



Research Article

QUANTIFICATION AND BIOACCESSIBILITY OF β -SITOSTEROL IN *LASUNA* (*Allium sativum* LINN) BEFORE AND AFTER PROCESSED WITH *TAILA* AND ITS BIOAVAILABILITY USING CACO-2 CELL LINES

Gayathri J^{1*}, Priya S², Priyalatha B³, Raiby P. Paul³

*¹PG Scholar, ²Professor & HOD, ³Professor, Department of Dravyaguna Vigyana, Amrita School of Ayurveda, Amritapuri, Amrita Vishwa Vidyapeetham, Amaravati, Andhra Pradesh, India.

Article info

Article History:

Received: 16-02-2025

Accepted: 19-03-2025

Published: 10-04-2025

KEYWORDS:

Taila, *Lasuna*,
Allium sativum, β -
sitosterol,
Bioavailability,
Bioaccessibility,
CACO-2 Cell Lines.

ABSTRACT

Lasuna (*Allium sativum* Linn) is a potent herb, which is widely used in food and in various Ayurvedic formulations. *Acharya* recommends the use of *Lasuna* in raw and in processed form. *Acaryas* have advised to process *Lasuna* along with *Taila* in various contexts. As per the studies, the active phytoconstituents of *Lasuna* tends to decrease as a result of thermal processing. Previous studies reveal the abundance of a phytosterol named β -sitosterol in *Lasuna*. Based on the recent studies, β -sitosterol possess thermal stability and hydrophobicity and are less bioavailable. β -sitosterol was found to a lipophilic phytonutrient. Since lipophilic phytonutrients are absorbed only after emulsification and micellization, it is necessary to add the raw drug into a suitable vehicle for facilitating the solubility of the lipophilic phytonutrient for improving the solubility and bioavailability. This research aims to determine if the processing of *Lasuna* with *Taila* possess the ability to enhance the quantity, bioaccessibility and Bioavailability of β -sitosterol.

INTRODUCTION

Ayurveda considers *Lasuna* as both *Ahara* (food) and *Oushadha* (medicine) and it has been used in various forms. The *Karmas* of *Lasuna* includes, *Hṛdya*, *Rasāyana*, *Medhya*, and so on. *Ācārya* recommends the use of *Lasuna* processed with *taila* in various contexts. According to the recent studies, the active ingredients in *Lasuna* may be lost, when it is heated by boiling, frying or blanching.^[1] *Lasuna* is found to possess significant level of a plant sterol, ' β -sitosterol'. This biomolecule can be obtained only from oral intake and cannot be synthesized in the body. The bioavailability of β - sitosterol is highly challenging due to its low solubility in water and increased biliary excretion. This emphasizes the need for physical modification of β -sitosterol to mitigate the solubility issues. In Ayurveda, various processing methods are available, in which *Sneha Kalpana* is one such method, that helps to enhance the availability of fat-soluble active principles.^[2,3]

The purpose of this study is to evaluate how processing *Lasuna* with *Taila* can improve the quantity, bioaccessibility and bioavailability of β -sitosterol.

AIM

To quantify and to assess the bioaccessibility of β -sitosterol in *Lasuna* (*Allium sativum* Linn) before and after processed with *Taila* and its bioavailability using Caco-2 cell lines.

OBJECTIVES

1. To evaluate the pharmacognostic, physicochemical and phytochemical analysis of the bulb of *Allium sativum* Linn.
2. To evaluate the physicochemical and phytochemical analysis of bulb of *Allium sativum* Linn. processed with *Taila*.
3. To identify and quantify the amount of β -Sitosterol in the bulb of *Allium sativum* Linn before processed with *Taila* using HPTLC and after processed with *Taila* using GC-MS.
4. To identify and quantify the amount of β -Sitosterol in *Tila taila* using GC-MS.
5. To assess the bioaccessibility of β -Sitosterol in *Allium sativum* Linn bulb before and after processed with *Taila* using static invitro gastrointestinal INFOGEST protocol (Minekus et Al.).

Access this article online

Quick Response Code



<https://doi.org/10.47070/ijapr.v13i3.3599>

Published by Mahadev Publications (Regd.)
publication licensed under a Creative Commons
Attribution-NonCommercial-ShareAlike 4.0
International (CC BY-NC-SA 4.0)

6. To quantify the amount of β -Sitosterol in bioaccessible fraction of *Allium sativum* Linn bulb before and after processed with *Taila* using HPTLC and GC- MS respectively.
7. To assess the bioavailability of β -Sitosterol in bioaccessible fraction of *Allium sativum* Linn before and after processed with *Taila*.
8. To quantify the amount of β -Sitosterol in Caco-2 cell lines using GC-MS.

MATERIALS AND METHODS

Materials for the study

Allium sativum Linn bulb, *Tila taila*, INFOGEST digestion materials, Caco-2 (Human colorectal adenocarcinoma cells) – NCCS Pune

Methodology

Identification and Collection of *Lasuna* (*Allium sativum* Linn) and *Tila*

The drug for the study were procured from Poombarai Village, Kodaikkanal, Tamilnadu, at the month of March 2023. The herbarium of the plant was prepared and submitted to JNTBGRI, Thiruvananthapuram, Kerala which has been identified by Botanic professionals as per ICN and Voucher specimen number 103519 was obtained. *Tila taila* prepared using geotagged Onattukara Tila was procured from Onattukara, FPO, Alappuzha.

Pharmacognostical Evaluation

Organoleptic evaluation, microscopy (Fig 3) and powder microscopy (Fig 4) of the bulb of *Allium sativum* Linn is done.

Preparation of *Lasuna* processed with *Taila*

Lasuna was processed with *Taila* based on *Taila pāka vidhi* mentioned in *Śāraṅgadhara Samhita. Madhyama Khānda*. It was prepared at Rasa Shastra and Bhaishajya Kalpana Lab, Amrita School of Ayurveda in 3 days.

Materials

- *Lasuna Swarasa*- 4000g
- *Lasuna Kalka*- 125.5g
- *Tila taila*-1500g

Procedure

Tila taila along with *Lasuna Swarasa* and *Lasuna Kalka* was taken in the above-mentioned quantity. It was subjected to mild to moderate fire. Constant stirring was done to avoid sticking of *Kalka dravya*. The mixture was allowed to boil for one hour for the first 2 days. It was properly covered with clean white cloth after self-cooling. On the 3rd day, the process of heating was continued for 5 hours. After *Sneha Siddhi Lakshana*, the vessel was taken out and *Taila* was filtered. After self-cool, the sample was weighed and stored in an air tight container. The obtained oil was 779ml.

Preliminary Phytochemical analysis

Phytochemical analysis for *Lasuna*, *Taila* and *Lasuna* processed with *Taila* includes tests for carbohydrates, flavonoids, phenols, saponins, glycosides, alkaloids, tannins and phytosterols.

Physicochemical Analysis

Physicochemical analysis of *Lasuna* (*Allium sativum* Linn) includes pH, LOD, total ash, acid insoluble ash, water insoluble ash, water soluble extractive, alcohol soluble extractive.

Physicochemical analysis of *Taila* and *Lasuna* processed with *Taila* includes LOD, specific gravity, refractive index, peroxide value, acid value, saponification value, iodine value and viscosity. organoleptic evaluation of *Taila* and *Lasuna* processed with *Tila taila* are done.

Quantification of β -sitosterol in *Lasuna* before and after processed with *Taila*

β -Sitosterol in *Lasuna* (*Allium sativum* Linn) was quantified using HPTLC and GC-MS analysis was done to quantify β -Sitosterol in *Taila* and *Lasuna* processed *Taila*.

Organoleptic evaluation of *Lasuna* before and after processed with *Taila*

Organoleptic properties are very important for the evaluation of quality. Organoleptic characters like colour, odour, state and taste and characters are observed with the naked eye.

Bioaccessibility of β -sitosterol before and after processed with *taila* using Invitro Simulated digestion using INFOGEST protocol followed by quantification.

The INFOGEST protocol was used to perform simulated digestion on samples of *Lasuna*, *Taila* and *Lasuna* processed with *Taila*. The invitro digestion includes three stages viz, oral phase, gastric phase and intestinal phase. salivary, gastric and intestinal fluids were prepared and adjusted to specific pH levels. The oral phase involved mixing liquid samples with simulated salivary fluid and adjusted to pH 7. The gastric phase involved mixing the oral bolus with simulated gastric fluid and adjusting to pH 3.0. The intestinal phase involved mixing gastric chyme with simulated intestinal fluid and adjusting to pH 7. After digestion, samples were centrifuged and extracted for the quantification of β -sitosterol. The bioaccessible fraction of β -sitosterol in *Lasuna* is quantified using HPTLC and in *Taila* and *Lasuna* processed with *Taila* GC-MS is conducted. [4]

Bioavailability of β -sitosterol before and after processed with *Taila* using Caco-2 cell analysis followed by quantification

Cell Culture Media and Maintenance

Cells were cultured in a DMEM media supplemented with 10% heat inactivated FBS and a

1% antibiotic cocktail containing penicillin (100U/ml), Streptomycin (100/ml), and Amphotericin B (2.5g/ml). The cell containing tissue culture flasks (25cm²) were incubated at 37°C in a 5% CO₂ environment with humidity using a Galaxy 170 Eppendorf cell culture incubator.^[5]

Cell line Preparation

Cells (0.3 x 10⁶ cells/well) were seeded on six-well plates and acclimatized for 24 hours at 37°C, 5% CO₂. Digested samples of *Lasuna*, *taila* and *Lasuna* processed with *Taila*, initially filtered through a 0.2µm Millipore syringe filter, were added to six-well sterile

microtiter plates at concentration of 200µL in DMEM medium. Untreated cells served as controls. ^[5]

Quantification

After a 24hr incubation, the cells are lysed using T-X-100 and ethanol. Once the cells are lysed, the lysate are transferred into microcentrifuge tubes and centrifuged at 3500 rpm for 12 mins at 4°C. The supernatants were collected and GC-MS analysis was carried out to quantify the bioavailable β-sitosterol. ^[6]

RESULTS

Organoleptic evaluation of *Lasuna* before and after processed with *Taila*

The organoleptic parameters are summarized below.

