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ORIGINAL ARTICLE

Isolation, biological evaluation and validated HPTLC-quantification of the marker constituent of the edible Saudi plant *Sisymbrium irio* L.

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KEYWORDS

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Cytotoxic;
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Abstract Phytochemical investigation and chromatographic purification of the *n*-hexane fraction of the aerial parts of the edible Saudi plant *Sisymbrium irio* led to the isolation of β-sitosterol (**1**), stigmasterol (**2**) and β-sitosterol-β-d-glucoside (**3**). The cytotoxic effects of the *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol fractions were tested against three cancer cell lines viz., MCF-7, HCT-116 and HepG2, using the crystal violet staining (CVS) method, while the antibacterial activity against a number of pathogenic bacterial strains, was also estimated using the broth microdilution assay. The *n*-hexane fraction showed potent cytotoxic activities against all tested human cancer cell lines (IC_{50}: 11.7–13.4 μg/mL), while the dichloromethane fraction was particularly potent against HCT-116 cells (IC_{50}: 5.42 μg/mL). On the other hand, the *n*-hexane and EtOAc fractions demonstrated significant inhibitory activities against the Gram positive bacteria *S. pyogenes* and *C. perfringens*; and the Gram negative bacterium *S. enteritidis*. Our results warrant the therapeutic potential of *S. irio* as nutritional supplement to reduce the risk of contemporary diseases. Additionally, a validated high performance thin-layer chromatography (HPTLC) method was developed for the quantitative analysis of biomarker β-sitosterol glucoside (isolated in high quantity) from the *n*-hexane fraction. The system was found to furnish a compact, sharp, symmetrical and high resolution band for β-sitosterol glucoside (R_f = 0.43 ± 0.002). The limit of detection (LOD) and limit of quantification (LOQ) for β-sitosterol glucoside was found to be 21.84 and

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66.18 ng band^{-1}, respectively. β-sitosterol glucoside was found to be present only in *n*-hexane fraction (2.10 μg/mg of dried fraction) while it was absent in the other fractions of *S. irio* which validated the high cytotoxic and antibacterial activity of *n*-hexane fraction of *S. irio*.

1. Introduction

Family Brassicaceae formerly known as Cruciferae, (cabbage family) is one of the most wide spread families in the plant kingdom (Wang et al., 2015). Most members of Brassicaceae plants are of economical importance, such as cabbage, purple cabbage, cauliflower, broccoli and mustard, being either very common edible plants or important source for oil production from the seeds (Hall et al., 2002; Song and Thornalley, 2007). The edible plant *Sisymbrium irio* L., commonly known as London rocket, is a member of Brassicaceae family, widely distributed in different areas in Saudi Arabia (El-Meligy et al., 2015). It is an annual herb above three feet in height with open, slender stem branches and small pale yellow petals. The basal leaves are broad and often lobed, while the upper leaves are linear in shape and up to four inches long (Ray et al., 2005). The Middle Eastern folk healers use the aerial parts of *S. irio* to detoxify liver and spleen, treat cough and chest congestion and rheumatism, reduce swelling and clean wounds (Lev, 2003), while seeds are used as expectorant and in treatment of voice disorders (Shah et al., 2014). *S. irio* is used for dietary purposes, and the leaves of the plant are frequently incorporated in salads and mixed with yogurt in certain recipes, by natives in some countries in the Mediterranean region (Al-Jaber, 2011). Different extracts and isolated compounds from several species of this genus were also reported to have antipyretic, analgesic, antimicrobial and antioxidant activities (Al-Qudah and Abu Zarga, 2010; Vohora et al., 1980). Literature review revealed that genus *Sisymbrium* contains several classes of secondary metabolites such as flavonoids, alkaloids, anthraquinones, steroids and fatty acids (Al-Jaber, 2011; Al-Qudah and Abu Zarga, 2010; Vohora et al., 1980). It is well known that main volatile constituents of Brassicaceae plants, including genus *Sisymbrium*, are glucosinolate degradation products, mostly isothiocyanates (ITC) and nitriles, obtained by enzymatic and thermal degradation of glucosinolates. Contrary to intact glucosinolates, these breakdown products are responsible for various biological effects of Brassicaceae plants (Di Sotto et al., 2010). In fact, isothiocyanates and nitriles have been reported to have a broad-spectrum activity against fungal and bacterial pathogens, insects, nematodes and weeds (Conrad et al., 2013). However, the limited researches on *S. irio* encouraged us to investigate the chemical composition of its aerial parts and to evaluate the antibacterial and cytotoxic potentials of the *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol fractions of the aforementioned plant. Additionally, we developed a simple and sensitive validated HPTLC method for quantification of biomarker, β-sitosterol glucoside, obtained as a major component from the biologically-active *n*-hexane fraction.

