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ORIGINAL ARTICLE

Assessment of β-Sitosterol in *Capsicum Annuum Grossum*: A Phytochemical Approach

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Abstract

Background/Aim: *Capsicum annuum Grossum* Senise, a widely cultivated sweet pepper in Iraq contains a diverse array of bioactive compounds such as polyphenols, carotenoids and capsinoids, known for their beneficial effects on health. The study aimed to employ advanced analytical techniques to elucidate the compound's chemical properties and concentration within this specific *Capsicum* cultivar, contributing to the growing body of knowledge regarding plant-derived sterols with potential nutraceutical application.

Methods: This study focused on the extraction, isolation, purification and quantitative analysis of β-sitosterol from Iraqi *Capsicum annuum Grossum*. Advanced chromatographic and spectroscopic techniques were employed to ensure accurate quantification and structural characterisation.

Results: The chloroform fraction exhibited a high β-sitosterol concentration (0.492 mg/mL dry weight), suggesting efficient biosynthesis of this secondary metabolite, possibly due to favourable biochemical and environmental factors.

Conclusion: The findings underscore the potential of Iraqi-grown *Capsicum annuum Grossum* as a valuable source of β-sitosterol, supporting its use in nutraceutical and pharmaceutical applications. Further research is warranted to explore its mechanistic pathways and therapeutic efficacy in clinical settings.

Key words: β-sitosterol; *Capsicum, annuum Grossum*; Pharmacognosy; Phytosterols.

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Introduction

Capsicum, a genus within the Solanaceae family, comprises five cultivated species: *Capsicum annuum, Capsicum chinense, Capsicum baccatum, Capsicum frutescens* and *Capsicum pubescens*.[1] In Iraq, only *Capsicum annuum Grossum* and *Capsicum frutescens* are cultivated.[2] These species share similarities in fruit shape and colouration, though *Capsicum annuum Grossum* is particularly notable for its rich content of essential nutrients and pharmacologically active compounds that contribute to various health benefits such as vitamins A, C and E, carotenoids and capsaicinoids, which contribute to various health benefits including antioxidant activity, immune system support, anti-inflammatory effects and potential roles in pain relief and metabolic regulation.[3] Chemical analysis reveals that *Capsicum annuum Grossum* fruits contain 9.92 % dry matter, with

nutritional components per 100 g serving including 0.33 g total fat, 0.99 g protein, 10.63 g carbohydrates, 2.73 g dietary fibre and 133.00 mg vitamin C, providing 195.58 kJ (46.79 kcal) of energy.[4] The characteristic pungency of peppers stems primarily from capsaicinoids, with capsaicin and dihydrocapsaicin constituting approximately 90 % of these compounds, accompanied by at least nine minor capsaicinoids such as nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nornorcapsaicin and vanillylamide.[5] Comprehensive phytochemical investigations of *Capsicum annuum Grossum* have identified approximately 33–34 polar and non-polar acidic compounds, 32–33 lipid constituents and 88–103 non-lipid bioactive compounds, reflecting the plant's complex and diverse chemical composition.[6] The anti-inflammatory potential of *Capsicum annuum Grossum* has been demonstrated through its *in vitro* inhibition of soybean lipoxygenase, with extracts applied in solution form. Among the tested varieties, green *Capsicum annuum Grossum* extracts showed the highest inhibition at 46.12 %, followed by yellow at 44.09 % and red at 32.18 %.[7] Parallel studies on *C frutescens* ethyl acetate extracts revealed comparable anti-inflammatory effects to diclofenac in rat models of egg albumin-induced paw oedema.[8]