Table 1: Organoleptic parameters of all the samples

Characters	<i>Lasuna</i>	<i>Tila taila</i>	<i>Lasuna</i> processed with <i>taila</i>
Colour	White to off white	Reddish orange	Light brown
Odour	Off, pungent and penetrating	Off odour	Off odour (unpleasant)
State	White to off white bulbs about 2-3cm in length	Liquid	Liquid
Taste	Pungent and astringent	-	-

Phytochemical and Physicochemical analysis of *Lasuna* before and after processed with *Taila*

Table 2: Physicochemical evaluation of *Allium sativum* Linn bulb

Parameters	<i>Lasuna</i> (%)
pH (10%) solution	5.75
Loss on drying	5.69
Total ash	2.14
Acid insoluble ash	0.38
Water insoluble ash	0.36
Alcohol soluble extractive	4.41
Water soluble extractive	30.86

Table 3: Physicochemical evaluation of *Tila taila* and *Lasuna* processed with *Taila*

Parameters	<i>Tila taila</i>	<i>Lasuna</i> processed with <i>Taila</i>
Loss on drying at 110°C	0.34	0.20
Specific gravity at 27°C	0.912	0.916
Refractive index at 40°C	1.4667	1.4620
Peroxide value	0.6	Nil
Acid value	25.05	27.35
Saponification value	139	156
Iodine value	104.4	128
Viscosity at 27cp	44cp to 45cp	48 cp to 49 cp
Rancidity	Not rancid	Not rancid

Phytochemical screening of *Lasuna*, *Tila taila* and *Lasuna* processed with *Taila*

Table 4: Phytochemical screening of all the samples

S.No	Parameters	<i>Lasuna</i>	<i>Tila taila</i>	<i>Lasuna</i> processed with <i>Taila</i>
1	Carbohydrates	Present	Absent	Absent
2	Flavonoids	Present	Absent	Present
3	Phenol	Present	Absent	Present
4	Saponins	Present	Absent	Present
5	Glycosides	Absent	Present	Present
6	Alkaloids	Absent	Present	Present
7	Tannins	Absent	Absent	Absent
8	Phytosterols	Absent	Present	Present

Quantification**Table 5: Quantification of β -Sitosterol in *Lasuna* before and after processed with *taila* using HPTLC and GC-MS analysis**

Sample	Analysis	Parameters	Result		
<i>Lasuna</i> (ppm)	HPTLC	β -Sitosterol	21.18	21.46	22.16
<i>Taila</i> (mg/100g)	GC-MS	β -Sitosterol	274.20	270.9	277.8
<i>Lasuna</i> processed with <i>Taila</i> (mg/100g)	GC-MS	β -Sitosterol	48.49	47.18	47.51

Table 6: Quantification of β -Sitosterol in Bioaccessible fraction of *Lasuna* before and after processed with *taila* using HPTLC and GC-MS analysis.

Sample	Analysis	Parameters	Result		
<i>Lasuna</i> (ppm)	HPTLC	β -Sitosterol	21.06	18.84	19.23
<i>Taila</i> (mg/L)	GC-MS	β -Sitosterol	6.7217	7.3020	7.2769
<i>Lasuna</i> processed with <i>Taila</i> (mg/L)	GC-MS	β -Sitosterol	15.3535	18.7649	19.7572

Table 7: Quantification of β -Sitosterol in Bioavailable fraction in final digesta of *Lasuna* before and after processed with *Taila* using GC-MS analysis.

Sample	Analysis	Parameters	Result		
<i>Lasuna</i> (ppm)	GC-MS	β -Sitosterol	7.3957	7.9215	7.0200
<i>Taila</i> (mg/L)	GC-MS	β -Sitosterol	5.7145	5.3681	5.5627
<i>Lasuna</i> processed with <i>Taila</i> (mg/L)	GC-MS	β -Sitosterol	7.6236	8.0826	7.6123

Statistical Analysis**Table 8: Statistical analysis of β -Sitosterol before and after Processing**

Group	β -Sitosterol		P Value
	Mean	SD	
<i>Lasuna</i>	21.26	0.17	0.0001*
<i>Taila</i>	2740.67	38.02	
<i>Lasuna</i> processed with <i>Taila</i>	477.27	6.81	

The mean β -Sitosterol in *Lasuna*, *Taila* and *Lasuna* processed with *Taila* was 21.26 ± 0.17 , 2740.67 ± 38.02 and 477.27 ± 6.81 respectively. There is a statistically, significant difference between the three groups. ($p \leq 0.05^*$). The amount of β -Sitosterol was found to be significant in *Lasuna* processed with *Taila* compared to *Lasuna*, and it was significant in *Taila* when compared to *Lasuna* and *Lasuna* processed with *Taila*.

Table 9: Statistical analysis of Bioaccessibility of β -Sitosterol Before and After Processing

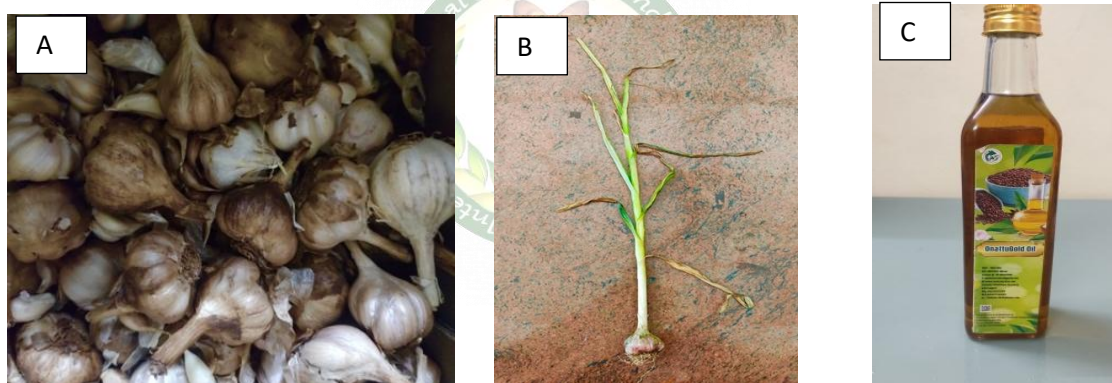
Group	β -Sitosterol		P Value
	Mean	SD	
<i>Lasuna</i>	19.71	1.18	0.001*
<i>Taila</i>	7.10	0.33	
<i>Lasuna</i> processed with <i>taila</i>	17.95	2.31	

The mean bioaccessibility of β -Sitosterol in *Lasuna* (*Allium Sativum* Linn) Bulb, *Taila* and *Lasuna* processed *Taila* was 19.71 ± 1.18 , 7.10 ± 0.33 and 17.95 ± 2.31 respectively. There is a statistically, significant difference between the three groups. ($p \leq 0.05^*$). The amount of Bioaccessible β -Sitosterol was found to be statistically significant in *Lasuna* than *taila* and *Lasuna* processed with *taila*

Table 10: Statistical analysis of Bioavailability of β -Sitosterol Before and After Processing

Group	β -Sitosterol		P Value
	Mean	SD	
<i>Lasuna</i>	7.44	0.45	0.0001*
<i>Taila</i>	5.54	0.17	
<i>Lasuna</i> processed with <i>Taila</i>	7.77	0.26	

The mean bioavailability of β -Sitosterol in *Lasuna* (*Allium sativum* Linn) bulb, *Taila* and *Lasuna* processed with *Taila* was 7.44 ± 0.45 , 5.54 ± 0.17 and 7.77 ± 0.26 respectively. There is statistically, significant difference exists between the three groups. ($p \leq 0.05^*$). The amount of bioavailable β -Sitosterol in *Lasuna* processed with *Taila* was found to be statistically significant compared to *Lasuna* and *Taila*.