2. Materials and methods

2.1. General experimental procedures

Column chromatographic separations were performed on silica gel 60 (230–400 μm, Merck, Darmstadt, Germany). All chemicals and solvents were purchased from Sigma Chemical Company (St Louis, MO, USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker spectrometer at NMR Unit, College of Pharmacy, King Saud University operating at 500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively. Resolution Mass Spectrophotometer was used for accurate mass determination. Electron Impact mode with ionization energy of 70 eV was accustomed.

2.2. Plant material

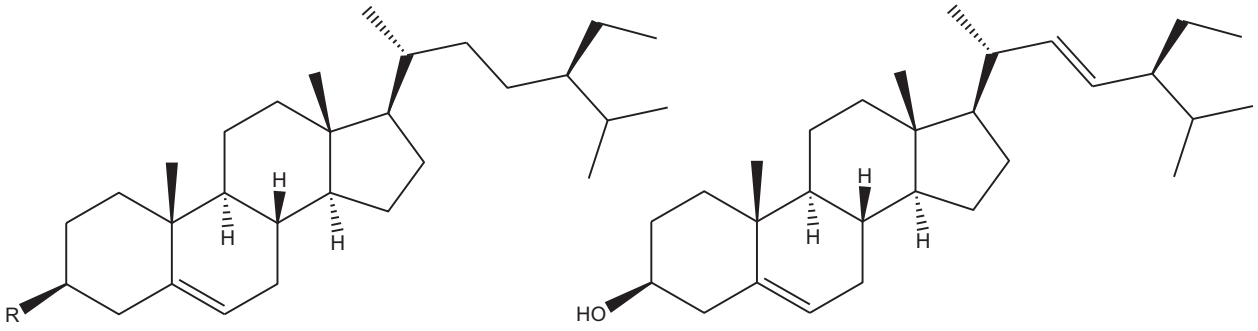
The aerial parts of *S. irio* L. were collected from a local farm in Riyadh city located in Najd region in February 2015 and, kindly identified by a taxonomist at the Pharmacognosy Department, College of Pharmacy, King Saud University. A voucher specimen has been deposited in the herbarium of Pharmacognosy Department, College of Pharmacy.

2.3. Extraction, fractionation and isolation

The air-dried powdered aerial parts of *S. irio* (250 g) were extracted by cold maceration with 85% ethanol till exhaustion. The ethanolic extract was dried in a rotary evaporator to give a dark residue (20 g). Subsequent fractions were obtained by dispersing the total ethanolic extract in 200 ml of distilled water followed by successive extraction with *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). All fractions were filtrated over anhydrous sodium sulfate and evaporated till dryness to yield fractions **A** (*n*-hexane, 2 g), **B** (DCM, 3.5 g), **C** (EtOAc, 2.5 g) and **D** (*n*-BuOH, 6.0 g). After comparing the results obtained from cytotoxicity and antibacterial for different fractions, part of fraction **A** (1 g) was subjected to silica gel column chromatography (100 g × 100 cm × 1.5 cm) using chloroform/methanol gradients to obtain seven subfractions (Si-1–7). Subfraction Si-3 was re-chromatographed on another silica gel column using chloroform/methanol in gradient mode of elution analysis. Fraction eluted by 2% MeOH afforded compounds **1** (50 mg) and **2** (30 mg) upon direct crystallization, while elution by 20% MeOH afforded compound **3** (60 mg).

2.4. Cytotoxic activity assay

The cytotoxic activities, of the prepared fractions, were evaluated on three cancer cell lines (MCF-7, HCT-116 and



1, β-sitosterol, R= OH
3, β-sitosterol glucoside, R= O-Glc

2, stigmasterol

Figure 1 Chemical structures of isolated compounds from *S. irio*.

