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BIOLOGICAL PROPERTIES OF STIGMASTEROL AND OTHER PHYTOSTEROLS

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REVIEW ARTICLE

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Abstract

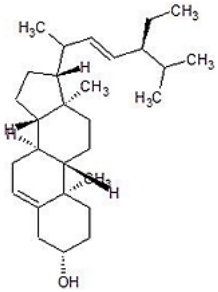
Natural products have long been recognized for their use in traditional medications in mankind. Some of the chemical compounds derived from plants which exhibit high therapeutic values include alkaloids, terpenoids, flavonoid, and phytosterol. Among phytosterols that have been studied, stigmasterol has shown to exhibit significant pharmacological therapeutic values. In this review, the overview of various derivatives' biological properties in the class of phytosterol compounds, especially stigmasterol, will be discussed. A systematic search was employed using google scholar (1977-present) and PubMed (1977- present) based on keywords such as "stigmasterol", "cancer", "structural", "mechanism", "phytosterols", "anticancer", "antimicrobial", "antioxidant", "cardioprotective", "anti- osteoarthritis", "neuroprotective", "anti-inflammatory", and "antidiabetic" to retrieve all relevant research papers published on different biological activities by stigmasterol and its derivatives. This group of compounds exhibits multiple biological effects. Stigmasterol and other identified phytosterol compounds showed promising therapeutic value and high potential candidate drug for various pathological disorders.

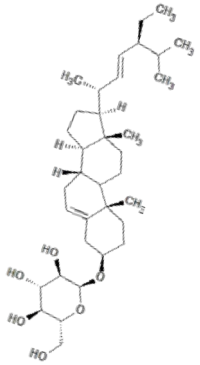
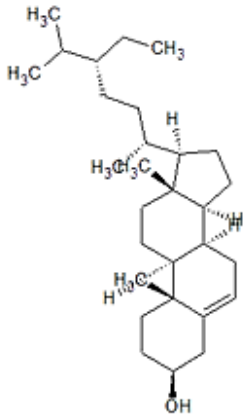
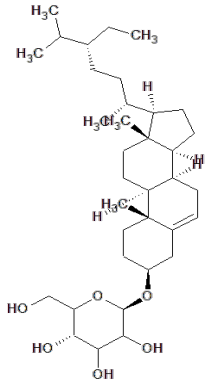
INTRODUCTION

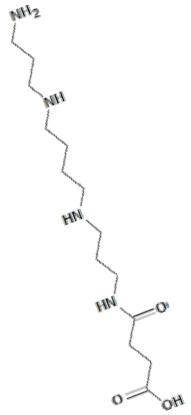
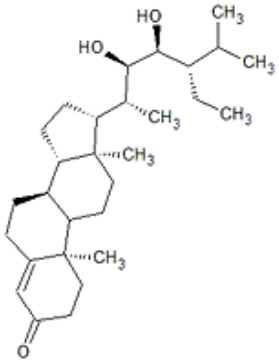
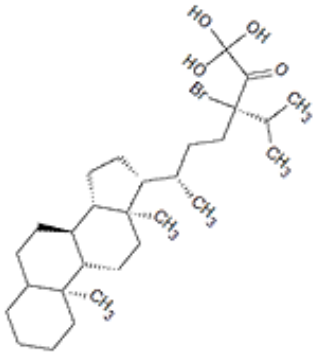
Plants, animals and several microorganisms have been extensively used as traditional medicinal products by the majority of native communities across the globe since plant resources are abundant, affordable and accessible [1-5]. Plant-derived analogues and phytochemicals from various parts of plants have been extensively studied via molecular modelling, combinatorial chemistry and other synthetic methods to identify the significant roles of phytochemicals in exhibiting pharmacological activities [1, 6-8]. Moreover, drug development derived from plants with bioactive compounds is highly preferred due to the abundance of primary and secondary metabolites [1, 9-11]. In nature, phytochemicals function as pollination and protection against environmental threats in plants [12-14]. Among the compounds of phytochemicals, phytosterols, also known as plant sterols or sterol esters, are triterpene families, commonly secreted as free sterols, fatty acid esters, non-acylated and acylated sterol glycosides [15-17]. Plants require phytosterols as they are vital to the growth and roles in regulating membrane-associated metabolism, fluidity, permeability and also act as precursor for signalling molecules synthesis [15, 18-23]. Over 200 phytosterols, with few being extensively studied such as β-sitosterol, stigmasterol, campesterol, lupeol and brassicasterol [15]. For stigmasterol, it is found in oils of soybean, calabar bean, rapeseed, vegetables, whole grains and legumes [6, 10]. Stigmasterol is physiologically essential as a precursor for progesterone and amalgamation of vitamin D3 [6, 24, 25]. Therefore, proven in numerous researches for its therapeutic values [1, 6, 9, 11, 26-31].