**Fig 1: Raw drugs**

- A- *Lasuna* bulb
 B- *Lasuna* plant
 C- *Tila taila*

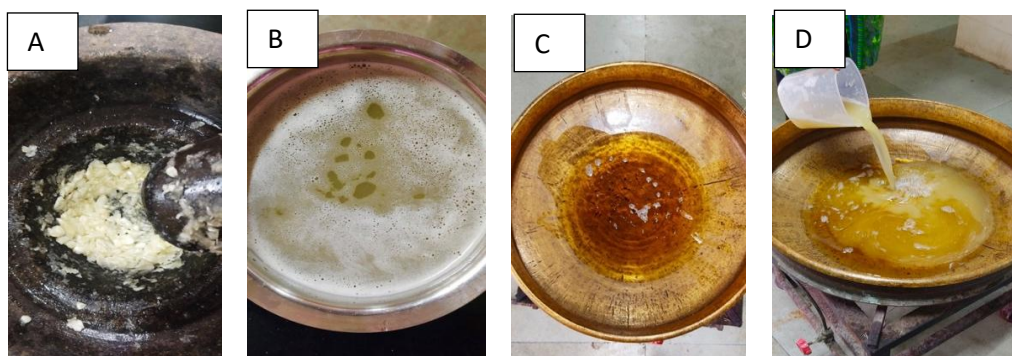




Fig 2: Preparation of Lasuna processed with Taila

- | | |
|--|--|
| A- Pounding <i>Lasuna</i> | F- Adding <i>Kalka</i> to the mixture |
| B- Expressed <i>Swarasa</i> | G- : Day 1- After boiling |
| C- Pouring <i>Taila</i> to the vessel | H- Day 2- After boiling |
| D- Adding <i>Swarasa</i> to the <i>Taila</i> | I- <i>Phenodgama</i> |
| E- Weighed <i>Kalka</i> | J- <i>Kalka</i> rolled into <i>Varti</i> |
| | K- End product |

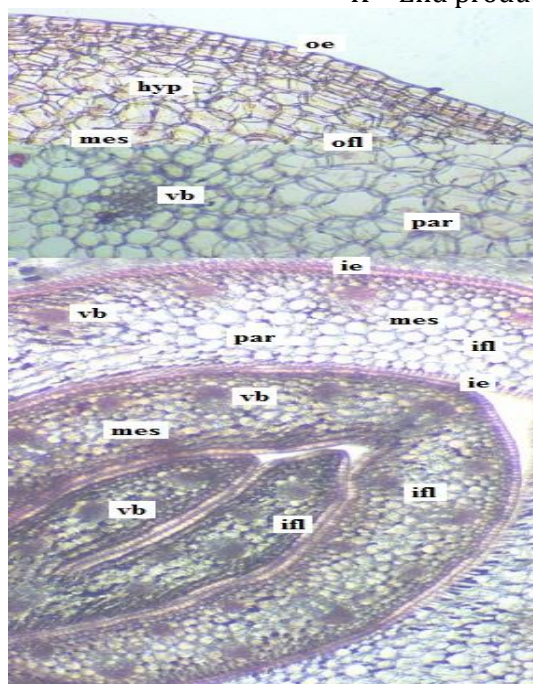


Fig 3: Microscopy of Lasuna

oe.: outer epidermis; **hyp.:** hypodermis; **par.:**parenchyma cells; **mes.:** mesophyll; **vb.:** vascular bundle; **ie.:** inner epidermis; **ofl.:** outer fleshy leaves; **infl.:** inner fleshy leaves.

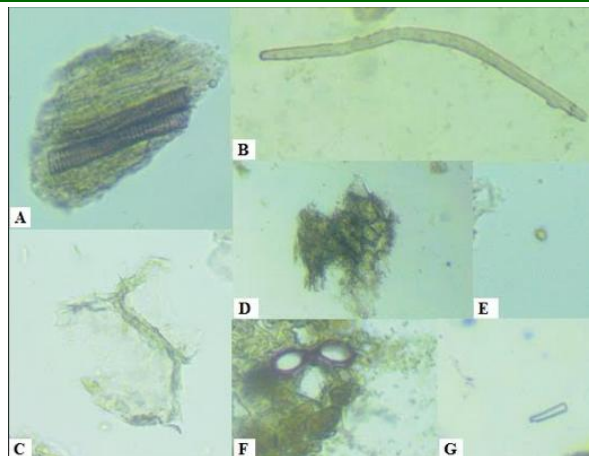


Fig 4: Powder microscopy of *Lasuna*

A.: reticulate vessel; B.: non-lignified fibre; C.: parenchymatous cells; D.: epidermal cells surface view; E.: yellow coloured content; F.: vascular bundles; G.: crystals of calcium oxalate.

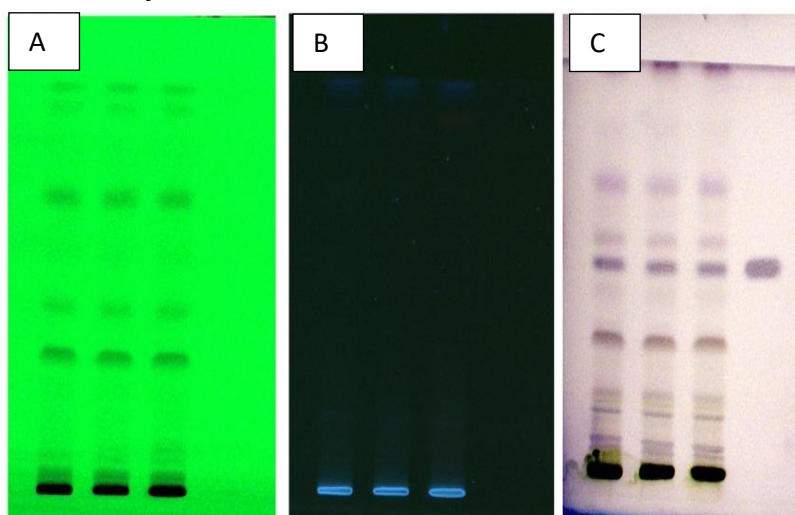


Fig 3: HPTLC profile of *Lasuna*

A- At 254 nm; At 366 nm; After derivatization

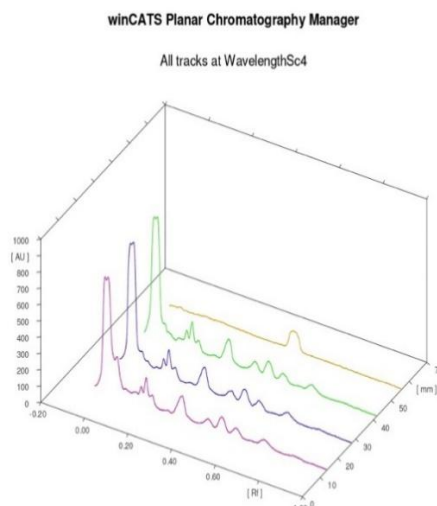


Fig 4: Densitogram of *Lasuna*

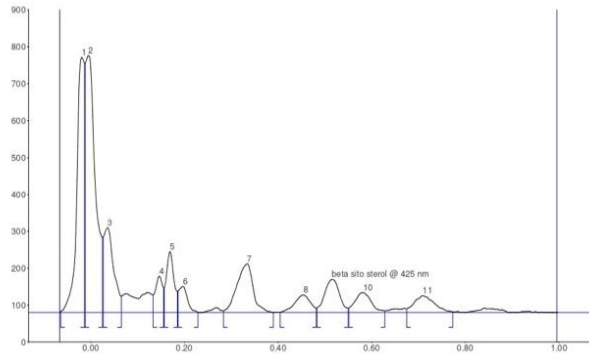
Track 1: Methanolic extract of *Allium sativum* Linn bulb at 20.0 μ L

Track 2: Methanolic extract of *Allium sativum* Linn bulb at 20.0 μ L

Track 3: Methanolic extract of *Allium sativum* Linn bulb at 20.0 μ L

Track 4: Methanolic extract of β -Sitosterol standard at 2.0 μ L

Track 1, ID: LASUNA (ALLIUM SATIVUM LINN) RAW

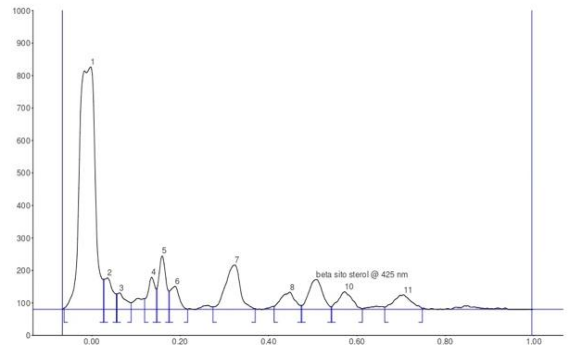


winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	3.7	-0.02	691.8	29.82	-0.01	675.4	9738.2	22.49	unknown *
2	-0.01	675.8	-0.01	696.4	30.02	0.03	203.6	13135.1	30.34	unknown *
3	0.03	205.2	0.04	229.5	9.89	0.07	44.1	4333.1	10.01	unknown *
4	0.13	47.8	0.15	98.6	4.25	0.16	65.7	1358.0	3.14	unknown *
5	0.16	66.3	0.17	164.5	7.09	0.19	57.6	2355.4	5.44	unknown *
6	0.19	58.2	0.20	70.5	3.04	0.23	0.3	1149.6	2.66	unknown *
7	0.28	4.9	0.33	131.9	5.69	0.39	0.0	3961.8	9.15	unknown *
8	0.41	1.2	0.45	47.4	2.04	0.48	12.3	1408.2	3.25	unknown *
9	0.48	12.8	0.52	89.9	3.87	0.55	12.2	2495.1	5.76	beta sito sterol
10	0.55	12.5	0.58	54.0	2.33	0.63	4.9	1639.3	3.79	unknown *
11	0.68	10.2	0.71	45.4	1.96	0.77	1.7	1719.5	3.97	unknown *