Phytosterols, structurally like mammalian cholesterol, represent another important class of bioactive compounds in *Capsicum species*. The major phytosterols include β-sitosterol (65 %), campesterol (30 %) and stigmasterol (3 %), which are widely distributed in plant-based food such as nuts, seeds, legumes and olive oil.[9] β-sitosterol has been particularly well-studied and is recognised for its safety and absence of harmful side effects in pharmaceutical applications.[10] Extensive research has documented the diverse pharmacological properties of β-sitosterol, including neuropharmacological effects such as anxiolytic, sedative[12] and analgesic activities;[11, 13] immunomodulatory[14] and antimicrobial properties;[15] anticancer potential through tumour inhibition[16] and anti-inflammatory effects via lipoxygenase (LOX) activity. Notably, LOX suppression has been primarily associated with the anti-inflammatory action of β-sitosterol, rather than being a central target in its other pharmacological effects.[17] Additionally, β-sitosterol exhibits metabolic regulatory effects including protection against non-alcoholic fatty liver disease,[18] lipid-lowering properties,[19] hepatoprotective[20] and respiratory protective effects,[21] as well as wound healing capacity.[22] Its antioxidant[23] and antidiabetic activities[24] further contribute to its therapeutic profile, along with protective effects against various digestive disorders such as gastric ulcers, irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) including Crohn's disease and ulcerative colitis (Table 1).[25] These findings highlight the significant nutritional and pharmacological value of *C annuum*, particularly its β-sitosterol content, which holds considerable promise for development into nutraceutical and pharmaceutical products.

Table 1: Biological activity of β-sitosterol[12-25]

N	Biological activity	Study type	Experimental model
1	Anxiety reduction and calming effects	*In vivo*	Male Swiss Webster mice
2	Pain relief and inflammation suppression	*In vivo*	Swiss strain male albino mice and Wistar albino rats
3	Immune system modulation	*In vitro* and *in vivo*	Human PBMCs and male swine
4	Bacterial growth inhibition	*In vitro*	*Staphylococcus aureus* and *Escherichia coli*
5	Tumour suppression	*In vivo*	Wistar albino rats
6	Inflammation reduction	*In vivo*	Male Wistar rats
7	Prevention of fatty liver disease	*In vivo*	Murine models
8	Cholesterol reduction	*In vivo*	Male mice
9	Liver cell protection	*In vivo*	Male Sprague-Dawley rats
10	Lung fibrosis mitigation	*In vitro*	A549 human lung cancer cells
11	Tissue repair enhancement	*In vitro*	Human fibroblast cultures
12	Oxidative stress reduction	*In vivo*	Wistar strain male albino rats

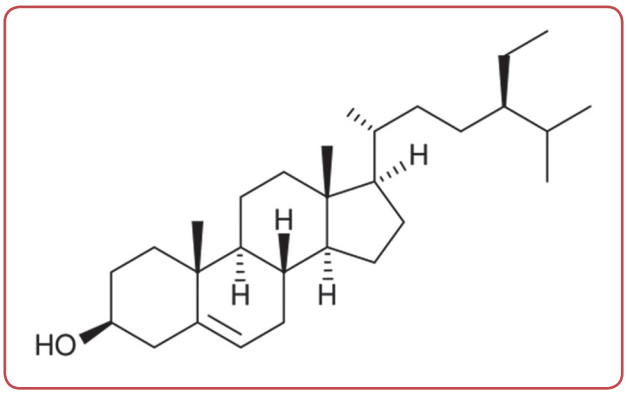

Figure 1: Chemical structure of β-sitosterol[28]

β-Sitosterol is a key constituent of plant cell membranes, essential for regulating their flexibility and selective permeability. This phytosterol presents as a white, waxy powder characterised by a distinctive aromatic profile. While structurally analogous to cholesterol, β-sitosterol is distinguished by the presence of an additional ethyl group at the C-24 position.[26] The compound is known by several systematic designations including (3β)-stigmast-5-en-3-ol, 22:23-dihydro stigmasterol, α-dihydrofucosterol, cinchol, cupreol, rhamnol, quebrachol and sitosterin. It exhibits three distinct crystalline forms depending on hydration state: anhydrous, hemihydrate and monohydrate configurations (Figure 1).[27, 28] The current investigation focuses on the precise quantification, isolation, purification and comprehensive chemical characterisation of β-sitosterol derived from Iraqi-origin *Capsicum annuum Grossum*, a variety particularly rich in this bioactive phytosterol. The study employed advanced analytical techniques to elucidate the compound's chemical properties and concentration within this specific *Capsicum* cultivar, contributing to the growing body of knowledge regarding plant-derived sterols with potential nutraceutical applications.