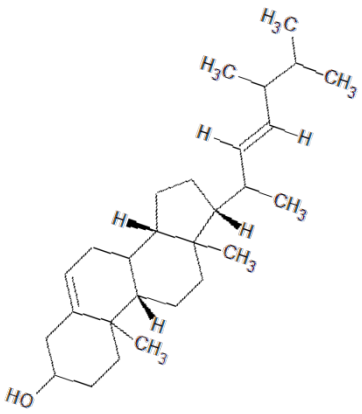
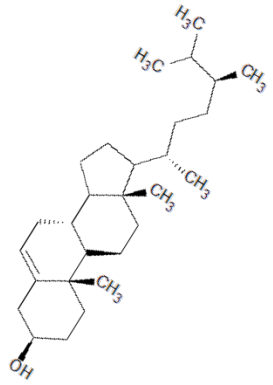
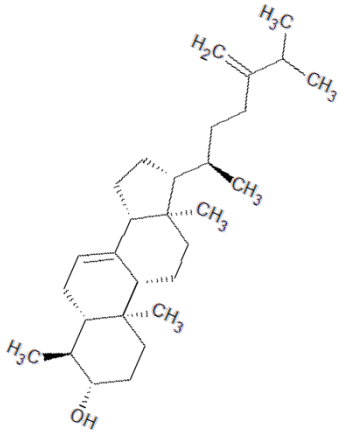
This review summarises various pharmacological activities of phytosterol compounds especially stigmasterol which are depicted in Table 1.

Table 1. Biological properties of stigmasterol and other phytosterol compounds designed using *Chemsketch 2.0* in reference to National Center for Biotechnology Information PubChem Database.

Compound	Biological Model	Potential Activity	References
Stigmasterol [C29H48O] [32]	***In Vitro Model***		
	HepG2	Anti-proliferation Apoptotic induction Cell cycle arrest	[33] [33, 34] [33]
	HUVEC	Anti-angiogenesis	[27]
	MCF-7 HT29	Apoptotic induction Antioxidant Cell cycle arrest Anti-angiogenesis	[28]
	U-87MG PC-3	Apoptotic induction Antioxidation	[28, 34]
	NOZ	Apoptotic induction	[35]
	Escherichia coli *Staphylococcus aureus* *Streptococcus pneumonia* *Salmonella pullorum*	Antibacterial	[36]
	Trypanosoma congolense	Antiparasitic	[37]
	Phytophthora infestans	Antifungal Antioxidation	[38] [39]
	SH-SY5Ywt transfected with APP695 or SPC99	Neuroprotective	[40]
	SH-SY5Y transfected with APP695 or SPC99		[40]
	In Vivo Model		
	BALB/c mice	Antioxidation	[41]
	NAFLD-induced mice WKY rats	Anti-hypercholesterolemic	[42] [43, 44]
	C58BL/6 mice		[42]
	C58BL/6J mice		[45]
	Sprague-Dawley rat fetus-exracted primary hippocampal cells	Neuroprotective	[46]
	Male Wistar rat w. surgically induced ischaemic injury		[47]
	Newborn swiss micecartilage	Antiosteoarthritic	[30]
	KK-Ay diabetic mice	Antidiabetic	[31]
	Streptozotocin induced diabetic Wistar mice	Antioxidation Anti-proliferation Anti-inflammation	[48] [27] [27]
	Alloxan-induced diabetic Wistar albino rats	Antihyperglycemic Hepatoprotective Antioxidation	[49] [49] [49]
	EAC Swiss albino mice	Apoptotic induction	[50]
	DMBA-induced skin cancer Swiss albino mice	Antioxidation Antigenotoxic	[51]