A

Track 2, ID: LASUNA (ALLIUM SATIVUM LINN) RAW

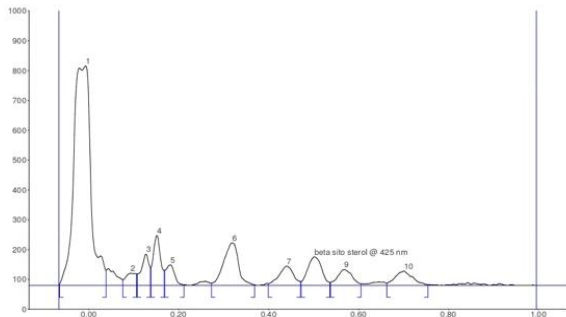


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	4.1	-0.00	746.4	46.31	0.03	92.7	22988.4	55.35	unknown *
2	0.03	92.9	0.04	97.2	6.03	0.06	47.7	1654.5	3.98	unknown *
3	0.06	47.9	0.06	51.0	3.16	0.09	20.9	879.7	2.12	unknown *
4	0.12	33.2	0.14	99.0	6.14	0.15	62.7	1425.8	3.43	unknown *
5	0.15	65.7	0.16	165.0	10.23	0.17	56.1	2309.3	5.56	unknown *
6	0.18	56.4	0.19	71.2	4.42	0.22	0.8	1233.6	2.97	unknown *
7	0.27	8.1	0.32	137.1	8.51	0.37	2.1	3965.2	9.55	unknown *
8	0.41	9.7	0.45	53.3	3.31	0.47	12.2	1486.6	3.58	unknown *
9	0.48	12.3	0.51	92.1	5.71	0.54	8.1	2528.1	6.09	beta sito sterol
10	0.54	8.6	0.57	54.5	3.38	0.61	2.4	1485.3	3.58	unknown *
11	0.66	7.5	0.71	45.2	2.80	0.75	3.3	1579.3	3.80	unknown *

B

winCATS Planar Chromatography Manager

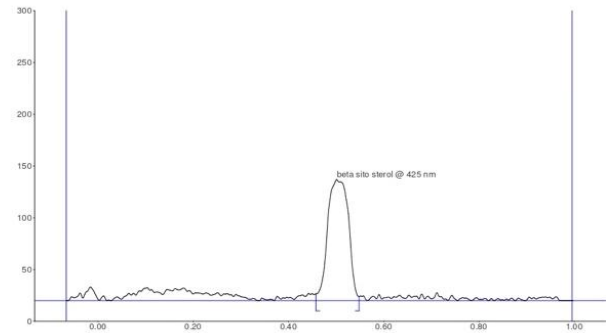
Track 3, ID: LASUNA (ALLIUM SATIVUM LINN) RAW



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	3.5	-0.01	736.3	48.31	0.04	50.8	24537.2	58.03	unknown *
2	0.08	19.5	0.09	40.8	2.68	0.11	39.3	835.7	1.98	unknown *
3	0.11	39.0	0.13	104.7	6.87	0.14	59.9	1582.1	3.74	unknown *
4	0.14	60.7	0.15	167.7	11.01	0.17	50.4	2424.9	5.74	unknown *
5	0.17	50.8	0.18	68.7	4.51	0.21	2.2	1175.8	2.78	unknown *
6	0.27	7.3	0.32	142.9	9.37	0.37	2.2	4088.6	9.67	unknown *
7	0.40	5.8	0.44	65.0	4.26	0.47	13.3	1845.3	4.36	unknown *
8	0.47	13.3	0.50	96.0	6.30	0.54	10.1	2611.5	6.18	beta sito sterol
9	0.54	10.2	0.57	53.5	3.51	0.61	5.8	1522.8	3.60	unknown *
10	0.66	7.6	0.70	48.6	3.19	0.76	2.6	1657.7	3.92	unknown *

C

Track 4, ID: BETA SITO STEROL



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.46	6.7	0.50	117.1	100.00	0.55	4.1	4454.3	100.00	beta sito sterol

D

HPTLC peaks at 366 nm A) Lasuna B) Lasuna C) Lasuna D) β -Sitosterol

GC-MS Quantification profile of *Tila taila*

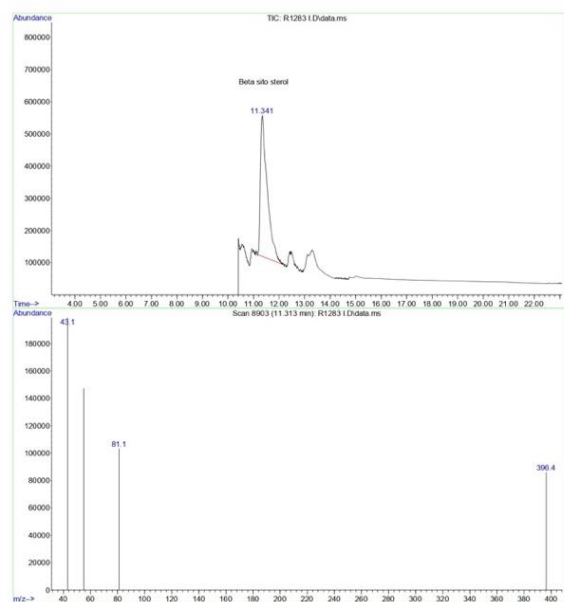


Fig 5: GC-MS profile of *Tila taila*

- A- Mass spectra of Track 1- *Tila taila*; Mass spectra of Track 2- *Tila taila*
 B- Mass spectra of Track 3- *Tila taila*; Mass spectra of Track 4- β -Sitosterol standard

Mass spectra of Lasuna processed with Taila using GC-MS

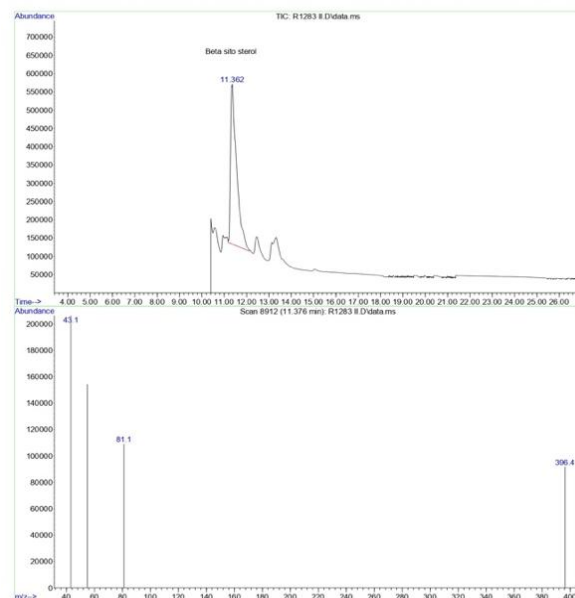
Instrument : GCMS
Sample Name: Lasuna Processed with taila 1
Misc Info :
Vial Number: 3



Sample : Lasuna Processed with taila 1
ALS Vial : 3
Method : D:\2024\METHOD\BETA SITOSTEROL.M

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	11.341	8888	8907	8998	rVB	449399	8237584	100.00%	95.106%

Instrument : GCMS
Sample Name: Lasuna Processed with taila 2
Misc Info :
Vial Number: 4

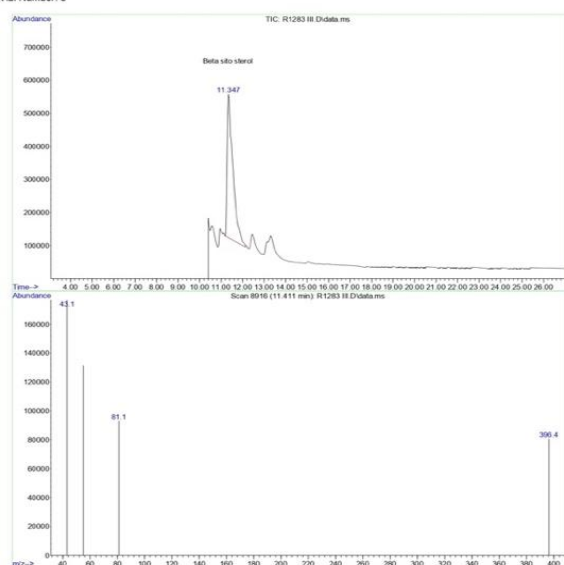


Sample : Lasuna Processed with taila 2
ALS Vial : 4
Method : D:\2024\METHOD\BETA SITOSTEROL.M

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	11.362	8890	8910	9042	rVB3	464412	7942546	100.00%	92.299%

A**B**

Instrument : GCMS
Sample Name: Lasuna Processed with taila 3
Misc Info :
Vial Number: 5