Methods

Plant material

The plant material was collected in Baghdad, Iraq and its authenticity was verified by the Pharmacognosy Department at the University of Baghdad's College of Pharmacy. Approximately 250 g of the aerial parts of the plants was subjected to extraction using a Soxhlet apparatus. The extraction was carried out using 85 % methanol, after which the solution was filtered and concentrated under vacuum. The obtained crude methanol extract was then dispersed in distilled water and subjected to liquid-liquid partitioning in a separatory funnel with chloroform. The extraction employed 85 % methanol for Soxhlet extraction and chloroform partitioning, a cost-effective method that efficiently disrupts plant matrices to extract both polar metabolites and non-polar compounds like β-sitosterol. The isolated fraction was subsequently dehydrated using anhydrous sodium sulphate, filtered and completely evaporated under reduced pressure using a rotary evaporator. The dried residue was then measured and preserved for subsequent analysis.

Preliminary phytochemical screening

The presence of phytosterols in *Capsicum Annuum Grossum* was detected through preliminary phytochemical screening using the Liebermann-Burchard test. A small portion of the chloroform layer was mixed with 10 drops of acetic anhydride, followed by the addition of 2 drops of concentrated sulfuric acid, leading to the development of a characteristic bluish-green colour indicative of phytosterols.[29]

Isolation and purification of β-sitosterol via preparative high-performance liquid chromatography (Prep-HPLC)

Prep-HPLC (Shimadzu, Japan) is a well-established technique for separating complex mixtures. In this study, a methanol-acetonitrile mobile phase was used in a gradient solvent system, initially set at 30:70, which was gradually increased in a linear gradient to 30:70 for 20 minutes, 0:100 for 10 minutes and finally 30:70 for 15 minutes. The UV detection was performed at 210 nm, with a flow rate of 5 mL/min. The purification process involved multiple purification cycles to obtain β-sitosterol in its pure form.[30]

Quantification of isolated β-sitosterol by high-performance thin-layerchromatography (HPTLC)

The chloroform fraction was identified and quantified using a pre-validated HPTLC method. The HPTLC conditions included a mobile phase of toluene: ethyl acetate: chloroform (50:10:4), thin-layer chromatography was conducted on silica gel 60 F254 pre-coated plates (20×10 cm, 0.2 mm layer thickness). Detection was performed under UV

254 nm and 366 nm and derivatisation was carried out using 5 % H_2SO_4 for visualisation.

Identification and characterisation of the isolated β-sitosterol

Fourier transform infrared (FT-IR) spectroscopy

Characterisation by FT-IR spectroscopy was carried out at the Baghdad National Centre for Drug Control and Research using Bruker instrumentation. Compound structures were verified by interpreting their distinctive spectral absorption patterns, consistent with the findings presented in the results.

Nuclear magnetic resonance (NMR) analysis

NMR spectroscopy was performed to further characterise the isolated β-sitosterol. The ^{1}H-NMR spectra were recorded using a Euro-vector EA 3000A NMR spectrometer (Italy, 300 MHz). The sample was dissolved in dimethyl sulfoxide-d6 (DMSO-d6; Sigma-Aldrich, USA) and tetramethylsilane (TMS; Merck, Germany) was used as the internal reference standard at 0 ppm. The chemical shifts (δ values) were analysed for structural confirmation.

Results

Preliminary phytochemical screening

The results of phytochemical screening assay for *Capsicum annuum Grossum* indicate the presence of sterols and steroids by production bluish green colour indicates the presence of steroidal nucleus.

Isolation of β-sitosterol through Prep-HPLC

The resulting chromatogram revealed multiple peaks, each representing a distinct compound, with a prominent peak appearing at 16.3 minutes and identified as β-sitosterol based on comparison with a reference standard. The target compound was isolated using a fraction collector, with collection intervals carefully timed to match the retention times of each resolved peak, as shown in Figure 2. To confirm the identity of the β-sitosterol peak, a UV absorbance spectrum was recorded using a diode array detector (DAD) at a wavelength of 210 nm, showing a spectral match with the β-sitosterol standard, Figure 3.

