Critical Analysis on Characterization, Systemic Effect, and Therapeutic Potential of Beta-Sitosterol: A Plant-Derived Orphan Phytosterol

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Abstract: Beta-sitosterol (BS) is a phytosterol, widely distributed throughout the plant kingdom and known to be involved in the stabilization of cell membranes. To compile the sources, physical and chemical properties, spectral and chromatographic analytical methods, synthesis, systemic effects, pharmacokinetics, therapeutic potentials, toxicity, drug delivery and finally, to suggest future research with BS, classical as well as on-line literature were studied. Classical literature includes classical books on ethnomedicine and phytochemistry, and the electronic search included Pubmed, SciFinder, Scopus, the Web of Science, Google Scholar, and others. BS could be obtained from different plants, but the total biosynthetic pathway, as well as its exact physiological and structural function in plants, have not been fully understood. Different pharmacological effects have been studied, but most of the mechanisms of action have not been studied in detail. Clinical trials with BS have shown beneficial effects in different diseases, but long-term study results are not available. These have contributed to its current status as an "orphan phytosterol". Therefore, extensive research regarding its effect at cellular and molecular level in humans as well as addressing the claims made by commercial manufacturers such as the cholesterol lowering ability, immunological activity etc. are highly recommended.

Keywords: beta-sitosterol; orphan phytosterol; characterization; therapeutic potential; drug delivery

1. Introduction

Beta-sitosterol (BS) is one of the several phytosterols with a chemical structure similar to that of cholesterol [1]. It is a natural micronutrient in higher plants and is found in the serum and tissues of healthy individuals at a concentration 800–1000 times lower than that of endogenous cholesterol. Its glycoside, sitosterolin, is also present in serum, but in lower concentration [2]. These molecules are synthesized in plants; whereas animals obtain them through diet [1].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) published scientific opinions on phytosterols without specific directives on BS separately [3]. In a series of scientific publications by the European Food Safety Authority (EFSA), BS was also not mentioned singly [4]. BS is generally considered as a safe, natural, and effective nutritional supplement and has been shown to have many potential benefits. Administration of BS in rats is found not to cause genotoxicity and cytotoxicity [5]. BS possesses antioxidant, antimicrobial, angiogenic, antioxidant, immunomodulatory, antidiabetic,

anti-inflammatory, anticancer, and antinociceptive activities without major toxicity. There are some nutraceutical preparations available on the market which contain BS. Their manufacturers claim many beneficial effects without substantial experimental evidence. The recent pace of research with BS has been slowed down significantly and has left this molecule as an "orphan phytosterol" [1,6–8].

There are some reviews regarding the effects of phytosterols on health [9–11] and some reviews on the beneficial effects of phytosterol on a specific disease [12], but there is no single review regarding critical analysis of the current knowledge, the gaps in the knowledge on BS, and the necessity of future research to be conducted with BS to fill those gaps. The purpose of this review is to describe the known sources, characteristics, biosynthesis, chemical synthesis, pharmacological and toxicological effects of BS in order to emphasize its significance as well as the limitation of information on BS in order to set an avenue for further study with BS.

2. Sources

BS has been reported to be present in various dietary and non-dietary plants [13]. It exists in different plant parts such as leaves [14], rhizomes [15], and fruits [16]. It has also been reported to be present in different plant tissue cultures [17]. Studies have been reported regarding its membrane stabilizing effect on cell membrane [18], but its role in the cytoplasm and chloroplast has not been studied yet [19,20]. BS-derived phytoecdysteroid is higher in plant tissues which are the most important chemical substance for plant survival, but whether or not BS has a significant role in plant protection needs further research [21].

3. Characterization

3.1. Physical and Chemical Properties

BS (Figure 1) is a white, waxy powder with a characteristic odor. Its molecular formula is $C_{29}H_{50}O$, melting point is 139–142 °C and PubChem CID is 222284 (PubChem, 2015). It is thermally unstable and converted to oxidized products [22].
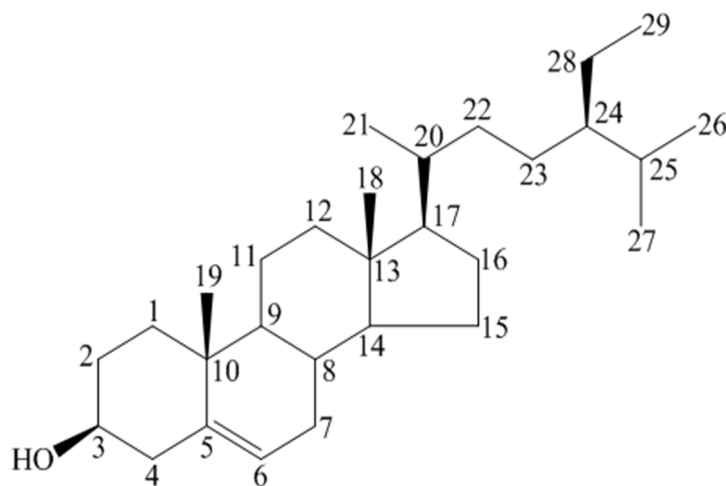

Figure 1. Beta-sitosterol(BS) [3] (Drawn by using *ChemDraw* software).

Although there is information on the oxidized products, only limited information regarding the physiological, pharmacological and pathological effects of those products is available [23,24]. It is hydrophobic and soluble in alcohols but has been observed to exist in three different forms based on the number of water molecules added: anhydrous, hemihydrate, and monohydrate. Monohydrate BS forms needle-shaped crystals instead of plate-like anhydrous crystals and structured suspensions with shear thinning behavior [25]. Its IUPAC name

is 17-(5-Ethyl-6-methyl heptane-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H
-cyclopenta[a]phenanthren-3-ol.　　BS is also mentioned as β-sitosterol, (3β)-stigmast-5-en-3-ol,
22:23-dihydro stigmasterol, α-dihydro fucosterol, cinchol, cupreol, rhamnol, quebrachol, and sitosterin.
Some other most prevalent plant sterols are campesterol (methyl group at C24), sitosterol (ethyl group
at C24), brassicasterol (methyl group at C24, Δ22), and stigmasterol (ethyl group at C24, Δ22) [26].
The molecular formula of Stigmasterol is $C_{29}H_{48}O$ (Figure 2), its melting point is 170 °C, and the
PubChem CID is 5280794. For Campesterol, the molecular formula is $C_{28}H_{48}O$ (Figure 3) and the
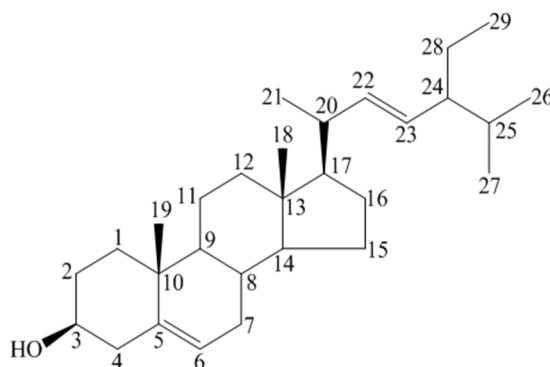PubChem CID is 173183 (PubChem, 2015).

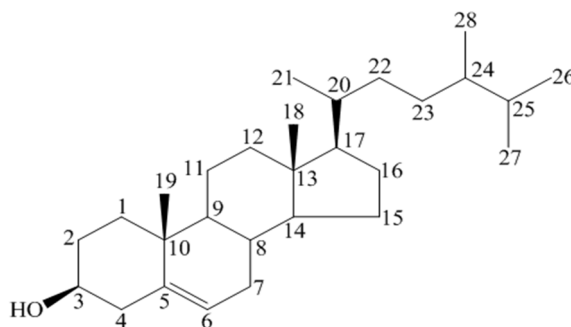**Figure 2.** Stigmasterol [3] (Drawn by using *ChemDraw* software).

Figure 3. Campesterol [3] (Drawn by using *ChemDraw* software).

3.2. Spectral Analysis

For the detection of BS, various methods have been developed to analyze singly or simultaneously
with food [27], vegetable oil [28], plasma [29,30], and dosage form [31].

3.2.1. IR Spectral Analysis

The IR spectral analysis reveals a broad peak at 3549.99 cm^{-1} for the OH group, 2935.73 cm^{-1} for
the CH2 group, 2867.38 cm^{-1} for CH group, 1637.63 cm^{-1} for the C=C group, and 1063.34 cm^{-1} for
the C–O group. The molecular weight determination indicates $C_{29}H_{50}O$ as its molecular formula [32].
Similar results were observed in which IR peaks were obtained at 3426.89, 2924.52, 2855.1, 1738.51,
and 1057.31 cm^{-1} [33].

3.2.2. NMR Spectral Analysis

The ^{1}H NMR spectrum showed the presence of an olefinic signal (δH 5.08), indicating a >C=C<
system in the ring. A one proton broad multiplet at δH 4.44 showed a cross peak with C-2 protons
and a C-4 proton in HETCOR and this signal was assigned to a C-3 methine proton. A plethora of
multiplets was found in the range δH 1.1–2.14 which was informative in the presence of different

methylene and methine protons of the steroidal structure. The other proton resonances were allocated to the glucopyranoside. Further evidence was also provided by the ^{13}C NMR (detail is provided in ref. [34]) that showed resonances for [35] carbon atoms.

The C3 carbon resonated at 71.73 ppm. The anomeric and the oxygenated methylene carbons of the sugar appeared at 100 and 61 ppm respectively. Thus on the basis of the spectral data, the structure of the compound was elucidated as stigmast-5-en-3-*O*-β-D-glucopyranoside (β sitosterol glucoside) [34].

Another study showed that the ^{1}H NMR spectrum (400 MHz, CDCl$_3$) of BS (Table 1) shows a proton corresponding to the proton connected to the C-3 hydroxyl group which appears as a triplet of a doublet of doublets at δ 3.53, the position and multiplicity of which is indicative of the steroid nucleus. The typical olefinic H-6 of the steroid skeleton is evident as a triplet (*J* = 6.4) at δ 5.36 that integrates for one proton. The spectrum further reveals two singlets at δ 0.68 and 1.01 ppm each of three proton intensity, assigned to two tertiary methyl groups at C-18 and C-19, respectively. The three proton intensity, is assigned to two tertiary methyl groups at C-18 and C-19, respectively. The NMR spectrum also displays two doublets (*J* = 6.4) at δ 0.83 and 0.81 which is attributable to two methyl groups at C-26 and C-27. The doublets (*J* = 6.5) at δ 0.93 are ascribed to a methyl group at C-21. On the other hand, the triplet at δ 0.84 (*J* = 7.2) of three proton intensity is assigned to the primary methyl group attached to C-29 [35].

Table 1. ^{1}H and ^{13}C NMR chemical shift values for BS * [35].

Position	^{1}H	^{13}C
3	3.53 (tdd, 1H, *J* = 4.5, 4.2, 3.8 Hz)	72.0
5	5.36 (t, 1H, *J* = 6.4 Hz)	140.9
18	1.01 (s, 3H)	12.0
19	0.68 (s, 3H)	19.0
21	0.93 (d,3H, *J* = 6.5 Hz)	19.2
26	0.83 (d, 3H, *J* = 6.4 Hz)	20.1
27	0.81 (d, 3H, *J* = 6.4 Hz)	19.6
29	0.84 (t, 3H, *J* = 7.2 Hz)	12.2

* Assignments made on the basis of COSY, HMQC, and HMBC correlations; Chemical shift values are in δ (ppm); Coupling constants are in Hz.

The ^{13}C NMR together with COSY, HMQC, and HMBC shows twenty-nine carbon signals including six methyls, eleven methylenes, ten methane, and three quaternary carbons [35].

3.3. Chromatographic Analysis

3.3.1. Thin Layer Chromatography (TLC)

In Thin layer chromatography (TLC) analysis of BS revealed a R_f value of 0.55 when the crystals were reconstituted in chloroform and spotted on the TLC plate in *n*-hexane: acetone (80:20) mobile phase system [32].

3.3.2. Gas Layer Chromatography

To analyze BS, a Gas-liquid chromatography (GLC) method using the butyl ester of BS was implemented [27]. It was evaluated and found that an immobile phase of 1% SE-30 coated on 100–120 mesh Gas-Chrom Q packed in a 6 × 4 mm id glass column operated at 255 °C is the most efficient column.

3.3.3. High-Performance Liquid Chromatography

Different High-Performance Liquid Chromatography (HPLC) methods have been developed for analyzing BS such as a narrow-bore HPLC-UV method [28]; a high-performance thin-layer chromatography densitometric method [28]; an HPLC method for qualitative analysis [36]; an HPLC method with online evaporative light scattering detector (ELSD) [31]; an HPLC/MS method using solvent combination of water/methanol vs. methanol/acetone/n-hexane applied on a Purospher Star RP-18e (125 × 2 mm, 3 micron) column [29].

3.3.4. Gas Chromatography Mass Spectrometry (GCMS)

Ahmida et al. (2006) developed capillary gas chromatography coupled to mass spectrometry (GC-MS) for simultaneous detection of BS from other sterols by multiple selected ion monitoring. This method is based on the alkaline hydrolysis of sterol esters, extraction of free sterols and derivatization. The recovery of all sterols was in the range 76%–101% [30]. Most of these techniques are laborious and time-consuming [23] except for a recent method described by Srividya et al. (2014) [37]. Alkaline hydrolysis and liquid–liquid extraction followed by parallel detection on GC-FID and GC-MS is proposed as an ideal methodology for the bio-analysis of phytosterols [38]. Therefore, there is plenty of scopes to improve the efficiency as well as the limit of detection and quantification.