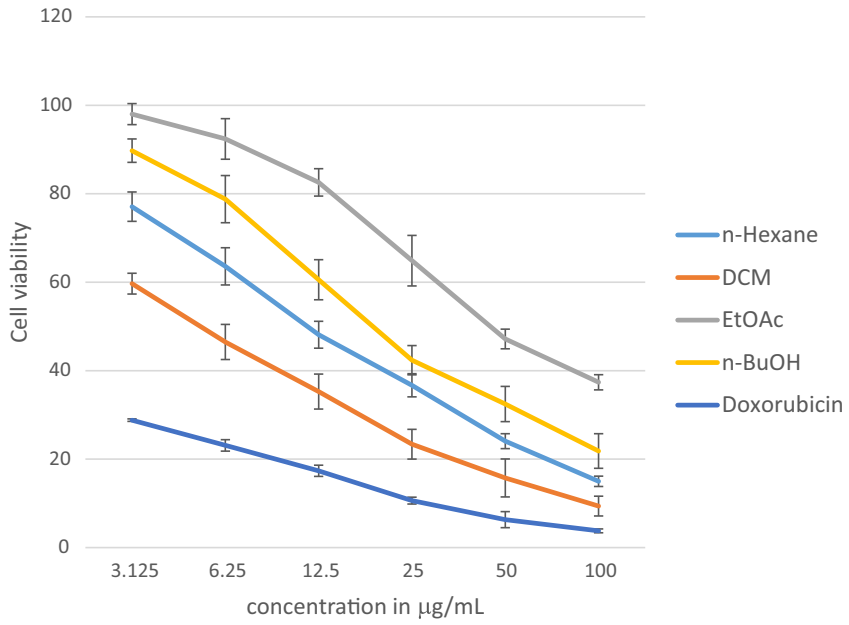


Figure 2 Cytotoxicity assessment by CVS assay in HCT-116 tumor cell line. Values are the mean ± SE of three assays, $p < 0.01$, compared to the reference drug.

HepG-2). The mammalian cell lines MCF-7 cells (human breast cancer), HepG-2 (human liver cancer) and HCT-116 (human colon cancer) were obtained from the American Type Culture Collection (ATCC). The cells were proliferated in Dulbecco's modified Eagle's Medium (DMEM) complemented with fetal bovine serum (10%), L-glutamine, HEPES buffer (1%) and gentamicin (50 µg/mL) (Sigma Chemical Co.). The cells were maintained with 5% CO_2 at 37 °C and were subcultured two times a week.

The cytotoxic activity was assessed by the crystal violet staining (CVS) method (Itagaki et al., 1991; Saotome et al., 1989) using a 96-well tissue culture micro plate seeded with cells at concentration of 1 × 104 cells in 100 µL of medium per well. After 24 h, different concentrations of *S. irio* fractions (**A**–**D**), were added. Sequential set of dilutions of each fraction (100, 50, 25, 12.5, 6.25 and 3.125 µg) was added into a flat bottomed 96-well microtiter plates and incubated with 5%CO_2 at 37 °C. Three wells were used for each concentration of the test sample. The control cells were incubated without a test sample and with or without DMSO. After 48 h, the media were removed and crystal violet solution (1%) was added to each well for 30 min. After that, the stain was removed by rinsing the plates with distilled water. For quantitative evaluation, the absorbance was measured in an automatic Microplate reader (TECAN, Inc., San Jose, CA, USA) at 595 nm for colorimetric estimation of fixed cells. The effect on cell growth was estimated by measuring the difference in absorbance percentage in the presence and absence of the tested fractions and presented in a dose-response curve. The concentration that inhibited cell growth by 50% (IC_{50}) was obtained. Doxorubicin was used as standard antitumor drug.