3-O-β-glucopyranosyl- stigmasterol [C35H58O6][52] 	***In Vitro Model***		
	RAW264.7	Anti-inflammation	[53]
β-sitosterol [C29H50O][54] 	***In Vitro Model***		
	U-87MG	Apoptotic induction	[28]
	PC-3	Antioxidation	
	HT-29		
	MCF-7	Apoptotic induction Antioxidation DNA fragmentation Cell cycle arrest	[28] [55]
	HCT116	DNA fragmentation	[55]
	HeLa	Cell cycle arrest	
	Phytophthora infestans	Antifungal	[38]
		Antioxidation	[39]
	SH-SY5Y wt transfected w/ APP695 or SPC99	Neuroprotective	[40]
	SH-SY5Y transfected w/ APP695 or SPC99		
	In Vivo Model		
	Type-2 diabetic rat	Antidiabetic	[56]
3-O-β-glucopyranosyl-β- sitosterol [C35H60O6][57] 	***In Vitro Model***		
	RAW264.7	Anti-inflammation	[53]
	In Vivo Model		
	Streptozotocin- induced diabetic Wistar mice	Antioxidation	[48]

Spermine 4-(12-amino-4,9-diazadodecylamino-4- oxobutanoate [C10H26N4][58] 	***In Vitro Model*** *Staphylococcus aureus*	Antibacterial	[59]
(22S,23S)-22,23-dihydroxystigmast-4-en-3-one [C13N4O3H28][60] 	***In Vitro Model*** *HSV-1*	Antiviral	[61]
(22S,23S)-3$\beta\beta$-bromo-5$\alpha\alpha$,22,23-trihydroxystigmastan-6-one [C29H49BrO4] 	***In Vitro Model*** *HSV-1*	Antiviral	[61]

Brassicasterol [C28H46O][62] 	***In Vitro Model*** SH-SY5Y wt transfected w/ APP695 or SPC99 SH-SY5Y transfected w/ APP695 or SPC99	Antioxidation Neuroprotective	[39] [40]
Campesterol [C28H48O][63] 	***In Vitro Model*** SH-SY5Y wt transfected w/ APP695 or SPC99 SH-SY5Y transfected w/ APP695 or SPC99	Neuroprotective	[40]
Gramisterol [C29H48O][64] 	***In Vivo Model*** WEHI-3	Genotoxic Apoptosis induction Cell cycle arrest Anti-proliferation Immuno- proliferation Spleno-hypotrophy Hepato-hypotrophy	[65]

RESEARCH METHODOLOGY

A systematic search was performed to identify all relevant research papers published on different biological activities by stigmasterol and its derivatives using google scholar (1977- present). PubMed (1977-present) was used as alternatives to ensure the inclusion of all relevant papers. The search strategy was performed using several keywords to track down the relevant research articles including "stigmasterol", "cancer", "structural", "mechanism", "anticancer", "antimicrobial", "antioxidant", "cardioprotective", "anti-osteoarthritis", "neuroprotective", "anti-inflammatory", and "antidiabetic".

Chemical Structure of Stigmasterol and Its Derivatives

Phytosterol compounds were generally constructed from stigmastane carbon skeleton (Table 1), which principally bears similarities towards cholestane moiety with adjoining ethyl or methyl group C-24, as shown in Figure 1 [6, 66-69]. Their thermal stabilities are based on numbers of unsaturation bonds and side chain length [33, 70].