4. Synthesis

4.1. Biosynthesis

The exact biosynthetic mechanism of BS varies according to organisms, but generally, it follows the mevalonate pathway [39]. BS is biologically synthesized from both mevalonate and deoxyxylulose pathways [20] but prioritizes both or one of the pathways based on the external environment. Using ^{13}C-labeling approach, the mechanism of BS biosynthesis was studied and it was proposed that isopentenyl diphosphate (IPP) combines with dimethylallyl diphosphate (DMAPP) to form farnesyl diphosphate (FPP) and then two molecules of FPP combine tail-to-tail to form Squalene, a triterpene and then cycloartenol which eventually forms BS by methylation, hydride shift, reduction, and slight modification in the beta-ring [19].

4.2. Comparison of Biosynthesis of BS and Cholesterol

Both the biosynthesis of BS and Cholesterol follow the same direction till the formation of Squalene (Figure 4). However, the fate of Squalene varies due to the different target product. In the case of the biosynthesis of BS, Squalene forms cycloartenol through a cyclization reaction with 2,3-oxidosqualene. The double bond of cycloartenol is methylated by *S*-Adenosyl methionine (SAM) to give a carbocation that undergoes a hydride shift and loses a proton to yield an intermediate compound with a methylene side-chain. Both of these steps are catalyzed by sterol C-24 methyltransferase. The intermediate compound is then catalyzed by sterol C-4 demethylase and loses a methyl group to produce cyclo-eucalenol. Subsequent to this, the cyclopropane ring is opened with cyclo-eucalenol cyclo-isomerase to form another intermediate compound. This intermediate compound then loses a methyl group and undergoes an allylic isomerization to form gramisterol. This step is catalyzed by sterol C-14 demethylase, sterol Δ14-reductase, and sterol Δ8-Δ7-isomerase. The last methyl group is removed by sterol demethylase to form episterol. Finally, episterol is converted to β-sitosterol through methylation by SAM, reduction by NADPH, and modifications in the β-ring. Here 24-methylenesterol *C*-methyltransferase plays a very important role [40] (Figure 5).

The synthesis of cholesterol occurs in three stages, with the first stage taking place in the cytoplasm and the second and third stages occurring in the endoplasmic reticulum. The stages are (1) Synthesis of isopentenyl pyrophosphate, the "building block" of cholesterol; (2) Formation of squalene via the condensation of six molecules of isopentenyl phosphate and; (3) Conversion of squalene to cholesterol via several enzymatic reactions [41] (Figure 6).
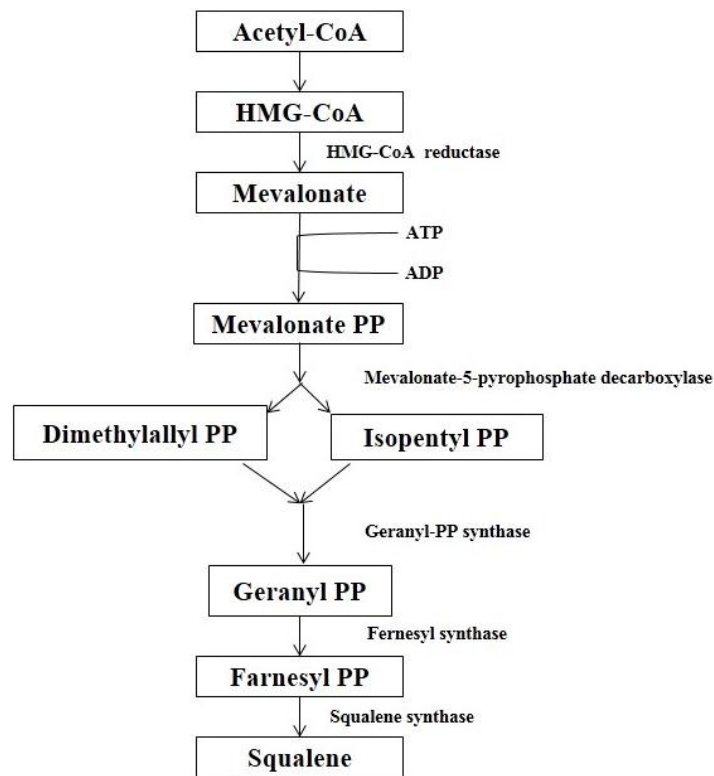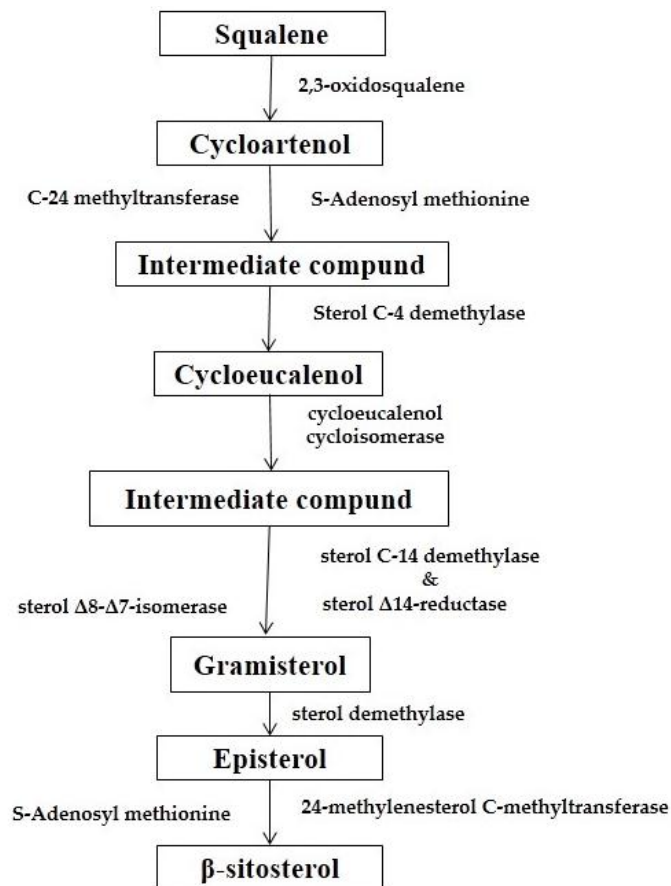
Figure 4. Formation of squalene [42].

Figure 5. Synthesis of BS [40].

Farnesyl pyrophosphate
↓ Squalene synthase
Squalene
↓ Squalene epoxidase
Squalene epoxide
↓ Squalene epoxidase
Lanosterol

Lanosterol 14α-demethylase → **4,4-Dimethyl-14 o-hydroxymethyl 5α-cholesta-8,24-dien-3β-ol**

24-dehydrocholesterol reductase → **24,25-Dihydrolanosterol**

4,4-Dimethyl-14 o-hydroxymethyl 5α-cholesta-8,24-dien-3β-ol
↓ C-4 methyl oxidase
4α-Carboxy-5α-cholesta-8,24-dien-3β-ol
↓ NAD(P) dependent steroid dehydrogenase
5α-cholesta-8,24-dien-3-one
↓ C-3 ketoreductase
Zymosterol
↓ Sterol 8-isomerase
5α-cholesta-7,24-dien-3β-ol
↓ C-5 desaturase
7-dehydrodesmosterol
↓ Δ7-dehydrocholesterol reductase
Desmosterol

24,25-Dihydrolanosterol
↓ Lanosterol 14α-demethylase
4,4-Dimethyl-14 o-hydroxymethyl 5α-cholesta-8-en-3β-ol
↓ C-4 methyl oxidase
4α-hydroxymethyl-4β-methyl-5α-cholesta-8-ien-3β-ol
↓ C-3 ketoreductase
Zymostenol
↓ Sterol 8-isomerase
Lathosterol
↓ C-5 desaturase
7-Dehydrocholesterol

Desmosterol *Cholesterol 24-dehydro Cholesterolvreductase* → **Cholesterol** ← *7-dehydro-cholesterol reductase* **7-Dehydrocholesterol**
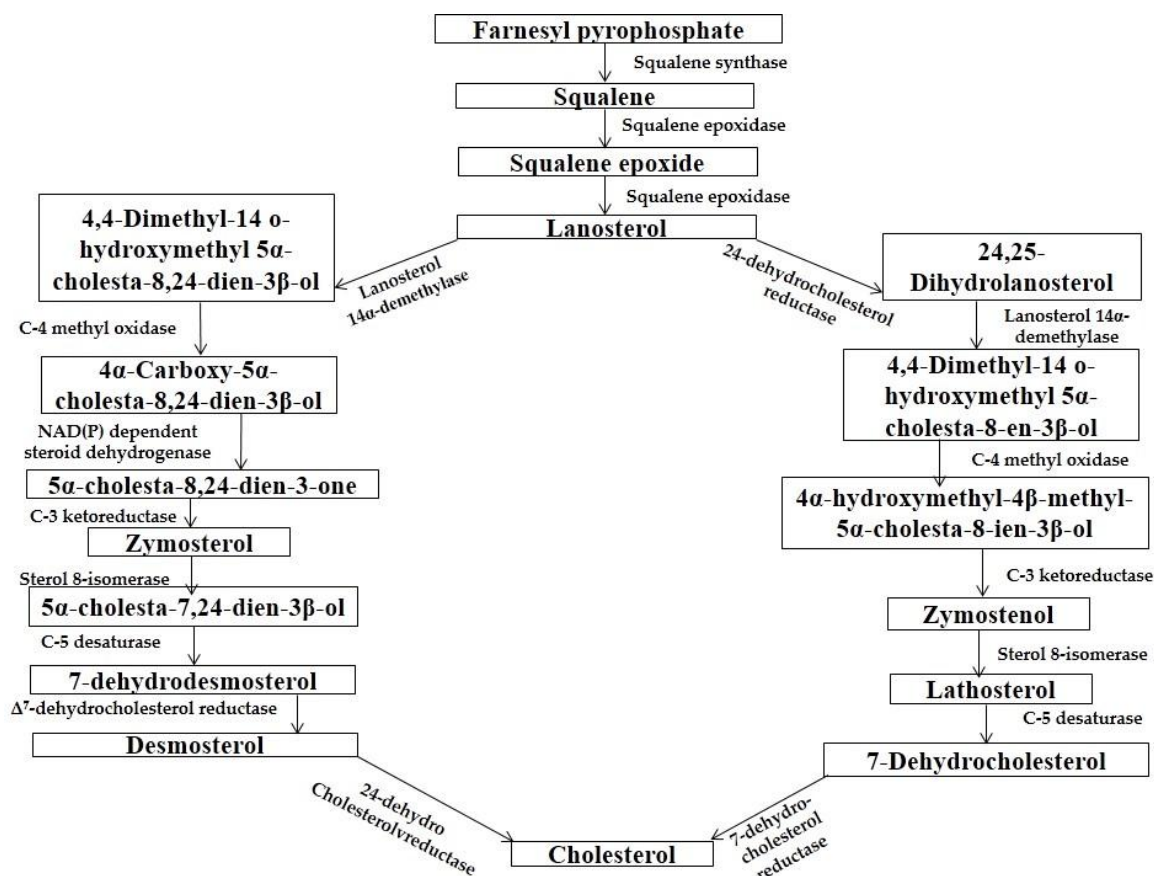
Figure 6. Synthesis of cholesterol [41].

4.3. Chemical Synthesis

Different routes have been reported for the synthesis of BS over more than 50 years. The selective hydrogenation of the stigmasterol side chain Δ^{20-21} alkene was found to produce BS contaminated with varying amounts of recovered stigmasterol as well as the fully saturated stigmastanol [43,44]. In another approach, the synthesis of sitosterol and related sterols circumvents the need for selective hydrogenation for protecting the Δ^{5-6} alkene as cyclopropyl carbinyl ether. Following hydrogenation of the Δ^{22-23} double bond, solvolysis of the cyclopropane reintroduces both the C3-alcohol and the Δ^{5-6} alkene [45,46]. Recently a new strategy for the synthesis of the side chain has modified phytosterols based upon the protection of the Δ^{5-6} alkene as an epoxide [47]. However, neither a complete BS biosynthetic pathway nor a total chemical synthesis for BS has been reported yet. Therefore, we propose a further study to understand the underlying mechanism of BS biosynthesis in different plants as well as an economic and efficient chemical synthesis process. The former will serve the purpose of finding the role of cytosolic and plastid BS in plants and may also produce clues for the economic and efficient chemical synthesis of BS.

5. Systemic Effect

5.1. Central Nervous System

BS containing plants show antinociceptive [48], anxiolytic, and sedative effects [49] in rats, but such findings in humans are not available. Neither the brain region nor the pathway affected by BS has been studied extensively yet. It has been shown that the effect of BS is somewhat similar to diazepam but whether the mechanism of action is similar or not has not been studied [50]. It has been proposed that BS is effectual by interacting with GABAA receptor, but there is no confirmatory

evidence for this claim [49]. BS has been shown to potentiate the binding of other compounds to muscarinic receptors [51]. However, whether or not BS binds to the muscarinic receptor itself is not known. Studies in immortalized mouse hippocampal cell line HT22 showed that BS prevents oxidative damage and neurotoxicity [52] and a series of other studies showed the beneficial effect in preventing neuronal damage [53,54]. There is evidence that BS crosses the blood brain barrier (BBB), but fundamental studies regarding the efficacy and efficiency of BS to cross BBB have not been undertaken. A comparative study has been done with other phytosterols like campesterol and sitosterol to check the efficiency in passing the brain endothelial monolayer where the reason behind the irreversible passage of the plant sterols across the endothelial monolayer was found to be the molecular complexity of the sterol side chain. A possible explanation for the difference of phytosterols in passing the BBB may be the different esterification rate within the endothelial cells [26]. Recent studies have shown that BS alone [55] or as extract [56] increases neural stem cell proliferation. However, further studies are recommended for potential applications in tissue engineering.

5.2. Skin

According to the Norwegian Food Safety Authority (Mattilsynet, NFSA, Oslo, Norway), BS has a skin conditioning effect and is used in sunscreen, moisturizer, body wash, and anti-aging cosmetic preparations (NFSA, 2012). Skin is one of the paths of BS excretion. It has been reported that BS inhibits the production and mRNA expression of thymic stromal lymphopoietin through blocking of caspase-1 and nuclear factor-kB (NkB) signal pathways in the stimulated human mast cell line, HMC-1 cells. Even though this study showed the potential therapeutic effect against atopic dermatitis, studies on long-term use of BS on the skin need to be conducted [57].