2.5. Antibacterial activity assay

To assess the antibacterial activity, four Gram positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Clostridium perfringens*) and four Gram negative bacteria (*Neisseria gonorrhoeae*, *Klebsiella pneumoni*, *Shigella*

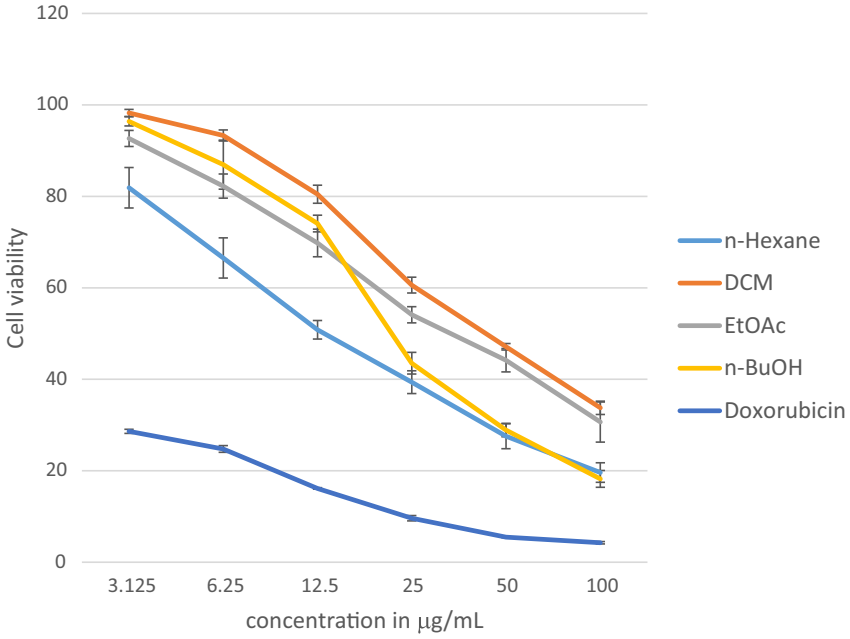


Figure 3 Cytotoxicity assessment by CVS assay in MCF-7 tumor cell line. Values are the mean ± SE of three assays, $p < 0.01$, compared to the reference drug.

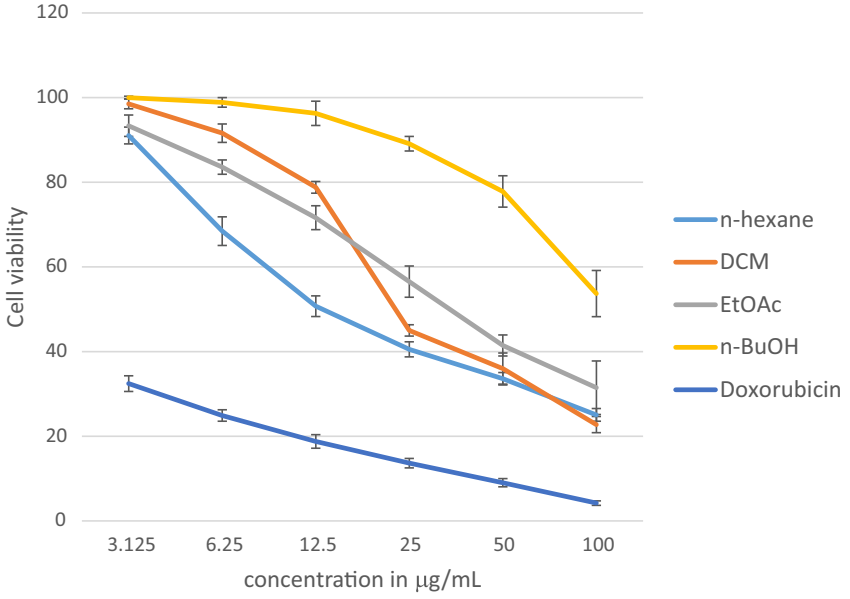


Figure 4 Cytotoxicity assessment by CVS assay in HepG-2 tumor cell line. Values are the mean ± SE of three assays, $p < 0.01$, compared to the reference drug.

Table 1 IC_{50}[a] (µg/mL) of *S. irio* fractions on tested cell lines.

Tumor cell line	*n*-Hexane	DCM	EtOAc	*n*-BuOH	Doxorubicin
HCT-116	11.7	5.42	46	19.8	0.47
MCF-7	13.4	44.7	35.4	22.3	0.44
HepG-2	13.3	23.2	35.8	> 50	0.469

[a] IC_{50}: concentration of fraction required to reduce cell survival by 50%.

Table 2 Antibacterial activities (mean zone of Inhibition, mm ± SD) of *S. irio* fractions against selected clinical pathogens.