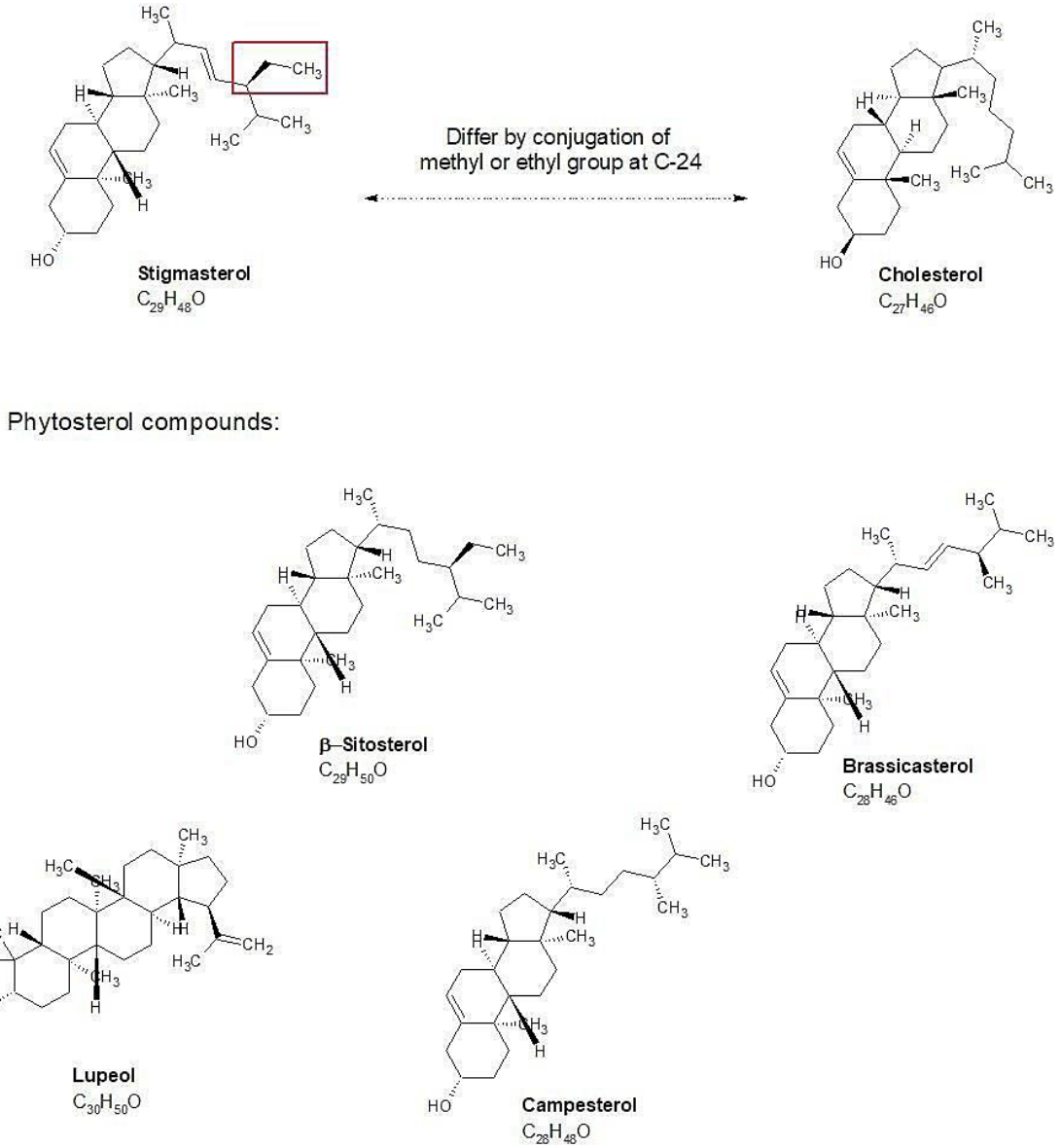


Figure 1. Chemical structure variation between stigmasterol, cholesterol, and other phytosterols. Illustration was created using ChemSketch 2.0. [Adapted from NIST Standard Reference Database 69, version 2018; RN 83-48-7 (Stigmasterol), RN 57-88-5 (Cholesterol), RN 83-46-5 (β-Sitosterol), RN 474-67-9 (Brassicasterol), RN 545-47-1 (Lupeol), RN 474-62-4 (Campesterol)] [82].

