The Story of Beta-sitosterol - A review

ABSTRACT

Aims: Phytosterols are a subgroup of the steroids, as an important class of bioorganic molecules, widespread in plants, animals, marines as well as fungi, and have similarity to cholesterol in structure. These compounds have a long history of consumption as food or pharmaceutical products, and generally recognized as safe without undesirable side effects.

Place and Duration of Study: Medicinal plants Research Center and Pharmaceutical Sciences Research Center, between March 2013 and May 2013.

Results: Among them, β-sitosterol is usually used for heart disease, hypercholesterolemia, modulating the immune system, prevention of cancer, as well as for rheumatoid arthritis, tuberculosis, cervical cancer, hair loss and benign prostatic hyperplasia. Furthermore, diverse biological activities whereby natural compounds or the extracts were considered while trypanocidal, mosquito larvicidal even neutralization of viper and cobra venom characteristics was recorded.

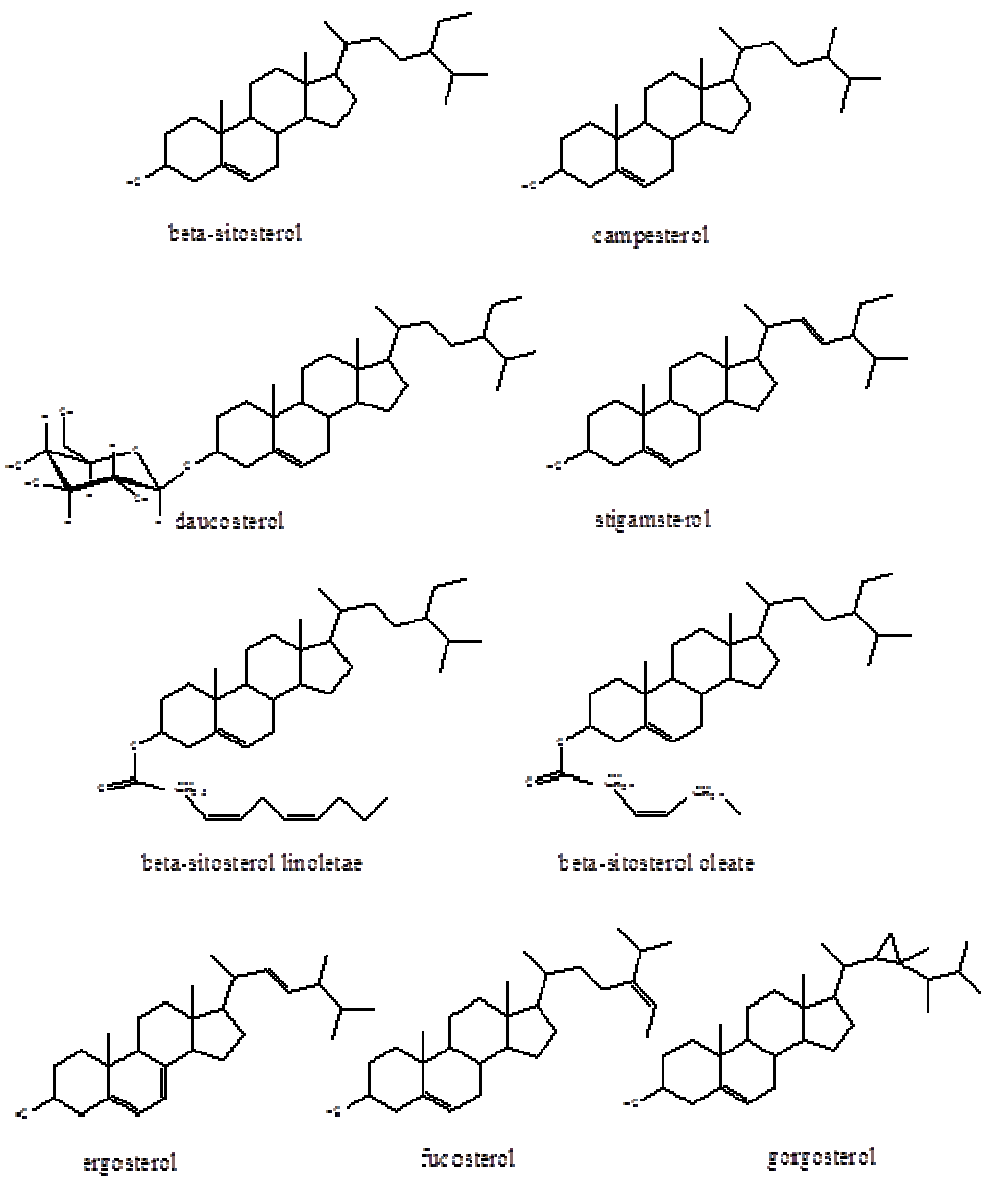
Conclusion: Some of the above indications are evidence based, but others are still in doubt and need more investigations to confirm its efficacy and safety. Regarding to the importance of these natural sterols and β-sitosterol as the most abundant of them, the main pharmacological and biological activities together with their clinical trials is reviewed here.

Keywords: β-sitosterol; Phytosterols; Pharmacological activities; Biological activities.

1. INTRODUCTION

A term “Phytochemicals’” (plant based chemicals), was introduced to the world in1994 and promptly became a trend and frontier for researchers and scientists, of which phytosterols are a subgroup of the steroids as an important class of bioorganic molecules [1, 2]. Phytosterols are widespread in plants and animals as well as fungi, and have structural similarity to cholesterol. Phytosterols play essential roles in the physiology of eukaryotic organisms. For instance, cholesterol is the main part of the cellular membrane in animals, affecting the cell membrane's fluidity and serving as secondary messenger in developmental signaling [2]. The most important benefit for these natural metabolites is their enrolment amongst the health promoting constituents of natural foods which contains them. The European Foods Safety Authority (EFSA) recommends consuming about 1.5 - 2.4 g/day of phytosterols and/or stanols in order to reduce blood cholesterol [3]. Furthermore, FDA has approved the role of foods containing phytosterol esters inside a low saturated fat and cholesterol diet in reducing the risk of heart disease, especially consumption of at least 1.3 g/day sterols, twice a day [4]. The natural foods and high phytosterol-containing dietary has been continuously marketed for decades in diverse countries. Vegetable oils and products made from them, nuts, cereal products, vegetables, fruit and berries have been classified as richest or significantly rich sources of phytosterols [5]. Three phytosterols including β-sitosterol, campesterol and stigmasterol (Figure 1) are predominant sterols in the human herbal nutrition forming 65%, 30% and 3% of diet contents, respectively [6]. Phytosterols, with a long history of consumption as food or pharmaceutical products, have generally recognized as safe (GRAS), and no undesirable side effects have been reported. An exception is an illness named “phytosterolaemia”, a genetically disease, related to some mutations in the ABCG5/G8 proteins which play the role of protein pump to enter the sterols into enterocytes and hepatocytes [7, 8].

Fig.1: Chemical structures of some phytosterols.



2. SYNTHESIS OF β-SITOSTEROL

Although β-sitosterol has not been completely synthesized so far, it has been produced from pure stigmasterol via two ways. In the first rout, the side chain Δ22–23 alkene is selectively hydrogenated to produce β-sitosterol together with diverse levels of stigmasterol and fully saturated stigmastanol, while this selective hydrogenation accompanied by protection of Δ5–6 alkene to cyclopropyl carbinyl ether is purposed in the second approach. This process should follow by hydrogenation of the Δ22–23 double bond and also solvolysation of the cyclopropane in order to produce the C3-alcohol and Δ5–6 alkene again. The latter method seems very useful due to achievement of β-sitosterol in high purity. As a fact, semi-synthesis of β-sitosterol is still a challenge because of producing the methyl ether by products, whose removal is difficult [9, 10].