5.3. Cardiovascular System

BS has beneficial effects on the cardiovascular system and prevents different cardiovascular diseases except for patients with ABCG5 and ABCG8 mutation [11,58]. However, there is no study regarding its effects on cells within the heart: the cardiomyocytes and the cardiac pacemaker cells. Although some studies point to the possibility that elevated plasma phytosterol concentrations could contribute to the development of premature coronary artery diseases, extensive safety evaluation studies have been conducted for these compounds, and they are considered safe [59].

5.4. Liver

BS containing diets change the live ultra-structure and such differences are observed in both young and adult mice fed with BS [60]. Pathophysiology of the liver is also affected by BS. For example, BS prevents gallstone formation and decreases serum and liver cholesterol [61], but such preventive effects are observed only at high doses [62]. The effect of BS on different metabolizing enzymes has not been studied and therefore sufficient information regarding the metabolism of drugs that are affected by BS is not available.

5.5. Endocrine System

BS possesses a weakly estrogenic effect and alone or in combination with progesterone, it inhibits the expression of intercellular adhesion molecule-1 [63] and testosterone propionate induced prostate hyperplasia [64] as well as reducing pregnenolone production [65]. Even though the molecular effect of BS on the tonicity of the uterus has been studied [66], the long-term effect of BS on different hormones has not been studied and therefore further study is required.

5.6. Reproductive System

The effect of BS on the reproductive system is contradictory. Study on American mink shows increased male fertility due to BS intake [67], but other studies in male rats [68] and goats [69] show

the opposite effect on reproduction. The level of sex hormones such as testosterone in males and estradiol in females is increased due to BS intake in rats [70]. Whether or not this increase has any clinical significance has not yet been studied.

5.7. Wound Healing

Different plants containing phytosterols like *Mimosa tenuiflora* have been used for decades as a remedy in the treatment of wounds and burns of the skin. This can be explained by the re-epithelialization process in wounded areas which is believed to be aided by BS. So, the ability to heal, together with the anti-inflammatory and antimicrobial activity of BS demonstrate its potential in tissue engineering applications [71].

6. Pharmacokinetic Studies

Pharmacokinetics and the bioavailability of BS (Figure 7) have been reported both in animal [72,73] and human [74,75], alone as well as with other compounds [76], including sex hormones [77] and cholesterol [78] mostly. Reports in diseased humans have also been reported [79]. Even though the metabolism of BS was described 45 years ago [80], the detailed metabolic turnover, absolute oral bioavailability, clearance, and volume of distribution for BS measured in healthy subjects have been reported only recently [75]. Generally, it has been considered that BS interrupts the recirculation of bile acids and/or reduces the absorption of cholesterol in the gut [81–85]. However, substantial experimental evidence is needed to propose the primary molecular mechanism about the physicochemical competition between cholesterol and BS and other phytosterols for micellar incorporation and uptake at the gut lumen [86].
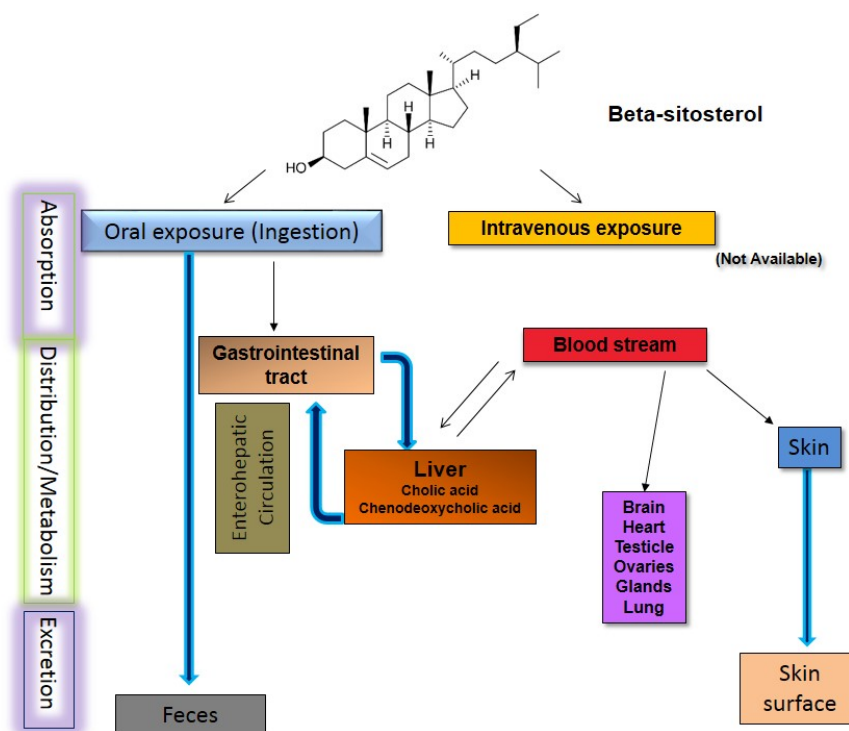

Figure 7. Pharmacokinetics of beta-sitosterol.

Structurally cholesterol and BS are different from each other only by the additional ethyl group at the C-24 position in the latter. The absorption of BS is one-fifth of that of cholesterol [87]. Field and Mathur (1983) attributed the inadequate esterification of BS to poor absorption, but there is no experimental evidence to this claim [88]. BS absorption is higher in females than males [89], but no

satisfactory explanation has been provided until now. The study shows that efflux transporters play a role in absorption of BS and it has been shown that loci on chromosomes 14 and 2 in rats play a role in the concentration of BS but further study is not available [90]. BS is distributed in the adrenal glands, ovaries, brain, testicles, as well as skin [75,89,91]. BS is metabolized to different compounds in the liver and other tissues forming different compounds. BS is converted to autoxidation products in the GI tract as well as before excretion via feces [92]. A general study regarding hepatic enzyme metabolism is available [93,94] but until now there has not been any study regarding the differential roles of different hepatic enzymes in its metabolism even though the role of BS has been studied while studying the effect of different hepatic enzymes on other drugs [95]. Bacterial conversion products from BS that have easily been identified and measured in the gut are 24-ethyl-coprostanol, as the most prominent component, as well as coprostanol, and 24-ethyl-coprostanone as minor components [96]. About 80% of the absorbed BS is excreted via feces [80] and the rest is excreted via skin [97]. Generally, excretion via feces is rapid but differential excretion is observed based on the disease condition of the colon [98] and physical stress [99]. Coeliac disease has been attributed to impaired BS absorption [100] but whether or not the presence of higher BS in the colon has a causal effect on coeliac disease needs to be studied.

7. Therapeutic Potentials

7.1. Antioxidant Activity

Several findings suggest that BS has antioxidant property [5,101]. It has also been shown to modulate antioxidant enzymes and human estrogen receptor [92]. It has been reported from a study that BS reduced Oxygen free radical and Hydrogen Peroxide levels in Phorbol myristate acetate (PMA) stimulated RAW 264.7 cells but does not function as a radical scavenger [102]. Glutathione peroxidase (GSH) and Mn superoxide dismutase (SOD) activities are decreased significantly by BS treatment [103]. BS does not affect Cu-Zn SOD activity, but whether BS promotes up-regulation of Mn-SOD needs further investigation.

7.2. Angiogenic Effect

BS plays a role in blood vessel formation and thus possesses potentials in wound healing [104]. However, there has been no experimental study on the mechanism of wound healing until now. Choi et al. (2002) shows blood vessel formation in ischemia, but further study regarding the feasibility of using BS as a therapeutic agent for ischemic stroke has not been conducted [105].

7.3. Antihyperlipidemic and Anti-Atherosclerosis Effects

BS is recommended for the prevention of different cardiovascular diseases [106–108] and the FDA has approved BS for the treatment of hyperlipidemia [109]. It prevents the absorption of cholesterol by displacing it from micelles [110] and thereby decreasing the amount in plasma [83,84,111–114]. In combination with other statins, it increases the potency of those statins [115]. Still further studies are required to resolve existing debate regarding its role in treating hypercholesterolaemia [116]. Study regarding the role of BS on the upregulation of paraoxonase-2 needs to be conducted in order to substantiate the claim made by Rosenblat et al. (2013) regarding the beneficial effect of simvastatin in combination with BS [117]. BS has also been related to sitosterolemia but not as a causal agent for the development of coronary heart disease in sitosterolemic patients [118]. Further experimental evidence is required.

7.4. Antipyretic Activity

A study on rats has shown that the antipyretic effect of BS is comparable to that of aspirin [119]. The preparations and extracts of plants containing BS have also been shown to have antipyretic

activity [14,120]. This effect is comparable to that of the standard antipyretic drug, aspirin, but the detailed mechanism has not yet been studied.

7.5. Anti-Inflammatory Activity

BS possesses anti-inflammatory activity in human aortic cells [121] as well as in rats [122,123]. Several studies in animals have indicated that BS reduces the secretion of pro-inflammatory cytokines, TNF-α as well as edema [119,124,125] and increases anti-inflammatory cytokines [126]. Chronic treatment with BS reduces its anti-inflammatory potential [127] and it does not affect the mast cells in histamine release [128] and the arachidonic acid pathway [129]. Whether BS inhibits either myeloperoxidase or adenosine deaminase activity or both needs further investigation. Again, whether BS inhibits or not IL-1β and TNF-α levels by increasing calcium uptake in activated neutrophils in a concentration- and time-dependent manner through L-type voltage-dependent calcium channels, phosphoinositide kinase-3, intracellular calcium and microtubule modulation, and thus promotes the anti-inflammatory activity as proposed by Liz et al. (2013) [130], requires experimental evidence. Even though Mahanjan and Mehta (2011) have shown therapeutic potential in allergic asthma by a chronic study in pigs it has not been used for clinical trials in humans [131].

7.6. Anti-Arthritic Activity

It has been reported from a study that the plant extract containing BS has significant anti-arthritic activity [132]. According to Moreau et al. (2002), BS decreases the activation of NF-κB transcription factor in PMA-stimulated macrophage cells [133]. However, further investigations are required regarding the therapeutic potential of BS to treat arthritis.

7.7. Immune Modulation and Anti-HIV Effect

BS has been shown to act as a powerful immune modulator [134]. BS exhibits immune-modulating activities in HIV-infected patients [135]. 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 [135,136]. In another study it was observed that BS maintains stable CD 4 cell counts in AIDS, declines apoptosis of CD 4 lymphocytes slightly, thereby slowing HIV. A significant decrease in IL-6 levels in the same study leads to a further claim that there is slowing down of viral replication rates in infected cells thereby decreasing viral load [137]. Neurath et al. (2005) proposes BS as an envelope virus neutralizing compound (EVNC) and thus acting as an HIV-1 entry inhibitor [138]. This claim has been substantiated by the fact that the EVNCs in the body fluid neutralize viruses in the blood stream and elicit an immune response to the neutralized authentically folded virus particle [139,140]. Even though the effect of BS on entry and exit out of the cell is not available, it is evident that BS facilitates the development of a potentially protective immunity against HIV. However, further study for considering BS as potential therapeutic agent has not progressed. Therefore, extensive study is suggested.

7.8. Anti-Cancer Effect

Experimental and epidemiological studies have shown the efficacy of BS in treating different types of cancer via different pathways. One recent review documented in detail regarding this [141]. However, most studies have been carried out with different cancer cell lines, where different cellular factors are affected by BS, but ultimately cell lines undergo apoptosis. For breast cancer, MDA-MB-231 [142], U937 [143], HL60 [144], MCF-7 [145]; for colon cancer, HT-29 [146,147], HT116 [105], COLO 320 DM [148], Caco 2 [149]; for prostate cancer, LNCaP [142], PC-3 [150], 22Rv1 [151], DU145 [151]; for fibrosarcoma MCA-102 [152]; for uterine cervix cancer, SiHa cells [153]; for larynx carcinoma, Heps [154] have been studied. Studies on the antitumor effect of BS in animals are relatively few. For colon carcinogenesis, studies were done on rodents and on rat prostate [155,156]. For the former, the result is positive, but for the later the result is negative. These studies with BS are extensive and explain the anticancer mechanism of action. For example, several studies

have indicated that BS inhibits the growth of various cultured cancer cell lines that are associated with the activation of the sphingomyelin cycle [147,157,158]; cell cycle arrest [150,159], and the stimulation of apoptotic cell death [105,160]. BS isolated from various plants promotes apoptosis by increasing first apoptosis signal (Fas) levels and caspase-8 activity [8], phosphorylation of extracellular signal-regulating kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) [161], inhibition of cancer cell proliferation, even at low concentrations with no cytotoxic effect on noncancerous cells [152], modulation of antioxidant enzyme levels in pathogenesis [103], arresting of cells at G2/M phase in cancer cells [150], and decreasing free radical generation in vitro [102,162]. BS induces a reduction in membrane sphingomyelin and an increase in the ceramide levels in some tumor cells [147,157]. The efficiency of ceramide playing a role in the activation of the extrinsic pathway as suggested by observations of death receptor clustering in ceramide-rich lipid rafts has not been studied for experimental evidence [163,164]. In addition to the negative effect of BS on cell growth, BS treatment of tumor cells is associated with increased apoptosis [165]. Even with these extensive studies, there is still very little translational research for treating different cancers. One possible explanation could be its lower efficacy and another could be fewer chances of patents by pharmaceutical research organizations. Therefore, research in an academic setting is needed.