Stigmasterol is a steroid derivative characterized by hydroxyl positioned at C-3 of stigmastane, unsaturated bonds at C5-6 of the β-ring, and alkyl substituent at C22-23. For its derivatives, brassicasterol prominent structural variation differs by having methyl group side chain at C24 and C22 [70-72]. Campesterol comprised of free alcohol, steryl-ferulate and a methyl group at C24 of stigmastane [24], which possess selective anti-inflammatory and anticancer properties as well as a strong competitive binding inhibitor against cholesterol [27, 28, 33-35, 38-40, 49, 51, 53, 55, 56, 70-72]. Structural difference between cholesterol and phytosterols resulted in variation towards bioabsorption [24, 73]. Similarly, stigmasterol shown to hinder its absorption in the bloodstream due to low fat solubility and low activity [74]. Conjugation with oximido side branch exhibited greater inhibition tumourigenicity due to reductive activity of chlorine atom [41]. In addition to that, it also induces antiestrogenic property through substitution and reduction of alkaline phosphatase [75]. β-sitosterol exhibited physicochemical property of sterol mobility as proportionally exhibited number of double bond side chains which affects its alcohol solubility, hydrophobicity and oxidization [19, 70, 71, 76]. Studies had shown that the composition of β-sitosterol and campesterol could effectively decrease cholesterol concentration by lowering cholesterol synthesis [71, 77-80]. Also, (22E)-ergost- 22-ene-1a,3b-diol showed high potency in reducing cholesterolemic level through genetic modification [81]. Gramisterol (24-methylenelophenol), on the other hand, is a moiety distinguished between other phytosterols by methyl group conjugation at C4 stigmastane with presence in the form of free alcohol as trans- and cis-ferulate, which ascertained its biological properties such as anti-inflammatory, anti-viral, and hepatoprotective properties [65, 66].

Biological Activities of Stigmasterol and Its Derivatives

Anticancer Activity

Stigmasterol and gramisterol were discussed extensively on their cellular apoptotic activity against tumourigenic cells [24, 28, 33, 55]. The anticancer action could be elucidated by upregulation of pro-apoptotic genes such as Bax and p53 [24, 33]. Tumourigenesis process was effectively hindered by inhibition of production of tumour biomarkers such as tumour necrosis factor-α (TNF-α), downstream effectors (eg. Src, Akt, PCL, and FAK) in VEGFR-2 signalling pathways protein expression level as well as histological representation such as attenuation in CD-31-positive microvessel density content and promote macrophages recruitment [27]. It was also elucidated that improved blood circulation could be associated with stigmasterol's ability to attenuate tumour growth [49, 83]. Stigmasterol demonstrated downregulation of signalling protein Jab1 which activates caspase-3 in mitochondrial apoptosis signalling pathway and resulted in the elevation of p27 protein genetic expression and induced antiproliferative effect [17, 35, 72].

Antimicrobials Activity

Phytosterol generally exhibited various potential against bacteria and viruses [84]. Recent research found that combinatory use of stigmasterol with beta-lactam antibiotic ampicillin showed greater bactericidal against antimicrobial resistance (AMR) strain bacteria [85, 86]. Another study uses α-spinasterol and ceftiofur usage together exhibited lower minimum inhibitory concentration (MIC) efficacy over treatment done with individual compounds against pathogenic strains of *Salmonella pullorum CVCC533*, *Streptococcus pneumoniae CAU0070*, *Escherichia coli*, and *Staphylococcus aureus* [36]. Interestingly, phytosterols such as spermine have a selective inhibitory effect on Gram-positive bacteria [59]. African trypanosomiasis or known as 'sleeping sickness,' is transmitted by Tsetse fly infected with parasite *Trypanosoma congolense* could lead to the production of free radicals and sialidase enzyme that causes organ damage and induce anaemia. Recent research has indicated that stigmasterol possesses anti-trypanosomal activity, which can ameliorate the antiproliferative activity of parasites [37, 83, 87, 88]. Furthermore, ubiquitous and highly resistant strain of herpes simplex virus type-1 (HSV-1) mostly caused by viral-containing mucocutaneous oral contact [61, 89, 90]. Designed phytosterols (22S,23S)-22,23-dihydroxystigmast-4-en-3-one and (22S,23S)-3$\beta\beta$-bromo-5$\alpha\alpha$,22,23-trihydroxystigmastan-6- one respectively were capable inhibitor of HSV-1 replication and demonstrated significant low cytotoxicity against nervous cells [61]. Additionally, antifungal resistance and the harmful effect of synthetic fungicides on humans have led to the development of fungicides derived from phytosterols or combinatory therapy of stigmasterol and β-sitosterol to exhibit antifungal effect, albeit weakly against various plant disease [38].