3. BIOSYNTHESIS OF β-SITOSTEROL

Biosynthesis of the phytosterols is regulating during membrane biogenesis. The literature showed that β-sitosterol is biologically synthesized from both mevalonate and deoxyxylulose pathways. Using 13C-labeling approach, the mechanism of β-sitosterol biosynthesis has been studied and although varies found according to the organism used, cycloarteol has been identified as an initial substrate. Actually, one molecule of isopentenyl diphosphate (IPP) joins to two molecules of dimethylallyl diphosphate (DMAPP) to produce farnesyl diphosphate (FPP). Two of the later molecule (FPP) are then combined tail-to-tail to result in formation of squalene, as a triterpene and finally cycloartenol [11].

4. PHARMACOLOGICAL ACTIVITIES

4.1. Anti-inflammatory Activity

Prieto et al., (2006) reported the *in vivo* effect of β-sitosterol in a model of delayed-type hypersensitivity (DTH). They revealed that this compound can modulate a cell-mediated edema but it was not effective on the arachidonate pathway of intact cells and did not inhibit the leukocyte infiltration measured as myeloperoxidase activity in biopsies. They emphasized that its response to oxazolone might be due to a different pathway independent of interleukin-4. Moreover, β-sitosterol was not able to inhibit the cyclooxigenase (COX) pathway responsible for prostaglandin E2 (PGE2) synthesis [12].

In another study, Loizou et al., (2010) determined the activity of β-sitosterol (dose ranged: 0.1-200 μM) on the expression of vascular adhesion and intracellular adhesion molecule 1 employing ELISA, alongside the monocyte attachment (U937 cells) in tumor necrosis factor-alpha (TNF-alpha)-stimulated human aortic endothelial cells (HAECs) using adhesion assay. They concluded that β-sitosterol was able to inhibit both vascular adhesion and intracellular adhesion molecule 1 expression in TNF-alpha-stimulated HAEC. Moreover, this compound acts as an inhibitor on phosphorylation of NFκB [13]. In fact, β-sitosterol reduces the NFκB transcription factor activity in macrophage cells.

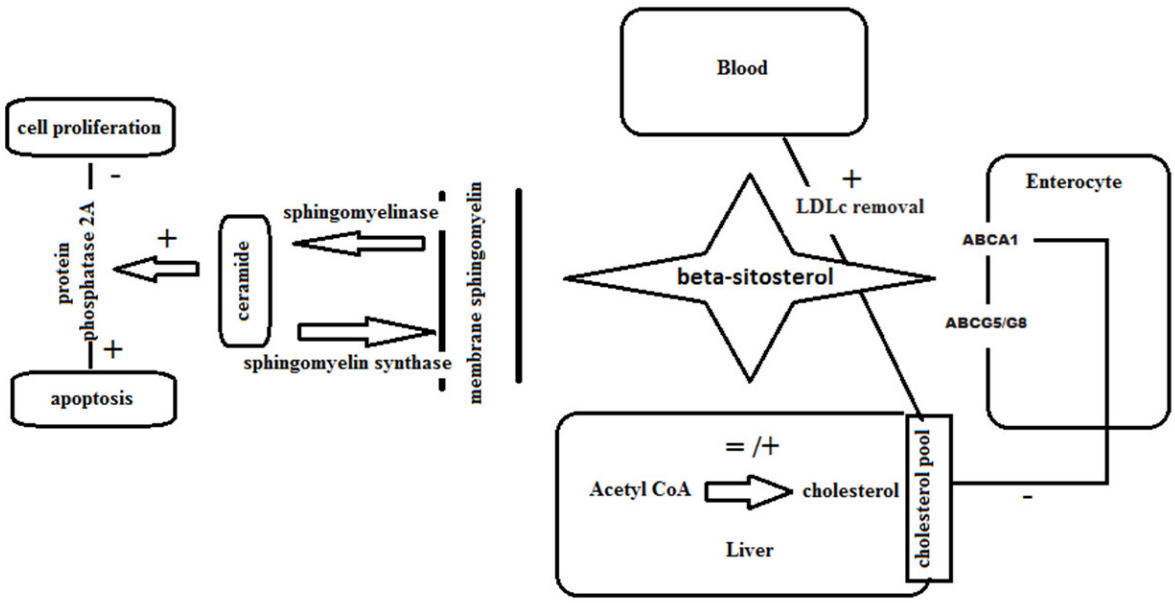
4.2. Inducing Apoptosis

Chai et al., (2008) reported that β-sitosterol could inhibit the proliferation of MCF-7 cells, in a dose-dependent manner. The above mentioned cell line was employed due to the presence of estrogenic receptors involved in breast cancer. The authors revealed a higher caspase activity (detected by increasing of DEVDase activity) after adding β-sitosterol to the cell line, resulted in caspase-induced apoptosis [14].Besides, the compound also showed antiproliferative and apoptosis activities in human leukemic U937 cells by activating of caspase-3 and Bax/Bcl-2 ratio [15]. However, the results of a study showed that β-sitosterol showed a stimulatory effect on MCF-7 cells *in vitro* while daucosterol did not affect the mentioned cells [16].Treatment of β-sitosterol on MDA-MB-231 human breast cancer cells increased apoptosis in cell culture and inhibited tumor growth indicating its beneficial effect in prevention of breast cancer [17]. Cytotoxicity of β-sitosterol and its glycoside, daucosterol, were examined against cancers cell lines by MTT assay. The results indicated that β-sitosterol inhibits the HT-29 cell line (colon carcinoma) while, daucosterol was more active against K-562 cell line (leukemia) [18].

4.3. Chemoprotective or Chemopreventive Effects

In a review published by Ovesna et al., (2004), they recorded the experimental inhibition of colon and breast cancer development by taraxasterol and β-sitosterol. They stated that these compounds can affect different levels of tumor development, such as their inhibitory effects on creation, promotion and induction of cancerous cells, as well as inhibition of tumor cells invasion and metastasis [19]. Dietary supplement of β-sitosterol decreased circulating 17β-estradiol (E2) levels as well as E2-induced MCF-7 tumor growth in ovariectomized athymic nude mice, which suggested that high dietary supplement of phytosterol may have beneficial effect in women with breast cancer [16]. A schematic diagram for simplifying its mode of action in anticancer activity is shown in Figure 2.

Fig.2: Schematic diagram of anticancer activity of β-sitosterol.



4.4. Hypocholesterolemic Activity

Sugano et al., (1977), compared the hypocholesterolemic activity of β-sitosterol and its hydrogenated product, β-sitostanol (Figure1) in young male rats. They demonstrated that although hypocholesterolemic activity of sitostanol was significantly greater than sitosterol, but their effects on liver concentration of cholesterol and triglyceride were similar. Furthermore, sitostanol exhibited a high plasma triglyceride. Just apposite of sitostanol, sitosterol was decomposed by mean fecal recovery around 85% - 92%. The authors concluded that hydorgenation of plant sterols is a new achievement, because it would improve their hypocholesterolemic activity without effect on their safety as regards to initial sterols [20]. Dietary supplement containing pytosterols in 28 patients with primary hyperlipoproteinaemia caused decrease in cholesterol concentration in plasma and in HDL followed bythe apolipoprotein B (apo-B) concentration in LDL [21].

4.5. Angiogenic Effect

Angiogenesis is a noteworthy mechanism for wound healing activity of *Aloe vera* gel. It is demonstrated that its extracts exhibited an angiogenic activity on the chorioallantoic membrane (CAM) of chick embryo. β-sitosterol, recognized as the main compound of this gel, exhibited strong angiogenic effects in the CAM assay. This approach was obtained using neovascular stimulation in the mouse Matrigel plug examination and detection of human endothelial cells motility in an *in vitro* wound migration bioassay [22].

4.6. Genotoxicity Effect

Genotoxic assays are used to determine how much hurts is sustained on DNA by xenobiotics, which consequently may ikluence on human exposed to them. Paniagua-Perez et al., (2005) reported the genotoxicity of β-sitosterol including the acute toxicity assay, which showed low lethal potential (38%) of this compound. The results indicated that no SCE (sister chromatid exchanges) increase was induced by tested doses (200, 400, 600, and 1000 mg/kg), as well as no changes in the cellular proliferation kinetics, or in the mitotic index. In that report, the highest applied dose showed 80% of the LD50. For this reason, β-sitosterol is not considered as genotoxic and/or cytotoxic. The safety of this compound encourages the scientists to perform more pharmacological investigations on this sterol [23].

