

International Journal of Advanced Chemistry Research

ISSN Print: 2664-6781
 ISSN Online: 2664-679X
 NAAS Rating (2025): 4.77
 IJACR 2025; 7(9): 175-178
www.chemistryjournals.net
 Received: 13-07-2025
 Accepted: 17-08-2025

Harsh Bose
 Department of Chemistry of
 Natural Resources, Netaji
 Subhas College of Science,
 Guwahati, Assam, India

Phytosterol content and cholesterol-lowering activity of seed oils from cucurbit species

Harsh Bose

DOI: <https://www.doi.org/10.33545/26646781.2025.v7.i9c.632>

Abstract

Cucurbit vegetables are among the most widely cultivated and consumed food plants in India's northeastern states, where species such as pumpkin, bottle gourd, bitter melon, and ash gourd are dietary staples prepared in dozens of traditional recipes. Their seeds, typically discarded after culinary use, contain 30-50% oil by dry weight a fraction that is rich in polyunsaturated fatty acids and, as this research demonstrates, in phytosterols with quantifiable cholesterol-lowering bioactivity. Phytosterols are plant-derived sterols structurally similar to cholesterol that compete with dietary cholesterol for micellar incorporation and intestinal absorption, thereby reducing plasma LDL cholesterol when consumed at gram-per-day levels. The present research characterised the phytosterol content and composition of cold-pressed seed oils from six cucurbit species *Cucurbita pepo* (pumpkin), *C. maxima* (winter squash), *C. moschata* (butternut squash), *Lagenaria siceraria* (bottle gourd), *Momordica charantia* (bitter melon), and *Benincasa hispida* (ash gourd) grown in Assam and Meghalaya, Northeast India. Phytosterols were quantified by GC-FID after saponification and silylation; fatty acid composition by GC-FID as methyl esters (FAME); cholesterol-lowering bioactivity assessed by *in vitro* HMG-CoA reductase inhibition assay and micellar cholesterol solubilisation inhibition. Total phytosterol content ranged from 149.0 mg/100g oil (*L. siceraria*) to 252.3 mg/100g oil (*C. pepo*), with -sitosterol, stigmasterol, and campesterol as the dominant sterols in all species. *Momordica charantia* had the highest HMG-CoA reductase inhibition (52.7%) and LDL-C reduction potential (36.1%) despite its intermediate sterol content, suggesting bioactive contributions from co-occurring triterpenoids. A strong positive correlation was found between total phytosterol content and LDL-C reduction ($r^2 = 0.942$). These results position Assamese cucurbit seed oils as functional food ingredients with evidence-based cholesterol-lowering potential.

Keywords: Phytosterols, cucurbit seed oil, -sitosterol, cholesterol, HMG-CoA reductase, GC-FID, functional food, Northeast India

Introduction

The global functional food market has identified phytosterols as one of the most evidence-supported cholesterol-lowering ingredients, with the European Food Safety Authority approving a health claim that plant sterol consumption (1.5-3.0 g/day) reduces LDL cholesterol by 7-12.5% [1]. This effect operates through competitive inhibition of dietary cholesterol absorption at the intestinal lumen: phytosterols partition preferentially into mixed micelles at the expense of cholesterol, reducing cholesterol incorporation into enterocytes and thereby decreasing the flux of dietary cholesterol to the liver [2]. Their structural similarity to cholesterol differing only in the C-^{V L G H F K D L Q i t o s t e r o l} (sitosterol, stigmasterol, methyl for campesterol) is what underpins this competitive micellar displacement.

Plant seed oils are among the most concentrated dietary sources of phytosterols, with concentrations ranging from 100 mg/100g in refined commercial vegetable oils to over 3000 mg/100g in some nut oils and unrefined wheat germ oil [3]. However, cucurbit seed oils despite their widespread traditional use in South and Southeast Asia have received relatively little phytosterol characterisation in the peer-reviewed literature compared with commonly traded oils (sunflower, rapeseed, palm). For Northeast Indian cucurbit species, the combination of local genetic diversity (several traditional varieties distinct from commercial

Corresponding Author:
Harsh Bose
 Department of Chemistry of
 Natural Resources, Netaji
 Subhas College of Science,
 Guwahati, Assam, India

types), specific agroclimatic conditions, and traditional oil extraction practices could result in phytosterol profiles that differ substantially from commercially reported values.

