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An Extensive Evaluation of Semi-Synthetic Production, The Importance of Pharmacological Effects, and Analytical Techniques for Identifying the Compounds Found in Phytosterols–B-Sitosterol

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ABSTRACT:

β-Sitosterol, a key phytosterol derived from plants, bears structural similarities to cholesterol and is commonly found in various foods like vegetable oils, nuts, and avocados. It demonstrates a wide range of biological activities, encompassing cholesterol reduction, anti-inflammatory properties, antioxidant effects, antimicrobial actions, and anticancer capabilities, along with modulation of the immune system and potential advantages in conditions such as diabetes, arthritis, and pulmonary tuberculosis. Despite its abundance in nature, obtaining pure β-sitosterol is difficult because of the presence of similar phytosterols, leading to interest in semi-synthetic methods like the tosylation of stigmasterol and green chemistry approaches for synthesizing derivatives, including enzyme-mediated transformations. This review offers an in-depth examination of β-sitosterol, addressing its natural presence, semi-synthetic and biocatalytic production methods, green chemistry applications, and analytical characterization techniques (FTIR, NMR, GC-MS), as well as its pharmacological significance. The article discusses current challenges such as low bioavailability, the difficulty of purification, and the limitations associated with large-scale production, while also highlighting emerging opportunities in sustainable synthesis, advanced purification technologies, and biotechnological innovations. By incorporating recent developments and identifying research gaps, this article emphasizes the potential of β-sitosterol as a promising candidate for applications in pharmaceuticals, nutraceuticals, and functional foods.

Keywords: β-Sitosterol, plant sterols, semi-synthetic processes, green chemistry, therapeutic effects, and analytical method

1. INTRODUCTION:

Phytosterols are plant-derived compounds resembling steroids, characterized by the presence of alcohol groups. Common examples include stigmasterol, sitosterol, spinasterol, and campesterol. Among these, β-sitosterol is the most prevalent variant, available in several forms—α, β, and γ—each differing in their side chains. Similar in structure to cholesterol, β-sitosterol is distinguished by an ethyl group located at the 24-position, making it 24-ethyl-5-cholestene-3-ol. This compound is utilized extensively for producing steroid medications, cosmetics, and wound healing products, and also serves as an anti-inflammatory agent. Additionally, it is used as a food additive with hypolipidemic properties.

When undergoing oxidation, β-sitosterol can convert into cholesterol and various oxidation products. However, the oxidation of cholesterol can lead to adverse health issues, such as atherosclerosis, often linked to elevated levels of oxidized phytosterols in the bloodstream. β-sitosterol can be sourced from foods like avocados, vegetable oils, and nuts such as soybeans. Research has indicated that it possesses anti-inflammatory, hypolipidemic, anti-cancer, and angiogenic properties, alongside antibacterial effects.

As a hypolipidemic agent, β-sitosterol effectively lowers cholesterol levels by inhibiting its absorption in the intestines in humans. Both laboratory and animal studies have shown its potential to inhibit the growth of cancer cells in conditions like colon, prostate, and breast cancers. Despite its presence in natural sources, the efficient and cost-effective extraction of phytosterols remains a challenge, often resulting in a mixture of compounds. Consequently, synthetic approaches for these compounds have been explored. This study intends to thoroughly examine the chemical synthesis, biosynthesis, pharmacological relevance, and analytical techniques related to β-sitosterol.[1],[2],[3],[4,],[5].

1.2. STRUCTURE OF β-SITOSTEROL:

The molecular formula is C29H50O.

Molecular weight is 414.71 g.

IUPAC name is (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3-ol.