Quantification of isolated β-sitosterol

The presence of β-sitosterol in the chloroform-soluble fraction was confirmed by matching both the retardation factor (R_f = 0.63) and the UV absorption profile with those of the authentic standard, as illustrated in Figure 4 (HPTLC plate). For quantitative analysis, a standard calibration curve was constructed using five concentrations of authentic β-sitosterol (ranging from 0.1 to 1.0 mg/mL), with

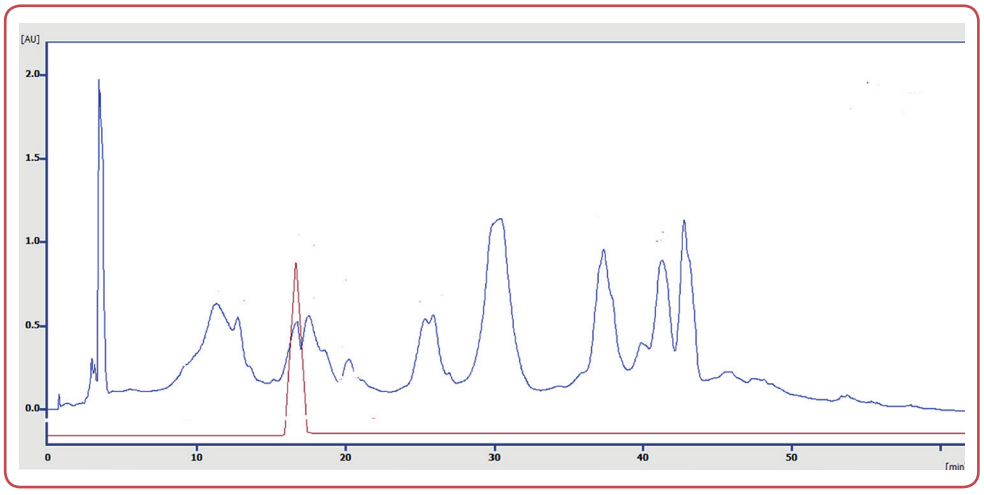

Figure 2: Preparative high-performance liquid chromatography (Prep-HPLC) chromatogram for β-sitosterol standard with the matched peak chloroform fraction

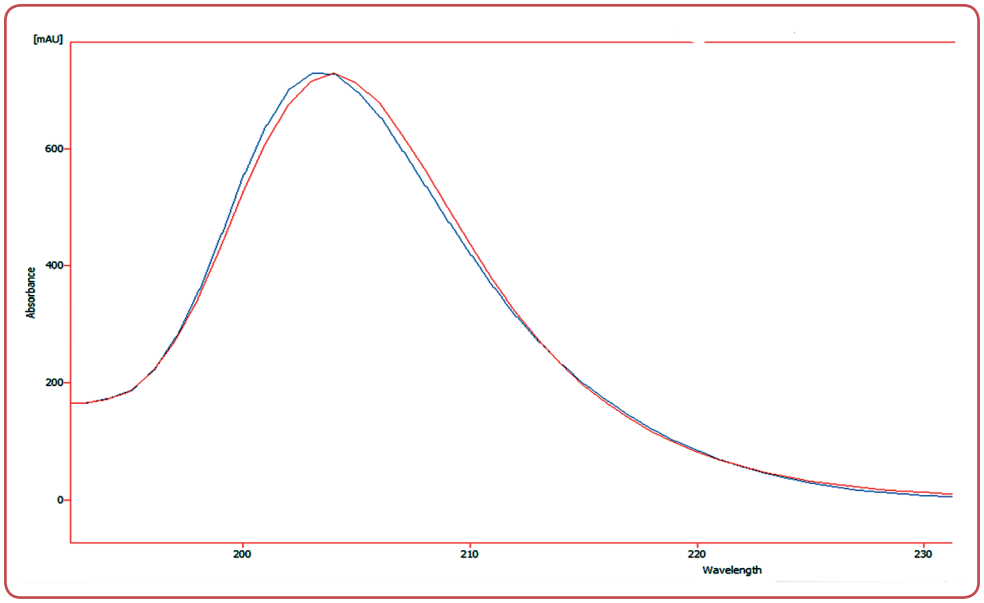

Figure 3: UV spectrum for β-sitosterol

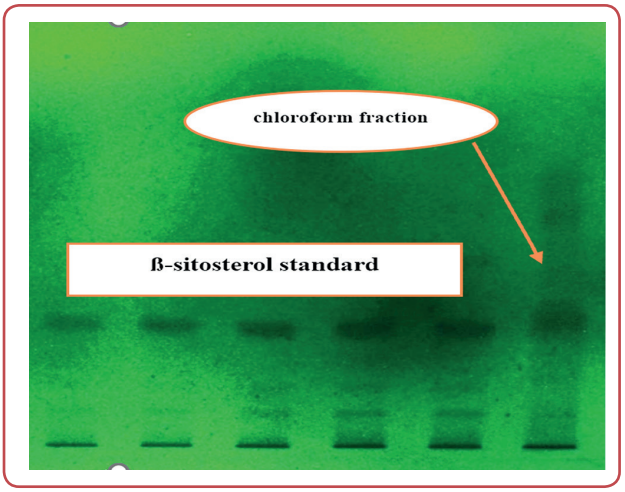

Figure 4: High-performance thin-layer chromatography (HPTLC) plate of chloroform fraction