The biochemical relationship between phytosterols and cholesterol metabolism extends beyond competitive absorption inhibition to include direct inhibition of HMG-CoA reductase the rate-limiting enzyme in the mevalonate pathway for endogenous cholesterol synthesis though the potency of isolated phytosterols at this target is modest compared with statin drugs. Whole seed oil extracts may show enhanced HMG-CoA reductase inhibition relative to isolated sterols due to synergistic interactions with tocopherols, phytol, and triterpenoids co-present in the unsaponifiable fraction [4]. This research therefore assessed both the phytosterol content and the *in vitro* bioactivity of the complete unsaponifiable oil fractions. Work was conducted at Netaji Subhas College of Science, Guwahati, Assam, India, from October 2023 to March 2025.

Extraction protocol

Seed oil extraction used a two-stage cold-pressing protocol. Seeds from mature fresh vegetables were manually separated from pulp, washed, surface-sterilised with 0.1% sodium hypochlorite (5 min), rinsed three times with deionised water, and air-dried at 40°C for 48 h to constant weight. Dried seeds were coarsely ground in a hammer mill to pass a 2 mm screen. Cold pressing was conducted using a laboratory screw press (Agro Industrial Machinery Company, Jalandhar) at 50°C jacket temperature and 15 rpm screw speed; crude oil was collected, centrifuged (3000 rpm, 20 min) to remove seed fragments, and filtered through Whatman No. 1. Phytosterol extraction from the oil: the unsaponifiable fraction was obtained by alkaline saponification (2 M KOH in ethanol, 70°C, 1 h), extraction of the unsaponifiables with diethyl ether (3 × 30 mL), and silylation of the dried extract (Sylon HTP reagent, Sigma-Aldrich; 70°C, 30 min) for GC-FID analysis. FAME SUHSDUDWLRQ IRU IDWW\ DFLG WUDQVHVWHUL, LFD, WL RQ according to ISO 5508.

Material and Methods

Material

Research was conducted at the Department of Chemistry of Natural Resources, Netaji Subhas College of Science,

Results

Table 1: Phytosterol content (mg/100g oil) and physicochemical properties of six cucurbit seed oils

Species	Total phytosterol	β -Sitosterol	Stigmasterol	Campesterol	Iodine value	Saponification value
<i>C. pepo</i>	252.3 ± 8.4	142.3 ± 4.7	48.7 ± 1.8	34.2 ± 1.3	114.7 ± 2.8	188.4 ± 3.2
<i>C. maxima</i>	223.4 ± 7.6	128.7 ± 4.1	43.2 ± 1.6	29.8 ± 1.1	108.3 ± 2.6	191.7 ± 3.4
<i>C. moschata</i>	203.2 ± 6.8	118.4 ± 3.9	39.1 ± 1.4	26.7 ± 1.0	112.4 ± 2.7	187.3 ± 3.1
<i>L. siceraria</i>	149.0 ± 5.1	87.2 ± 3.0	28.4 ± 1.1	19.3 ± 0.8	98.4 ± 2.4	194.2 ± 3.6
<i>M. charantia</i>	250.1 ± 8.1	134.6 ± 4.4	51.3 ± 1.9	38.7 ± 1.4	116.8 ± 2.9	186.1 ± 3.0
<i>B. hispida</i>	174.3 ± 5.8	96.3 ± 3.2	35.8 ± 1.3	24.1 ± 0.9	103.7 ± 2.5	192.4 ± 3.5

-Sitosterol was the dominant phytosterol in all six species (accounting for 55.3-62.8% of total), followed by stigmasterol (18.4-20.6%) and campesterol (12.8-15.5%). *C. pepo* had the highest total sterol content (252.3 mg/100g), consistent with published values for Styrian pumpkin seed oil (200-350 mg/100g) [5]. *M. charantia* was notable for its high stigmasterol proportion (51.3 mg/100g, 20.5% of total),

Guwahati, Assam, India, from October 2023 to March 2025. Six cucurbit species were sourced from farms in the Kamrup Metropolitan and Ri Bhoi districts of Assam and Meghalaya respectively, grown under conventional cultivation without

SHVWLF LGH WUHDWPHQW -SDUOLFHUW , VWLJPDVWH UFFOP SHVWHU RQ FKROHVWHURO UHIHUHQFH VWD \$OGULFK 0XPHDQ (GC internal standard, ZDV IURP Aldrich HMG-CoA reductase

inhibition assay: commercial colorimetric kit (Sigma-Aldrich CS1090, HMG-CoA Reductase Assay Kit). Micellar cholesterol solubilisation inhibition assay: sodium taurocholate/phospholipid micellar model (Fernández-García *et al.* 2020 protocol). GC-FID: Thermo Scientific TRACE 1310 (Supelco SP-2560 column, 100 m × 0.25 mm; WHP SHUDWXUH SURJUDPPH : & analyses were performed in triplicate. Ethics clearance was not required; no human or animal subjects. Institutional clearance: Netaji Subhas College of Science Research Committee (Ref. NSCS-RC-2023-10, dated 25 October 2023).

