

# Revelation of $\beta$ -sitosterol from *Benincasa hispida* Seeds, *Carissa congesta* Roots and *Polyalthia longifolia* Leaves by High Performance Liquid Chromatography

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## ABSTRACT

**Background:** Fruit juice of *Benincasa hispida* (BH) is regarded as *Vrindamadhava* that is recommended for internal use in snake's bites. The fruits have been reported to contain good amount of proteins, enzymes, Vitamin B<sub>1</sub> and C, flavonoid C-glycoside, terpenes, phenolic acids and free sugars such as glucose, rhamnose, mannitol, uronic acid, astilbin, catechin, naringenin, pectic polysaccharides and even some trace metals. *Polyalthia longifolia* (PL) has been well known for its ayurvedic preparations such as *Sitaphala kula*, *Kaphapitashama*, *Anulomak* and *Krimighna*. PL leaves has been reported to contain phytoconstituents like polylongine, Aporphine-N-oxide alkaloids, allo-aromadendrene, caryophyllene oxide,  $\beta$ -caryophyllene,  $\beta$ -selinene,  $\alpha$ -humulene,  $\alpha$ -pinene and camphene. *Carissa congesta* (CC), known as *Bengal currant*, have yielded 2-acetyl phenol, carissone, carindone and Des-*n*-methylnoracronycine.  $\beta$ -sitosterol, an important phytoconstituent recognized from all these selected plants has been well known for its ethnopharmacological importance. In this new study, the research team members has focused on determining the percentage of the  $\beta$ -sitosterol present in the BH seeds, CC roots and PL leaves by subjecting the ex-

tract to High Performance Liquid Chromatography (HPLC). **Materials and Methods:** BH, CC and PL plants were shade-dried and extracted by suitable extraction methods. In HPLC, peaks obtained in the extracts were compared with the standard by matching their retention time. **Results:** The amounts of  $\beta$ -sitosterol present in the BH seeds, CC roots and PL leaves extracts obtained at 254 nm were found to be 36.00, 7.46 and 3.21% w/w respectively. **Conclusion:** Thus, BH, PL and CC extracts were said to contain  $\beta$ -sitosterol as a key constituent.

**Key words:** *Benincasa hispida*, *Carissa congesta*, HPLC, *Polyalthia longifolia*,  $\beta$ -sitosterol.

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## INTRODUCTION

In today's scenario, currently much of the phytochemical research is propagated in higher plants and shrubs. Crude plant extracts were initially assayed for their particular phytoconstituents as well as active fractions. The targets are thought to have immense potential in health care system.<sup>1</sup> The major advantage of the phytochemistry in correlation to HPLC is to carry out the investigation even if the component present in the extract is present in minute amounts.<sup>2</sup>

*Benincasa hispida* has been stated in Indian system of medicine as *Brihatrayee* and *Bhavaprakasha nighantu*.<sup>3,4</sup> It has been reported to contain proteins, enzymes, vitamin B<sub>1</sub> and C, flavonoids, terpenes, phenolic acids, glucose, rhamnose, mannitol, uronic acid as well as trace metals. Sequential extraction and high-speed counter current chromatography have yielded components of peptic polysaccharides and astilbin, catechin and naringenin respectively.<sup>5-10</sup>

In ayurveda, *Polyalthia longifolia* is well known its preparations such as *Kula*, *Sitaphala kula*, *Kaphapitashama*, *Anulomak*, *Krimighna*, *Pramehahara* and many other allied remedies.<sup>11-13</sup> Various constituents have been identified from the leaves like as azafluorene and Aporphine N-oxide alkaloids.<sup>14</sup> PL has been reported for the presence of flavonoids and other associated constituents such as quercetin-3-*o*- $\beta$ -glucopyranoside, kaempferol-3-*o*- $\alpha$ -rhamnopyranosyl- $\beta$ -glucopyranoside and kaempferol-3-*o*- $\alpha$ -rhamnopyranosyl-(1-6)  $\beta$  gluco pyranoside.<sup>15</sup> Ethanolic extract has showed the presence of triterpenoids components.<sup>10</sup>

*Carissa congesta* is well known as *Bengal currant*, Christ's thorn, *Karaunda*.<sup>16</sup> The roots have yielded volatile components such as 2-acetylphenol, lignans like carinol and mixtures of sesquiterpenes as carissone and carindone. In addition, Des-*n*-methylnoracronycine and lupeol have been reported.<sup>17-20</sup>

Though numbers of researchers have scrutinized presence of  $\beta$ -sitosterol by various chromatographic techniques, no studies on HPLC reported to guide fractions for  $\beta$ -sitosterol from these plant extracts.  $\beta$ -sitosterol has been reported to enhance the *in vitro* proliferative activity of T-lymphocytes and to act as chemopreventive agent in colon and breast cancer.<sup>21,22</sup> Taking snapshot of above lookouts, our research article focuses on estimation of  $\beta$ -sitosterol estimation in the petroleum ether extracts of BH seeds and CC roots and ethanolic extract of PL leaves ethanolic extract by one of the most precise and sensitive method i.e. HPLC.