Tested microorganisms	Sample				
	n-Hexane	DCM	EtOAc	*n*-BuOH	Ampicillin
Gram positive bacteria					
Staphylococcus aureus (RCMB 010027)	21.3 ± 0.23	NA	18.3 ± 0.34	NA	28.9 ± 0.14
Staphylococcus epidermidis (RCMB 010024)	18.0 ± 0.13	NA	17.7 ± 0.16	NA	25.4 ± 0.18
Streptococcus pyogenes (RCMB 010015)	21.3 ± 0.15	NA	21.7 ± 0.32	NA	26.4 ± 0.34
Clostridium perfringens (RCMB 010034)	15.3 ± 0.32	NA	13.7 ± 0.14	NA	18.4 ± 0.34
Gram negative bacteria					Gentamycin
Neisseria gonorrhoeae (RCMB 010076)	NA	NA	NA	NA	19.9 ± 0.18
Klebsiella pneumoniae (RCMB 0010093)	17.7 ± 0.11	12.3 ± 0.12	21.0 ± 0.11	16.0 ± 0.10	26.3 ± 0.15
Shigella flexneri (RCMB 0100542)	16.7 ± 0.14	12.7 ± 0.16	18.3 ± 0.31	15.3 ± 0.41	24.8 ± 0.24
Salmonella enteritidis (RCMB 010084)	21.0 ± 0.24	15.7 ± 0.21	22.3 ± 0.16	17.3 ± 0.18	25.3 ± 0.18

NA: not active, data are expressed in the form of mean ± S.D.

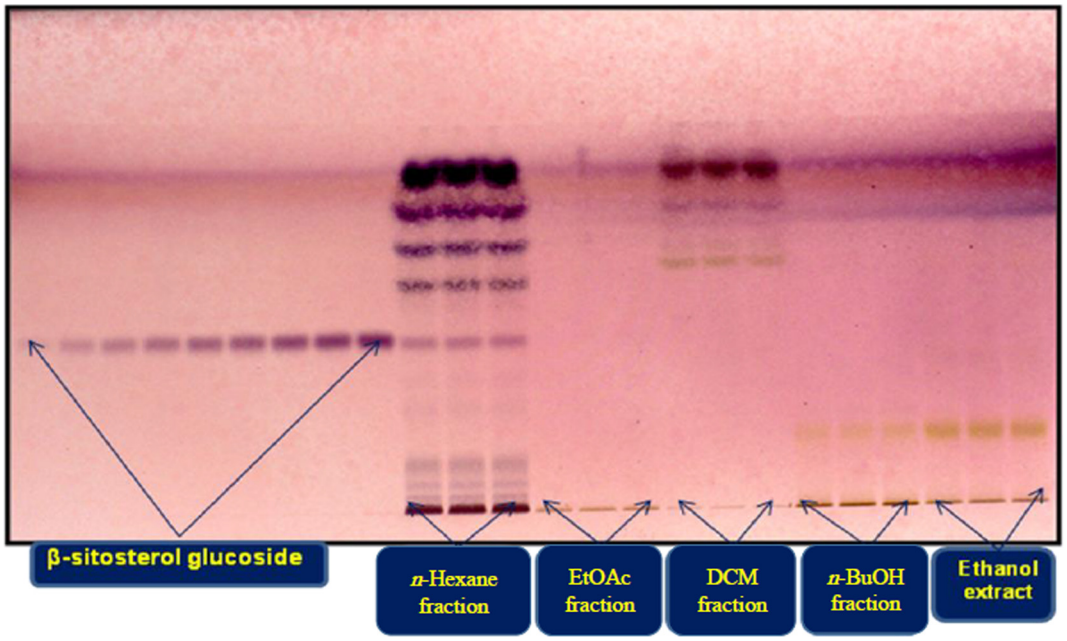


Figure 5 Picture of the developed TLC plate derivatized with p-anisaldehyde reagent at daylight; mobile phase:chloroform:methanol (16:4).

flexner and *Salmonella enteritidis*) were selected. The different strains of microbes were acquired from the American Type Culture Collection (ATCC).