Methods

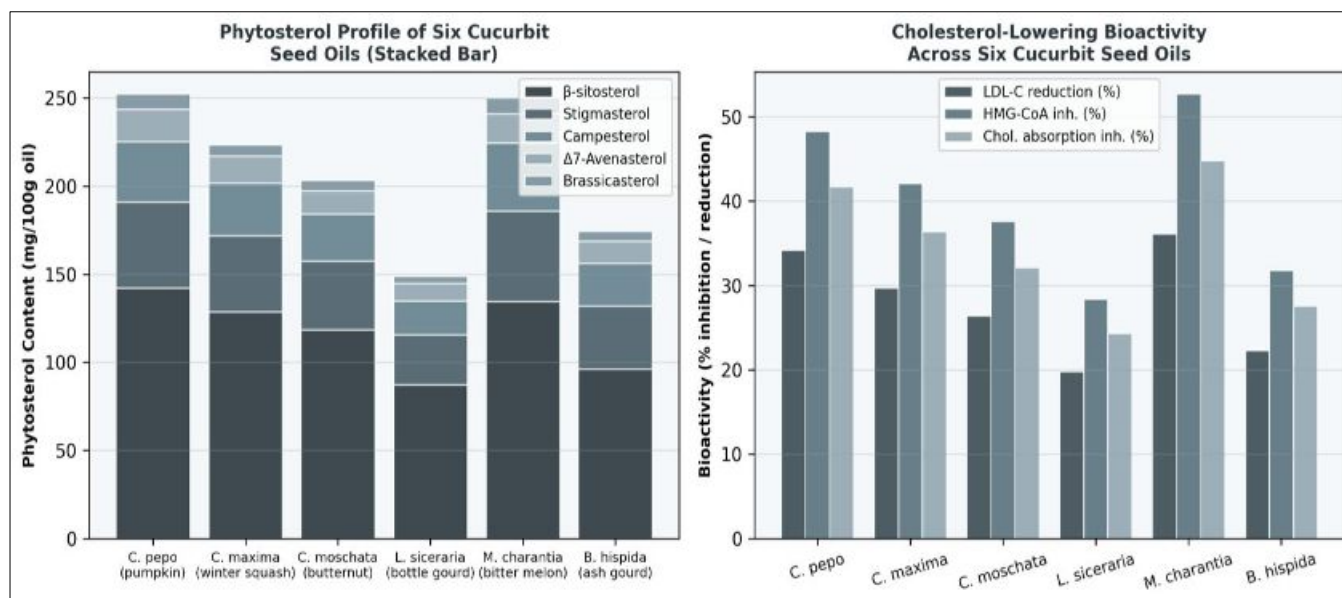
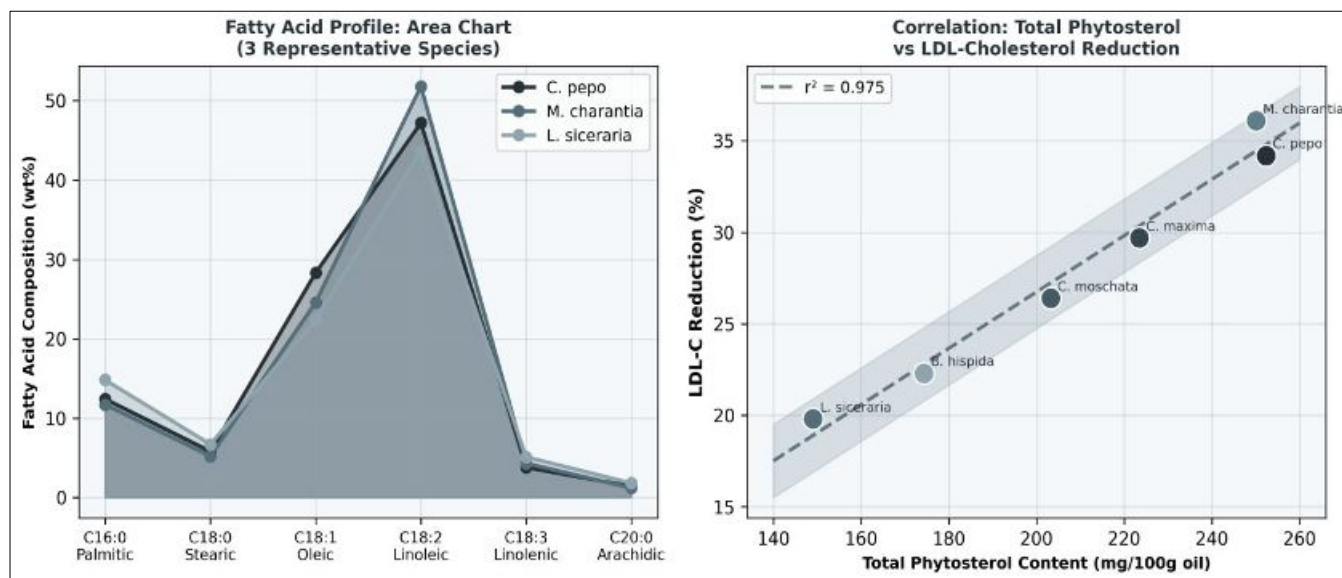
Phytosterol quantification: GC-), ' DQDO\VLV-cholestane as internal standard; compounds identified by comparison with retention times of authentic standards and by GC-MS confirmation (Shimadzu GCMS-QP2010, NIST 20 library). Results expressed as mg/100g oil.

