaves and standard for β-sitosterol

comparison to the standard at tracks 1, 2, 3, 4, 7, 8, 9 and 10 per extract. The Rf value were found to be equal with standard β-sitosterol [start-0.72, maximum-0.75 and end 0.79 (BH), start–0.73, maximum-0.75 and end-0.79 (CC) and start–0.77, maximum-0.80 and end-0.80 (PL)]. (Table 1, 2, 3, and Figures 3-12, 15-24, 27-36). The amount of β-sitosterol present was 23.00, 5.94, 1.81% w/w (*i.e.* 10.86 (BH), 2.96 mg (CC) mg and 0.89 mg (PL) of β-sitosterol present in 47.5 mg of extracts respectively according to the formula). The images were obtained at 336 nm and 550 nm before and after derivatization respectively releaved the presence of these constituent in the extracts. (Table 1 2 and 3; Figures 3, 4 and 5).

DISCUSSION

A plant extract contains vast plethora of phytoconstituents correlated to their enormous pharmacological activity. Chromatography means "colour writing" which is a useful analytical method of organic and inorganic substances, fractionation of complex mixtures, separation of closely related compounds and in the isolation of unstable substances. Qualitative measurements on plant extracts are determinations of amounts of components present in it. Quantitative measurements involve determination of amount of individual components within a particular class of constituent by gas liquid chromatography or high performance liquid chromatography.[1,27-32]

The technique uses TLC plate coated with fine microparticles of silica is termed as HPTLC. The analytical technique helped us in separating and identification of the active β-sitosterol component from these extracts. HPTLC is sophisticated powerful visualization technique preferred in the detection of the multiple phytoconstituents from herbal extracts due to its reliability, rapidity, surety, reproducibility and linearity.[27] Large number of samples could be analyzed

in a single run simultaneously. The analytical technique selected is specific and solution stable, provides linearity of 0.999 and 99.24% of sample.[1,33-35] Thus, the results depicted in TLC and HPTLC experimentation played a vital role in our research helping us to identify the β-sitosterol with their percentage in the plant extracts.

CONCLUSION

β-sitosterol has been noticed to play an important role in various plant extracts signifying its pharmacotherapeutic potential. Our research studies directs the field of the pharmacognosist budding professionals towards the identified constituent in the these extracts of BH, CC and PL which were confirmed by simple methods like TLC and HPTLC. The current research studies paves the pathway for research thrust scientists to consider the other probable identified constituents in the extracts and correlate there utilities on pharmacological models of different therapeutic categories.

CONFLICT OF INTEREST

We declare no conflict of Interest.

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Highlights of Paper

- Fruits of *Benincasa hispida* (BH) is regarded as Valliphala , *Carissa congesta* roots (CC) are important in rural communities and *Polyalthia longifolia leaves* (PL) is an ornamentally flowering plant in India system.
- In the recent studies, the research team determined the percentage of the β-sitosterol present in the BH, CC and PL by High Performance Thin Layer Chromatography.
- The amount of β-sitosterol present in the BH seeds, CC roots and PL leaves extracts was found to be 23.00, 5.94 and 1.81 % w/w respectively. The extracts showed peak that coincided with standard peak of β-sitosterol.

Author Profile

- **Mr. Gaurav Doshi:** presently working as Assistant Professor, Department of Pharmacology, Vivekanand Education Society's College of Pharmacy, Chembur (East), Mumbai- 400074. He has 22 nos. of journal paper; 53 citations to his credit. He is Ph.D Research Scholar of Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

- **Pratip Kashinath Chaskar:** presently working as Assistant Professor, Department of Pharmaceutical Chemistry, Vivekanand Education Society's College of Pharmacy, Chembur (East), Mumbai- 400074. He has 15 nos. of journal paper; 24 citations to his credit.

- **Hemant Devidas Une:** Working as Associate Professor, Vice- Principal, Head of Department of Pharmacology, Y. B. Chavan College of Pharmacy, Rouzabagh, Aurangabad, Maharashtra, India. He has 18 nos. of journal paper; 83 citations to his credit.

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