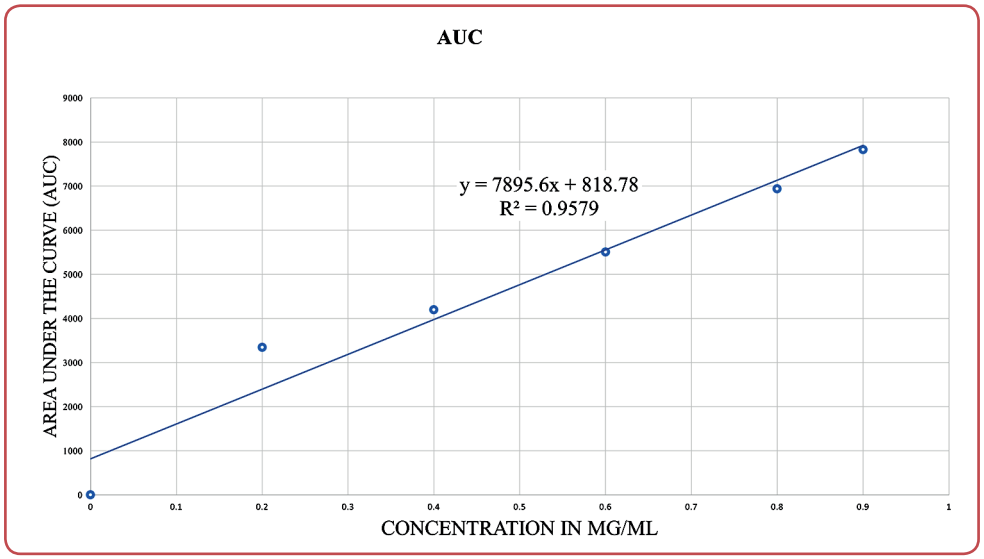

Figure 5: Calibration curve of β-sitosterol

linear regression analysis yielding a correlation coefficient (R^2) of 0.9579, indicating excellent linearity (Figure 5). Based on this calibration, the β-sitosterol content in the chloroform fraction was determined to be 0.492 mg/mL dry weight, reflecting a significant concentration of the compound in the extract.

Identification and characterisation of the isolated β-sitosterol

The FT-IR of the isolated β-sitosterol showed identical spectrum to that reported in literature[31] as shown in Figure 6. The characteristic bands of β-sitosterol are listed in Table 2.