7.9. Anti-Diabetic Effect

Oral treatment with BS increases the fasting plasma insulin levels. There is a corresponding decrease in fasting glycemia when BS is administered orally. In addition, it improves the oral glucose tolerance test with an increase in glucose-induced insulin secretion [166]. These effects are comparable to that of the standard anti-hyperglycemic drug Glibenclamide. However, the hypoglycemic effect manifested by BS by increasing circulating insulin levels and the mechanism of this increase need further study. A study has shown that treatment of diabetic rats with BS prevents the development of diabetes as well as ameliorating diabetic complications along with other diseases such as arthritis [101]. The same study showed that BS increases glucose uptake in adipocytes and stimulates adipogenesis in differentiating preadipocytes. Paradoxically, it also induces lipolysis in adipocytes which have not been attenuated by insulin and co-incubation with epinephrine. Like insulin, it down-regulates GLUT4 gene expression, but a confirmatory study is required to ensure that elevation of glucose uptake by BS in adipocytes is unrelated to the de novo synthesis of GLUT4 and whether lipolysis is associated with down-regulation of Akt and PI3K genes. Even though due to the unique effects of BS on the regulation of glucose uptake, adipogenesis, and lipolysis in adipocytes supports its potential to be utilized in diabetes and weight management [167], no clinical study has yet progressed. Furthermore, a study should be conducted on whether or not BS has any role in insulin sensitivity and glucagon secretion.

7.10. Anti-Pulmonary Tuberculosis Effect

According to the double-blind, randomized, placebo-controlled trial conducted by Donald et al. (1997) on culture-positive pulmonary tuberculosis patients, BS has been found to have a significant improvement in weight that is lost due to pulmonary tuberculosis [168]. The same study showed that patients receiving BS exhibit notable differences in certain hematological parameters, including increased lymphocyte, eosinophil, and monocyte counts. The detailed mechanism of this effect has not yet been studied. The efficiency of BS as immune modulating agent in case of multi-drug-resistant tuberculosis needs further investigation.

7.11. Antimicrobial Activity

BS obtained from different plants shows antibacterial and antifungal activity without toxicity in brine shrimp lethality assay [169–171]. The formulation or plant extract containing BS shows mosquito larvicidal activities [172] and antitrypanosomal activities [173]. BS has been reported to have antibacterial activity with a comparable zone of inhibition to other standard antimicrobial agents [32,174]. The prime limitation of these studies is the inadequacy in explaining the mechanism of

actions. Kanokmedhakul et al. (2005) attributed this to the ability of BS to inhibit bacterial cell surface protein, "sortase" thus preventing transpeptidation [175]. Betasitosterol-3-*O*-glucopyranoside (BSG), a derivative of BS, inhibits bacterial cell adhesion to a fibronectin indicating that modification of BS is needed to exert its antibacterial effect [176]. However, no study has been conducted regarding the mechanism of anti-protozoal, anti-larvicidal or anti-fungal effects. Again, no study has been run to ensure any effect of BS on the ribosome, RNA transcription, DNA replication or the enzymes involved in central dogma. A detailed study is proposed with a hope of obtaining a good alternative to the antimicrobial agent in this current era of antimicrobial resistance.

7.12. Miscellaneous

BS has been reported to have anthelmintic properties alone [177] and in combination with one of its derivatives [178]. The mechanism is not well defined and no study has been conducted yet on this. Various plant extracts containing BS can neutralize different snake venoms [179]. However, the mechanism has not yet been discovered with experimental evidence. BS also has a significant role in the treatment of androgenic alopecia [180,181] and studies with human clinical trials have shown positive results [182]. There are some marketed preparations with BS claiming its efficacy in this case, but long term safety data is not available. There are also some marketed preparations that claim beneficial effects in benign prostate hyperplasia (BPH) [183–185] and on lower urinary tract infection [186]. However, the molecular mechanism of any of these claims has not yet been established. Lomenick et al. (2015) discovered some protein receptors of BS, but more research in required [187].

8. Toxicity

Even though the United States National Toxicology Program (NTP) reviewed toxicological effects of BS about 18 years ago (NTP, 1997), many study results need to be re-evaluated based on the latest findings. A high level of BS concentrations in blood has also been correlated with increased severity of heart disease in men who have previously been suffering from heart attacks [188]. There are drug interactions of BS with Ezetimibe and atorvastatin, pravastatin, simvastatin, and lovastatin or fluvastatin [189,190]. Ezetimibe inhibits the uptake of BS which provides the molecular basis for the treatment of sitosterolemia with ezetimibe [191]. Short-term repeated administration of BS in rats has been reported not to produce gross or microscopic lesions in liver or kidney [68] but such a report on humans taking BS for a long time has not been produced. An extensive toxicological study has shown high LD_{50} in rats (>2 gm/kg) [5]. According to JECFA (2009), acceptable daily intake (ADI) is 40 mg/kg·BW/day; No-Observed-Adverse-Effect-Level (NOAEL) is 4200 mg/kg·BW/day; Margin of Safety (MOS) is 210 mg/kg·BW/day and 8.3 mg/kg·BW/day for systemic and cosmetic products respectively. These values are calculated approximately from phytosterol mixtures, not directly from BS solely and therefore values based on BS are highly recommended. BS inhibits mutagenicity [177], prevents chromosomal breaks [192], and shows no genotoxic effects [193]. Even though one study found its potentially harmful effect on the reproductive system [194], later study found that it does not have an effect on the reproductive system [195]. However, there is no study regarding the long-term effect of BS in the human reproductive system. In a recent study, it was shown that high exposure of BS is related to impaired hepatic and intestinal ATP-binding cassette transporters G5/8 and possesses potential risks of blood-brain barrier integrity in diabetic rats [196]. Another main limitation of BS toxicity study is the unavailability of its readily oxidized products.

9. Drug Delivery with Beta-Sitosterol

Side chain double bonds increased sterol mobility considerably in HPLC, which reflected decreased hydrophobicity of the molecule. However, the change in hydrophobicity depended upon the position of the side chain double bond: sterols with double bonds at the C22 position were more hydrophobic than sterols with double bonds at the C24 position. Increases in the side chain length, by the addition of methyl or ethyl groups, resulted in decreased HPLC mobility and

increased hydrophobicity, whereas the insertion of a double bond in one or both fatty acyl chains decreased hydrophobicity [197]. BS has poor absorptivity and therefore additives for enhancing its bioavailability or drug delivery with different dosage forms did not progress extensively even though its pharmacokinetic and bioavailability data was reported long ago [72,73,80]. Liposomal BS has been reported along with its ability to increase natural killer cell activity and decrease metastatic colonies in the lungs significantly in comparison to the control group [198]. BS has been reported to act as a model drug or substance for a novel formulation [199] and to test the efficacy in emulsion form [200]. It has also been reported as a formulation additive for stable micellar formulation [201], and for novel bio-active lipid nanocarriers for stabilization and sustained release [202]. It enhances drug release from a gel preparation [203], activity in phyto-vesicle preparation [181], oral absorption efficiency [204] and the sustained release of hormone [205]. More works have been conducted with BSG for enhancing absorption of a different formulation of genes [206,207] and drugs for skin [208]. In combination with another drug, BS has been reported to enhance nasal [209] and intestinal absorption [210], or to deliver the drug to the specifically targeted organ, such as liver [207,211]. However, no clinical trial has been conducted with any of these formulations. Designing intelligent drug delivery for increasing intestinal BS absorption is promising, especially for site-specific therapy of cancer, because of the non-toxic nature of BS to non-cancer cells. Therefore, we propose clinical trials of BS liposomal drug delivery for breast cancer, colon cancer etc.

10. Future Research Directions

BS has been reported to have beneficial effects in different diseases, but it has not developed as an independent drug mostly because of its relatively lower efficacy and the development of other drugs with higher efficacy. For example, both BS and glucocorticoid, dexamethasone (DX) have anti-inflammatory effects, but DX has obtained unprecedented approval being a standard drug since its inception [212] even though it lacks sufficient clinical trials [213]. Now research with BS which has fewer side effects might lead to the development of a newer anti-inflammatory drug. New study design should be made on drug delivery to compensate its lower efficacy and poor absorptivity. Over-generalization of systemic pharmacological effects of all phytosterols by regulatory agencies such as EFSA, WHO, FAO and attributing generalized statements on BS is also considered a big challenge. BS is one of the phytosterols which is structurally different from other phytosterols such as campestral, brassicasterol, ergosterol etc. It is highly likely that phytosterols have differences in their effects, at least in their efficacy. EFSA, USFDA, joint FAO/WHO published a report on phytosterols as food supplements or additives without any specific emphasis on any individual compound. This tendency to generalize the effect of phytosterols has limited the study of the individual effect of different phytosterols. This effect is also observed in many clinical trials. However, most of the trials do not categorize phytosterols, but rather administer a mixture of phytosterols. Such oversimplified statements are vague and do not lead to the development of newer therapeutics. However, several clinical trials that have been carried out with BS are multicentric, placebo-controlled, double-blind [183] or simple comparative study [15,186,214] for the treatment of BPH [183–185]; lower urinary tract infection [186]; hypercholesteremia [214], immunosuppression and inflammation [135], rheumatoid arthritis [215] and androgenetic alopecia [182]. The results have shown some beneficial effects, but neither long-term safety data nor clinical trials with drug delivery aiming to overcome its lower efficacy are available. This is mostly due to lack of sufficient research-based information on BS needed for such official publication. Even the Norwegian Food Safety Authority (NFSA, 2012) published a risk profile of BS but its directives are mostly based on studies on phytosterols in general, not BS alone. General directives for phytosterols may serve as a guideline for the use of a phytosterol mixture, but it cannot serve the purpose when BS is used and marketed alone. Even though the FDA has approved the manufacturer's claim of the beneficial effects of BS against coronary heart disease, most of the manufacturers commercialize it to treat alopecia and BPH, even though there is still no long-term convincing result regarding the efficacy of BS against alopecia and BPH. Pharmaceutical research

organizations have a relatively low interest in research with BS. Therefore, research in an academic setting as well as through funding from national and international organizations such as FAO, WHO, EFSA etc. to find its long term effects at the molecular and cellular level is recommended. Research for improving efficacy via chemical modifications or via intelligent drug delivery to improve absorptivity and specificity is also recommended. Such research is urgent for at least two reasons: one is caution and another is hope. The caution is regarding its safety for chronic public use, either systemically or topically. The hope is for the pharmaceutical research organizations to set newer avenues to find out modern alternatives to current therapeutic agents. Even though BS has many important roles in different diseases, it has been neglected mostly because of its lower potency in most of these cases. However, the fact is that its relatively higher safety in comparison to other available drugs being used to treat different diseases has been ignored. An extensive risk-benefit study, at least in the academic setting, is therefore highly recommended.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bouic, P.; Clark, A.; Lamprecht, J.; Freestone, M.; Pool, E.; Liebenberg, R.; Kotze, D.; van Jaarsveld, P. The effects of B-sitosterol (BSS) and B-sitosterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: Inhibition of post marathon immune suppression and inflammation. *Int. J. Sports Med.* **1999**, *20*, 258–262. [CrossRef] [PubMed]

2. Pegel, K.H. The importance of sitosterol and sitosterolin in human and animal nutrition. *S. Afr. J. Sci.* **1997**, *93*, 263–268.

3. Schneider, K.; DiNovi, M.; Baines, J.; Schlatter, J. Phytosterols, phytostanols and their esters. *Saf. Eval. Certain Food Addit.* **2009**, *60*, 117–157.

4. Raport, E. Consumption of food and beverages with added plant sterols in the European Union. *EFSA J.* **2008**, *133*, 1–21.

5. Paniagua-Pérez, R.; Madrigal-Bujaidar, E.; Reyes-Cadena, S.; Molina-Jasso, D.; Gallaga, J.P.; Silva-Miranda, A.; Velazco, O.; Hernández, N.; Chamorro, G. Genotoxic and cytotoxic studies of beta-sitosterol and pteropodine in mouse. *BioMed Res. Int.* **2005**, *2005*, 242–247. [CrossRef] [PubMed]

6. Wilt, T.J.; Ishani, A.; Rutks, I.; MacDonald, R. Phytotherapy for benign prostatic hyperplasia. *Public Health Nutr.* **2000**, *3*, 459–472. [CrossRef] [PubMed]

7. Shefer, S.; Salen, G.; Nguyen, L.; Batta, A.; Packin, V.; Tint, G.; Hauser, S. Competitive inhibition of bile acid synthesis by endogenous cholestanol and sitosterol in sitosterolemia with xanthomatosis. Effect on cholesterol 7 alpha-hydroxylase. *J. Clin. Investig.* **1988**, *82*, 1833–1839. [CrossRef] [PubMed]

8. Awad, A.; Chinnam, M.; Fink, C.; Bradford, P. B-sitosterol activates fas signaling in human breast cancer cells. *Phytomedicine* **2007**, *14*, 747–754. [CrossRef] [PubMed]

9. Alemany, L.; Barbera, R.; Alegría, A.; Laparra, J. Plant sterols from foods in inflammation and risk of cardiovascular disease: A real threat? *Food Chem. Toxicol.* **2014**, *69*, 140–149. [CrossRef] [PubMed]

10. Vanmierlo, T.; Bogie, J.F.; Mailleux, J.; Vanmol, J.; Lütjohann, D.; Mulder, M.; Hendriks, J.J. Plant sterols: Friend or foe in CNS disorders? *Prog. Lipid Res.* **2015**, *58*, 26–39. [CrossRef] [PubMed]

11. Mannucci, L.; Guardamagna, O.; Bertucci, P.; Pisciotta, L.; Liberatoscioli, L.; Bertolini, S.; Irace, C.; Gnasso, A.; Federici, G.; Cortese, C. Beta-sitosterolaemia: A new nonsense mutation in the ABCG5 gene. *Eur. J. Clin. Investig.* **2007**, *37*, 997–1000. [CrossRef] [PubMed]

12. Mooradian, A.D.; Haas, M.J.; Wong, N.C. The effect of select nutrients on serum high-density lipoprotein cholesterol and apolipoprotein A-I levels. *Endocr. Rev.* **2006**, *27*, 2–16. [CrossRef] [PubMed]

13. Ulbricht, C.E. An evidence-based systematic review of beta-sitosterol, sitosterol (22,23-dihydrostigmasterol, 24-ethylcholesterol) by the natural standard research collaboration. *J. Diet. Suppl.* **2015**, *13*, 35–92. [PubMed]

14. Khan, A.; Rahman, M.; Islam, S. Antipyretic activity of *Peperomia pellucida* leaves in rabbit. *Turk. J. Biol.* **2008**, *32*, 37–41.