4.7. Analgesic Bioassay

Villasenor et al., (2002) reported that the number of writhes for some fractions of *Mentha cordifolia* was decreased. This observation was recorded in levels comparable to the positive standard, mefenamic acid. They found that both β-sitosterol and its glucoside decreased the number of squirms (70% and 73% for each compound, respectively), which were induced using acetic acid [24].

4.8. Anthelminthic and Anti-mutagenic Activities

Villasenor et al., (2002) have also reported β-sitosterol as an anthelminthic constituent of *M. cordifolia*. They employed *in vitro* tests by *Ascaris suum*, which resulted in the similar behavior of worms treated with β-sitosterol alongside the positive controls, combantrin and antiox. They claimed that β-sitosterol (by 0.5 mg /kg mouse administration), indicated anti-mutagenic activity and act as an inhibitor of tetracycline mutagenesis by 65.3%. Furthermore, administration of this compound alone, did not change the number of MN-PCE (micronucleated polychromatic erythrocytes) regarding to the control but differed from tetracycline [24].

4.9. Immunomodulatory Activity

Extremely little doses of β-sitosterol and daucosterol (its 3-O-D-glucoside) have been reported to elevate the *in vitro* proliferative activity of T-lymphocytes, when they were stimulated by phytohaemagglutinin (PHA) in the lower concentrations than optimum. Essential sterolin formulation (ESF) caused a significant augmentation in the expression of CD25 and HLA-Dr antigens on T- lymphocytes and also a growth in the secretion of IL-2 and gamma interferon. Either β-sitosterol or daucosterol increased the activity of NK-cells, while ESF showed a higher activity [25].

4.10. Effect on Benign Prostatic Hyperplasia (BPH)

In a systematic review by Wilt et al., (1999), four double-blind clinical trials were reported with lasting around 4-26 weeks. β-sitosterol alone was administered in three trials and a formulation of daucosterol (its glucoside) was consumed in

another study. They concluded that β-sitosterol could improve the urinary symptom and flow in comparison of placebo, and did not decrease prostate size. In only trial with pure daucosterol no improvement in urinary flow was observed. Moreover, men who consumed β-sitosterol alone did not show different withdrawal rates from placebo. However, the duration of those studies was short and for this reason, probably effect of β-sitosterol in elongated period, its safety and capacity to prevent the complications of BPH are still in doubt [26].

4.11. Prostatic Cancer Treatment

In a study by Jourdain et al., (2006) the effect of several cocoa extracts, containing each of polyphenols or β-sitosterol, on two human prostate cancer cell lines (nonmetastatic and metastatic) as well as one normal cell line has been determined. The results revealed that cocoa extracts with polyphenol alone exhibited a potent and rapid reduction on cell growth compared to those contained β-sitosterol alone. They reported neither synergism nor additional activity by adding β-sitosterol to the cocoa polyphenols extract [27].

Another study undertaken by von Holtz et al., (1998) investigated the activity of two nutritional sterols (β-sitosterol from herbal sources and cholesterol from animals) on prostate cancer cells regarding to the evaluation of cell growth, differentiation, apoptosis, and sphingomyelin cycle intermediates. A decrease in cell growth (24%) and induction of apoptosis (fourfold) followed by cell rounding, also an enhancement in ceramide production (Figure 2) was considered by β-sitosterol (16 μM). Cell differentiation (evaluated by prostate-specific antigen and prostatic acid phosphatase) showed no alteration, nevertheless total acid phosphatase was elevated by treating for one week. The researchers suggested that those observations have been created by activating the sphingomyelin cycle [28].

4.12. Anti-oxidant Effects

The results of a study showed that β-sitosterol in 1,2-dimethylhydrazine-induced colon carcinogenesis in rats caused elevation in enzymatic and non-enzymatic antioxidant, which recommended the compound as an effective chemopreventive drug for colon carcinogenesis [29]. β-sitosterol stimulates antioxidant enzymes by activation of estrogen receptor/PI3-kinase-dependentpathway.The GSH and GSH/total glutathione ratio recovered after treatment by β-sitosterol suggesting that this phytosterol could be a ROS scavenger [30].

4.13 Neuroprotection

Glucose oxidase-induced oxidative stress and lipid peroxidation could be prevented by incorporation of β-sitosterol into cell membrane that revealed valuable effect of the compound in the neurodegenerative disorders like Alzheimer disease [31].

4.14 Anti-diabetic Effects

Administration of β-sitosterol reduced levels of glucose, nitric oxide(NO) and HbA1c in stereptozocin induced diabetic rats followed by increase in insulin level. It also showed protective effect on pancreatic tissue with enhancement of pancreatic antioxidant [32]. However, β-sitosterol and stigmasterol revealed no hypoglycemic effect on alloxan induced diabetic rats, while the mixture of them produced hyperglycemia in experimental diabetic animals [33]. β-sitosterol administration could promote sensitivity to insulin may be trough increasing NO levels in high fat diet rats [34].

5. DISTRIBUTION OF β-SITOSTEROL IN PLANTS AND ALGAE

β-sitosterol is an ancient molecules in plants kingdom. Simple sterols have evolved into more complex forms from single cellular organisms to vascular plants. As shown in the literature, fungi, algae and protozoa, synthesize 24β- methyl sterols or ergosterols, while plants synthesize 24 α- ethyl sterols like sitosterols [35]. Literature review revealed that β-sitosterol has been isolated and purified by different chromatographic methods from diverse plant families. Some important plant and marine sources of this compound have been summarized in Table 1. Distribution of this compound and its derived components consists of a wide range of plant families, and the plants discussed here are just some well-known sources.

Table 1. Some important plant and marine sources of β-sitosterol and/or its glucoside and/or esters.

Plant family	Sources	Reference
Lamiaceae (Labiatae)	*Hymenocrather calycinus*[*]	[36]
	Salvia hypoleuca[*]	[37]
	Salvia macrosiphon[*]	[38]
	Salvia limbata[*, **]	[39]
	Satureja khuzistanica[**]	[40]
	Satureja sahendica[*]	[41]
	Satureja spicigera[*]	[42]
	Lagochilus cabulicus[*, ***]	[43]
	Dracocephalum kotschyi[*]	[44]
Asteraceae (Compositae)	*Achillea talagonica*[*]	[45]
	Achillea tenuifolia[*]	[46]
Apiaceae (Umbelliferae)	*Lomatopodium staurophyllum*[*]	[47]
	Ferulago subvelutina[***]	[48]
Rosaceae	*Geum iranicum*[*, ***]	[49]
Rubiaceae	*Knoxia valerianoids*[*]	[50]
Fabaceae (Leguminisae)	*Tephrosia uniflora*[*]	[51]
	Tephrosia purpurea[*]	[52]
	Tephrosia candida[*]	[53]
Gracilariaceae (marine algae)	*Gracilariopsis persica*[*]	[54]
	Gracilaria salicornia[*]	[55]
Zingiberaceae	*Alpinia galangal*[*]	[56]
Tiliaceae	*Tilia americana*[*]	[57]
Cucurbitaceae	*Momordica charantia*[*]	[58]
	Coccinia indica[*]	[59]
Solanaceae	*Solanum xanthocarpum*[*]	[60]
	Lycium chinensis[**]	[61]
Thymelaeaceae	*Thymelea hirsute*[**]	[62, 63]
	Aquilaria sinensis[*, **]	
Acanthaceae	*Hygrophila spinosa*[*]	[64]
Moraceae	*Ficus chlamydocarpa*[*]	[65]
	Ficus cordata[**]	
Rhamnaceae	*Zizyphus spina-christi*[*]	[66]
Polygonaceae	*Coccoloba acrostichoides*[*]	[67]
	Coccoloba excoriate[*]	
Vitaceae	*Vitis vinifera*[*]	[68]

** Source of β-sitosterol, ** source of β-sitosterol glucoside, *** source of β-sitosterol estersl.*

Glycine max (soybean, Fabaceae) is a valuable medicinal food plant and well-known for high contents of its phytosterols. A non-polar extract of *G. max* contains at least 13 main sterol components. The literature showed that C4-desmethyl

Delta (5)-sterols (e.g. β-sitosterol) were the predominant sterols found in shoots of *G. max*, while cycloartenol and 24(28)-methylene cycloartanol were mainly compacted in seeds [69]. As it is revealed in the literature, the entrance of foods containing soy products in human nutrition decreases the risk of mortality and recurrence of breast and colorectal cancer especially during menopause in women [70, 71].