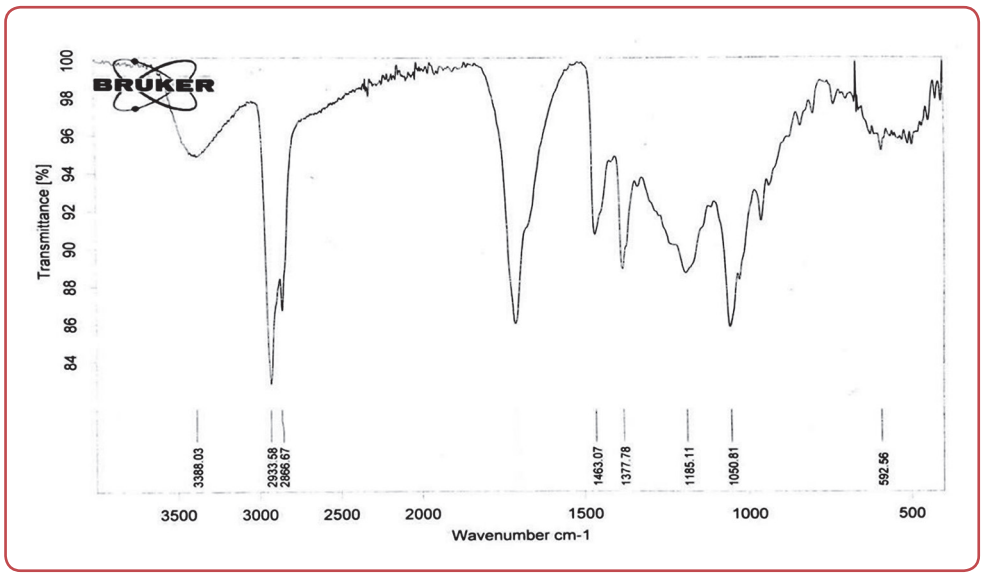

Figure 6: Fourier transform infrared (FT-IR) spectra of isolated β-sitosterol

Table 2: Distinctive Fourier transform infrared (FT-IR) spectral peaks (cm^{-1})

Functional group	Group frequency wave number (cm^{-1}) Isolated β-sitosterol	Main attributed
O-H	3388.03	O-H stretching vibration
C-H	2933.58 2866.67	Aliphatic C-H stretching
C=C	1694	C=C stretching
C-H	1463	Bending frequencies of cyclic (CH_2)
CH2-CH3	1377.78	CH (CH_3)$_2$ stretching
O-H	1185.11	O-H bending
C-C	1050.81	C-C stretching
C-H	832, 794, 621	C-H out plane bending vibration

NMR is the agreed to be the optimal approach for compound characterisation and structural determination, for isolated β-sitosterol the result showed that the NMR spectral data were closely similar to that reported in literatures for β-sitosterol,32, 33 as shown in Figure 7 and Table 3.

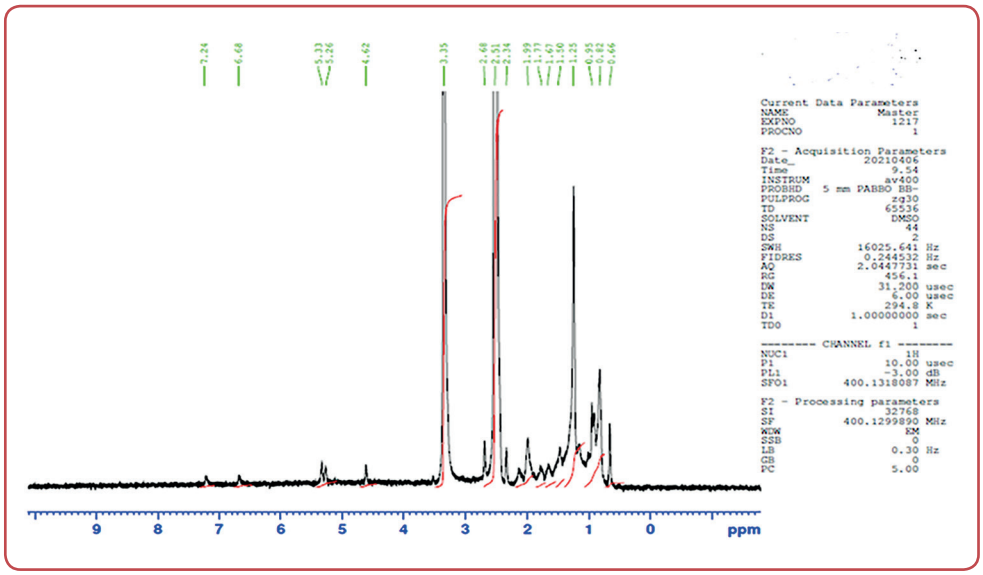

Figure 7: ^{1}*H-NMR spectrum*

Table 3: Nuclear magnetic resonance (NMR) spectral data

Carbon atom	Ppm experimental	Integration	Multiplicity	Assignment
C-3	7.24	1H	Singlet	OH
C-6	5.26	1H	Multiplet	CH
C-3	3.35	1H	Multiplet	CH
C-21	1.25	3H	Doublet	CH3
C-19		2H	Singlet	
C-29	0.95	3H	Triplet	CH3
C-26, C-27	0.82	3H	Doublet	CH3
C-18	0.66	1H	Singlet	CH3

Discussion

The successful isolation and characterisation of β-sitosterol from *Capsicum annuum Grossum* grown in Iraq represents a significant contribution to the understanding of phytochemical qualification of this plant. The quantified concentration of 0.492 mg/mL in the chloroform fraction highlights the potential of Iraqi-grown *Capsicum annuum Grossum* as a rich source of this bioactive phytosterol. This finding is particularly relevant given the well-documented pharmacological properties of β-sitosterol, including its anti-inflammatory, antioxidant and lipid-lowering effects. The structural confirmation through FT-IR and NMR spectroscopy aligns with previous reports, demonstrating characteristic functional groups and proton signals consistent with β-sitosterol's known structure. These results not only validate the identity of the isolated compound but also reinforce the reliability of the analytical methods employed in this study.