## EXPERIMENTAL

### Part A: Collection, authentication and extraction

All the studies undertaken in Part A have been previous been reported by us when we subjected our extracts to Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC).<sup>10,23-25</sup>

### Part B: Reagents and Biomarker

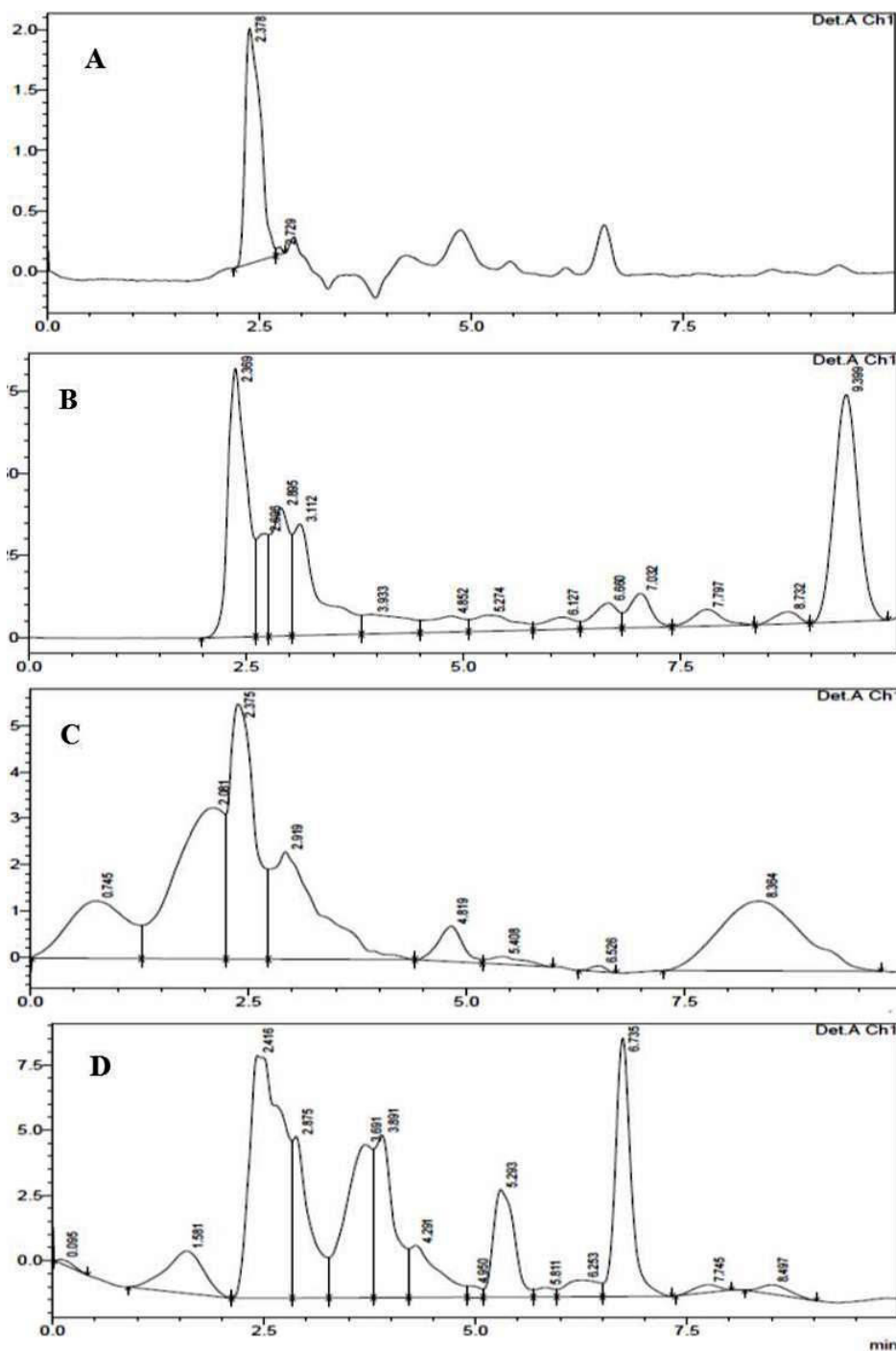
Standard biomarker of  $\beta$ -sitosterol was procured from Sigma-Aldrich Private Ltd., Bangalore, India, and solvents from Merck Ltd., Mumbai, India.