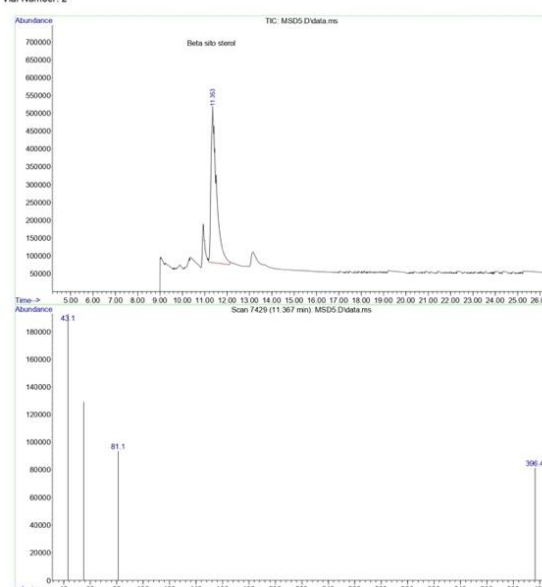


Sample : Lasuna Processed with taila 3
ALS Vial : 5
Method : D:\2024\METHOD\BETA SITOSTEROL.M

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	11.347	8889	8907	9041	rVB2	467996	8032237	100.00%	100.000%

C

Instrument : GCMS
Sample Name: STD(BETA SITO STEROL)
Misc Info :
Vial Number: 2



Sample : STD(BETA SITO STEROL)
ALS Vial : 2
Method : D:\2024\METHOD\BETA SITOSTEROL.M

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	11.353	7401	7427	7529	rM	447632	7745137	100.00%	100.000%

D**Fig 6: GC-MS profile of Lasuna processed with taila**

- A- Mass spectra of Track 1- *Lasuna* processed with *Taila*
B- Mass spectra of Track 2- *Lasuna* processed with *Taila*
C- Mass spectra of Track 3- *Lasuna* processed with *Taila*
D- Mass spectra of Track 4 - β -Sitosterol standard

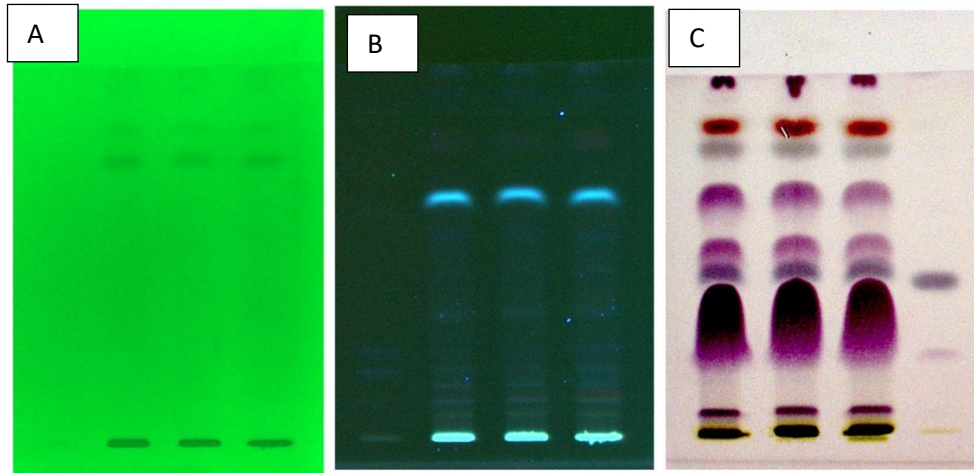


Fig 7: HPTLC profile of digested *Lasuna*
A- At 254 nm; At 366 nm; After derivatization

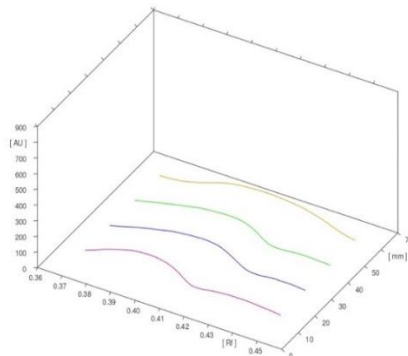


Fig 8: Densitogram of digested *Lasuna*

Track 1: Methanolic extract of digested *Allium sativum* Linn bulb at 20.0 μ L
Track 2: Methanolic extract of digested *Allium sativum* Linn bulb at 20.0 μ L
Track 3: Methanolic extract of digested *Allium sativum* Linn bulb at 20.0 μ L
Track 4: Methanolic extract of β -Sitosterol standard at 2.0 μ L