β-sitosterol is an unsaturated sterol with one double bond in a sterol ring structure.[6]

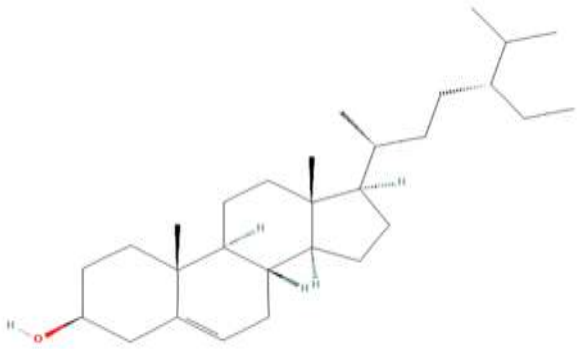

Fig no.1

1.3 PHYSICHEMICAL PROPERTIES:[6]

S. No.	Characteristics	
1	Formula	$C_{29}H_{50}O$
2	Number. heavy atoms	30
3	Molecular weight	414.7g/mol
4	Number. aromatic heavy atoms	0
5	Number. rotatable bonds	6
6	H-bond acceptors' count	1
7	Number. H-bond count	1
8	Molar refractivity	133.23
9	Number of violations (nVio)	1
10	Melting temperature	140˚C
11	Bioavailability score	0.55
12	Gastrointestinal absorption	Low
13	Blood-brain barrier (BBB) permeant	Nos
14	Nuclear receptor ligand	0.73
15	Enzyme inhibitor	0.51

2. SEMI-SYNTHETIC SYNTHESIS OF β-SITOSTEROL:[8]

To obtain pure β-sitosterol 2, the initial step involves synthesizing stigmasterol tosylate 8 from stigmasterol 1 via standard tosylation methods. This tosylate is then reacted with anhydrous methanol and pyridine, leading to the formation of i-stigmasterol methyl ether 9 as a viscous solid with a yield of 74%. Alongside this product, stigmasterol methyl ether 10 is produced in a 5:1 ratio, which was previously not identified as a by-product. While the usual practice involves purifying this mixture through chromatography, it was used without purification, and the minor component was removed in a later step.

The hydrogenation process follows established techniques that date back to 1963, utilizing palladium on carbon (Pd/C) and ethyl acetate as the solvent. However, recent investigations have noted isomerization during the hydrogenation of campesterol, prompting the examination of various catalysts such as platinum dioxide (PtO2) and 5% Pd/C. After removing the double bond in the B-ring, β-sitosterol was achieved with a purity of 85.9% as confirmed by gas chromatography-mass spectrometry (GC-MS). Although PtO2 was found to enhance hydrogenation selectivity for campesterol, it did not significantly affect the hydrogenation outcomes for β-sitosterol, irrespective of the solvents used. Other catalysts, including Raney nickel and Wilkinson's catalyst, showed no reactivity even under excess and varied conditions.

Initially, ethyl acetate was used, but this led to the creation of an unidentified isomer, possibly due to double bond shifts influenced by residual acetic acid. Hydrogenation using a base resulted in no reduction. The process then switched to ethanol, which, despite initial solubility challenges, allowed the substrate to dissolve completely as the reaction progressed. Post-deprotection, high-purity β-sitosterol was verified using NMR, which showed no alkene signals. The 13C NMR spectrum confirmed the stereochemistry at C-24, identifying the (24-R)-epimer. The purity of the product was validated by GC-MS analysis of TMS-ethers.

The by-product appears to be an isomer of stigmasterol, which undergoes slow reaction under hydrogenation, even under rigorous conditions. The use of ethanol helped minimize isomerization issues, although some heating was necessary due to solubility concerns. It's also likely that residual campesterol remained from the initial stigmasterol. A scaled-up hydrogenation process (8g) yielded consistent purity and yield, which was later utilized to synthesize phytosterol oxides.