Antioxidant Activity

Oxidative stress inflicted by reactive oxygen species (ROS) induces a significant extent of damages from genetic DNA to functional organs, which generally contributed towards ageing, degenerative diseases, cancer, and organs impairment [41, 91, 92]. Phytochemicals demonstrated hindrance against lipid peroxidation (LPO) by increasing the antioxidative activity of glutathione (GSH), superoxide dismutase (SOD) and catalase enzymes. The elevated endogenous antioxidant level strengthened immunity against oxidative infliction via neutralisation of ROS [51, 91-93]. Stigmasterol, on the other hand, demonstrated modest ROS scavenging activity over lipid peroxidation [39, 41, 91, 93-95]. β-sitosterol, stigmasterol, campesterol, sitostenone, 3-O-β-glucopyranosyl-β-sitosterol, cycloeucalenol and 24-methylenecycloartenol were characterised by their potential

in ROS regulatory mechanism to evaluate their effectiveness as anti-inflammatory agents [94].

Anti-hypercholesterolemic Activity

Structural variation of stigmasterol at C-22 with the presence of unsaturated bonds had contributed to the relatively lowers the cardioprotective activity of stigmasterol in comparison with β-sitosterol structure presentation [72, 96]. Oral administration of stigmasterol noted suppression on HMG-CoA reductase activity via competitive inhibition, which hinders the synthesis of plasma cholesterol and cholesterol absorption, hence greatly reducing hepatic cholesterol and bile acid synthesis [43, 44]. Furthermore, stigmasterol proved to hinder cholesterol, bile acid and dietary lipids absorption thus lead to reduced body weight, suppressed lipogenic gene expression, lowered circulating CM level and regulate ceramide and genes involved in lipid metabolism in hypercholesterolemic disorders such as non-alcoholic fatty liver disease (NAFLD) [42, 45]. Moreover, stigmasterol and β-sitosterol evidently inhibited colon shortening and reduced faecal haemoglobin [45].

Neuroprotective Activity

The generation of membrane microdomain lipid raft with the presence of cholesterol has been implicated towards amyloidogenic activity, which attributed towards neurodegenerative diseases development [40, 41, 97]. Stigmasterol, β-sitosterol, campesterol, and brassicasterol showed association with elevated activity in integral transmembrane proteases of β- and $\gamma\gamma$-secretase which are effective against plaques formation. Stigmasterol has demonstrated to replace cholesterol in lipid rafts which hinders localisation of amyloid-β (Aβ) plaques in the brain. Whereas β-sitosterol showed an increment of β-secretase complex expressing gene BACE1 but reduced the formation of $\gamma\gamma$-secretase complex, resulted to the inhibition of Aβ plaques deposition [40]. Furthermore, neuroprotective property of stigmasterol was identified to be effective against inflammatory hippocampal region via reduction of the transport of vesicular glutamate (VGULT1), synaptic vesicle pool size, expression of GluN2B synaptic protein, and ROS rate of production [46].

Stigmasterol also showed to induce autophagy via elevated level in autophagy marker LC3 BII and adaptor protein P62, also increased mitophagy rate via increment in phosphatase and tensin homolog-induced protein kinase 1 (PINK1). Competitive inhibition binding interaction between hippocampal protein molecules LXRβ with stigmasterol showed great potential in regulating ischaemic injury pathways in neuroinflammation through autophagy inhibition mTOR/AMPK and JNK pathways [46, 47].

Anti-Osteoarthritic Activity

Osteoarthritis is a chronic degenerative disorder characterised by the imbalance between anabolism and catabolism of articular cartilage tissues [29, 98]. An *in vitro* study by Chen et al. shown that stigmasterol treatment of stigmasterol effectively regulated disease-associated protein expression of matrix-degrading enzyme matrix metalloproteinase (MMP-1, MMP-3, and MMP-13), tissue inhibitors of metalloproteinase (TIMP-1), and proinflammatory inhibitors PGE2 expression level. Stigmasterol showed close interaction in the proinflammatory modulation mechanism of NF-kappa B pathway as the molecule strongly binds to chondrocyte membrane compared to other phytosterols. As a result, the phytosterol demonstrated a downregulation effect on the expression of MMP and PGE2 utilizing NF-kappa B pathway inhibition [29, 30].