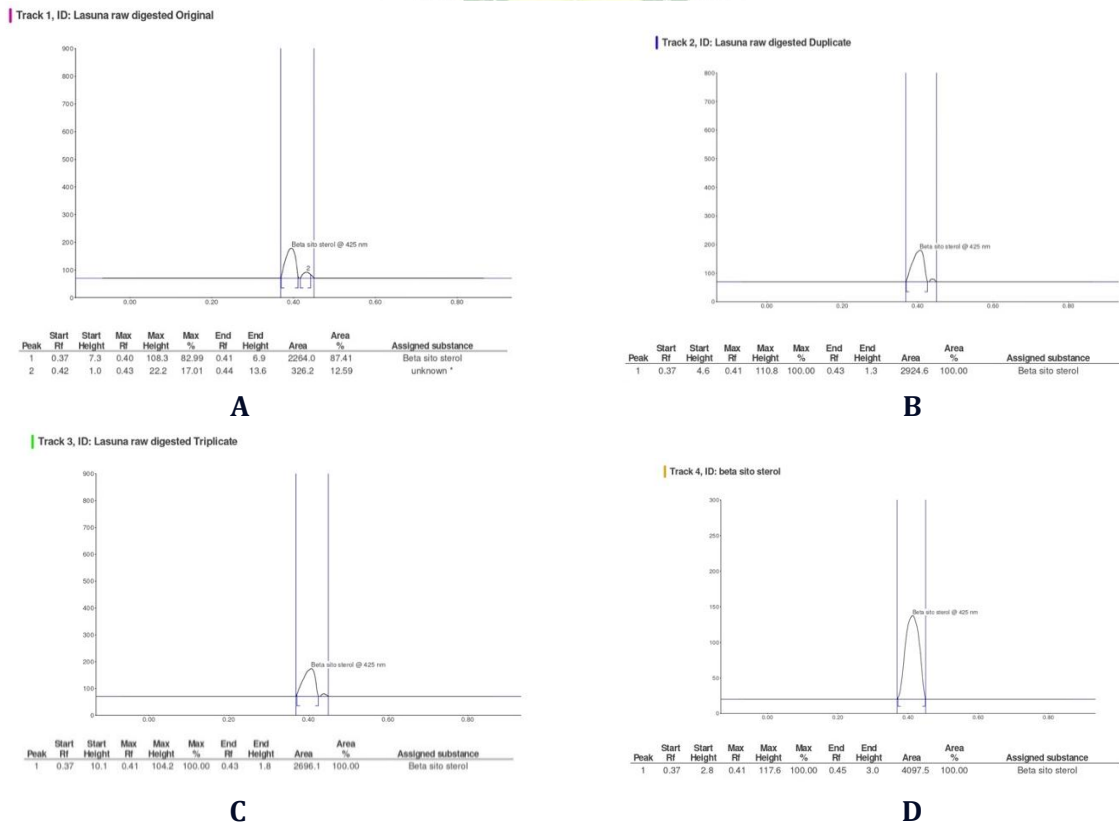


Fig 9: HPTLC profile digested *Lasuna*

GC-MS quantification of digested *Tila taila*

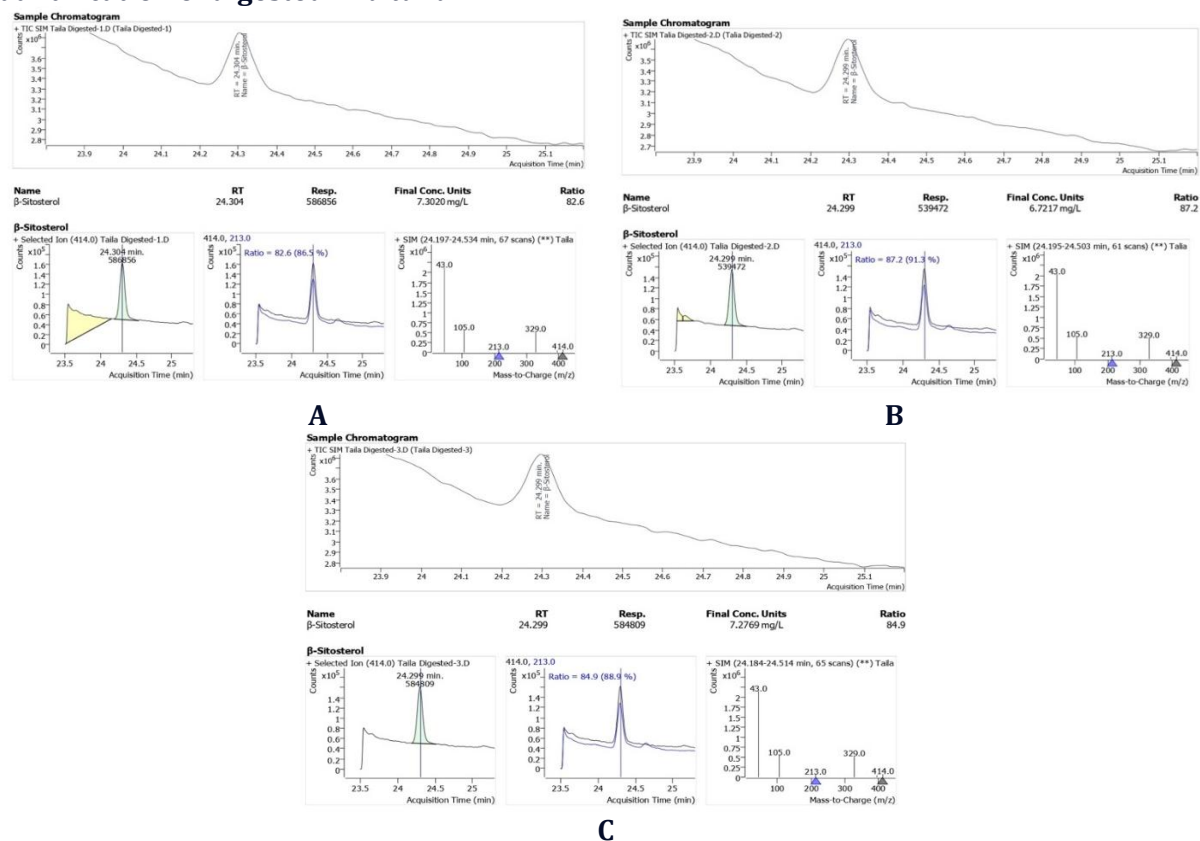


Fig 10: GC-MS profile of digested *Tila taila*

- A- Mass spectra of Track 1- *Tila taila* digested
- B- Mass spectra of Track 2- *Tila taila* digested
- C- Mass spectra of Track 3 - *Tila taila* digested

GC-MS quantification of digested *Lasuna* processed with *taila*

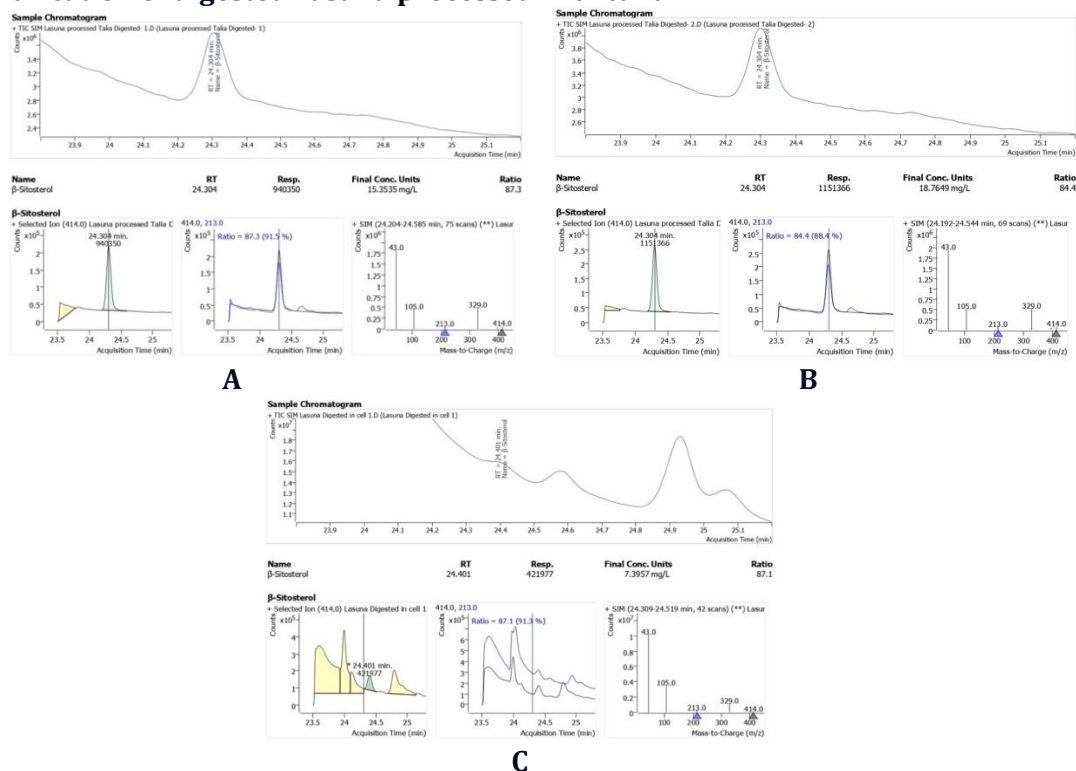


Fig 11: GC-MS profile of digested *Lasuna* processed with *Taila*

- A- Mass spectra of Track 1- *Lasuna* processed with *Taila* digested
- B- Mass spectra of Track 2 - *Lasuna* processed with *Taila* digested
- C- Mass spectra of Track 3 - *Lasuna* processed with *Taila* digested