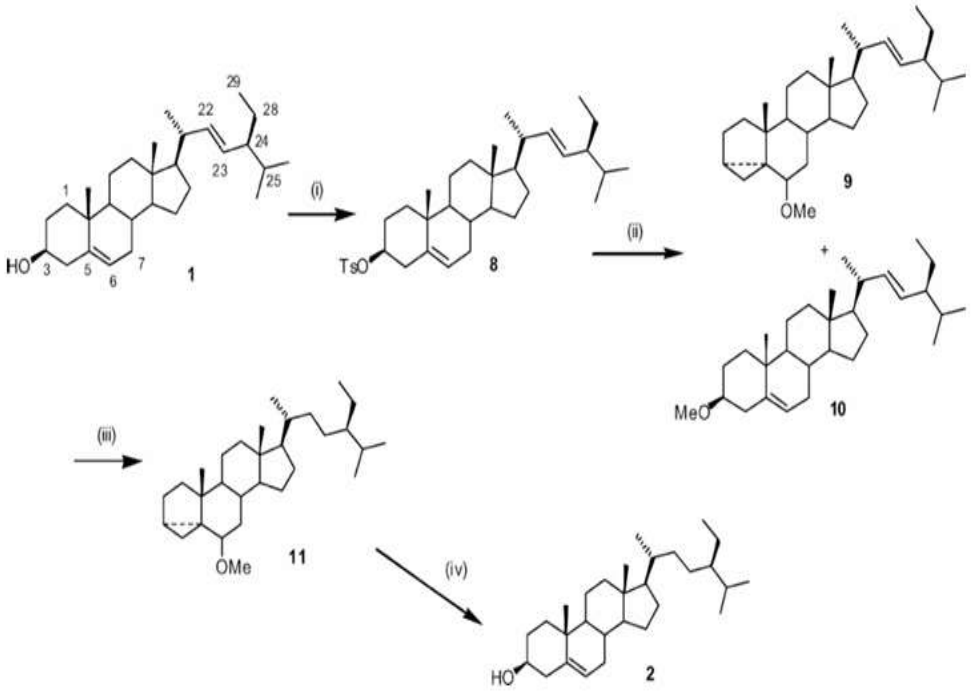

Fig. no.2 Reagents and conditions:(i)p-TsCl, DMAP, pyridine (90%); (ii) MeOH (anhydrous), pyridine (74%); (iii) H_2, catalyst and solvent (iv) p-TsOH, aq. Dioxane, 80 ◦C (55% for 2 steps) [8]

GREEN CHEMISTRY BASED SYNTHESIS OF B-SITOSTEROL DERIVATIVES:[9]

B-SITOSTEROL OLEATE:

The study investigated the production of β-sitosterol oleate using free and immobilized Candida rugosa lipase (CRL), focusing on factors such as temperature, substrate molar ratios, enzyme dosage, reaction duration, and agitation speed. Initial tests revealed that β-sitosterol solubility was inadequate at molar ratios of 1:2 to 1:6, leading to the selection of 1:8, 1:10, and 1:12 for further experiments at 40 °C. With free CRL, conversion rates ranged from 17% to 85%, while immobilized CRL only showed 11% to 27%, indicating that the experimental conditions significantly impacted the results.

Analysis of variance (ANOVA) and Fisher's test highlighted that enzyme loading and reaction duration greatly influenced conversion rates for both CRL forms, emphasizing the need for optimization due to the high cost of enzymes and maintenance. Temperature and agitation did not significantly affect conversion rates; however, temperature control aids solubilization and collision likelihood between enzyme and substrate. A consistent agitation speed of 500 rpm was sufficient for optimal mixing.