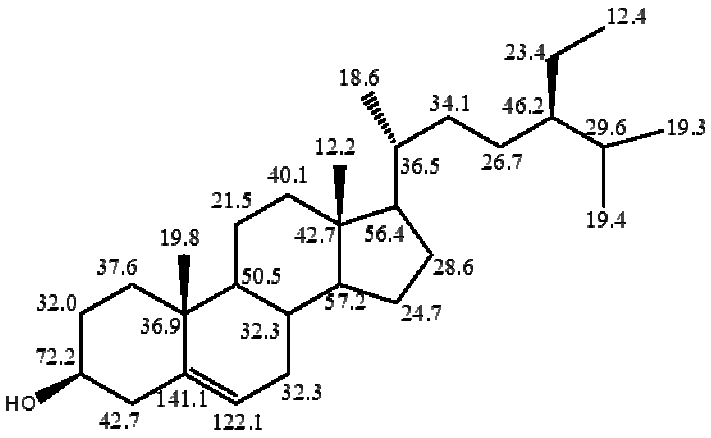
6. ISOLATION AND IDENTIFICATION OF β-SITOSTEROL FROM HERBAL EXTRACTS

β-sitosterol is the dominant phytosterol, which may undergo oxidative process just like cholesterol, resulting in β-sitosterol oxides. This makes isolation of pure β-sitosterol a challenge due to presence of sitosterol oxides [72]. The common isolation procedure is preparing a chloroform extract from a plant, then performing various chromatographic separations on silica gel column and monitoring the fractions on TLC. Sometimes, the fraction containing β-sitosterol is dissolved in a mixture of chloroform: ethanol (2:3) followed by heating on a water bath. Needle crystals might be appeared by leaving the solution undisturbed in a refrigerator [69].

HPLC with reverse phase stationary phase (RP-18) is one of the most applied chromatographic techniques for this purpose. Capillary gas chromatography-mass spectrometry (GC/MS) technique is also employed to determine either sitosterol oxides in vegetable oils or sterol esters [73, 74]. Moreover, plant sterols could be analyzed using high performance liquid chromatography-atmospheric pressure chemical ionization mass spectroscopy (HPLC-APCI-MS) [75]. Nevertheless β-sitosterol-D-glycoside is more polar than β-sitosterol itself, it has been reported to separate from petroleum ether fraction of *Ocimum sanctum*, as a yellow amorphous solid, soluble in petroleum ether, ethyl acetate, chloroform and dichloromethane [76]. When an herbal extract contains both β-sitosterol and stigmasterol, isolation of these similar analogs is not simple. However, β-sitosterol and stigmasterol are frequently isolated and purified from petroleum ether fraction of crude methanol extract via chromatography methods [77].

When the hexane or petroleum ether extract of a plant containing sterols subject to thin layer chromatography, using normal phase silica gel as stationary phase and petroleum ether: chloroform, hexane: ethyl acetate or chloroform: methanol as mobile phase, the chromatograms would show identical zones for steroidal nucleus with Liebermann - Buchard, vanillin – sulfuric acid or anisaldehyde- sulfuric acid visualizing reagents. Structural elucidation of β-sitosterol is commonly carried out by various spectral data from 1H- and 13C-NMR, IR and Mass spectroscopy, like other plant sterols. Concise data for 13C-NMR of β-sitosterol are indicated in Figure 3. This compound usually forms a white crystal with a melting point around 138 ℃ and also has no absorption under UV-Vis Lamp (254 and 366 nm), whereas its λ max in ethanol is at 206 nm and its main IR bands may appear at 3549 (OH), 2935 (CH2), 2867 (CH), 1637 (C=C) and finally 1063 (C-O), (all absorptions are in cm-1). High-resolution Mass spectra of β-sitosterol confirm its molecular mass at m/z: 414.7, which would be related to the molecular formula C29H50O. Characteristic fragments observed in EI-Mass are at m/z: 414, 396, 381, 329, 289, 273, 255, 213, 199 and 173. NMR spectrum of this compound shows the presence of six methyl groups, eleven methylene and three quaternary carbons together with a hydroxyl group. The olephinic carbons are appeared at 140.7 (C-5) and 121.7 (C-6) ppm. The number of carbons, extracted from ^{13}C-NMR, may reveal the structure of a sterol with 27 carbons (Figure 3). Comparison of the experimental data with those reported in the literature supports the proposed structure of this compound (Figure 3) [78-83].

Fig.3: Chemical shifts of ^{13}C-NMR in the structure of β-sitosterol.



7. OTHER BIOLOGICAL ACTIVITIES OF β-SITOSTEROL

Abdul Rahuman et al., (2008) reported the moderate larvicidal activity of five herbal medicines including: *Jatropha gossypifolia*, *Abutilon indicum*, *Aegle marmelos*, *Euphorbia thymifolia*, and *Solanum torvum* on the larvae of *Culex quinquefasciatus*. Interestingly, the main isolated compound from the petroleum ether extract of *A. indicum* (as the most active plant) was identified as β-sitosterol, which introduces this natural compound as a novel mosquito larvicidal sterol [84].

In another study by Gomesdaubet al., (2007), β-sitosterol is reported as a neutralizing agent on viper and cobra venom. First, they found this activity in methanol extract of the roots of *Pluchea indica* (Asteraceae), growing wildly in India, then they isolated a mixture of β-sitosterol and stigmasterol (low percentage) using bioactivity guided fractionation. The authors followed anti-snake venom activity by study on experimental animals, which revealed the possible mechanism of action via antagonizing venom-induced changes in lipid peroxidation and superoxide dismutase activity [85].

Moreover the above mentioned effects, is found as an antibacterial and antifungal agent separated from methanol extract of *Senecio lyratus* belonging to Asteraceae family [86]. Antimicrobial activity of pure β-sitosterol has been also reported using agar disk diffusion method. The authors claimed antibacterial activity ranged 10-14 mm for *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumonia*, approximately equal to the standard Gentamicin [58].On the other hand, β-sitosterol isolated from *C. acrostichoides* was not active against *M. luteus*, *S. aureus*, *A. niger* and *F. oxysporum* by agar diffusion method [67].Antimicrobial activity of β–sitosterol is still in doubt, because the results of the various studies are controversial and there are some reports which did not demonstrate such activity from this compound alongside the reported active components [36, 87]. For instance, antimicrobial activity of β–sitosterol and β-sitosterol-3-O-D-glucoside has been evaluated on *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and two fungi, *A. niger* and *C. albicans*, leading to no effects with MICs above 200 µg/ml [88].

Furthermore, Nweze et al., (2011) reported an *in vitro* trypanocidal activity from seeds of *Buchholzia coriacea* (Capparaceae family), which has been applied for fever in African folklore medicine. The authors claimed that β-sitosterol was the active components against bloodstream forms of *Trypanosoma brucei* S427 [89]. Although trypanocidal activity of β-sitosterol was observed against *T. brucei* (the causative agent of African Trypanosomiasis or sleeping sickness), we did not find it effective against epimastigotes of *T. cruzi*, the etiological agent of American Trypanosomiasis or Chagas disease [44].

Beside the mentioned activities, the effect of β-sitosterol on diabetic rats and also its antioxidant activity were examined by Gupta et al., (2011). They revealed that administration of this compound in diabetic rats lead to less glycated hemoglobin, serum glucose, and nitric oxide. Additionally, β-sitosterol enhanced the pancreatic antioxidant levels, and reduced thiobarbituric acid-reactive substances [90].