The antibacterial activity was determined by agar well diffusion method performed in accordance with the National Committee Clinical Laboratory Standards (NCCLS) criteria (Wayne, 2002, 2004). Bacterial suspension was prepared at 0.5 McFarland standard turbulence in a volume of 100 µL and was cultivated on Mueller-Hinton agar media punched with 6-mm diameter wells. Then, 100 µl of 10% *S. irio* fractions was added to the wells, while 10% DMSO was used as the negative control. Ampicillin and gentamicin (30 µg/mL) were used as standard agents against the Gram positive bacteria and Gram negative bacteria, respectively. The antibacterial activity was estimated, after incubation of the plates at 37 °C for 18–24 h, by calculating the diameter of inhibition zones (mm). Each test was done three times and the mean of the results was determined.

2.6. Standardization of different fractions of S.irio by validated HPTLC method

2.6.1. HPTLC instrumentation and conditions

A validated high-performance thin layer chromatography (HPTLC) method was used to standardize the total ethanolic extract, *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol fractions of *S. irio*. The chromatography was performed on 20 × 10 cm precoated silica gel F_{254} HPTLC plate, using β-sitosterol glucoside as the marker compound. Numerous combination of mobile phases was tried to get good separation and resolution of various compounds present in *S. irio*. Based on our observations, chloroform and methanol in the ratio of 16:4 were selected as mobile phase to accomplish the standardization. The marker along with different samples was applied by CAMAG automatic TLC sampler-4 on the HPTLC plate. CAMAG Automated Developing Chamber-2 was used to develop the HPTLC plate under controlled condi-

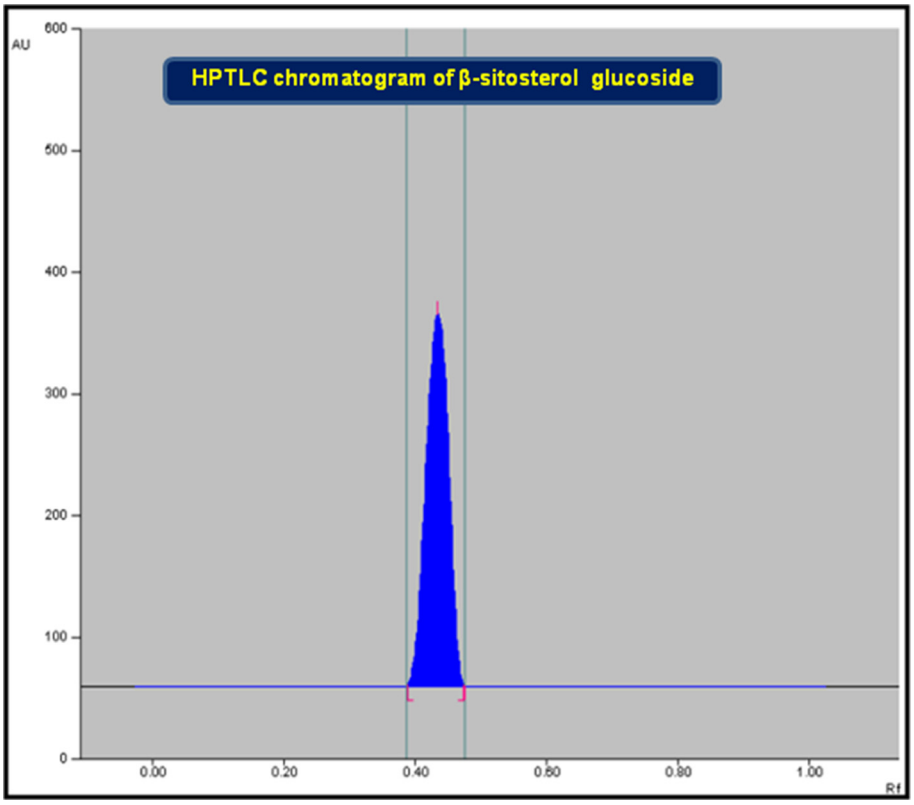


Figure 6 Chromatogram of standard β-sitosterol glucoside (R_f = 0.43; 500 ng/spot) at 600 nm; mobile phase:chloroform:methanol (16:4).

Table 3 R_f, linear regression data for the calibration curve of β-sitosterol glucoside (n = 6).