When compared to other plant sources, the β-sitosterol yield from *Capsicum annuum Grossum* is competitive, with a quantified concentration of 0.492 mg/mL in the chloroform-soluble fraction. This yield reflects a substantial content relative to commonly reported levels in other medicinal plants, highlighting *C annuum* var *Grossum* as a valuable source of β-sitosterol, suggesting that this cultivar may be particularly efficient as a source for synthesis of secondary metabolite. The observed concentration exceeds levels found in some common dietary sources, such as nuts and seeds, which underscores the nutritional and

therapeutic potential of *C annuum*. However, it is important to note that variations in extraction methodologies and growing conditions can significantly influence phytosterol yields. The use of Soxhlet extraction and chloroform partitioning in this study, while effective, may not capture all sterol derivatives, such as glycosides or esterified forms, which could be explored in future research. The β-sitosterol yield from Iraqi *Capsicum annuum Grossum* (0.492 mg/mL) is notably higher than many Capsicum species like *Capsicum frutescens* ~0.17 mg/mL (ethyl acetate fraction) and common dietary sources like Almonds, Pumpkin seeds and Soybean oil supporting its nutraceutical potential.[34, 35]

The anti-inflammatory and antioxidant properties of β-sitosterol, as inferred from its high concentration in *C annuum*, support its potential applications in nutraceutical and pharmaceutical formulations. Previous studies have demonstrated β-sitosterol's efficacy in mitigating inflammation and modulating lipid metabolism, which aligns with the pharmacological profile observed in *C annuum* extracts.[36] However, the current study's reliance on *in vitro* and phytochemical assays limits direct extrapolation to *in vivo* or clinical efficacy. Future research should focus on investigating the bioavailability, pharmacokinetics and mechanistic pathways of β-sitosterol from *C annuum* using animal models or human trials to fully elucidate its therapeutic potential.

While the current study confirms β-sitosterol's presence and references its known pharmacological effects, further *in vivo* validation is proposed. This includes anti-inflammatory testing using carrageenan-induced paw oedema in rats,[17] a hypolipidemic study in high-fat diet-induced hyperlipidaemic models[19] and a bioavailability assessment through oral administration and plasma monitoring via HPLC.[10] These experiments aim to substantiate the compound's therapeutic relevance beyond *in vitro* analysis and support its potential for clinical or industrial application.

Despite these promising findings, several limitations must be acknowledged. The extraction process, while robust, may not be optimal for all sterol derivatives and alternative techniques, such as supercritical fluid or ultrasound-assisted extraction, could enhance yields. Additionally, the study used market-sourced samples, which may introduce variability due to differences in growing conditions, post-harvest handling and storage. These factors could affect the consistency of metabolite profiles and should be controlled in future studies.

Conclusion

In conclusion, this study demonstrates that *Capsicum annuum Grossum* from Iraq is a valuable source of β-sitosterol, with significant potential for nutraceutical and pharmaceutical applications. The Iraqi cultivar *Capsicum annuum var grossum* demonstrates unique advantages, including a high β-sitosterol yield (0.492 mg/mL), surpassing many other Capsicum species and dietary sources. Its adaptation to Iraq's semi-arid climate enhances secondary metabolite production, while its wide availability, short growth cycle and suitability for large-scale cultivation make it an ideal candidate for regional phytopharmaceutical development. The findings contribute to the growing body of knowledge on plant-derived sterols and highlight the importance of further research to optimise extraction methods and validate the observed bioactivities in clinical settings. By addressing the current limitations and exploring the synergistic effects of β-sitosterol with other bioactive compounds in Capsicum, future studies can unlock the full therapeutic potential of this promising phytosterol.

Ethics

This study did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request. The raw data include chromatographic (Prep-HPLC and HPTLC), spectroscopic (FT-IR and ^{1}H-NMR) output files, calibration curves and quantified values of β-sitosterol from *Capsicum annuum Grossum*.

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