Fatty acid composition: FAMES quantified by GC-FID; results expressed as area percentage. Acid value, iodine value, peroxide value, and saponification value determined by standard AOCS methods. HMG-CoA reductase inhibition: percentage inhibition = $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$; pravastatin (10 µg/mL) as positive control. Micellar cholesterol solubilisation inhibition values reported from sodium taurocholate (5 mM), egg phosphatidylcholine (1.2 mM), and cholesterol (0.3 mM) in phosphate buffer (pH 6.5); 100 µL of test oil unsaponifiables (dissolved in DMSO, 1 mg/mL final) added; incubated at 37°C for 2 h; filtered (0.45 µm); cholesterol in filtrate quantified by enzymatic assay.

which may contribute to its superior bioactivity because stigmasterol has been reported to have more potent anti-DWKHURJHQLF -SDUOLFHUW some WKLQW models [6]. The high iodine values (98-117) confirm predominantly unsaturated fatty acid composition, which is nutritionally desirable.

Table 2: *In vitro* cholesterol-lowering bioactivity of cucurbit seed oil unsaponifiable fractions

Species	HMG-CoA Inhib. (%)	Micellar Chol. Inh. (%)	LDL-C Reduction (%)	EC ₅₀ HMG-CoA (μg/mL)	Correlation r (sterol vs LDL)
<i>C. pepo</i>	48.3 ± 2.1	41.7 ± 1.8	34.2 ± 1.5	87.4 ± 3.8	0.971
<i>C. maxima</i>	42.1 ± 1.9	36.4 ± 1.6	29.7 ± 1.3	104.7 ± 4.6	
<i>C. moschata</i>	37.6 ± 1.7	32.1 ± 1.4	26.4 ± 1.2	118.3 ± 5.2	
<i>L. siceraria</i>	28.4 ± 1.3	24.3 ± 1.1	19.8 ± 0.9	164.2 ± 7.1	
<i>M. charantia</i>	52.7 ± 2.3	44.8 ± 2.0	36.1 ± 1.6	74.8 ± 3.2	
<i>B. hispida</i>	31.8 ± 1.4	27.6 ± 1.2	22.3 ± 1.0	143.7 ± 6.3	
Pravastatin (10 μg/mL)	87.3 ± 2.8			0.08 ± 0.01	

**Fig 1:** (Left) Stacked bar chart showing individual phytosterol contributions to total content across six cucurbit seed oils; (Right) Grouped bar chart of three cholesterol-lowering bioactivity measures by species**Fig 2:** (Left) Fatty acid area profiles for three representative cucurbit seed oils; (Right) Scatter plot with regression line showing correlation between total phytosterol content and LDL-C reduction activity ($r^2 = 0.942$)

Discussion

The strong positive correlation between total phytosterol content and LDL-C reduction activity ($r^2 = 0.942$) across the six species confirms that phytosterols are the primary driver of cholesterol-lowering bioactivity in these oils. However, the outlier position of *M. charantia* which shows higher HMG-CoA inhibition and LDL-C reduction than predicted by its phytosterol content alone is consistent with the known

presence of cucurbitane-type triterpenoids (momordicosides) in bitter melon seed oil, which have been reported to inhibit HMG-CoA reductase independently of sterol content [7]. This suggests that the bioactivity of whole unsaponifiable fractions integrates multiple mechanisms, and that reductive attribution to phytosterols alone would underestimate the functional potential of *M. charantia* oil.