3. BIOSYNTHESIS OF β-SITOSTEROL:

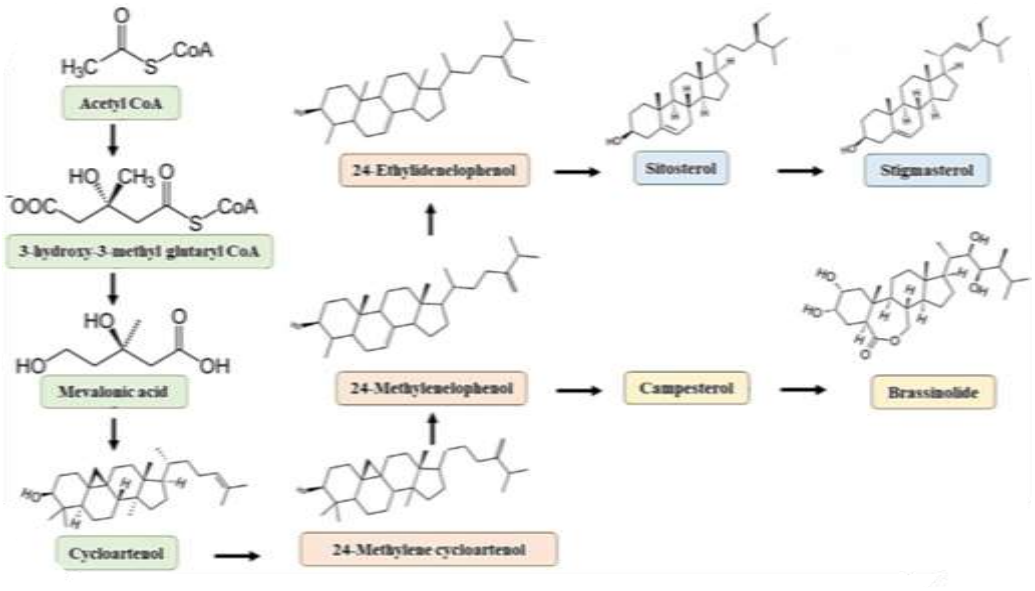

Figure 3: Biosynthesis pathway of sitosterol [6]

4. ANALYTICAL METHODS:[8]

4.1. Identification of Functional Groups in Compound TPH-F-7.1

The IR spectrum for Compound TPH-F-7.1 exhibits characteristic peaks at 3444 cm-1 (indicating O-H stretching), 2953, 2924, and 2870.1 cm-1 (related to aliphatic C-H bonds), 1640 cm−1 (representing C=C bonds), 1458 cm−1 (linked to CH2 groups), 1377 cm-1 (indicating the presence of OH), 1100 cm-1 (associated with cycloalkane structures), and 797 cm-1 (as illustrated in Fig 4). The broad O-H absorption at 3444 cm-1 signifies the presence of hydroxyl groups, while the C-H peaks are indicative of long aliphatic chains that contain both methylene and methyl groups. The presence of C=C at 1640 cm-1 supports the existence of conjugated sterol rings. Bending vibrations observed at 1377 cm-1 and 1458 cm-1 pertain to the CH3 and CH2 groups, respectively. The C-O stretch observed around 1100 cm-1 further confirms the presence of alcohol functionality. These FTIR bands suggest that β-sitosterol is present, which typically shows a weak absorption near 1640 cm-1 due to its non-conjugated C=C bond. It is also noted that atmospheric CO2 can introduce a common artifact at 2320 cm-1.

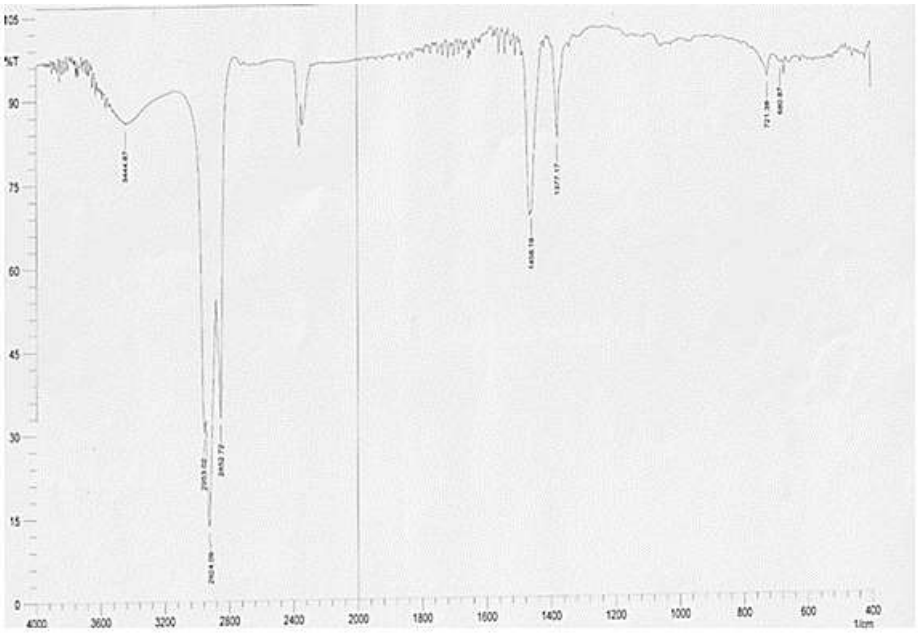

Fig.4 FTIR spectrum of the compound TPH-F-7.1

4.2. Determination of the chemical environment of atoms in TPH-F-7.1

1H-NMR data analysis:

The 1H-NMR spectrum of the compound TPH-F-7.1, recorded at 400 MHz in CDCl3, is illustrated in Figure 5(a). It displays the following chemical shifts: δ 1.47 (2H, t, H-1), 1.57 (2H, m, H-2), 3.55 (1H, m, H-3), 2.30 (2H, d, H-4), 5.37 (1H, t, H-6), 2.04 (2H, t, H-7), 1.68 (1H, m, H-8), 1.56 (2H, m, H-9), 1.54 (2H, m, H-11), 1.52 (2H, t, H-12), 1.50 (1H, m, H-14), 1.66 (2H, m, H-15), 1.86 (2H, m, H-16), 1.46 (1H, m, H-17), 0.71 (3H, s, H-18), 1.03 (3H, s, H-19), 1.59 (1H, m, H-20), 0.95 (1H, d, H-21), 0.92 (2H, m, H-22), 1.16 (2H, m, H-23), 1.39 (1H, m, H-24), 1.70 (1H, m, H-25), 0.85 (3H, d, H-26), 0.85 (3H, d, H-27), 1.11 (2H, m, H-28), 0.83 (3H, t, H-29), along with a broad signal at δ 4.85 ppm attributed to -OH. The spectrum integrates to a total of fifty hydrogen atoms, which include six methyl (CH3), eleven methylene (CH2), nine methine (CH), and one hydroxyl (-OH) group. Singlets observed at δ 0.71 ppm and 1.03 ppm indicate the presence of two CH3 groups bonded to quaternary carbons. Also, complex multiplets at the same shifts suggest two CH2 groups adjacent to carbons that are linked to an OH group. The multiplet at δ 3.55 ppm typically corresponds to a proton attached to a carbon that has an OH group. Moreover, an overlapping triplet at δ 5.37 ppm points to the existence of an olefinic proton.