15. Pateh, U.; Haruna, A.; Garba, M.; Iliya, I.; Sule, I.; Abubakar, M.; Ambi, A. Isolation of stigmasterol, β-sitosterol and 2-hydroxyhexadecanoic acid methyl ester from the rhizomes of *Stylochiton lancifolius* Pyer and Kotchy (Araceae). *Niger. J. Pharm. Sci.* **2009**, *7*, 19–25.

16. Khanam, S.; Sultana, R. Isolation of β-sitosterol & stigmasterol as active immunomodulatory constituents from fruits of *Solanum xanthocarpum* (Solanaceae). *IJPSR* **2012**, *3*, 1057–1060.

17. Sarin, R.; Bansal, N. Phytosterol from in vivo and in vitro cultures of two medicinal plants viz. *Adhatoda Vasica* **2011**, *2*, 927–930.

18. Ryan, E.; Galvin, K.; O'Connor, T.; Maguire, A.; O'Brien, N. Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *Int. J. Food Sci. Nutr.* **2006**, *57*, 219–228. [CrossRef] [PubMed]

19. Kongduang, D.; Wungsintaweekul, J.; de-Eknamkul, W. Biosynthesis of β-sitosterol and stigmasterol proceeds exclusively via the mevalonate pathway in cell suspension cultures of *Croton stellatopilosus*. *Tetrahedron Lett.* **2008**, *49*, 4067–4072. [CrossRef]

20. De-Eknamkul, W.; Potduang, B. Biosynthesis of β-sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. *Phytochemistry* **2003**, *62*, 389–398. [CrossRef]

21. Klein, R. Phytoecdysteroids. *J. Am. Herbalists Guild* **2004**, *5*, 18–28.

22. Xu, G.; Guan, L.; Sun, J.; Chen, Z.-Y. Oxidation of cholesterol and β-sitosterol and prevention by natural antioxidants. *J. Agric. Food Chem.* **2009**, *57*, 9284–9292. [CrossRef] [PubMed]

23. Zhang, X.; Julien-David, D.; Miesch, M.; Geoffroy, P.; Raul, F.; Roussi, S.; Aoudé-Werner, D.; Marchioni, E. Identification and quantitative analysis of β-sitosterol oxides in vegetable oils by capillary gas chromatography–mass spectrometry. *Steroids* **2005**, *70*, 896–906. [CrossRef] [PubMed]

24. Zhang, X.; Geoffroy, P.; Miesch, M.; Julien-David, D.; Raul, F.; Aoudé-Werner, D.; Marchioni, E. Gram-scale chromatographic purification of β-sitosterol: Synthesis and characterization of β-sitosterol oxides. *Steroids* **2005**, *70*, 886–895. [CrossRef] [PubMed]

25. Christiansen, L.I.; Rantanen, J.T.; von Bonsdorff, A.K.; Karjalainen, M.A.; Yliruusi, J.K. A novel method of producing a microcrystalline β-sitosterol suspension in oil. *Eur. J. Pharm. Sci.* **2002**, *15*, 261–269. [CrossRef]

26. Vanmierlo, T.; Weingärtner, O.; Pol, S.; Husche, C.; Kerksiek, A.; Friedrichs, S.; Sijbrands, E.; Steinbusch, H.; Grimm, M.; Hartmann, T.; et al. Dietary intake of plant sterols stably increases plant sterol levels in the murine brain. *J. Lipid Res.* **2012**, *53*, 726–735. [CrossRef] [PubMed]

27. Hubbard, W.; Sheppard, A.; Newkirk, D.; Prosser, A.; Osgood, T. Comparison of various methods for the extraction of total lipids, fatty acids, cholesterol, and other sterols from food products. *J. Am. Oil Chem. Soc.* **1977**, *54*, 81–83. [CrossRef] [PubMed]

28. Careri, M.; Elviri, L.; Mangia, A. Liquid chromatography–UV determination and liquid chromatography–atmospheric pressure chemical ionization mass spectrometric characterization of sitosterol and stigmasterol in soybean oil. *J. Chromatogr. A* **2001**, *935*, 249–257. [CrossRef]

29. Nagy, K.; Jakab, A.; Pollreisz, F.; Bongiorno, D.; Ceraulo, L.; Averna, M.R.; Noto, D.; Vekey, K. Analysis of sterols by high-performance liquid chromatography/mass spectrometry combined with chemometrics. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2433–2440. [CrossRef] [PubMed]

30. Ahmida, H.M.; Bertucci, P.; Franzò, L.; Massoud, R.; Cortese, C.; Lala, A.; Federici, G. Simultaneous determination of plasmatic phytosterols and cholesterol precursors using gas chromatography–mass spectrometry (GC–MS) with selective ion monitoring (SIM). *J. Chromatogr. B* **2006**, *842*, 43–47. [CrossRef] [PubMed]

31. Nair, V.; Kanfer, I.; Hoogmartens, J. Determination of stigmasterol, β-sitosterol and stigmastanol in oral dosage forms using high performance liquid chromatography with evaporative light scattering detection. *J. Pharm. Biomed. Anal.* **2006**, *41*, 731–737. [CrossRef] [PubMed]

32. Patra, A.; Jha, S.; Murthy, P.N.; Manik; Sharone, A. Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa*. *Int. J. Pharm. Sci. Res.* **2010**, *2*, 95–100.

33. Karan, S.K.; Mishra, S.K.; Pal, D.; Mondal, A. Isolation of β-sitosterol and evaluation of antidiabetic activity of *Aristolochia indica* in alloxan-induced diabetic mice with a reference to in vitro antioxidant activity. *J. Med. Plant Res.* **2012**, *6*, 1219–1223.

34. Ahmed, K.B.A.; Subramaniam, S.; Veerappan, G.; Hari, N.; Sivasubramanian, A.; Veerappana, A. β-sitosterol-D-glucopyranoside isolated from *Desmostachya bipinnata* mediate photoinduced rapid green synthesis of silver nanoparticles. *RSC Adv.* **2014**, *4*, 59130–59136. [CrossRef]

35. Chaturvedula, V.S.P.; Prakash, I. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus*. *Int. Curr. Pharm. J.* **2012**, *1*, 239–242. [CrossRef]

36. Gao, Y.; Xu, H.; Lu, Z.; Xu, Z. Quantitative determination of steroids in the fruiting bodies and submerged-cultured mycelia of *Inonotus obliquus*. *Chin. J. Chromatogr.* **2009**, *27*, 745–749.

37. Srividya, N.; Heidorn, D.B.; Lange, B.M. Rapid purification of gram quantities of β-sitosterol from a commercial phytosterol mixture. *BMC Res. Notes* **2014**, *7*. [CrossRef] [PubMed]

38. Mackay, D.S.; Jones, P.J.H.; Myrie, S.B.; Plat, J.; Lütjohann, D. Methodological considerations for the harmonization of non-cholesterol sterol bio-analysis. *J. Chromatogr. B* **2014**, *957*, 116–122. [CrossRef] [PubMed]

39. Dewick, P.M. *Medicinal Natural Products: A Biosynthetic Approach*; John Wiley & Sons: Hoboken, NJ, USA, 2002.

40. Dewick, P.M. *Medicinal Natural Products: A Biosynthetic Approach*, 3rd ed.; John Wiley & Sons Ltd.: Chichester, UK, 2009; p. 539.

41. Joffrion, T.M.; Cushion, M.T. Sterol biosynthesis and sterol uptake in the fungal pathogen *Pneumocystis carinii*. *FEMS Microbiol. Lett.* **2010**, *311*, 1–9. [CrossRef] [PubMed]

42. Fernández-Checa, J.C. *Free Cholesterol—A Double-Edge Sword in Alzheimer Disease—Challenges for the Future*; Inga, Z., Ed.; Georg August University: Göttingen, Germany, 2015; pp. 141–166.

43. Geoffroy, P.; Julien-David, D.; Marchioni, E.; Raul, F.; Aoudé-Werner, D.; Miesch, M. Synthesis of highly pure oxyphytosterols and (oxy)phytosterol esters: Part I. Regioselective hydrogenation of stigmasterol: An easy access to oxyphytosterols. *Steroids* **2008**, *73*, 702–707. [CrossRef] [PubMed]

44. Foley, D.A.; O'Callaghan, Y.; O'Brien, N.M.; McCarthy, F.O.; Maguire, A.R. Synthesis and characterization of stigmasterol oxidation products. *J. Agric. Food Chem.* **2009**, *58*, 1165–1173. [CrossRef] [PubMed]

45. Khripach, V.A.; Zhabinskii, V.N.; Konstantinova, O.V.; Khripach, N.B.; Antonchick, A.V.; Antonchick, A.P.; Schneider, B. Preparation of (25*R*)-and (25*S*)-26-functionalized steroids as tools for biosynthetic studies of cholic acids. *Steroids* **2005**, *70*, 551–562. [CrossRef] [PubMed]

46. McCarthy, F.O.; Chopra, J.; Ford, A.; Hogan, S.A.; Kerry, J.P.; O'Brien, N.M.; Ryan, E.; Maguire, A.R. Synthesis, isolation and characterisation of β-sitosterol and β-sitosterol oxide derivatives. *Org. Biomol. Chem.* **2005**, *3*, 3059–3065. [CrossRef] [PubMed]

47. Hang, J.; Dussault, P. A concise synthesis of β-sitosterol and other phytosterols. *Steroids* **2010**, *75*, 879–883. [CrossRef] [PubMed]

48. Santos, A.; Niero, R.; Yunes, R.; Pizzolatti, M.; Delle Monache, F.; Calixto, J. Antinociceptive properties of steroids isolated from *Phyllanthus corcovadensis* in mice. *Planta Medica* **1995**, *61*, 329–332. [CrossRef] [PubMed]

49. López-Rubalcava, C.; Piña-Medina, B.; Estrada-Reyes, R.; Heinze, G.; Martínez-Vázquez, M. Anxiolytic-like actions of the hexane extract from leaves of *Annona cherimolia* in two anxiety paradigms: Possible involvement of the GABA/benzodiazepine receptor complex. *Life Sci.* **2006**, *78*, 730–737. [CrossRef] [PubMed]

50. Aguirre-Hernández, E.; Rosas-Acevedo, H.; Soto-Hernández, M.; Martínez, A.L.; Moreno, J.; González-Trujano, M.E. Bioactivity-guided isolation of beta-sitosterol and some fatty acids as active compounds in the anxiolytic and sedative effects of *Tilia americana* var. Mexicana. *Planta Medica* **2007**, *73*, 1148–1155. [CrossRef] [PubMed]

51. Zhu, M. CNS active principles from *Alangium plantanifolium*. *Planta Medica* **1998**, *64*, 8–11. [CrossRef] [PubMed]

52. Brimson, J.M.; Brimson, S.J.; Brimson, C.A.; Rakkhitawatthana, V.; Tencomnao, T. *Rhinacanthus nasutus* extracts prevent glutamate and amyloid-β neurotoxicity in ht-22 mouse hippocampal cells: Possible active compounds include lupeol, stigmasterol and β-sitosterol. *Int. J. Mol. Sci.* **2012**, *13*, 5074–5097. [CrossRef] [PubMed]

53. Shi, C.; Liu, J.; Wu, F.; Zhu, X.; Yew, D.T.; Xu, J. B-sitosterol inhibits high cholesterol-induced platelet β-amyloid release. *J. Bioenerg. Biomembr.* **2011**, *43*, 691–697. [CrossRef] [PubMed]

54. Shi, C.; Wu, F.; Xu, J. Incorporation of β-sitosterol into mitochondrial membrane enhances mitochondrial function by promoting inner mitochondrial membrane fluidity. *J. Bioenerg. Biomembr.* **2013**, *45*, 301–305. [CrossRef] [PubMed]

55. Hamedi, A.; Ghanbari, A.; Saeidi, V.; Razavipour, R.; Azari, H. Effects of β-sitosterol oral administration on the proliferation and differentiation of neural stem cells. *J. Funct. Foods* **2014**, *8*, 252–258. [CrossRef]

56. Hamedi, A.; Ghanbari, A.; Razavipour, R.; Saeidi, V.; Zarshenas, M.M.; Sohrabpour, M.; Azari, H. Alyssum homolocarpum seeds: Phytochemical analysis and effects of the seed oil on neural stem cell proliferation and differentiation. *J. Nat. Med.* **2015**, *69*, 1–10. [CrossRef] [PubMed]

57. Han, N.-R.; Kim, H.-M.; Jeong, H.-J. The β-sitosterol attenuates atopic dermatitis-like skin lesions through down-regulation of TSLP. *Exp. Biol. Med.* **2014**, *239*, 454–464. [CrossRef] [PubMed]

58. Su, Y.; Wang, Z.; Yang, H.; Cao, L.; Liu, F.; Bai, X.; Ruan, C. Clinical and molecular genetic analysis of a family with sitosterolemia and co-existing erythrocyte and platelet abnormalities. *Haematologica* **2006**, *91*, 1392–1395. [PubMed]

59. Brufaua, G.; Canelab, M.A.; Rafecas, M. Phytosterols: Physiologic and metabolic aspects related to cholesterol-lowering properties. *Nutr. Res.* **2008**, *28*, 217–225. [CrossRef] [PubMed]

60. Tamura, M.; Suzuki, H.; Itoh, K. Effect of beta-sitosterol on ultrastructure of liver cells in young and aged mice. *Int. J. Vitam. Nutr. Res.* **1997**, *68*, 146–148.