GC-MS quantification of *Lasuna* digested in cell

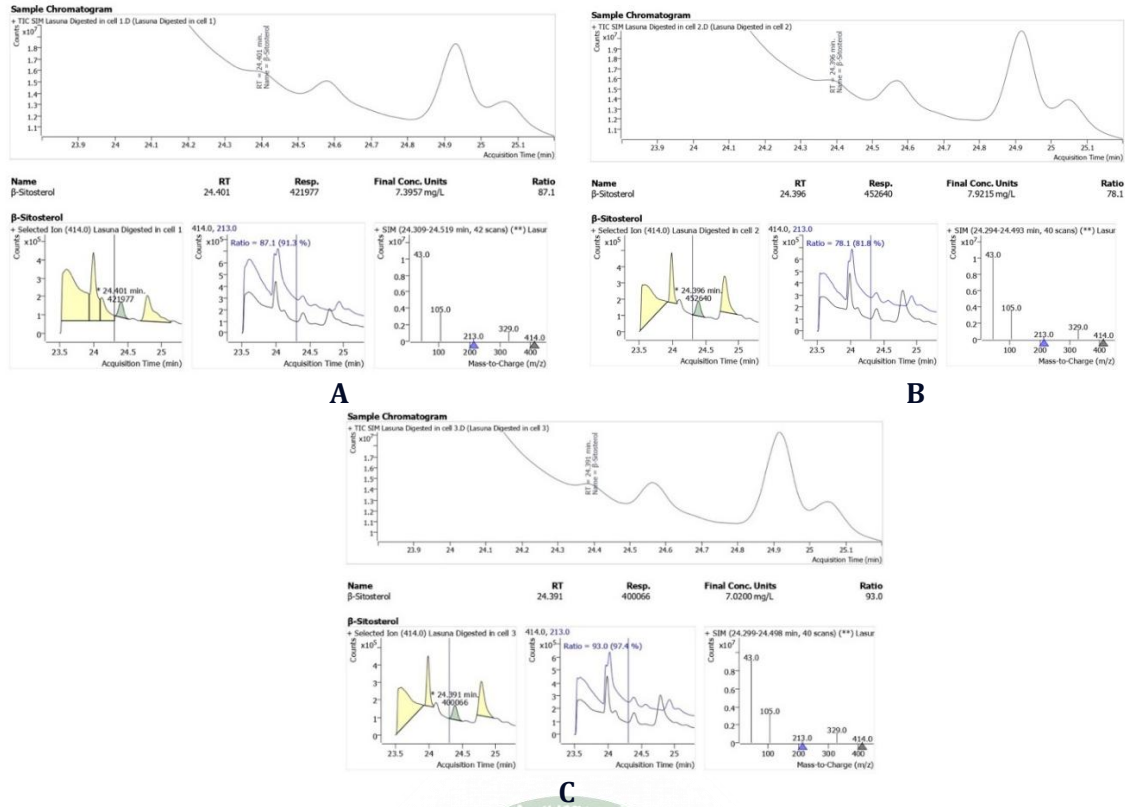


Fig 11: GC-MS profile of *Lasuna* digested in cell

A- Mass spectra of Track 1 – *Lasuna* digested in cell

B- Mass spectra of Track 2 – *Lasuna* digested in cell

C- Mass spectra of Track 3 – *Lasuna* digested in cell

GC-MS quantification of *Tila taila* digested in cell

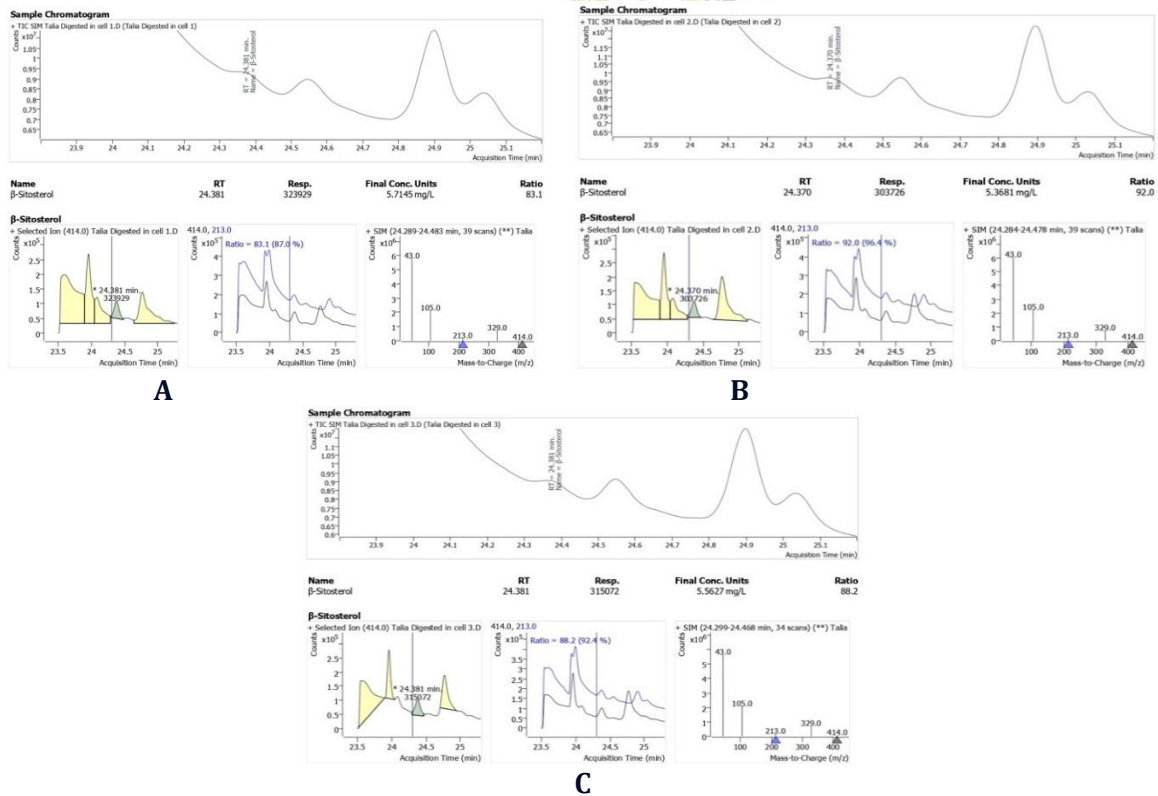
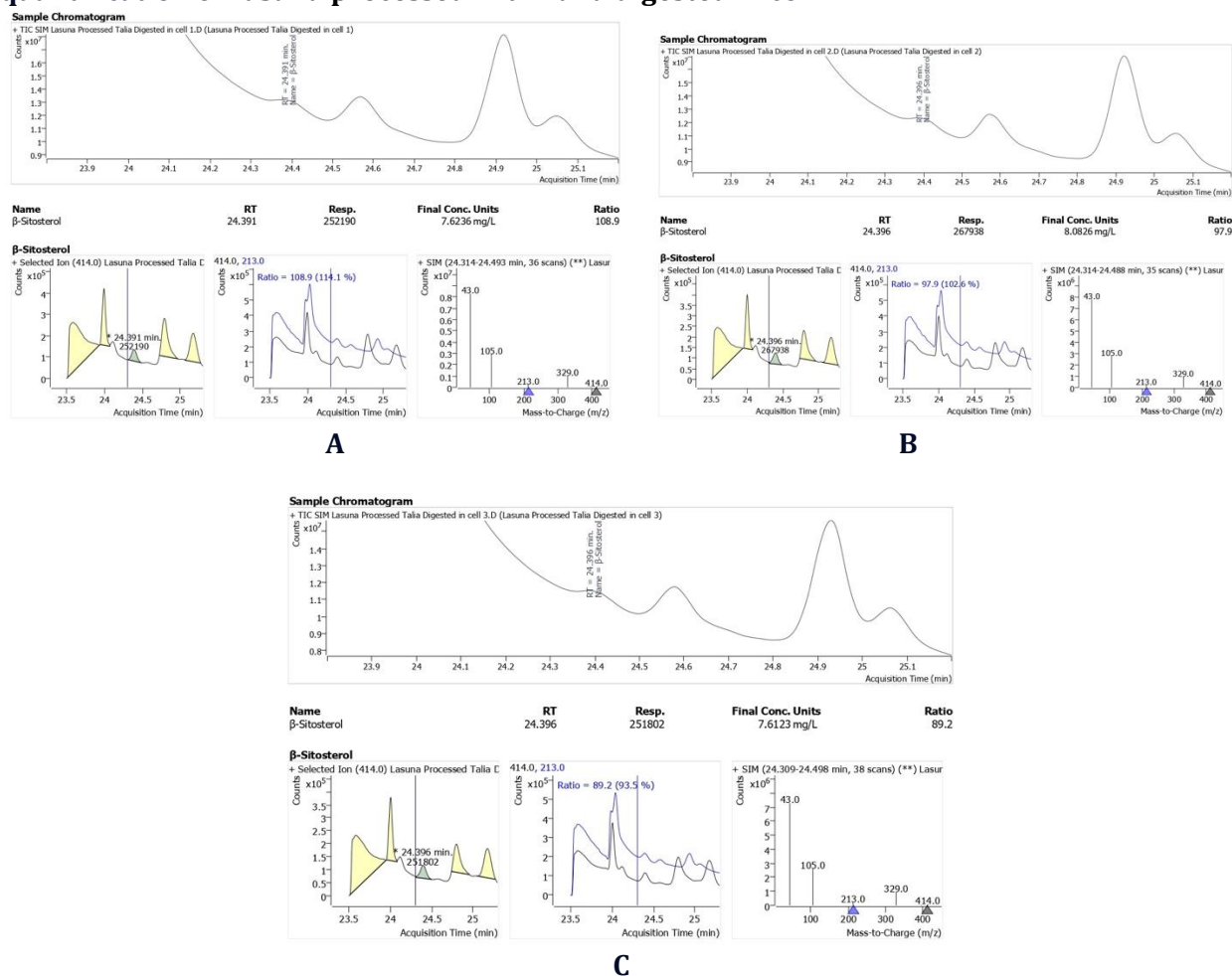
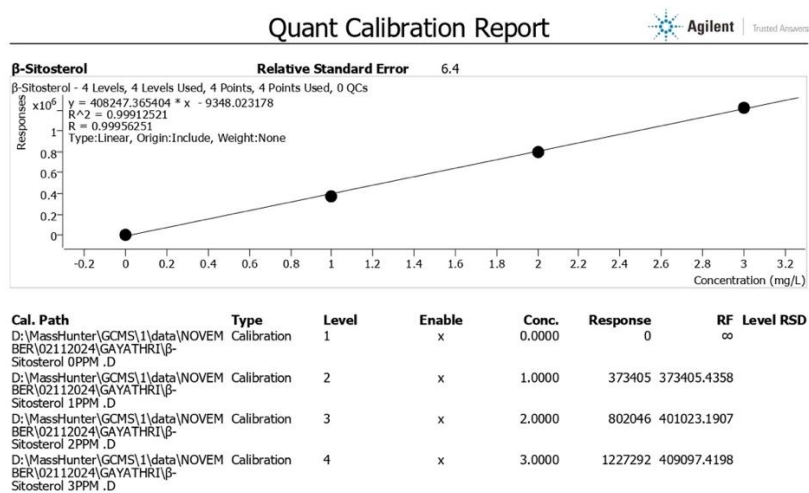


Fig 12: GC-MS profile of *Tila taila* digested in cell

A- Mass spectra of Track 1 – *Tila taila* digested in cell

B- Mass spectra of Track 2 – *Tila taila* digested in cell

C- Mass spectra of Track 3 – *Tila taila* digested in cell

GC-MS quantification of *Lasuna* processed with *Taila* digested in cell**Fig 12: GC-MS profile of *Lasuna* processed with *taila* digested in cell**A- Mass spectra of Track 1- *Lasuna* processed with *Taila* digested in cell.B- Mass spectra of Track 2 - *Lasuna* processed with *Taila* digested in cell.C- Mass spectra of Track 3 - *Lasuna* processed with *Taila* digested in cell.**Fig 13: Calibration curve of β -Sitosterol**