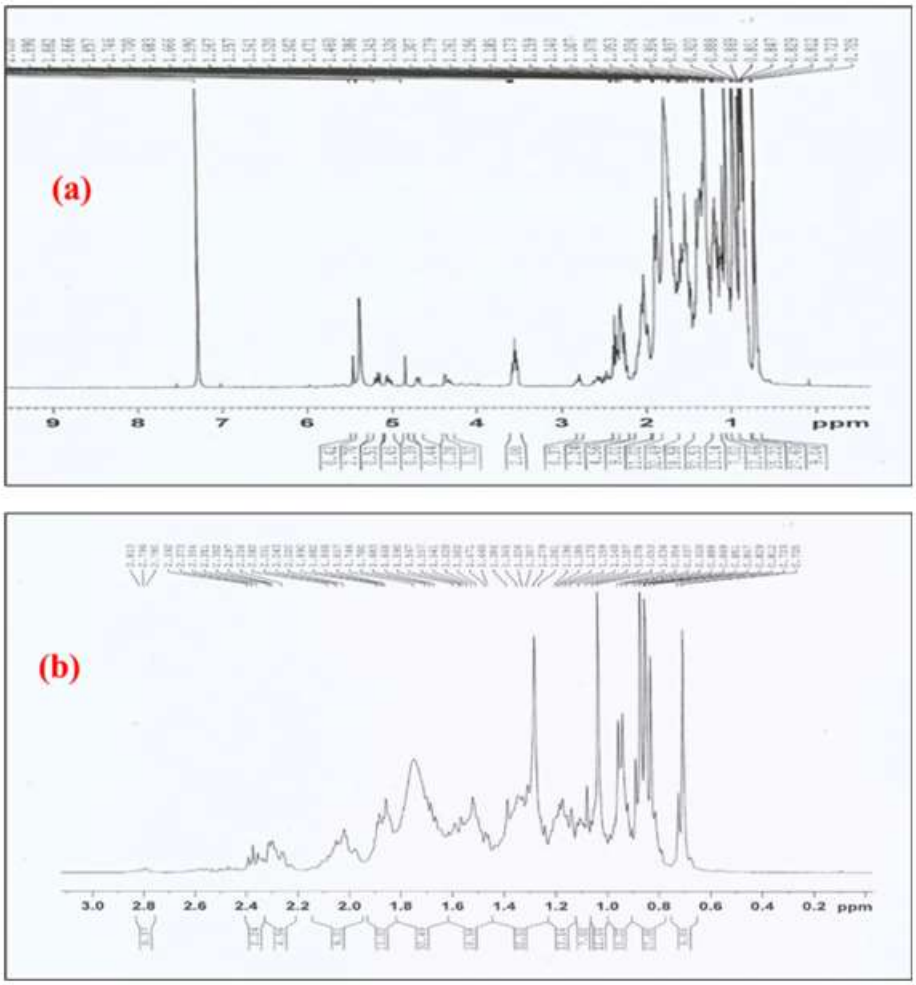

Fig no.5 (a) 1H-NMR spectrum of the compound TPH-F-7.1 in CDCl3, 5(b) 1H-NMR (expanded) spectrum of the com pound TPH-F-7.1

13C-NMR data analysis:

Figures 6a and 6b depict the 13C-NMR (400 MHz, CDCl3) spectrum for compound TPH-F-7.1. The spectrum reveals several carbon signals at δc (ppm): 37.266 for C-1, 33.964 for C-2, 71.829 for C-3, 42.316 for C-4, 140.77 for C-5, 121.732 for C-6, 31.681 for C-7, 31.922 for C-8, 50.152 for C-9, 36.519 for C-10, 21.096 for C-11, 39.790 for C-12, 42.337 for C-13, 56.783 for C-14, 26.100 for C-15, 28.253 for C-16, 56.074 for C-17, 11.860 for C-19, 18.787 for C-18, 36.154 for C-20, 19.402 for C-21, 29.695 for C-22, 24.312 for C-23, 45.858 for C-24, 29.175 for C-25, 19.821 for C-26, 19.043 for C-27, 23.084 for C-28, and 11.989 for C-29, totaling 29 carbon signals identified in the NMR analysis. The double bonds at C-5 and C-6 correspond to the prominent signals at 140.8 ppm and 121.7 ppm in the spectrum. C-15, the angular carbon, is represented by the signal at 26.1 ppm. The alkene carbons are indicated at 140.8 ppm and 121.7 ppm. The signal at δ 71.8 ppm is associated with C-3, which contains a hydroxyl (OH) group. The signals at δ 11.86 ppm and 18.78 ppm correspond to the methyl groups at C-19 and C-18, respectively. The γ-gauche interaction contributes to a lower chemical shift for C-18 due to enhanced shielding. In contrast, the absence of a hydrogen atom on C-6 leads to a decrease in the shielding effect for C-19, resulting in a higher frequency for its 13C-NMR chemical shift.