8. PHYTOSTEROL CONTAINING HERBAL MEDICINES AND DRUGS

β-sitosterol itself has a poor absorption from gastrointestinal track and it is essential to improve its pharmacokinetic behavior by enhancing the bioavailability in combination with phosphatidyl choline. This approach is employed to make a formulation as phyto-vesicles in treatment of alopecia [91]. However, several formulations of this compound or other phytosterols exist, which contain either plant extracts or pure sitosterol. In addition, the efficacy and safety of these preparations or formulations have been examined via various clinical trials. Here, a concise history of the mentioned trials has been gathered in Table 2.

Table 2. Clinical trials considering the diverse effects of β-sitosterol containing herbal drugs on various illnesses.

Type of study	Drugs & Doses	Observations	Ref.
randomized, double-blind, placebo-controlled trial for benign prostatic hyperplasia	Azuprostat, 130 mg (β-sitosterol) daily	Sig. improvements in tested groups who received drug., increase in Qmax (4.5 mL/s) and decrease in PVR (33.5 mL) in test groups.	[92]
double-blind, multicenter, placebo-controlled randomized trial for benign prostatic hyperplasia	saw palmetto extract (*Serenoa repens* berries); up to 3 times the standard dose (320 mg/day)	Dose enhancement of drug did not decrease lower urinary tract symptoms more than placebo.	[93]
open-label, multi-center study for benign prostatic hyperplasia	50 mg of tadenan (*Pygeum africanum* extract) twice a day	Drug induces sig. improvement in IPSS (40%) and uroflowmetry parameters. Satisfactory safety profile and substantial improvement in QoL (31%)	[94]
randomized, placebo-controlled, double-blind clinical trial for benign prostatic hyperplasia	20 mg β-sitosterol (which contains a mixture of phytosterols) three times/day	Sig. improvement in symptoms and urinary flow parameters, decrease in IPSS, increase in peak flow, and decrease of mean residual urinary volume	[95]
a clinical study divided into three periods of forty days: stabilization, treatment and wash out periods for hypercholesterolemia	formulation of soy proteins supplemented with isolated β-sitosterol in a ratio of 4:1, 10 g one time a day	Reduction in LDL-C, TG and apoB levels, low doses of soy protein & β-sitosterol was a safe alternative for patients suffered from modest reductions in LDL-C (< 15%)	[96]
a pilot placebo-controlled study on runners of an ultra-marathon in Cape Town for immunological activities	formulations of plant sterols and sterolins	Reduce neutrophilia, lymphopenia and leukocytosis in treated group, sig. augmentation in lymphocyte, reduction of the plasma level of IL6, sig. decrease in the cortisol and inflammatory response	[97]
a randomized, placebo-controlled clinical trial for rheumatoid arthritis	preparation of β-sitosterol and its glucoside	Less secretion of IL6 and TNF-alpha from activated monocytes, involved in the pathogenesis of RA.	[98]
randomized, double-blind, placebo-controlled trial for improving hair loss	extract of saw palmetto (400 mg) plus β-sitosterol (100 mg) daily (duration 5 month)	Improving the hair growth in 60% of the men in comparison to the beginning.	[99]

As previously stated here, β-sitosterol as well as other phytosterols act through multiple modes of action, including inhibition of cancer-cell growth, angiogenesis, invasion and metastasis, and also by promoting apoptosis in cancerous cells. Literature showed that SinnolZym is a potent anti-cancer drug consisting of an adaptogenic mixture of the fermented herbal compounds (two strong phytosterols, Cerulin and Zorvan) which are separated from well-known medicinal plants. Capsaicin (the main bitter component of *Capsicum* spp.) is reported to decrease the anti-cancer activity of SinnolZym. The activity of Capsaicin is mediated by vanilloid receptors and promoting the release of a protein called substance P, which causes pain and inflammation [100].

9. OTHER ANALOGUES OF β-SITOSTEROL

So far, more than 250 various phytosterols and related derivatives have been found in diverse plants and marines, divided into three sub-groups as: 4-desmethyl sterols, 4α-monomethyl sterols, and 4,4-dimethyl-sterols, of which two later classes are less identified than 4-desmethyl sterols [101]. β-sitosterol, campesterol, and stigmasterol are the most found phytosterols belong to the group of 4-desmethyl sterols. These compounds may find in the form of aceate, glucoside, oleate and linoleate esters, and also methyl and ethyl ethers. Even though β-sitosterol is well-known bioactive plant sterol, pharmacological and biological activities are reported from other related analogues in animals and human. Table 3; show a summary of diverse activities from these compounds.

Table 3. Biological and pharmacological activities of other analogues of β-sitosterol.

Type of phytosterol	Activities	References
Daucosterol (β-sitosterol-3-O-D-glucoside)	Protection of mice against candidiasis by the CD4+ Th1 immune response.	[102]
Stigmasterol	Inhibition of various pro-inflammatory degradation mediators involving in osteoarthritic-induced cartilage degradation, possible mechanism: inhibition of the NF-κB pathway.	[103]
	Significant antihypercholesterolemic activity without adverse effect on heart and liver. Inhibition of cholesterol absorption (54%). High doses (up to 52 mg/day) enhanced cholesterol, coprostanol and bile acid output.	[104]
	Inhibition of hepatic synthesis and intestinal absorption of cholesterol in the rat.	[105]
	Ameliorating effect on scopolamine-induced memory.	[106, 107, 108]
	Antiperoxidative, thyroid inhibition and hypoglycemic properties	[109]
Campesterol	Anti-angiogenic activity by inhibition of endothelial cell proliferation and capillary differentiation	[110]
	Decrease the biliary secretion in compared with cholesterol	[111]
Fucosterol	Antioxidant and hepatoprotective activities in rats were observed.	[112]
	Inhibition of histamine (97%) and acetylcholine (94%) induced contractions. Anti-diabetic activity: administration of fucosterol (30 mg/Kg in streptozotocin-induced diabetic rats) led to less serum glucose concentrations	[113, 114]
Gorgosterol and its oxygenated analogues	Weak antifungal activity	[115]

Ergosterol	Increase sensitivity of cells to amphotericin B	[116]
Ergosterol peroxide	Antibacterial activity on *M. tuberculosis,* only with the Bactec 460 system	[117]
	potent inhibitiotion on lipid peroxidation higher than α-tocopherol and thiourea	[118]

10. CONCLUSION

Phytosterols, found abundantly in non-polar fractions of plants and marines, are consumed (200-400 mg daily) in human diets. Some of these compounds are structurally resembled cholesterol (such as β-sitosterol, stigmasterol and their analogues) and be able to inhibit the absorption of cholesterol, cancer-cell growth, angiogenesis, invasion and metastasis. Moreover, diverse biological activities are observed using these natural compounds or the extracts, in which implicated, e.g. trypanocidal, mosquito larvicidal, and as neutralizing agent on viper and cobra venom. Among the above mentioned sterols, β-sitosterol is well-known natural sterol in composition of known herbal drugs for treatment of benign prostatic hyperplasia and prostate cancer. Besides, the compound elevated enzymatic and non-enzymatic antioxidant in cells making it effective anti-diabetic, neuroprotective and chemoprotective agent as well.High potential of this compound and its analogues in treatment of various illnesses, classifies this compound as the noteworthy drug of the future, although its role in treatment of BPH is now approved via clinical trial confirmations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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CONSENT (WHEREEVER APPLICABLE)

No patient was involved in this study.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

No human or animal subjects were involved in this study.

REFERENCES

[1] Begley S. Beyond Vitamins.Newsweek.1994;123:45-9.

[2] Akhisa T, Kokke W. Naturally occurring sterols and related compounds from plants. In: Patterson GW, Nes WD, editors. Physiology and Biochemistry of Sterols. Champaign, IL: American Oil Chemists' Society; 1991.

[3] Plant Sterols and Blood Cholesterol. The EFSA J. 2008;781:1-12.