61. Goswami, S.K.; Frey, C.F. Effect of beta-sitosterol on cholesterol-cholic acid-induced gallstone formation in mice. *Am. J. Gastroenterol.* **1976**, *65*, 305–310. [PubMed]

62. Singhal, A.K.; Cohen, B.I.; Finver-Sadowsky, J.; McSherry, C.K.; Mosbach, E.H. Role of hydrophilic bile acids and of sterols on cholelithiasis in the hamster. *J. Lipid Res.* **1984**, *25*, 564–570. [PubMed]

63. Bustos, P.; Duffau, C.; Pacheco, C.; Ulloa, N. B-sitosterol modulation of monocyte-endothelial cell interaction: A comparison to female hormones. *Maturitas* **2008**, *60*, 202–208. [CrossRef] [PubMed]

64. Wu, G.-X.; Lin, Y.-X.; Ou, M.-R.; Tan, D.-F. An experimental study (II) on the inhibition of prostatic hyperplasia by extract of seeds of *Brassica alba*. *Zhongguo Zhong Yao Za Zhi* **2003**, *28*, 643–646. [PubMed]

65. Leusch, F.D.; MacLatchy, D.L. In vivo implants of β-sitosterol cause reductions of reactive cholesterol pools in mitochondria isolated from gonads of male goldfish (*Carassius auratus*). *Gen. Comp. Endocrinol.* **2003**, *134*, 255–263. [CrossRef]

66. Promprom, W.; Kupittayanant, P.; Indrapichate, K.; Wray, S.; Kupittayanant, S. The effects of pomegranate seed extract and β-sitosterol on rat uterine contractions. *Reprod. Sci.* **2010**, *17*, 288–296. [CrossRef] [PubMed]

67. Nieminen, P.; Pölönen, I.; Mustonen, A.-M. Increased reproductive success in the white American mink (*Neovison vison*) with chronic dietary β-sitosterol supplement. *Anim. Reprod. Sci.* **2010**, *119*, 287–292. [CrossRef] [PubMed]

68. Malini, T.; Vanithakumari, G. Rat toxicity studies with β-sitosterol. *J. Ethnopharmacol.* **1990**, *28*, 221–234. [CrossRef]

69. Chattopadhyay, D.; Dungdung, S.R.; Mandal, A.B.; Majumder, G.C. A potent sperm motility-inhibiting activity of bioflavonoids from an ethnomedicine of onge, *Alstonia macrophylla* Wall ex A. Dc, leaf extract. *Contraception* **2005**, *71*, 372–378. [CrossRef] [PubMed]

70. Ryökkynen, A.; Käyhkö, U.-R.; Mustonen, A.-M.; Kukkonen, J.V.; Nieminen, P. Multigenerational exposure to phytosterols in the mouse. *Reprod. Toxicol.* **2005**, *19*, 535–540. [CrossRef] [PubMed]

71. Martel-Estrada, S.A.; Rodríguez-Espinoza, B.; Santos-Rodríguez, E.; Jiménez-Vega, F.; García-Casillas, P.E.; Martínez-Pérez, C.A.; Armendáriz, I.O. Biocompatibility of chitosan/*Mimosa tenuiflora* scaffolds for tissue engineering. *J. Alloy. Compd.* **2015**, *643*, 119–123. [CrossRef]

72. Ikeda, I.; Sugano, M. Comparison of absorption and metabolism of β-sitosterol and β-sitostanol in rats. *Atherosclerosis* **1978**, *30*, 227–237. [CrossRef]

73. Ritschel, W.; Kastner, U.; Hussain, A.; Koch, H. Pharmacokinetics and bioavailability of beta-sitosterol in the beagle dog. *Arzneim.-Forsch.* **1990**, *40*, 463–468.

74. Relas, H.; Gylling, H.; Miettinen, T.A. Fate of intravenously administered squalene and plant sterols in human subjects. *J. Lipid Res.* **2001**, *42*, 988–994. [PubMed]

75. Duchateau, G.; Cochrane, B.; Windebank, S.; Rudzinska, J.; Sanghera, D.; Burien, A.M.; Müller, M.; Zeitlinger, M.; Lappin, G. Absolute oral bioavailability and metabolic turnover of β-sitosterol in healthy subjects. *Drug Metab. Dispos.* **2012**, *40*, 2026–2030. [CrossRef] [PubMed]

76. Sudhop, T.; Sahin, Y.; Lindenthal, B.; Hahn, C.; Lüers, C.; Berthold, H.; von Bergmann, K. Comparison of the hepatic clearances of campesterol, sitosterol, and cholesterol in healthy subjects suggests that efflux transporters controlling intestinal sterol absorption also regulate biliary secretion. *Gut* **2002**, *51*, 860–863. [CrossRef] [PubMed]

77. Gilman, C.I.; Leusch, F.D.; Breckenridge, W.C.; MacLatchy, D.L. Effects of a phytosterol mixture on male fish plasma lipoprotein fractions and testis P450scc activity. *Gen. Comp. Endocrinol.* **2003**, *130*, 172–184. [CrossRef]

78. Ikeda, I.; Tanaka, K.; Sugano, M.; Vahouny, G.; Gallo, L. Discrimination between cholesterol and sitosterol for absorption in rats. *J. Lipid Res.* **1988**, *29*, 1583–1591. [PubMed]

79. Salen, G.; Shore, V.; Tint, G.; Forte, T.; Shefer, S.; Horak, I.; Horak, E.; Dayal, B.; Nguyen, L.; Batta, A. Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. *J. Lipid Res.* **1989**, *30*, 1319–1330. [PubMed]

80. Salen, G.; Ahrens, E., Jr.; Grundy, S.M. Metabolism of β-sitosterol in man. *J. Clin. Investig.* **1970**, *49*, 952. [CrossRef] [PubMed]

81. Sirtori, C.; Manzoni, C.; Lovati, M. Mechanisms of lipid-lowering agents. *Cardiology* **1991**, *78*, 226–235. [CrossRef] [PubMed]

82. Hauser, H.; Dyer, J.H.; Nandy, A.; Vega, M.A.; Werder, M.; Bieliauskaite, E.; Weber, F.E.; Compassi, S.; Gemperli, A.; Boffelli, D. Identification of a receptor mediating absorption of dietary cholesterol in the intestine. *Biochemistry* **1998**, *37*, 17843–17850. [CrossRef] [PubMed]

83. Plat, J.; Mensink, R.P. Plant stanol and sterol esters in the control of blood cholesterol levels: Mechanism and safety aspects. *Am. J. Cardiol.* **2005**, *96*, 15–22. [CrossRef] [PubMed]

84. Plat, J.; Nichols, J.A.; Mensink, R.P. Plant sterols and stanols: Effects on mixed micellar composition and LXR (target gene) activation. *J. Lipid Res.* **2005**, *46*, 2468–2476. [CrossRef] [PubMed]

85. Wang, X.; Huang, W.; Lei, L.; Liu, Y.; Ma, K.Y.; Li, Y.M.; Wang, L.; Huang, Y.; Chen, Z.-Y. Blockage of hydroxyl group partially abolishes the cholesterol-lowering activity of β-sitosterol. *J. Funct. Foods* **2015**, *12*, 199–207. [CrossRef]

86. Calpe-Berdiel, L.; Escolà-Gil, J.C.; Blanco-Vaca, F. New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. *Atherosclerosis* **2009**, *203*, 18–31. [CrossRef] [PubMed]

87. Ikeda, I.; Sugano, M. Some aspects of mechanism of inhibition of cholesterol absorption by β-sitosterol. *Biochim. Biophys. Acta (BBA)-Biomembr.* **1983**, *732*, 651–658. [CrossRef]

88. Field, F.J.; Mathur, S.N. Beta-sitosterol: Esterification by intestinal acylcoenzyme A: Cholesterol acyltransferase (ACAT) and its effect on cholesterol esterification. *J. Lipid Res.* **1983**, *24*, 409–417. [PubMed]

89. Sanders, D.; Minter, H.; Howes, D.; Hepburn, P. The safety evaluation of phytosterol esters. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat. *Food Chem. Toxicol.* **2000**, *38*, 485–491. [CrossRef]

90. Sehayek, E.; Duncan, E.M.; Lutjohann, D.; von Bergmann, K.; Ono, J.G.; Batta, A.K.; Salen, G.; Breslow, J.L. Loci on chromosomes 14 and 2, distinct from ABCG5/ABCG8, regulate plasma plant sterol levels in a C57bl/6J× CASA/Rk intercross. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16215–16219. [CrossRef] [PubMed]

91. Jansen, P.J.; Lütjohann, D.; Abildayeva, K.; Vanmierlo, T.; Plösch, T.; Plat, J.; von Bergmann, K.; Groen, A.K.; Ramaekers, F.C.; Kuipers, F. Dietary plant sterols accumulate in the brain. *Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids* **2006**, *1761*, 445–453. [CrossRef] [PubMed]

92. Song, Y.S.; Jin, C.; Park, E.-H. Identification of metabolites of phytosterols in rat feces using GC/MS. *Arch. Pharm. Res.* **2000**, *23*, 599–604. [CrossRef] [PubMed]

93. Aringer, L.; Eneroth, P. Formation and metabolism in vitro of 5,6-epoxides of cholesterol and β-sitosterol. *J. Lipid Res.* **1974**, *15*, 389–398. [PubMed]

94. Aringer, L.; Eneroth, P.; Nordström, L. Side chain hydroxylation of cholesterol, campesterol and beta-sitosterol in rat liver mitochondria. *J. Lipid Res.* **1976**, *17*, 263–272. [PubMed]

95. Miettinen, T.A.; Vanhanen, H. Dietary-sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atherosclerosis* **1994**, *105*, 217–226. [CrossRef]

96. Grundy, S.M.; Ahrens, E.H., Jr.; Salen, G. Dietary β-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **1968**, *9*, 374–387. [PubMed]

97. Bhattacharyya, A.K.; Connor, W.E.; Lin, D.S. The origin of plant sterols in the skin surface lipids in humans: From diet to plasma to skin. *J. Investig. Dermatol.* **1983**, *80*, 294–296. [CrossRef] [PubMed]

98. Miettinen, T.; Tarpila, S. Fecal β-sitosterol in patients with diverticular disease of the colon and in vegetarians. *Scand. J. Gastroenterol.* **1978**, *13*, 573–576. [CrossRef] [PubMed]

99. Sutherland, W.; Nye, E.; Macfarlane, D.; Williamson, S.; Robertson, M. Cholesterol metabolism in distance runners. *Clin. Physiol.* **1992**, *12*, 29–37. [CrossRef] [PubMed]

100. Vuoristo, M.; Tilvis, R.; Miettinen, T. Serum plant sterols and lathosterol related to cholesterol absorption in coeliac disease. *Clin. Chim. Acta* **1988**, *174*, 213–224. [CrossRef]

101. Gupta, R.; Sharma, A.K.; Dobhal, M.; Sharma, M.; Gupta, R. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. *J. Diabetes* **2011**, *3*, 29–37. [CrossRef] [PubMed]

102. Moreno, J.J.; Mitjavila, M.T. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (review). *J. Nutr. Biochem.* **2003**, *14*, 182–195. [CrossRef]

103. Vivancos, M.; Moreno, J.J. B-sitosterol modulates antioxidant enzyme response in raw 264.7 macrophages. *Free Radic. Biol. Med.* **2005**, *39*, 91–97. [CrossRef] [PubMed]

104. Moon, E.-J.; Lee, Y.M.; Lee, O.-H.; Lee, M.-J.; Lee, S.-K.; Chung, M.-H.; Park, Y.-I.; Sung, C.-K.; Choi, J.-S.; Kim, K.-W. A ncovel angiogenic factor derived from aloe vera gel: B-sitosterol, a plant sterol. *Angiogenesis* **1999**, *3*, 117–123. [CrossRef] [PubMed]

105. Choi, Y.H.; Kong, K.R.; Kim, Y.; Jung, K.-O.; Kil, J.-H.; Rhee, S.-H.; Park, K.-Y. Induction of bax and activation of caspases during β-sitosterol-mediated apoptosis in human colon cancer cells. *Int. J. Oncol.* **2003**, *23*, 1657–1662. [CrossRef] [PubMed]

106. Pollak, O.J.; Kritchevsky, D. *Sitosterol*; Karger: Basel, Switzerland, 1981.

107. Hu, F.B. Plant-based foods and prevention of cardiovascular disease: An overview. *Am. J. Clin. Nutr.* **2003**, *78*, 544S–551S. [PubMed]

108. Retelny, V.S.; Neuendorf, A.; Roth, J.L. Nutrition protocols for the prevention of cardiovascular disease. *Nutr. Clin. Pract.* **2008**, *23*, 468–476. [CrossRef] [PubMed]

109. Yeshurun, D.; Gotto, A. Drug treatment of hyperlipidemia. *Am. J. Med.* **1976**, *60*, 379–396. [CrossRef]

110. Jesch, E.D.; Carr, T.P. Sitosterol reduces micellar cholesterol solubility in model bile. *Nutr. Res.* **2006**, *26*, 579–584. [CrossRef]

111. Sugano, M.; Morioka, H.; Ikeda, I. A comparison of hypocholesterolemic activity of beta-sitosterol and beta-sitostanol in rats. *J. Nutr.* **1977**, *107*, 2011–2019. [PubMed]

112. Zak, A.; Zeman, M.; Vitkova, D.; Hrabak, P.; Tvrzicka, E. Beta-sitosterol in the treatment of hypercholesterolemia. *Casopis Lekaru Ceskych* **1990**, *129*, 1320–1323. [PubMed]

113. Martins, S.; Silva, H.F.; Novaes, M.; Ito, M.K. Therapeutic effects of phytosterols and phytostanols in cholesterolemia. *Arch. Latinoam. Nutr.* **2004**, *54*, 257–263. [PubMed]

114. Ellegard, L.H.; Andersson, S.W.; Normén, A.L.; Andersson, H.A. Dietary plant sterols and cholesterol metabolism. *Nutr. Rev.* **2007**, *65*, 39. [CrossRef] [PubMed]

115. Richter, W.O.; Geiss, H.C.; Sönnichsen, A.C.; Schwandt, P. Treatment of severe hypercholesterolemia with a combination of beta-sitosterol and lovastatin. *Curr. Ther. Res.* **1996**, *57*, 497–505. [CrossRef]

116. Weingärtner, O.; Böhm, M.; Laufs, U. Controversial role of plant sterol esters in the management of hypercholesterolaemia. *Eur. Heart J.* **2009**, *30*, 404–409. [CrossRef] [PubMed]

117. Rosenblat, M.; Volkova, N.; Aviram, M. Pomegranate phytosterol (β-sitosterol) and polyphenolic antioxidant (punicalagin) addition to statin, significantly protected against macrophage foam cells formation. *Atherosclerosis* **2013**, *226*, 110–117. [CrossRef] [PubMed]

118. Wilund, K.R.; Yu, L.; Xu, F.; Vega, G.L.; Grundy, S.M.; Cohen, J.C.; Hobbs, H.H. No association between plasma levels of plant sterols and atherosclerosis in mice and men. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 2326–2332. [CrossRef] [PubMed]

119. Gupta, M.; Nath, R.; Srivastava, N.; Shanker, K.; Kishor, K.; Bhargava, K. Anti-inflammatory and antipyretic activities of beta-sitosterol (*Cyperus*). *Planta Medica* **1980**, *39*, 157–163. [CrossRef] [PubMed]

120. Begum, T.N.; Ilyas, M.H.M.; Anand, A.V. Antipyretic activity of *Azima tetracantha* in experimental animals. *Int. J. Curr. Biomed. Pharm. Res.* **2011**, *1*, 41–44.