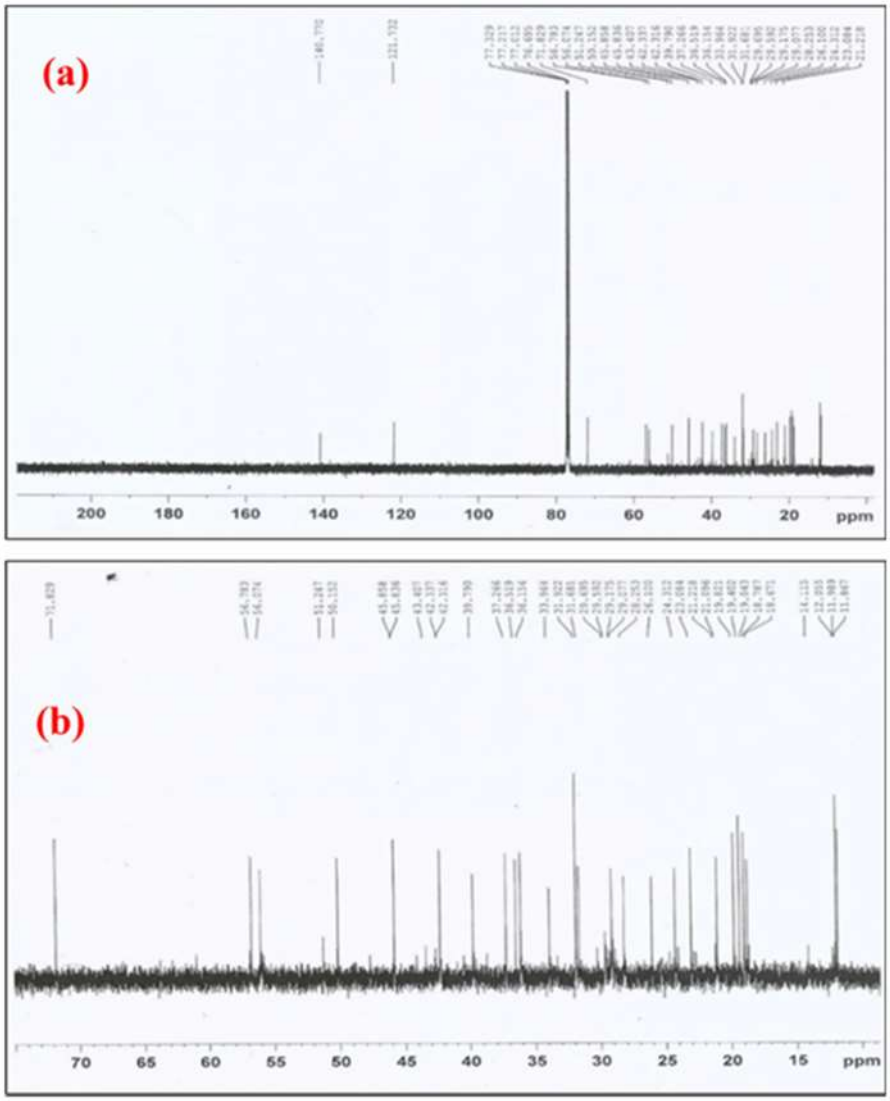

Fig no.6(a) 13C-NMR spectrum and 6(b) 13C-NMR (expanded) spectrum of the compound TPH-F-7.1 in CDCl3

5. PHARMACOLOGICAL EFFECTS OF B-SITOSTEROL:[7]

- **Anti-Cancer Effect:**

β-Sitosterol has demonstrated efficacy against several cancer types, such as breast, prostate, colon, lung, stomach, ovarian cancers, and leukemia. Research indicates that it impacts various signaling pathways, which play crucial roles in processes like cell cycle regulation, apoptosis, cell proliferation, survival, invasion, angiogenesis, metastasis, and inflammation.

- **Anti-Diabetic Effect:**

β-Sitosterol is known for its antioxidant properties. Several studies have shown its potential in managing diabetes. Oral administration of β-sitosterol has been demonstrated to increase fasting plasma insulin levels and enhance results of oral glucose tolerance tests, resulting in increased glucose-stimulated insulin secretion.

- **Anti-microbial Effect:**

β-sitosterol, derived from various plants, has demonstrated antibacterial and antifungal properties without toxicity in the brine shrimp lethality assay. Formulations or plant extracts containing β-sitosterol show mosquito larvicidal and antitrypanosomal activities. Additionally, it exhibits antibacterial effects with inhibition zones similar to those of standard antimicrobial agents.

- **Anti-Inflammatory Effect:**

β-sitosterol shows anti-inflammatory effects in human aortic cells and rats. Several animal studies have demonstrated that it lowers the secretion of pro-inflammatory cytokines, reduces edema, and increases anti-inflammatory cytokines.