### Part C: Instrumentation

- **Chemicals:**  $\beta$ -sitosterol Standard, buffers, ethanol, plant extracts of PL, BH and CC were used for the study.
- **HPLC instrument make:** Shimadzu LC-10 ATVP
- **Software:** Chromtech N 2000 data
- **Detector:** UV Wavelength 254 nm.
- **Flowrate:** 1 ml/min
- **Injection volume:** 20  $\mu$ l

**Table 1: HPLC analysis of *Benincasa hispida*, *Carissa congesta*, *Polyalthia longifolia* and standard  $\beta$ -sitosterol**

Details of standard and sample	Observation Parameter (Ret Time) Area	% of $\beta$ -sitosterol
BH extract	(2.369)1232761	36.00% w/v
CC extract	(2.416) 255510	7.46% w/v
PL extract	(2.375) 109900	3.21% w/v
Standard $\beta$ -sitosterol	(2.378) 3081902	
Dilution ratio (standard: sample)	1:1	



**Figure 1:** HPLC peaks of standard  $\beta$ -sitosterol (A), *Polyalthia longifolia* leaves (B), *Benincasa hispida* seeds (C) and *Carissa congesta* roots (D).

- Column dimensions: RP C-18, 250×4.6 mm, 5  $\mu$

## MATERIALS AND METHODS

In the current research article, our studies focuses on HPLC were done after we have reported identification of  $\beta$ -sitosterol in our previous studies by TLC and HPTLC.<sup>24-26</sup> The procedure comprises of Methanol: Phosphate Buffer (pH 3) as mobile phase in ratio 70:30. 2 mg of standard and 5 mg of the sample were dissolved in 2 ml and 5 ml of solvent respectively. The formula used was:

$$\text{Percentage of } \beta\text{-sitosterol} = \frac{\text{sample area} \times \text{standard} \times \text{purity}}{\text{standard area} \times \text{sample dilution}} \times 100$$

## RESULTS

### (a) Extraction and preliminary phytochemical analysis

The results of extraction yield, preliminary analysis of the extracts, TLC and HPTLC of  $\beta$ -sitosterol were been previously reported by us.<sup>23-25</sup>

### (b) High Performance Liquid Chromatography (HPLC)

The ethanolic extract of PL leaves and the petroleum ether extracts of BH seeds and CC roots have shown well-resolved peaks at 254 nm. Retention time of the extract was observed to be 2.3 min in comparison to standard  $\beta$ -sitosterol at a flow rate of 1 ml/min. (Tables 1 and Figure 1 (A, B, C and D).

## DISCUSSION

Plant extracts has shown the presence of various phytoconstituents identified by chromatographic techniques which are therapeutically and economically important. Chromatography has been regarded as one of the best tool in terms of separation of phytoconstituents by subjecting the extracts to analytical method development.<sup>2</sup>

HPLC is one of the most sensitive as well as powerful visualization technique preferred in the detection of the multiple phytoconstituents present in plant extracts due to its sensitivity, accuracy and precision. The analytical technique is selected as it is specific and solution stable, provides linearity of 0.98 and 99.20% of sample.<sup>26</sup> Thus, the results depicted in this research have provided us with a probable confirmation of  $\beta$ -sitosterol, which we had identified previously by HPTLC.

## CONCLUSION

Our current research studies direct the field of pharmacognosy researchers and herbal scientists towards  $\beta$ -sitosterol constituent identified from BH, PL and CC extracts by HPLC.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

We declare no conflict of Interest.

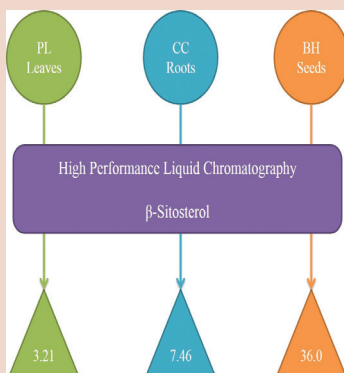
## ABBREVIATION USED

**BH:** *Benincasa hispida*; **CC:** *Carissa congesta*; **PL:** *Polyalthia longifolia*;  
**HPLC:** High Performance Liquid Chromatography.

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### PICTORIAL ABSTRACT



### SUMMARY

- The paper covers estimation of  $\beta$ -sitosterol present in the BH seeds, CC roots and PL leaves extracts by High Performance Thin Layer Chromatography.
- They were found to be 36.00, 7.46 and 3.21 % w/w at 254 nm respectively.
- Thus, extracts contains a key constituent such as  $\beta$ -sitosterol.

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