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Synthesis of *β*-Hydroxy-*β*-methylglutaryl- coenzyme A reductase inhibitors utilizing *in-situ* and *ex-situ* recovered phytosterols from *Gymnema sylvestre* waste

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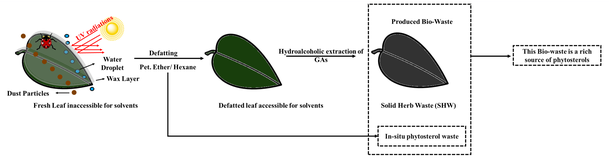
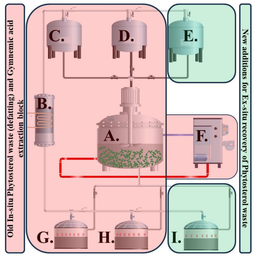
Introduction



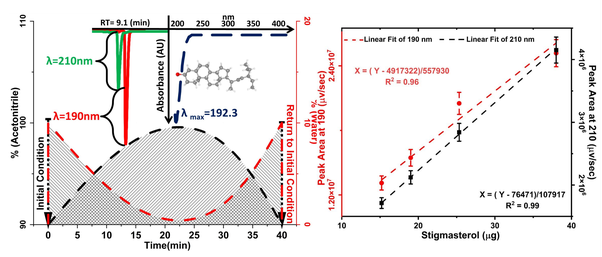
- Herbal medicine use is rapidly increasing worldwide, generating large quantities of processing waste.
- *Gymnema sylvestre* is widely used for producing hypoglycaemic extracts rich in Gymnemic acids.
- Industrial extraction generates phytosterol-rich waste streams and solid herb waste (SHW).
- These waste streams contain valuable phytosterols, particularly stigmasterol, that are often discarded.
- This study recovers stigmasterol from waste, synthesizes novel derivatives, and evaluates their potential as HMG-CoA reductase inhibitors.

Methodology for phytosterol recovery



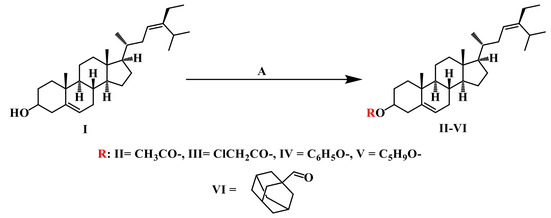
- An integrated industrial process was developed to recover phytosterols from both in-situ defatting waste and ex-situ solid herb waste generated during *Gymnema sylvestre* extract production.
- The recovered phytosterol fractions provided a sustainable source of stigmasterol for the synthesis of value-added bioactive molecules.

Analytical Assessment



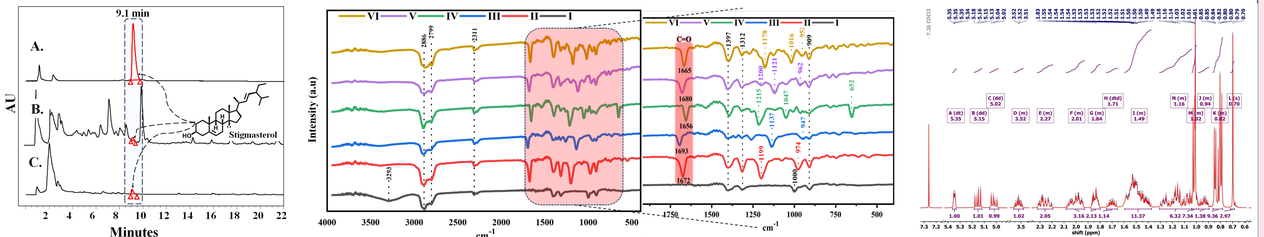
- A validated Rp-HPLC method was developed for quantitative estimation of stigmasterol in phytosterol waste (PW).
- Method validation demonstrated excellent sensitivity, precision, linearity, and chromatographic performance.
- Stigmasterol content in PW was determined using calibration curve-based quantification.

Isolation and derivatization of Stigmasterol

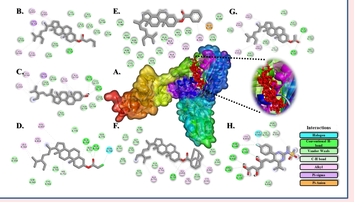


- Stigmasterol was isolated by normal phase column chromatography.
- Stigmasterol esters (II–VI) were synthesized by esterification and purified by column chromatography.
- TLC analysis confirmed product formation, purity, and reaction completion.
- TGA, FT-IR, and NMR analyses verified the structure and thermal stability of the synthesized compounds.

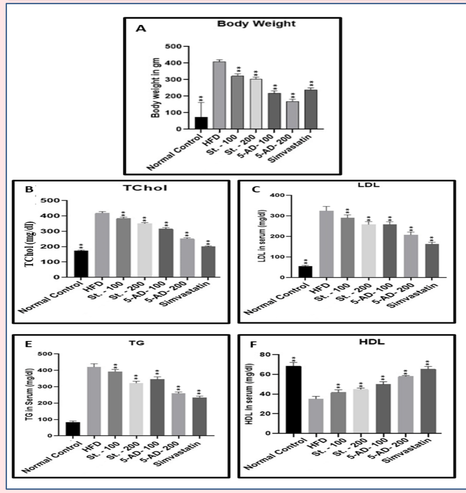
Introduction



- Industrial processing of Gymnema sylvestre generated phytosterol-rich waste streams, yielding 4.63% (PWin) and 4.49% (PWex) extractives.
- A validated Rp-HPLC method quantified stigmasterol at 0.67% in PWin and 0.72% in PWex.
- Bulk isolation of stigmasterol was successfully achieved using silica gel column chromatography.
- Five stigmasterol-derived esters (II–VI) were synthesized through esterification reactions and purified chromatographically.
- TLC, FT-IR, TGA, and NMR analyses confirmed the structure, purity, and thermal stability of the synthesized derivatives.
- Molecular docking identified Compound VI as the most potent HMG-CoA reductase inhibitor, exhibiting the highest binding affinity among all synthesized compounds.



In-vivo assessment



- High-fat diet (HFD) induced hypercholesterolemia, characterized by increased body weight, total cholesterol, LDL, and triglycerides, along with reduced HDL levels.
- Both stigmasterol and Compound VI significantly improved serum lipid profiles compared to the HFD control group.
- Compound VI demonstrated superior hypocholesterolemic activity, producing greater reductions in total cholesterol, LDL, and triglycerides, and a greater increase in HDL than stigmasterol.

Conclusion

Industrial phytowaste from Gymnema sylvestre can be transformed into value-added stigmasterol-derived therapeutics, with Compound VI emerging as a promising anti-hypercholesterolemic lead molecule.

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