121. Loizou, S.; Lekakis, I.; Chrousos, G.P.; Moutsatsou, P. B-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. *Mol. Nutr. Food Res.* **2010**, *54*, 551–558. [CrossRef] [PubMed]

122. Nirmal, S.A.; Pal, S.C.; Mandal, S.C.; Patil, A.N. Analgesic and anti-inflammatory activity of β-sitosterol isolated from *Nyctanthes arbortristis* leaves. *Inflammopharmacology* **2012**, *20*, 219–224. [CrossRef] [PubMed]

123. Prieto-Garcia, J.; Recio, M.C.; Giner, R.M. Anti-inflammatory activity of beta-sitosterol in a model of oxazolone-induced contact delayed type hypersensitivity. *Bol. Latinoam. Caribe Plantas Med. Aromát.* **2006**, *6*, 57–62.

124. Bouic, P.; Etsebeth, S.; Liebenberg, R.; Albrecht, C.; Pegel, K.; Van Jaarsveld, P. Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: Implications for their use as an immunomodulatory vitamin combination. *Int. J. Immunopharmacol.* **1996**, *18*, 693–700. [CrossRef]

125. Bouic, P.; Lamprecht, J.H. Plant sterols and sterolins: A review of their immune-modulating properties. *Altern. Med. Rev.* **1999**, *4*, 170–177. [PubMed]

126. Valerio, M.; Awad, A.B. B-sitosterol down-regulates some pro-inflammatory signal transduction pathways by increasing the activity of tyrosine phosphatase SHP-1 in J774A. 1 murine macrophages. *Int. Immunopharmacol.* **2011**, *11*, 1012–1017. [CrossRef] [PubMed]

127. Gomez, M.; Saenz, M.; Garcia, M.; Fernandez, M. Study of the topical anti-inflammatory activity of *Achillea ageratum* on chronic and acute inflammation models. *Z. Naturforschung C* **1999**, *54*, 937–941. [CrossRef]

128. Navarro, A.; De Las Heras, B.; Villar, A. Anti-inflammatory and immunomodulating properties of a sterol fraction from *Sideritis foetens* Clem. *Biol. Pharm. Bull.* **2001**, *24*, 470–473. [CrossRef] [PubMed]

129. Breu, W.; Hagenlocher, M.; Redl, K.; Stadler, F.; Wagner, H. Anti-inflammatory activity of sabal fruit extracts prepared with supercritical carbon dioxide. In vitro antagonists of cyclooxygenase and 5-lipoxygenase metabolism. *Arzneim.-Forsch.* **1992**, *42*, 547–551.

130. Liz, R.; Zanatta, L.; dos Reis, G.O.; Horst, H.; Pizzolatti, M.G.; Silva, F.R.M.B.; Fröde, T.S. Acute effect of β-sitosterol on calcium uptake mediates anti-inflammatory effect in murine activated neutrophils. *J. Pharm. Pharmacol.* **2013**, *65*, 115–122. [CrossRef] [PubMed]

131. Mahajan, S.G.; Mehta, A.A. Suppression of ovalbumin-induced Th2-driven airway inflammation by β-sitosterol in a guinea pig model of asthma. *Eur. J. Pharmacol.* **2011**, *650*, 458–464. [CrossRef] [PubMed]

132. Tatiya, A.U.; Saluja, A.K. Further studies on membrane stabilizing, anti-inflammatory and FCA induced arthritic activity of various fractions of bark of *Machilus macrantha* in rats. *Rev. Bras. Farmacogn.* **2011**, *21*, 1052–1064. [CrossRef]

133. Moreau, R.A.; Whitaker, B.D.; Hicks, K.B. Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses. *Prog. Lipid Res.* **2002**, *41*, 457–500. [CrossRef]

134. Fraile, L.; Crisci, E.; Córdoba, L.; Navarro, M.A.; Osada, J.; Montoya, M. Immunomodulatory properties of beta-sitosterol in pig immune responses. *Int. Immunopharmacol.* **2012**, *13*, 316–321. [CrossRef] [PubMed]

135. Bouic, P.J. The role of phytosterols and phytosterolins in immune modulation: A review of the past 10 years. *Curr. Opin. Clin. Nutr. Metab. Care* **2001**, *4*, 471–475. [CrossRef] [PubMed]

136. Breytenbach, U.; Clark, A.; Lamprecht, J.; Bouic, P. Flow cytometric analysis of the Th1–Th2 balance in healthy individuals and patients infected with the human immunodeficiency virus (HIV) receiving a plant sterol/sterolin mixture. *Cell Biol. Int.* **2001**, *25*, 43–49. [CrossRef] [PubMed]

137. Bouic, P.J. Immunomodulation in HIV/AIDS: The Tygerberg/Stellenbosch University Experience. *AIDS Bull.* **1997**, *6*, 18–20.

138. Neurath, A.R.; Strick, N.; Li, Y.Y.; Debnath, A.K. *Punica granatum* (Pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide. *Ann. N. Y. Acad. Sci.* **2005**, *1056*, 311–327. [CrossRef] [PubMed]

139. Kotwal, G.J. Genetic diversity-independent neutralization of pandemic viruses (eg HIV), potentially pandemic (eg H5N1 strain of influenza) and carcinogenic (eg HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCS). *Vaccine* **2008**, *26*, 3055–3058. [PubMed]

140. Kotwal, G.J.; Kulkarni, A.P. Antigenic variation in microbial evasion of immune responses. *eLS* **2013**. [CrossRef]

141. Bin Sayeed, M.S.; Ameen, S.S. Beta-sitosterol: A promising but orphan nutraceutical to fight against cancer. *Nutr. Cancer* **2015**, *67*, 1–7. [CrossRef] [PubMed]

142. Awad, A.; Downie, A.C.; Fink, C.S. Inhibition of growth and stimulation of apoptosis by beta-sitosterol treatment of MDA-MB-231 human breast cancer cells in culture. *Int. J. Mol. Med.* **2000**, *5*, 541–546. [CrossRef] [PubMed]

143. Park, C.; Moon, D.-O.; Rhu, C.-H.; Choi, B.T.; Lee, W.H.; Kim, G.-Y.; Choi, Y.H. Beta-sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. *Biol. Pharm. Bull.* **2007**, *30*, 1317–1323. [CrossRef] [PubMed]

144. Moon, D.-O.; Kim, M.-O.; Choi, Y.H.; Kim, G.-Y. B-sitosterol induces G 2/M arrest, endoreduplication, and apoptosis through the Bcl-2 and PI3K/Akt signaling pathways. *Cancer Lett.* **2008**, *264*, 181–191. [CrossRef] [PubMed]

145. Tasyriq, M.; Najmuldeen, I.A.; In, L.L.; Mohamad, K.; Awang, K.; Hasima, N. 7α-hydroxy-β-sitosterol from *Chisocheton tomentosus* induces apoptosis via dysregulation of cellular Bax/Bcl-2 ratio and cell cycle arrest by downregulating Erk1/2 activation. *eCAM* **2012**, *12*, 155–237.

146. Awad, A.; Chen, Y.; Fink, C.; Hennessey, T. Beta-sitosterol inhibits ht-29 human colon cancer cell growth and alters membrane lipids. *Anticancer Res.* **1995**, *16*, 2797–2804.

147. Awad, A.; Von Holtz, R.; Cone, J.; Fink, C.; Chen, Y. Beta-sitosterol inhibits growth of HT-29 human colon cancer cells by activating the sphingomyelin cycle. *Anticancer Res.* **1997**, *18*, 471–473.

148. Baskar, A.A.; Ignacimuthu, S.; Paulraj, G.M.; Al Numair, K.S. Chemopreventive potential of β-sitosterol in experimental colon cancer model-an in vitro and in vivo study. *BMC Complement. Altern. Med.* **2010**, *10*, 24. [CrossRef] [PubMed]

149. Normén, A.L.; Brants, H.A.; Voorrips, L.E.; Andersson, H.A.; van den Brandt, P.A.; Goldbohm, R.A. Plant sterol intakes and colorectal cancer risk in the netherlands cohort study on diet and cancer. *Am. J. Clin. Nutr.* **2001**, *74*, 141–148. [PubMed]

150. Awad, A.B.; Burr, A.T.; Fink, C.S. Effect of resveratrol and β-sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells. *Prostaglandins Leukot. Essent. Fatty Acids* **2005**, *72*, 219–226. [CrossRef] [PubMed]

151. Jourdain, C.; Tenca, G.; Deguercy, A.; Troplin, P.; Poelman, D. In Vitro effects of polyphenols from cocoa and β-sitosterol on the growth of human prostate cancer and normal cells. *Eur. J. Cancer Prev.* **2006**, *15*, 353–361. [CrossRef] [PubMed]

152. Jayaprakasha, G.; Mandadi, K.; Poulose, S.M.; Jadegoud, Y.; Gowda, G.N.; Patil, B.S. Inhibition of colon cancer cell growth and antioxidant activity of bioactive compounds from *Poncirus trifoliata* (L.) Raf. *Bioorg. Med. Chem.* **2007**, *15*, 4923–4932. [CrossRef] [PubMed]

153. Wang, L.; Yang, Y.; Chen, S.; Ge, X.; Xu, C.; Gui, S. Effects of beta-sitosterol on microtubular systems in cervical cancer cells. *Zhonghua Yi Xue Za Zhi* **2006**, *86*, 2771–2775. [PubMed]

154. Matos, M.; Leite, L.; Brustolim, D.; De Siqueira, J.; Carollo, C.; Hellmann, A.; Pereira, N.; da Silva, D. Antineoplastic activity of selected constituents of *Duguetia glabriuscula*. *Fitoterapia* **2006**, *77*, 227–229. [CrossRef] [PubMed]

155. Nair, P.P.; Turjman, N.; Kessie, G.; Calkins, B.; Goodman, G.T.; Davidovitz, H.; Nimmagadda, G. Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Dietary cholesterol, beta-sitosterol, and stigmasterol. *Am. J. Clin. Nutr.* **1984**, *40*, 927–930. [PubMed]

156. Carilla, E.; Briley, M.; Fauran, F.; Sultan, C.; Duvilliers, C. Binding of permixon, a new treatment for prostatic benign hyperplasia, to the cytosolic androgen receptor in the rat prostate. *J. Steroid Biochem.* **1984**, *20*, 521–523. [CrossRef]

157. Von Holtz, R.L.; Fink, C.S.; Awad, A.B. B-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. *Nutr. Cancer* **1998**, *32*, 8–12. [CrossRef] [PubMed]

158. Awad, A.B.; Fink, C.S. Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J. Nutr.* **2000**, *130*, 2127–2130. [PubMed]

159. Awad, A.B.; Williams, H.; Fink, C.S. Phytosterols reduce in vitro metastatic ability of MDA-MB-231 human breast cancer cells. *Nutr. Cancer* **2001**, *40*, 157–164. [CrossRef] [PubMed]

160. Awad, A.B.; Roy, R.; Fink, C.S. B-sitosterol, a plant sterol, induces apoptosis and activates key caspases in MDA-MB-231 human breast cancer cells. *Oncol. Rep.* **2003**, *10*, 497–500. [CrossRef] [PubMed]

161. Moon, D.-O.; Lee, K.-J.; Choi, Y.H.; Kim, G.-Y. B-sitosterol-induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. *Int. Immunopharmacol.* **2007**, *7*, 1044–1053. [CrossRef] [PubMed]

162. Moreno, J.J. Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages RAW 264.7. *Free Radic. Biol. Med.* **2003**, *35*, 1073–1081. [CrossRef]

163. Hueber, A. Role of membrane microdomain rafts in TNFR-mediated signal transduction. *Cell Death Differ.* **2003**, *10*, 7–9. [CrossRef] [PubMed]

164. Scheel-Toellner, D.; Wang, K.; Assi, L.; Webb, P.; Craddock, R.; Salmon, M.; Lord, J. Clustering of death receptors in lipid rafts initiates neutrophil spontaneous apoptosis. *Biochem. Soc. Trans.* **2004**, *32*, 679–681. [CrossRef] [PubMed]

165. Awad, A.; Chinnam, M.; Fink, C.; Bradford, P. Targeting ceramide by dietary means to stimulate apoptosis in tumor cells. *Curr. Top. Nutraceutical Res.* **2004**, *2*, 93–100.

166. Ivorra, M.; D'ocon, M.; Paya, M.; Villar, A. Antihyperglycemic and insulin-releasing effects of beta-sitosterol 3-beta-D-glucoside and its aglycone, beta-sitosterol. *Arch. Int. Pharmacodyn. Thér.* **1987**, *296*, 224–231.