- **Antioxidant Effect:**

Several studies suggest that β-sitosterol has an antioxidant effect. It has also been shown to modulate antioxidant enzymes and human estrogen receptors. A study has reported that BS reduced oxygen free radical and hydrogen peroxide levels in Phorbol myristate acetate (PMA) stimulated RAW 264.7 cells, but it does not function as a radical scavenger.

- **Immune Modulation and Anti-HIV Effects:**

β-sitosterol has been shown to act as a powerful immune modulator. It exhibits immune-modulating activities in HIV-infected patients. It has also been reported that BS targets specific T-helper (Th) lymphocytes, increasing Th1 activity and improving T-lymphocyte and natural killer (NK) cell activity. In another study, it was observed that BS maintains stable CD4 cell counts in AIDS, decreases the apoptosis of CD4 lymphocytes, thereby slowing HIV progression. A significant decrease in IL-6 levels in the same study suggests a slowing of viral replication rates in infected cells, thus reducing viral load.

- **Anti-Pulmonary Tuberculosis Effect:**

β-sitosterol has been shown to significantly improve weight loss caused by pulmonary tuberculosis. The study also noted that patients taking it displayed notable changes in some hematological parameters, such as higher lymphocyte, eosinophil, and monocyte counts. The specific mechanism behind this effect remains unclear. Further research is needed to confirm the role of β-sitosterol as an immune modulator in multidrug-resistant tuberculosis cases.

- **Anti-Arthritic Effect:**

A study has reported that a plant extract containing β-sitosterol exhibits notable anti-arthritic effects. It reduces the activation of the NF-κB transcription factor in PMA-stimulated macrophage cells. Nevertheless, additional research is necessary to assess the therapeutic potential of β-sitosterol for arthritis treatment.

- **Antipyretic Effect:**

The antipyretic effect of β-sitosterol is similar to aspirin. Plant preparations and extracts containing β-sitosterol have also demonstrated antipyretic activity, comparable to the standard drug aspirin.

6. CHALLENGES AND OPPORTUNITIES:

- **Challenges:**

The large-scale production of precursor phytosterols is limited. Recent studies show that semi-synthetic pathways deliver poor results. Purifying and separating them from phytosterol mixtures remains challenging.

- **Opportunities:**

There is potential to develop synthesis methods based on green chemistry that utilize safer solvents and reagents. Enzyme-mediated or biocatalytic transformations should be explored. Incorporating scalable purification methods such as chromatography and crystallization presents an opportunity.

7. CONCLUSION :

β-sitosterol is a plant sterol with a broad therapeutic profile, known for its potential health benefits, including cholesterol-lowering effects, anti-inflammatory properties, and immune-boosting capabilities. This compound can be synthesized in large quantities, making it an attractive candidate for further drug development and pharmaceutical applications. Its significance in the pharmaceutical industry cannot be overstated, as it is often utilized in nutraceuticals and dietary supplements aimed at promoting cardiovascular health and overall wellness.

Despite the abundance of β-sitosterol in various plant sources, purifying this compound presents significant challenges due to its coexistence with a complex mixture of other phytosterols. This complexity can complicate extraction and purification processes, leading to a need for more efficient methods to isolate β-sitosterol effectively.

This review focuses on recent advancements in synthesizing β-sitosterol through innovative green chemistry techniques and biotechnological approaches. Green chemistry emphasizes environmentally friendly methods that minimize waste and reduce toxic byproducts, which is essential for sustainable production practices. Notably, biotechnological advancements, such as the use of microbial fermentation or plant cell cultures, have shown promise in enhancing the yield and purity of β-sitosterol.

Such breakthroughs could revolutionize its commercial production, leading to more accessible and cost-effective therapeutic options. The integration of these advanced techniques highlights the ongoing need for research and development in the field, which could significantly impact the future of β-sitosterol utilization in medicine and health supplements. By improving the synthesis and purification processes, the pharmaceutical industry can leverage the full potential of this valuable compound, ultimately improving health outcomes for consumers.

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