[4 Department of Health and Human Services. Food Labeling; Health Claim; Phytosterols and Risk of Coronary Heart Disease; Proposed Rule. Federal Register. 2010;75:76526-71.

[5] Valsta LM, Lemstrom A, Ovaskainen ML, Lampi AM, Toivo J, Korhonen T, et al. Estimation of plant sterol and cholesterol intake in Finland: quality of new values and their effect on intake. Br J Nutr. 2004;92:671-8.

[6] Weihrauch JL, Gardner JM. Sterol content of foods of plant origin. J Am Diet Assoc. 1978;73:39-47.

[7] Sudhop T, Gottwald BM, von Bergmann K. Serum plant sterols as a potential risk factor for coronary heart disease. Metabolism. 2002;51:1519-21.

[8] US Food and Drug Administration. Federal Register. 1997;62:18938-60.

[9] Hang J, Dussault P. A concise synthesis of beta-sitosterol and other phytosterols. Steroids. 2010;75:879-83.

[10] Khripach VA, Zhabinskii VN, Konstantinova OV, Khripach NB, Antonchick AV, Antonchick AP, et al. Preparation of (25R)- and (25S)-26-functionalized steroids as tools for biosynthetic studies of cholic acids. Steroids. 2005;70:551-62.

[11] Dewick PM, editor. Medicinal natural products: A biosynthetic approach. United Kingdom cyclization: John Wiley & Sons Ltd.; 2009.

[12] Prieto JM, Recio MC, Giner RM. Anti-inflammatory activity of β-sitosterol in a model of oxazolone induced contact-delayed-type hypersensitivity. Bol Latinoam Caribe Plant Med Aromat. 2006;5:57-62.

[13] Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. Beta-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol Nutr Food Res. 2010;54:551-8.

[14] Chai JW, Kuppusamy UR, Kanthimathi MS. Beta-sitosterol induces apoptosis in MCF-7 cells. Malays J Biochem Molecular Bio. 2008;16:28-30.

[15] Park C, Moon DO, Rhu CH, Choi BT, Lee WH, Kim GY, Choi YH. β-sitosterol induces antiproliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. Biol. Pharm. Bull. 2007;30:1317-23.
[16] Ju YH, Clausen LM, Allred KF, Almada AL, Helferich WG. β-sitosterol, β-sitosterol glucoside, and a mixture of β-sitosterol and β-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells *in vitro* and in ovariectomized athymic mice.J Nutr. 2004;134:1145-51.
[17] Awad AB, Downie AC, Fink CS. 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: 541-6.
[18] Manayi A, Saeidnia S, Ostad SN, Hadjiakhoondi A, Shams Ardekani MR, Vazirian M,Akhtar Y, Khanavi M. Chemical constituents and cytotoxic effect of the main compounds of *Lythrum salicaria* L.Z. Naturforsch. 2013;68 c:367-75.
[19] Ovesna Z, Vachalkova A, Horvathova K. Taraxasterol and beta-sitosterol: new naturally compounds with chemoprotective/chemopreventive effects. Neoplasma. 2004;51:407-14.
[20] Sugano M, Morioka H, Ikeda I. A comparison of hypocholesterolemic activity of beta-sitosterol and beta-sitostanol in rats. J Nutr. 1977;107:2011-9.
[21] Zak A,Zeman M, VitkovaD, HrabakP, TvrzickaE. Beta-sitosterol in the treatment of hypercholesterolemia. Cas Lek Cesk.1990;129:1320-3.
[22] Moon EJ, Lee YM, Lee OH, Lee MJ, Lee SK, Chung MH, et al. A novel angiogenic factor derived from *Aloe vera* gel: beta-sitosterol, a plant sterol. Angiogenesis. 1999;3:117-23.
[23] Paniagua-Perez R, Madrigal-Bujaidar E, Reyes-Cadena S, Molina-Jasso D, Gallaga JP, Silva-Miranda A, et al. Genotoxic and cytotoxic studies of beta-sitosterol and pteropodine in mouse. J Biomed Biotechnol. 2005;2005:242-7.
[24] Villasenor IM, Angelada J, Canlas AP, Echegoyen D. Bioactivity studies on beta-sitosterol and its glucoside. Phytother Res. 2002;16:417-21.
[25] Bouic PJ, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP. 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.
[26] Wilt TJ, MacDonald R, Ishani A. Beta-sitosterol for the treatment of benign prostatic hyperplasia: a systematic review. BJU Int. 1999;83:976-83.
[27] Jourdain C, Tenca G, Deguercy A, Troplin P, Poelman D. In-vitro effects of polyphenols from cocoa and beta-sitosterol on the growth of human prostate cancer and normal cells. Eur J Cancer Prev. 2006;15:353-61.
[28] Holtz RL, Fink CS, Awad AB. Beta-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. Nutr Cancer. 1998;32:8-12.
[29] Baskar AA, Al Numair KS, Paulraj MG, Alsaif MA, Al Muamar M, Ignacimuthu S. *β*-Sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1,2-dimethylhydrazine-induced colon cancer. J Med Food. 2012;15:335-43.
[30] Vivancos M, Moreno JJ. β-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. Free Radical Biol Med. 2005;39:91-7.
[31] Shi C, Wu F, Zhu X, Xu J.Incorporation of β-sitosterol into the membrane increases resistance to oxidative stress and lipid peroxidation via estrogen receptor-mediated PI3K/GSK3β signaling. Biochim Biophysic Acta. 2013;1830:2538-44.
[32] Gupta A, Sharma AK, Dobhal MP, Sharma MC, Gupta RS.Antidiabetic and antioxidant potential of b-sitosterol in treptozotocin-induced experimental hyperglycemia.J Diabetes. 2011;3:29-37.
[33] Jamaluddin F, Mohamed S, Lajis MN. Hypoglycaemic effect of *Parma speciosa*seeds due to the synergistic action of β-sitosterol and stigmasterol.Food Chem. 1994;49:339-45.
[34] Radika MK, Viswanathan P, Anuradha CV. Nitric oxide mediates the insulin sensitizing effects of β-sitosterol in high fat diet-fed rats. Nitric Oxide. 2013;32:43-53.
[35] Neelakandan AK, Song Z, Wang J, Richards MH, Wu X, Valliyodan B, et al. Cloning, functional expression and phylogenetic analysis of plant sterol 24C-methyltransferases involved in sitosterol biosynthesis. Phytochemistry. 2009;70:1982-98.
[36] Gohari AR, Saeidnia S, Shahverdi AR, Yassa N, Malmir M, Mollazade K, et al. Phytochemistry and antimicrobial compounds of *Hymenocrater calycinus*. Eur Asia J BioSci. 2009;3:64-8.
[37] Saeidnia S, Ghamarinia M, Gohari AR, Shakeri A. Terpenes from the root of *Salvia hypoleuca* Benth. Daru. 2012;20:66.
[38] Gohari AR, Ebrahimi H, Saeidnia S, Foruzani M, Ebrahimi P, Ajani Y. Flavones and flavone glycosides from *Salvia macrosiphon* Boiss. Iran J Pharm Res. 2011;10:247-51.
[39] Saeidnia S, Gohari AR, Malmir M, Moradi-Afrapoli F, Ajani Y. Tryptophan and sterols from *Salvia limbata*. J Med. Plants. 2011;10:41-7.
[40] Moghaddam FM, Farimani MM, Salahvarzi S, Amin G. Chemical constituents of dichloromethane extract of cultivated *Satureja khuzistanica*. Evid Based Complement Alternat Med. 2007;4:95-8.
[41] Saeidnia S, Nourbakhsh MS, Gohari AR, Davood A. Isolation and identification of the main compounds of *Satureja sahendica* Bornm. Aust J Basic Appl Sci. 2011;5:1450-3.