Antidiabetic Effect

Phytosterols have drawn interest in developing more effective treatments against type-2 diabetes mellitus as they exhibited antidiabetic properties and were relatively safe with less side effects when compared to drugs [31, 48]. Stigmasterol treatment ameliorated body weight with reduced serum lipids other than high-density lipoprotein cholesterol (HDL-C), which was elevated. Stigmasterol exhibited antidiabetic action by targeting the expression of GLUT4 glucose transporter to increase insulin sensitivity [31]. Apart from stigmasterol, phytosterol compounds such as β-sitosterol showed increased uptake of glucose and hepato-oxidative activity, which elevates the expression level of insulin-mediated signalling molecules [56].

Anti-Inflammatory Effect

Treatment with 3-O-β-glucopyranosyl-β-sitosterol and 3-O-β-glucopyranosyl-stigmasterol exhibited inhibitory activity on nitric oxide (NO) production. The outcome may suggest property of anti-inflammation through selective inhibition of NO production [53]. Stigmasterol also exhibited anti-inflammatory through inhibition of proinflammatory mediators such as NO, tumour necrosis factor-α and interleukin-1β [99, 100]. Colonic inflammation was shown to improve with stigmasterol treatment acts by inhibiting the expression of NF-κB p65, COX-2 and CSF-1 were putative transcription factor involved in inflammatory signalling pathway [29, 45].

Drawbacks of Stigmasterol and Other Phytosterols Application

Stigmasterol and other phytosterols were well investigated over the years to elucidate their medicinal benefits. Despite that, there are still drawbacks that hindered transcribing their

therapeutic values into clinical application. Individuals with phytosterolaemia, also known as sitosterolaemia, a rare inherited lipid storage disorder genetically associated with mutations of adenosine triphosphate-binding cassette (ABC) transporter ABCG5 and ABCG8 genes [101], characterized by exponentiated uptake and reduced excretion of cholesterol and sitosterol, plausible contributing factors in the development of atherosclerosis [101-103]. Their known clinical representation of phytosterolaemia primarily involved cardiac fibrosis and inflammation, which showed no clinical improvement through statins treatments [103]. Due to that, combinatory consumption of phytosterol and statin showed lower total and low-density lipoprotein (LDL) cholesterol, but increased phytosterol serum level, which plausibly worsened the condition of coronary heart disease in patients with sitosterolaemia [104]. Moreover, observation from clinical trials showed that long-term consumption of plants sterols resulted in a small depression of vitamin D and E concentrations [105]. However, current studies elucidated consumption of phytosterol on short-term effects (e.g. up to 1 year) and consumption of phytosterols for long term was not evidently sufficient to describe it is safe for consumption [106]. Further studies should be done to support the long-term clinical endpoints of phytosterol as diet supplementation and better elucidate attenuation of heart diseases [104, 106].

There were no clinically significant impacts of consuming phytosterol enriched products such as margarine on a dietary basis, associated with hypercholesterolaemia [106]. Similarly, combinatory treatment such as oral administration of statins and phytosterols showed no clinical evidence to their effect in management of atherosclerosis [104, 106]. Most existing studies were limited to cellular and animal models, which do not show prominent clinical significance to be recommended as a complementary treatment or dietary supplementation, especially in phytosterolaemic patients [103, 106]. Due to that, current intervention using medicinal plants containing stigmasterol and other phytosterols have their limitation and may not be as effective as pharmaceutical drugs in terms of effectiveness and efficacy.

CONCLUSION

Stigmasterol and other variation of phytosterols have been generally recognised on their biological properties. Through various studies elucidated from past to present studies, elucidation on the bioactive potentiality of stigmasterol and its derivatives compounds showed possibility as candidate of pharmaceutical drugs comprising pharmacological properties such as antidiabetic, cardioprotective, antioxidant, and antimicrobial. Interestingly, possible application of mixture of phytosterols could possess relatively greater synergistic bioactivity, which could be safer than conventional treatments and yield promising therapeutic values. However, this review mainly evaluates preclinical studies from the present, thus treatment based on extracted stigmasterol influencing clinical efficacies and possible adverse effects from phytotherapy on human demand for acquiring in-depth discussion. Hence, the bioactivity of stigmasterol and its derivatives have shown to be promising, yet further investigation and refinement would be essential to formulate a clinically significant treatment.

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Author Contribution

The literature searches, data collection and manuscript draft were performed by G.C.C.K., and T.R. The manuscript was critically reviewed and edited by T.Y.Q., Y.S.W, A.C.Y.Y and Y.W.H. The project was conceptualized by T.Y.Q. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this manuscript.

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