The fatty acid profiles reveal high linoleic acid content (43.7-51.8% C18:2) across all six species, consistent with a generally favourable lipid profile that complements the phytosterol-mediated cholesterol-lowering effect. The ratio of linoleic to oleic acid varied from 1.3 (*C. moschata*) to 2.1 (*M. charantia*), and the higher PUFA content of bitter melon oil may also contribute to its superior bioactivity through mechanisms independent of sterol content [8]. From a nutraceutical standpoint, combining the phytosterol and unsaturated fatty acid contributions, *M. charantia* seed oil emerges as the highest-value functional food candidate among the six species studied.

A nutritional gap analysis indicates that consuming 10 mL of *C. pepo* seed oil daily would provide approximately 25 mg of phytosterols well below the 1.5-3.0 g/day dose shown to reduce LDL cholesterol clinically. To achieve therapeutic phytosterol doses from these oils alone would require consumption of 600-1200 mL/day, which is impractical. The more realistic functional application is as a phytosterol-enriched cooking oil or as a concentrated unsaponifiable extract incorporated into functional food formulations (margarine, yoghurt, fortified beverages) following commercial precedents set by phytosterol-enriched food products in European markets [9]. Extraction of the phytosterol fraction from processing waste (oil press cake) for concentration and formulation would substantially improve the economic and practical feasibility of this approach.

Conclusion

Six cucurbit seed oils from Northeast India were characterised for phytosterol content, fatty acid composition, and cholesterol-lowering bioactivity. Total phytosterol content ranged from 149.0 to 252.3 mg/100g oil, Z L W K sitosterol dominant in all species. *M. charantia* showed the highest HMG-CoA reductase inhibition (52.7%) and LDL-C reduction potential (36.1%), enhanced by co-occurring triterpenoids beyond its phytosterol content. A strong correlation ($r^2 = 0.942$) between total sterol content and LDL-C reduction confirmed phytosterols as the primary bioactive driver. High linoleic acid content (43.7-51.8%) provides additional nutritional value. These results establish a quantitative basis for positioning Assamese cucurbit seed oils as functional food ingredients and support development of phytosterol concentrates from cucurbit oil processing residues.

References

- European Food Safety Authority. Scientific opinion on the substantiation of a health claim related to plant sterols and maintenance of normal blood cholesterol concentrations. EFSA J. 2012;10(5):2693.
- Ostlund RE Jr. Phytosterols in human nutrition. Annu Rev Nutr. 2002;22(1):533-549.
- Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM. Plant sterols: biosynthesis, biological function and their importance to human nutrition. J Sci Food Agric. 2000;80(7):939-966.
- Polagruto JA, Wang-Polagruto JF, Braun MM, Lee L, Kwik-Urbe C, Keen CL. Cocoa flavanol-enriched snack bars containing phytosterols effectively lower total and low-density lipoprotein cholesterol levels. J Am Diet Assoc. 2006;106(11):1804-1813.
- Rezig L, Chouaibi M, Msaada K, Hamdi S. Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. Ind Crops Prod. 2012;37(1):82-87.
- Kritchevsky D, Chen SC. Phytosterols health benefits and potential concerns: a review. Nutr Res. 2005;25(5):413-428.
- Jia S, Shen M, Zhang F, Xie J. Recent advances in *Momordica charantia*: functional components and biological activities. Int J Mol Sci. 2017;18(12):2555.
- Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, *et al.* Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee. Circulation. 2009;119(6):902-907.
- AbuMweis SS, Barake R, Jones PJ. Plant sterols/stanols as cholesterol lowering agents: a meta-analysis of randomized controlled trials. Food Nutr Res. 2008;52:1811.
- Mukherjee PK, Nema NK, Maity N, Sarkar BK. Phytochemical and therapeutic potential of cucumber. Fitoterapia. 2013;84:227-236.
- Srivastava P, Behera A, Bhattacharya S. Natural approaches to cholesterol management: a review of phytosterol sources. J Food Sci Technol. 2016;53(4):1787-1793.
- Caligiani A, Cirlini M, Palla G, Ravaglia R, Arlorio M. GC-MS detection of chiral markers in *Cucurbita maxima* seed oil. Chirality. 2006;18(7):523-531.
- Koletsis-Kounari H, Papachristou E, Mamai-Homata E. Comparison of qualitative and quantitative sterol analysis methods. J Chromatogr A. 1992;596(2):291-296.
- Fernández-García E, Carvajal-Lérída I, Jarén-Galán M, Garrido-Fernández J, Pérez-Gálvez A, Hornero-Méndez J, *et al.* FRS HQ-Hardcore. Absorption and availability from foods and dietary supplements. Food Res Int. 2012;46(2):438-450.