[42] Gohari AR, Ostad SN, Moradi-Afrapoli F, Malmir M, Tavajohi S, Akbari H, et al. Evaluation of the cytotoxicity of *Satureja spicigera* and its main compounds. Scientific World Journal. 2012;2012:203861.
[43] Saeidnia S, Barari E, Shakeri A, Gohari AR. Isolation and identification of main compounds of *Lagochilus cabulicus*. Asian J Chem. 2013;25:1509-11.
[44] Saeidnia S, Gohari AR, Uchiyama N, Ito M, Honda G, Kiuchi F. Two new monoterpene glycosides and trypanocidal terpenoids from *Dracocephalum kotschyi*. Chem Pharm Bull (Tokyo). 2004;52:1249-50.
[45] Saeidnia S, Moradi Afrapoli F, Gohari AR, Malmir M. Cytotoxic flavonoids from *Achillea talagonica* Boiss. J Med Plants. 2009;8:52-6.
[46] Moradkhanian S, Ayatollahi AM, Ghanadian M, Moin MR, Razavizadeh M, Shahlaei M. Phytochemical analysis and metal-chelation activity of *Achillea tenuifolia* Lam. Iran J Pharm Res. 2012;11:177-83.
[47] Gohari AR, Saeidnia S, Malmir M, Yazdanpanah M, Ajani Y. Sterols and flavonoids of *Lomatopodium staurophyllum*. J Med Plants. 2011;10:43-8.
[48] Naseri M, Monsef-Esfehani HR, Saeidnia S, Dastan D, Gohari AR. Antioxidative coumarins from the roots of *Ferulago subvelutina*. Asian J Chem. 2013;25:1875-8.
[49] Shahani S, Monsef-Esfahani HR, Saeidnia S, Saniee P, Siavoshi F, Foroumadi A, et al. Anti-Helicobacter pylori activity of the methanolic extract of *Geum iranicum* and its main compounds. Z Naturforsch. C 2012;67:172-80.
[50] Ye JC, Chang WC, Hsieh DJY, Hsiao MW. Extraction and analysis of β-sitosterol in herbal medicines. J Med Plants Res. 2010;4:522-7.
[51] Abreua PM, Luisa MH. Constituents of *Tephrosia Uniflora*. Nat Prod Lett 1996;9:81-6.
[52] Rao EV, Ranga Raju N. Steroid constituents of *T. purpurea* root. Indian J Nat Prod. 1995;11:16-7.
[53] Andrei CC, Vieira PC, Fernandes JB, Da silva M, Fatima Das GF, Rodrigues Fo E. Dimethylchromene rotenoids from *T. candida*. Phytochemistry. 1997;46:1081-5.
[54] Saeidnia S, Permeh P, Gohari AR, Mashinchian-Moradi A. *Gracilariopsis persica*, from Persian Gulf, contains bioactive sterols. Iran J Pharm Res. 2012;11:845-9.
[55] Saeidnia S, Permeh P, Gohari AR, Mashinchian-Moradi A. *Gracilariopsis persica*, from Persian Gulf, Contains Bioactive Sterols. Iran J Pharm Res. 2012;11:845-9.
[56] Jaju SB, Indurwade NH, Sakarkar DM, Fuloria NK, Ali MD, Basu SP. Isolation of beta-sitosterol diglucosyl caprate from *Alpinia galanga*. Pharmacogn Res. 2010;2:264-6.
[57] Aguirre-Hernandez E, Rosas-Acevedo H, Soto-Hernandez M, Martinez AL, Moreno J, Gonzalez-Trujano ME. 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 Med. 2007;73:1148-55.
[58] Sen A, Dhavan P, Khukla KK, Singh S, Tejovathi G. Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from *Momordica charantia*. Sci Secure J Biotechnol. 2012;1:9-13.
[59] Gawade SP, Chandrashekar Rao MV. Antihepatotoxic activities of Ci Compound: β- sitosterol isolated from fruits and leaves of *Coccinia indica*. Indian J Pharmaceut Educ Res. 2012;46:7-11.
[60] Heble MR, Narayanaswami S, Chadha MS. Diosgenin and beta-Sitosterol: Isolation from *Solanum Xanthocarpum* Tissue Cultures. Science. 1968;161:1145.
[61] Imai S, Murata T, Fujioka S, Goto M. Isolation of beta-sitosterol-beta-Dglucoside from the leaves of *Lycium Chinense* Mill. Yakugaku Zasshi. 1963;83:1092.
[62] Rizk AM, Rimpler H. Isolation of daphnoretin and beta-sitosterol-beta-d-glucoside from *Thymelea hirsuta*. Phytochemistry. 1972;11:473-5.
[63] Gohari AR, Saeidnia S, Hadjiakhoondi A, Honda G. Isolation and identification of four sterols from Oud. J Med Plants. 2008;7:47-55.
[64]Patra A, Jha S, Murthy PN, Manik Sharone A. Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. Int J Pharma Sci Res. 2010;1:95-100.
[65] Kuetea V, Ngameni B, Fotso Simo CC, TankeuRK,Ngadjui BT, Meyer JJM, Lall N, Kuiatea JR. Antimicrobial activity of the crude extracts and compoundsfrom *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). J Ethnopharmacol.2008;120:17-24.
[66] Nazif NM. Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity. Food Chem. 2002;76:77-81.
[67] Cota BB, Oliveira AD,Souza-Filho JD, Braga FC. Antimicrobial activity and constituents of *Coccoloba acrostichoides*. Fitoterapia.2003;74:729-31.
[68] Ruggiero A, Vitalini S, Burlini N, Bernasconi S, Iriti M. Phytosterols in grapes and wine, and effects of agrochemicals on their levels. Food Chem. 2013;141:3473-79.
Rivero-Cruz JF, Zhu M, Kinghorn AD, Wua CD. Antimicrobial constituents of Thompson seedless raisins (*Vitis vinifera*) against selected oral pathogens. Phytochemistry Let. 2008;1:151-4.
[69] Marshall JA, Dennis AL, Kumazawa T, Haynes AM, Nes WD. Soybean sterol composition and utilization by *Phytophthora sojae*. Phytochemistry. 2001;58:423-8.
[70] Shu XO, Zheng Y, Cai H, Gu K, Chen Z, Zheng W, et al. Soy food intake and breast cancer survival. J Am Med Assoc. 2009;302:2437-43.