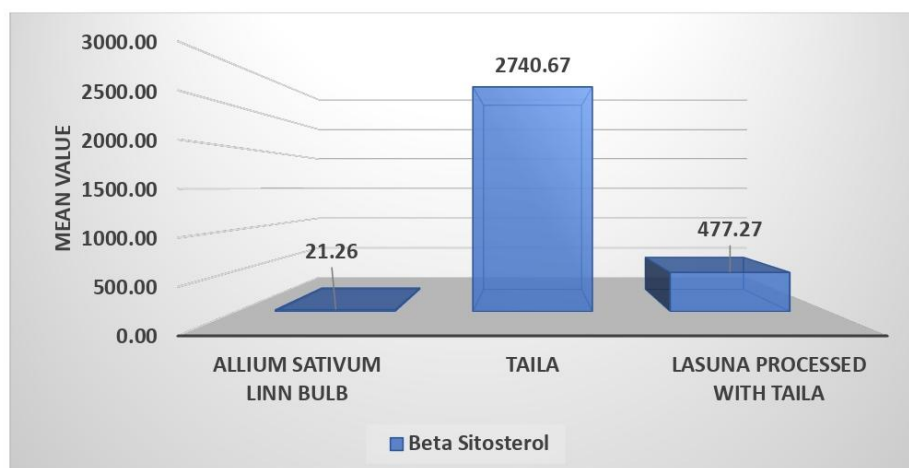


Fig 14: Bar diagram of β -Sitosterol before and after processing with *Taila*

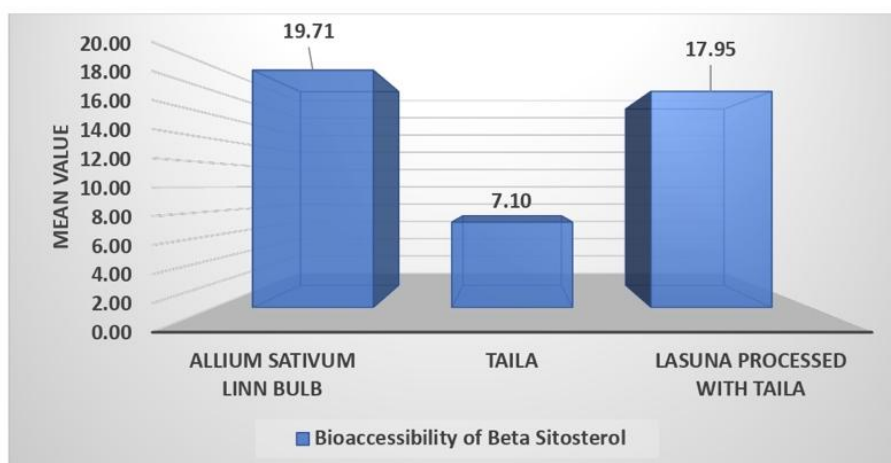


Fig 15: Bar diagram of Bioaccessibility of β -Sitosterol before and after processing

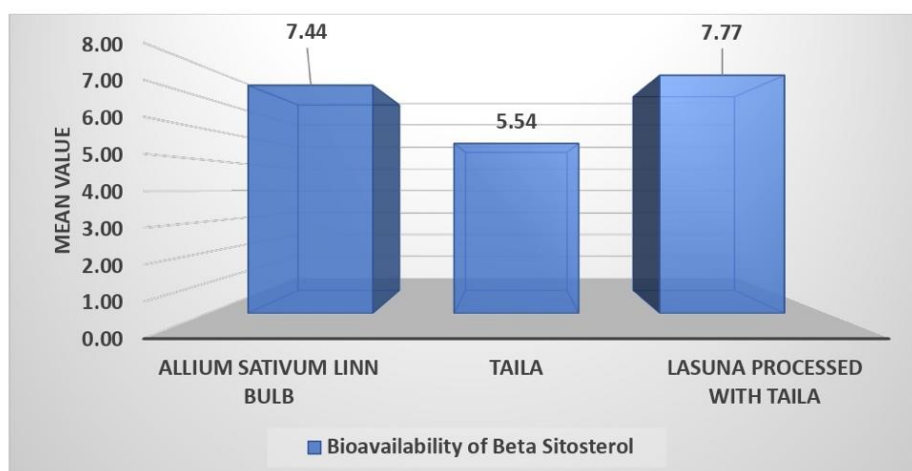


Fig 16: Bar diagram of Bioavailability of β -Sitosterol before and after processing

DISCUSSION

On preliminary phytochemical screening, flavonoids, phenols, saponins, glycosides, alkaloids and phytosterols were found to be present in *Laśuna* processed with *Taila* except carbohydrates and tannins. Among this, flavonoids, phenols and saponins were present in *Laśuna* alone were also found to be present in *Laśuna* processed with *Taila*. Glycosides and Alkaloids were found to be present in *Taila* alone were also found to be present in *Laśuna* processed *Taila*.

Carbohydrates present in *Laśuna* alone was found to be absent in processed one. Tannins were found to be absent in all the three samples. Phytosterols were found to be absent in *Laśuna*. It was found to be present in *Taila* and *Laśuna* processed with *Taila*.

On physicochemical evaluation, pH (5.69) of *Laśuna* which shows the acidic nature of the drug. The LOD (5.69) and total ash (2.14) of *Laśuna* was found to be corresponding with the API standards. Water

soluble extractive value (30.86) was found to be more than that of alcohol soluble extractive (4.41). Acid value (27.35) and saponification value (156) were found to be slightly higher. All other parameters were found to be corresponding with the API standards.

The initial amount of β -Sitosterol was found to be significant in *Lasuna* processed with *Taila* (477.27 ± 6.81) compared to *Lasuna* (21.26 ± 0.17). The amount of bioaccessible β -Sitosterol was found to be significant in *Lasuna* (19.71 ± 1.18) compared to *Taila* (7.10 ± 0.33) and *Lasuna* processed with *Taila* (17.95 ± 2.31). The amount of bioavailable β -Sitosterol was found to be significant in *Lasuna* processed with *Taila* (7.77 ± 0.26) compared to *Taila* (5.54 ± 0.17) and *Lasuna* (7.44 ± 0.45). It can be concluded from the results that *Taila* may be capable to improve the bioavailability of β -Sitosterol. This study reveals that the processing of *Lasuna* in *Taila* has increased the quantity of β -Sitosterol in *Lasuna* processed with *Taila*. Bioaccessible β -sitosterol was found in final digesta of *Lasuna* and *Lasuna* processed with *Taila*, significant elevation was noted in *Lasuna*. Bioavailable β -sitosterol was found in final digesta of *Lasuna* and *Lasuna* processed with *Taila*, significant increase was slightly elevated in *Lasuna* processed with *Taila*.

CONCLUSION

Since the bioavailability of β -Sitosterol was present both in *Lasuna* and *Lasuna* processed with *Taila*, and found to be slightly higher in *Lasuna* processed with *Taila*, *Taila* can be considered as an effective media in improving the Quantity and Bioavailability of β -Sitosterol present in *Lasuna* processed with *Taila*. Additionally, Phytochemical screening revealed that thermal processing of *Lasuna* in *Taila* has not affected the active constituents present in it. As per this study, the processing of *Lasuna* in *Taila* can be adopted as an effective media to improve the Bioavailability of β -Sitosterol.

ACKNOWLEDGEMENTS

We extend our sincere gratitude to the PG STAR TEAM, CCRAS, Ministry of AYUSH, Government of India for providing the financial support that is necessary for the research. We are also thankful to Dr. G. Rajkumar, Principal Scientist and Head, PS & ES

Division, KSCSTE-JNTBGRI, Palode, Trivandrum, Mr. Austin P., Founder and MD of Athmic Biotech Solutions Pvt Ltd, Ms. Pooja Suresh, Instrumentation Analyst, MG University, Kottayam, CARE- KERALAM, Koratty, Trissur.

REFERENCES

- De Queiroz YS, Antunes PB, Vicente SJ, Sampaio GR, Shibao J, Bastos DH. Bioactive compounds, in vitro antioxidant capacity and Maillard reaction products of raw, boiled and fried garlic (*allium sativum*L). *International Journal of Food Science & Technology*. 2013; 49(5): 1308–14.
- Bali S, Prasad S, Saini V. Ayurvedic lipid based rasayans- A perspective on the preparation and pharmacological significance of lipids on the bioavailability of phytoconstituents. *J Ayurveda Integr Med [Internet]*. 2022; 13(2): 100526. Available from: <http://dx.doi.org/10.1016/j.jaim.2021.09.004>
- Yang Y, McClements DJ. Vitamin E bioaccessibility: Influence of carrier oil type on digestion and release of emulsified α -tocopherol acetate. *Food Chem [Internet]*. 2013; 141(1): 473–81. Available from: <http://dx.doi.org/10.1016/j.foodchem.2013.03.033>
- Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, et al. A standardised static in vitro digestion method suitable for food– an international consensus. *Food Funct [Internet]*. 2014; 5(6): 1113–24. Available from: <http://dx.doi.org/10.1039/c3fo60702j>
- Lea T, Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C, Lea T, et al. Caco-2 Cell Line. In: *The Impact of Food Bioactives on Health: in vitro and ex vivo models*. Cham (CH: Springer; 2015).
- Elhady SS, Ibrahim EA, Goda MS, Nafie MS, Samir H, Diri RM, et al. GC-MS/MS quantification of EGFR inhibitors, β -sitosterol, betulinic acid, (+) eriodictyol, (+) epipinoresinol, and secoisolariciresinol, in crude extract and ethyl acetate fraction of *Thonningia sanguinea*. *Molecules [Internet]*. 2022; 27(13): 4109. Available from: <http://dx.doi.org/10.3390/molecules27134109>

Cite this article as:

Gayathri J, Priya S, Priyalatha B, Raiby P. Paul. Quantification and Bioaccessibility of β -Sitosterol in Lasuna (*Allium sativum* Linn) Before and After Processed with Taila and its Bioavailability Using CACO-2 Cell Lines. *International Journal of Ayurveda and Pharma Research*. 2025;13(3):16-31.

<https://doi.org/10.47070/ijapr.v13i3.3599>

Source of support: Nil, Conflict of interest: None Declared

*Address for correspondence

Dr. Gayathri J

PG Scholar

Department of Dravyaguna Vigyana,
Amrita School of Ayurveda,
Amritapuri, Amrita Vishwa
Vidyapeetham, India

Email: drgayathri59@gmail.com

Disclaimer: IJAPR is solely owned by Mahadev Publications - dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJAPR cannot accept any responsibility or liability for the articles content which are published. The views expressed in articles by our contributing authors are not necessarily those of IJAPR editor or editorial board members.