167. Chai, J.-W.; Lim, S.-L.; Kanthimathi, M.; Kuppusamy, U.R. Gene regulation in β-sitosterol-mediated stimulation of adipogenesis, glucose uptake, and lipid mobilization in rat primary adipocytes. *Genes Nutr.* **2011**, *6*, 181–188. [CrossRef] [PubMed]

168. Donald, P.; Lamprecht, J.; Freestone, M.; Albrecht, C.; Bouic, P.; Kotze, D.; Van Jaarsveld, P. A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. *Int. J. Tuberc. Lung Dis.* **1997**, *1*, 518–522. [PubMed]

169. Kiprono, P.C.; Kaberia, F.; Keriko, J.M.; Karanja, J.N. The in vitro anti-fungal and anti-bacterial activities of β-sitosterol from *Senecio lyratus* (Asteraceae). *Z. Naturforschung C* **2000**, *55*, 485–488.

170. Ajaiyeoba, E.; Onocha, P.; Nwozo, S.; Sama, W. Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia* **2003**, *74*, 706–709. [CrossRef]

171. Mishra, G.; Singh, P.; Verma, R.; Kumar, S.; Srivastav, S.; Jha, K.; Khosa, R. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. *Der Pham. Lett.* **2011**, *3*, 141–164.

172. Rahuman, A.A.; Gopalakrishnan, G.; Venkatesan, P.; Geetha, K. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) sweet. *Parasitol. Res.* **2008**, *102*, 981–988. [CrossRef] [PubMed]

173. Nweze, N.E. In vitro anti-trypanosomal activity of *Morinda lucida* leaves. *Afr. J. Biotechnol.* **2011**, *11*, 1812–1817.

174. Bumrela, S.; Naik, S. Hepato protective activity of methanolic extract of *Dipteracanthus patulus* (Jacq) Nees: Possible involvement of antioxidant and membrane stabilization property. *Int. J. Pharm. Pharm. Sci.* **2012**, *4*, 685–690.

175. Kanokmedhakul, K.; Kanokmedhakul, S.; Phatchana, R. Biological activity of anthraquinones and triterpenoids from *Prismatomeris fragrans*. *J. Ethnopharmacol.* **2005**, *100*, 284–288. [CrossRef] [PubMed]

176. Oh, K.-B.; Oh, M.-N.; Kim, J.-G.; Shin, D.-S.; Shin, J. Inhibition of sortase-mediated *Staphylococcus aureus* adhesion to fibronectin via fibronectin-binding protein by sortase inhibitors. *Appl. Microbiol. Biotechnol.* **2006**, *70*, 102–106. [CrossRef] [PubMed]

177. Villaseñor, I.M.; Angelada, J.; Canlas, A.P.; Echegoyen, D. Bioactivity studies on β-sitosterol and its glucoside. *Phytother. Res.* **2002**, *16*, 417–421. [CrossRef] [PubMed]

178. Deepak, M.; Dipankar, G.; Prashanth, D.; Asha, M.; Amit, A.; Venkataraman, B. Tribulosin and β-sitosterol-D-glucoside, the anthelmintic principles of *Tribulus terrestris*. *Phytomedicine* **2002**, *9*, 753–756. [CrossRef] [PubMed]

179. Gomes, A.; Saha, A.; Chatterjee, I.; Chakravarty, A. Viper and cobra venom neutralization by β-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less (Asteraceae). *Phytomedicine* **2007**, *14*, 637–643. [CrossRef] [PubMed]

180. Hu, Y.; Ye, W.; Yin, Z.; Zhao, S. Chemical constituents from flos *Sesamum indicum* L. *Yao Xue Xue Bao* **2007**, *42*, 286–291. [PubMed]

181. Upadhyay, K.; Gupta, N.K.; Dixit, V.K. Development and characterization of phyto-vesicles of β-sitosterol for the treatment of androgenetic alopecia. *Arch. Dermatol. Res.* **2012**, *304*, 511–519. [CrossRef] [PubMed]

182. Prager, N.; Bickett, K.; French, N.; Marcovici, G. A randomized, double-blind, placebo-controlled trial to determine the effectiveness of botanically derived inhibitors of 5-α-reductase in the treatment of androgenetic alopecia. *J. Altern. Complement. Med.* **2002**, *8*, 143–152. [CrossRef] [PubMed]

183. Klippel, K.; Hiltl, D.; Schipp, B. A multicentric, placebo-controlled, double-blind clinical trial of β-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. *Br. J. Urol.* **1997**, *80*, 427–432. [CrossRef] [PubMed]

184. Breza, J.; Dzurny, O.; Borowka, A.; Hanus, T.; Petrik, R.; Blane, G.; Chadha-Boreham, H. Efficacy and acceptability of tadenan®(*Pygeum africanum* extract) in the treatment of benign prostatic hyperplasia (BPH): A multicentre trial in central Europe. *Curr. Med. Res. Opin.* **1998**, *14*, 127–139. [PubMed]

185. Berges, R.; Windeler, J.; Trampisch, H.; Senge, T.; β-sitosterol study group. Randomised, placebo-controlled, double-blind clinical trial of β-sitosterol in patients with benign prostatic hyperplasia. *Lancet* **1995**, *345*, 1529–1532. [CrossRef]

186. Barry, M.J.; Roehrborn, C.G. Extracts from "clinical evidence": Benign prostatic hyperplasia. *BMJ* **2001**, *323*, 1042. [CrossRef] [PubMed]

187. Lomenick, B.; Shi, H.; Huang, J.; Chen, C. Identification and characterization of β-sitosterol target proteins. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4976–4979. [CrossRef] [PubMed]

188. Assmann, G.; Cullen, P.; Erbey, J.; Ramey, D.R.; Kannenberg, F.; Schulte, H. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: Results of a nested case-control analysis of the prospective cardiovascular münster (PROCAM) study. *Nutr. Metab. Cardiovasc. Dis.* **2006**, *16*, 13–21. [CrossRef] [PubMed]

189. Hedman, M.; Miettinen, T.A.; Gylling, H.; Ketomäki, A.; Antikainen, M. Serum noncholesterol sterols in children with heterozygous familial hypercholesterolemia undergoing pravastatin therapy. *J. Pediatr.* **2006**, *148*, 241–246. [CrossRef] [PubMed]

190. Thongtang, N.; Lin, J.; Schaefer, E.J.; Lowe, R.S.; Tomassini, J.E.; Shah, A.K.; Tershakovec, A.M. Effects of ezetimibe added to statin therapy on markers of cholesterol absorption and synthesis and LDL-C lowering in hyperlipidemic patients. *Atherosclerosis* **2012**, *225*, 388–396. [CrossRef] [PubMed]

191. Yamanashi, Y.; Takada, T.; Suzuki, H. Niemann-Pick C1-like 1 overexpression facilitates ezetimibe-sensitive cholesterol and β-sitosterol uptake in Caco-2 cells. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 559–564. [CrossRef] [PubMed]

192. Raj, A.; Katz, M. Corn oil and its minor constituents as inhibitors of DMBA-induced chromosomal breaks in vivo. *Mutation Res.* **1984**, *136*, 247–253. [CrossRef]

193. Maguire, L.; Konoplyannikov, M.; Ford, A.; Maguire, A.R.; O'Brien, N.M. Comparison of the cytotoxic effects of β-sitosterol oxides and a cholesterol oxide, 7β-hydroxycholesterol, in cultured mammalian cells. *Br. J. Nutr.* **2003**, *90*, 767–775. [CrossRef] [PubMed]

194. MacLatchy, D.; Peters, L.; Nickle, J.; van Der Kraak, G. Exposure to β-sitosterol alters the endocrine status of goldfish differently than 17β-estradiol. *Environ. Toxicol. Chem.* **1997**, *16*, 1895–1904. [CrossRef]

195. Nieminen, P.; Pölönen, I.; Ikonen, K.; Määttänen, M.; Mustonen, A.-M. Evaluation of reproductive safety of β-sitosterol on the American mink (*neovison vison*). *Chemosphere* **2008**, *71*, 493–499. [CrossRef] [PubMed]

196. Li, J.; Wang, X.; Liu, H.; Guo, H.; Zhang, M.; Mei, D.; Liu, C.; He, L.; Liu, L.; Liu, X. Impaired hepatic and intestinal ATP-binding cassette transporter G5/8 was associated with high exposure of β-sitosterol and the potential risks to blood–brain barrier integrity in diabetic rats. *J. Pharm. Pharmacol.* **2014**, *66*, 428–436. [CrossRef] [PubMed]

197. Armstrong, M.J.; Carey, M.C. Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. *J. Lipid Res.* **1987**, *28*, 1144–1155. [PubMed]

198. Imanaka, H.; Koide, H.; Shimizu, K.; Asai, T.; Kinouchi Shimizu, N.; Ishikado, A.; Makino, T.; Oku, N. Chemoprevention of tumor metastasis by liposomal. Beta-sitosterol intake. *Biol. Pharm. Bull.* **2008**, *31*, 400–404. [CrossRef] [PubMed]

199. Borowy-Borowski, H.; Sodja, C.; Docherty, J.; Roy Walker, P.; Sikorska, M. Unique technology for solubilization and delivery of highly lipophilic bioactive molecules. *J. Drug Target.* **2004**, *12*, 415–424. [CrossRef] [PubMed]

200. Sjöström, B.; Bergenståhl, B.; Kronberg, B. A method for the preparation of submicron particles of sparingly water-soluble drugs by precipitation in oil-in-water emulsions. II: Influence of the emulsifier, the solvent, and the drug substance. *J. Pharm. Sci.* **1993**, *82*, 584–589. [CrossRef] [PubMed]

201. Zhou, Z.; Li, L.; Yang, Y.; Xu, X.; Huang, Y. Tumor targeting by pH-sensitive, biodegradable, cross-linked *N*-(2-hydroxypropyl) methacrylamide copolymer micelles. *Biomaterials* **2014**, *35*, 6622–6635. [CrossRef] [PubMed]

202. Lacatusu, I.; Badea, N.; Stan, R.; Meghea, A. Novel bio-active lipid nanocarriers for the stabilization and sustained release of sitosterol. *Nanotechnology* **2012**, *23*, 455702. [CrossRef] [PubMed]

203. Farkas, E.; Schubert, R.; Zelkó, R. Effect of β-sitosterol concentration and high pressure homogenization on the chlorhexidine release from vesicular gels. *Int. J. Pharm.* **2006**, *307*, 51–55. [CrossRef] [PubMed]

204. Patel, S.P.; Jarowski, C.I. Oral absorption efficiency of acid-labile antibiotics from lipid-drug delivery systems. *J. Pharm. Sci.* **1975**, *64*, 869–872. [CrossRef] [PubMed]

205. Joseph, A.; Hill, J.; Patel, J.; Patel, S.; Kincl, F.A. Sustained-release hormonal preparations XV: Release of progesterone from cholesterol pellets in vivo. *J. Pharm. Sci.* **1977**, *66*, 490–493. [CrossRef] [PubMed]

206. Maitani, Y.; Yano, S.; Hattori, Y.; Furuhata, M.; Hayashi, K. Liposome vector containing biosurfactant-complexed DNA as herpes simplex virus thymidine kinase gene delivery system. *J. Liposome Res.* **2006**, *16*, 359–372. [CrossRef] [PubMed]

207. Zhang, Y.; Qi, X.R.; Gao, Y.; Wei, L.; Maitani, Y.; Nagai, T. Mechanisms of co-modified liver-targeting liposomes as gene delivery carriers based on cellular uptake and antigens inhibition effect. *J. Control. Release* **2007**, *117*, 281–290. [CrossRef] [PubMed]

208. Hiruta, Y.; Hattori, Y.; Kawano, K.; Obata, Y.; Maitani, Y. Novel ultra-deformable vesicles entrapped with bleomycin and enhanced to penetrate rat skin. *J. Control. Release* **2006**, *113*, 146–154. [CrossRef] [PubMed]

209. Maitani, Y.; Nakamura, K.; Suenaga, H.; Kamata, K.; Takayama, K.; Nagai, T. The enhancing effect of soybean-derived sterylglucoside and β-sitosterol β-D-glucoside on nasal absorption in rabbits. *Int. J. Pharm.* **2000**, *200*, 17–26. [CrossRef]

210. Nakamura, K.; Takayama, K.; Nagai, T.; Maitani, Y. Regional intestinal absorption of FITC–dextran 4,400 with nanoparticles based on β-sitosterol β-D-glucoside in rats. *J. Pharm. Sci.* **2003**, *92*, 311–318. [CrossRef] [PubMed]

211. Elmowafy, M.; Viitala, T.; Ibrahim, H.M.; Abu-Elyazid, S.K.; Samy, A.; Kassem, A.; Yliperttula, M. Silymarin loaded liposomes for hepatic targeting: In vitro evaluation and HepG2 drug uptake. *Eur. J. Pharm. Sci.* **2013**, *50*, 161–171. [CrossRef] [PubMed]

212. Hench, P.S.; Kendall, E.C.; Slocumb, C.H.; Polley, H.F. Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions: A study in clinical physiology. *Arch. Intern. Med.* **1950**, *85*, 545–666. [CrossRef]

213. Dixon, W.G.; Bansback, N. Understanding the side effects of glucocorticoid therapy: Shining a light on a drug everyone thinks they know. *Ann. Rheum. Dis.* **2012**, *71*, 1761–1764. [CrossRef] [PubMed]

214. Cicero, A.F.; Fiorito, A.; Panourgia, M.P.; Sangiorgi, Z.; Gaddi, A. Effects of a new soy/β-sitosterol supplement on plasma lipids in moderately hypercholesterolemic subjects. *J. Am. Diet. Assoc.* **2002**, *102*, 1807–1811. [CrossRef]

215. Louw, I.; Halland, A.; Bouic, P.; Freestone, M.; Lamprecht, J. A pilot study of the clinical effects of a mixture of beta-sitosterol and beta-sitosterol glucoside in active rheumatoid arthritis. *Am. J. Clin. Nutr.* **2002**, *75*, 351S.