[71] Yang G, Shu XO, Li H, Chow WH, Cai H, Zhang X, et al. Prospective cohort study of soy food intake and colorectal cancer risk in women. Am J Clin Nutr. 2009;89:577-83.
[72] McCarthy FO, Chopra J, Ford A, Hogan SA, Kerry JP, O'Brien NM, et al. Synthesis, isolation and characterisation of beta-sitosterol and beta-sitosterol oxide derivatives. Org Biomol Chem. 2005;3:3059-65.
[73] Kamm W, Dionisi F, Fay LB, Hischenhuber C, Schmarr HG, Engel KH. Analysis of steryl esters in cocoa butter by on-line liquid chromatography-gas chromatography. J Chromatogr A. 2001;918:341-9.
[74] Kuksis A, Marai L, Myher JJ. Plasma lipid profiling by liquid chromatography with chloride-attachment mass spectrometry. Lipids. 1991;26:240-6.
[75] Mezine I, Zhang H, Macku C, Lijana R. Analysis of plant sterol and stanol esters in cholesterol-lowering spreads and beverages using high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy. J Agric Food Chem. 2003;51:5639-46.
[76] Rahmana SMM, Mukta AZ, Hossain MA. Isolation and characterization of β-sitosterol-D-glycoside from petroleum extract of the leaves of Ocimum sanctum L. As. J Food Ag-Ind. 2009;2:39-43.
[77] Habib MR, Nikkon F, Rahman M, Haque ME, Karim MR. Isolation of stigmasterol and beta-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Pak J Biol Sci. 2007;10:4174-6.
[78] Goad LJ, Akihisa T. Analysis of sterols. London: Blackie Academic and Professional; 1997.
[79] Slomp G, Mackellar FA. Nuclear magnetic resonance studies on some hydrocarbon side chains of sterols. J Am Chem Soc. 1962;84:204-6.
[80] Sadikun A, Aminah I, Ismailand N, Ibrahim P. Sterols and sterol glycosides from the leaves of *Gynura procumbens*. Nat Prod Sci. 1996;2:19-23.
[81] Nes WD, Norton RA, Benson M. Carbon-13 NMR studies on sitosterol biosynthesized from [13C] mevalonates. Phytochemistry. 1992;31:805-11.
[82] Smith WB. Carbon-13NMR Spectroscopy of steroids. In: Webb GA, editor. Annual reports on NMR spectrorscopy. London: Academic Press inc; 1978.
[83] Agrawal PK, Jain DC, Gupta RK, Thakur RS. Carbon -13 NMR spectroscopy of steroidal sapgenins and steroidal saponins. Phytochem Res. 1985;24:2476-96.
[84] Abdul Rahuman A, Gopalakrishnan G, Venkatesan P, Geetha K. Isolation and identification of mosquito larvicidal compound from Abutilon indicum (Linn.) Sweet. Parasitol Res. 2008;102:981-8.
[85] Gomes A, Saha A, Chatterjee I, Chakravarty AK. Viper and cobra venom neutralization by beta-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less. (Asteraceae). Phytomed. 2007;14:637-43.
[86] Kiprono PC, Kaberia F, Keriko JM, Karanja JN. The *in vitro* anti-fungal and anti-bacterial activities of beta-sitosterol from *Senecio lyratus* (Asteraceae). Z Naturforsch C. 2000;55:485-8.
[87] Mishra BB, Singh DD, Kishore N, Tiwari VK, Tripathi V. Antifungal constituents isolated from the seeds of *Aegle marmelos*. Phytochemistry. 2010;71:230-4.
[88] Bayor MT, Gbedema SY, Anna K. The antimicrobial activity of Croton membranaceus, a species used in formulations for measles in Ghana. J Pharmacogn Phytother. 2009;1:47-51.
[89] Nweze NE, Anene BM, Asuzu IU. *In vitro* anti-trypanosomal activities of crude extracts, β-sitosterol and α-sulphur from *Buchholzia coriacea* seed. Afr J Biotechnol. 2011;10:15626-32.
[90] Gupta R, Sharma AK, Dobhal MP, Sharma MC, Gupta RS. Antidiabetic and antioxidant potential of beta-sitosterol in streptozotocin-induced experimental hyperglycemia. J Diabetes 2011;3:29-37.
[91] Upadhyay K, Gupta NK, Dixit VK. Development and characterization of phyto-vesicles of beta-sitosterol for the treatment of androgenetic alopecia. Arch Dermatol Res 2012;304:511-9.
[92] Klippel KF, Hiltl DM, Schipp B. A multicentric, placebo-controlled, double-blind clinical trial of beta-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. German BPH-Phyto Study group. Br J Urol 1997;80:427-32.
[93] Barry MJ, Meleth S, Lee JY, Kreder KJ, Avins AL, Nickel JC, et al. Effect of increasing doses of saw palmetto extract on lower urinary tract symptoms: a randomized trial. J Am Med Assoc 2011;306:1344-51.
[94] Breza J, Dzurny O, Borowka A, Hanus T, Petrik R, Blane G, et al. 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;14:127-39.
[95] Berges RR, Windeler J, Trampisch HJ, Senge T. Randomised, placebo-controlled, double-blind clinical trial of beta-sitosterol in patients with benign prostatic hyperplasia. Beta-sitosterol Study Group. Lancet 1995;345:1529-32.
[96] Cicero AF, Fiorito A, Panourgia MP, Sangiorgi Z, Gaddi A. Effects of a new soy/beta-sitosterol supplement on plasma lipids in moderately hypercholesterolemic subjects. J Am Diet Assoc 2002;102:1807-11.
[97] Bouic PJ, Clark A, Lamprecht J, Freestone M, Pool EJ, Liebenberg RW, et al. 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;20:258-62.
[98] Louw I, Halland A, Bouic PJ. A pilot study of the clinical effects of a mixture of beta-sitosterol and beta-sitosterol glucoside in active rheumatoid arthritis (RA) [abstract]. Am J Clin Nutr 2002;75:351S.

[99] 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-alpha-reductase in the treatment of androgenetic alopecia. J Altern Complement Med 2002;8:143-52.
[100] Yang X, Gong H, Liu Z, Liu H, Wang H, Li Z. Similar and different effects of capsaicin and resiniferatoxin on substance P release and transient receptor potential vanilloid type 1 expression of cultured rat dorsal root ganglion neurons. Methods Find Exp Clin Pharmacol. 2010;32:3-11.
[101] Brufau G, Canela MA, Rafecas M. Phytosterols: physiologic and metabolic aspects related to cholesterol-lowering properties. Nutr Res. 2008;28:217-25.
[102] Lee JH, Lee JY, Park JH, Jung HS, Kim JS, Kang SS, et al. Immunoregulatory activity by daucosterol, a beta-sitosterol glycoside, induces protective Th1 immune response against disseminated Candidiasis in mice. Vaccine. 2007;25:3834-40.
[103] Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, et al. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. Osteoarthr Cartil. 2010;18:106-16.
[104] Chandler RF, Hooper SN, Ismail HA. Antihypercholesterolemic studies with sterols: beta-sitosterol and stigmasterol. J Pharm Sci. 1979;68:245-7.
[105] Vahouny GV, Connor WE, Subramaniam S, Lin DS, Gallo LL. Comparative lymphatic absorption of sitosterol, stigmasterol, and fucosterol and differential inhibition of cholesterol absorption. Am J Clin Nutr. 1983;37:805-9.
[106] Andriamiarina R, Laraki L, Pelletier X, Debry G. Effects of stigmasterol-supplemented diets on fecal neutral sterols and bile acid excretion in rats. Ann Nutr Metab. 1989;33:297-303.
[107] Batta AK, Xu G, Honda A, Miyazaki T, Salen G. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. Metabolism. 2006;55:292-9.
[108] Park SJ, Kim DH, Jung JM, Kim JM, Cai M, Liu X, et al. The ameliorating effects of tigmasterol on scopolamine-induced memory impairments in mice. Eur J Pharmacol. 2012;676:64-70.
[109] Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia.2009;80 123-6.
[110] Choi JM, Lee EO, Lee HJ, Kim KH, Ahn KS, Shim BS, et al. Identification of campesterol from Chrysanthemum coronarium L. and its antiangiogenic activities. Phytother Res. 2007;21:954-9.
[111] Sudhop T, Sahin Y, Lindenthal B, Hahn C, Luers C, Berthold HK, et al. 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;51:860-3.
[102] Lee S, Lee YS, Jung SH, Kang SS, Shin KH. Anti-oxidant activities of fucosterol from the marine algae Pelvetia siliquosa. Arch Pharm Res. 2003;26:719-22.
[113] Kumar SS, Kumar Y, Khan MSY, Anbu J, De Clercq E. Antihistaminic, anticholinergic and antiviral activities of fucosterol from Turbinaria conoides (J. Agardh) Kutzing. Pharmacologyonline. 2009;1:1104-12.
[114] Lee YS, Shin KH, Kim BK, Lee S. Anti-diabetic activities of fucosterol from Pelvetia siliquosa. Arch Pharm Res. 2004;27:1120-2.
[115] Wright AD, Goclik E, Konig GM. Oxygenated analogues of gorgosterol and ergosterol from the soft coral Capnella lacertiliensis. J Nat Prod. 2003;66:157-60.
[116] Schiffman FJ, Fisher JM, Rabinovitz M. Ergosterol-conferred sensitivity to amphotericin B in cultured L1210 mouse leukemia cells. Biochem Pharmacol. 1977;26:177-80.
[117] Duarte N, Ferreira MJ, Martins M, Viveiros M, Amaral L. Antibacterial activity of ergosterol peroxide against Mycobacterium tuberculosis: dependence upon system and medium employed. Phytother Res. 2007;21:601-4.
[118] Kim SW, Park SS, Min TJ, Yu KH. Antioxidant Activity of Ergosterol Peroxide in *Armillariella mellea*.Bull Korean Chem Soc.1999;20:819-23.