Parameters	β-sitosterol glucoside
Linearity range (ng/spot)	100–900
Regression equation	$Y = 9.518x + 885.57$
Correlation (r^2) coefficient	0.9966 ± 0.0004
Slope ± SD	9.518 ± 0.062
Intercept ± SD	885.5 ± 13.77
Standard error of slope	0.0257
Standard error of intercept	5.623
R_f	0.43 ± 0.002
LOD	21.84 ng/band
LOQ	66.18 ng/band

tion. The developed plate was dried and derivatized by spraying with p-anisaldehyde reagent and scanned by CAMAG TLC Scanner-3 (λ = 600 nm).

2.6.2. Preparation of standard stock solutions

Standard stock solution of β-sitosterol glucoside (1 mg/mL) was prepared in methanol, following further dilution with methanol to furnish different concentrations ranging from 10 to 90 μg/mL. All the dilutions were applied (10 μL, each) through microliter syringe attached with the applicator on the HPTLC plate to furnish the linearity range of 100–900 ng/band.

2.6.3. Validation of method

Validation of the proposed HPTLC method was performed according to the ICH guidelines (ICH, 2005), for the determination of linearity range, limit of detection (LOD), limit of quantification (LOQ), precision, recovery as accuracy and robustness.

2.7. Statistical analysis

Data were expressed as means ± SD. The unpaired Student's t-test was conducted using the Graph Pad In Stat (ISI Software) computer program. Differences were considered significant at P values of less than 0.05.

Table 4 Recovery as accuracy studies of the proposed HPTLC method (n = 6).

Percent (%) of β-sitosterol glucoside added to analyte	Theoretical concentration of β-sitosterol glucoside (ng/ml)	Concentration of β-sitosterol glucoside found (ng/mL) ± SD	% RSD	SEM	% Recovery
0	200	197.15 ± 1.562	0.792	0.637	98.57
50	300	297.38 ± 2.439	0.821	0.995	99.12
100	400	395.45 ± 3.645	0.921	1.487	98.86
150	500	498.21 ± 4.951	0.993	2.021	99.64

Table 5 Precision of the proposed HPTLC method ($n = 6$).

Conc. of β-sitosterol glucoside (ng/band)	Intra-day precision			Inter-day precision		
	Average conc. found ± SD	%RSD	SEM	Average conc. found ± SD	%RSD	SEM
400	397.55 ± 1.851	0.465	0.755	394.40 ± 1.679	0.425	0.685
600	598.86 ± 2.931	0.489	1.196	591.50 ± 2.718	0.459	1.109
800	797.44 ± 3.871	0.485	1.581	795.34 ± 3.626	0.456	1.480

Table 6 Robustness of the proposed HPTLC method ($n = 6$).

Optimization condition	β-sitosterol glucoside (300 ng/band)		
	SD	%RSD	SEM
Mobile phase composition; (chloroform: methanol, v/v)			
16:4	1.526	0.501	0.622
15.8:4.2	1.589	0.523	0.648
16.2:3.8	1.685	0.556	0.687
Mobile phase volume (for saturation)			
18 ml	1.506	0.495	0.614
20 ml	1.529	0.504	0.624
22 ml	1.585	0.524	0.646
Duration of saturation			
10 min	1.496	0.493	0.610
20 min	1.517	0.501	0.619
30 min	1.535	0.525	0.646

3. Results

3.1. Identification of isolated compounds

The structures of the isolated compounds were elucidated by analyzing their spectroscopic data (1D, 2DNMR and MS) and by comparing these data with the literature as: β-sitosterol (**1**) (Habib et al., 2007), stigmasterol (**2**) (Kasahara et al., 1994) and β-sitosterol glucoside (**3**) (Rahman et al., 2009) (Fig. 1).

3.2. Biological studies

3.2.1. Cytotoxic activity

The cytotoxic activity was determined for the *n*-hexane, DCM, EtOAc and *n*-BuOH fractions (**A**–**D**) against HCT-116, MCF-7 and HepG-2 cancer cell lines, using the CVS method employing doxorubicin as a reference drug (Figs. 2–4). The IC_{50} was estimated for each cell line and results are shown in Table 1.

3.2.2. Antibacterial activity

The antibacterial activities for the obtained fractions (**A**–**D**) were evaluated, by determining their zone of inhibition, against four Gram-positive and four Gram-negative bacteria (Table 2). The tested pathogenic bacteria are known to cause many serious diseases, ranging from simple skin infections to life-threatening systemic diseases including skin and respiratory infections and food poisoning.

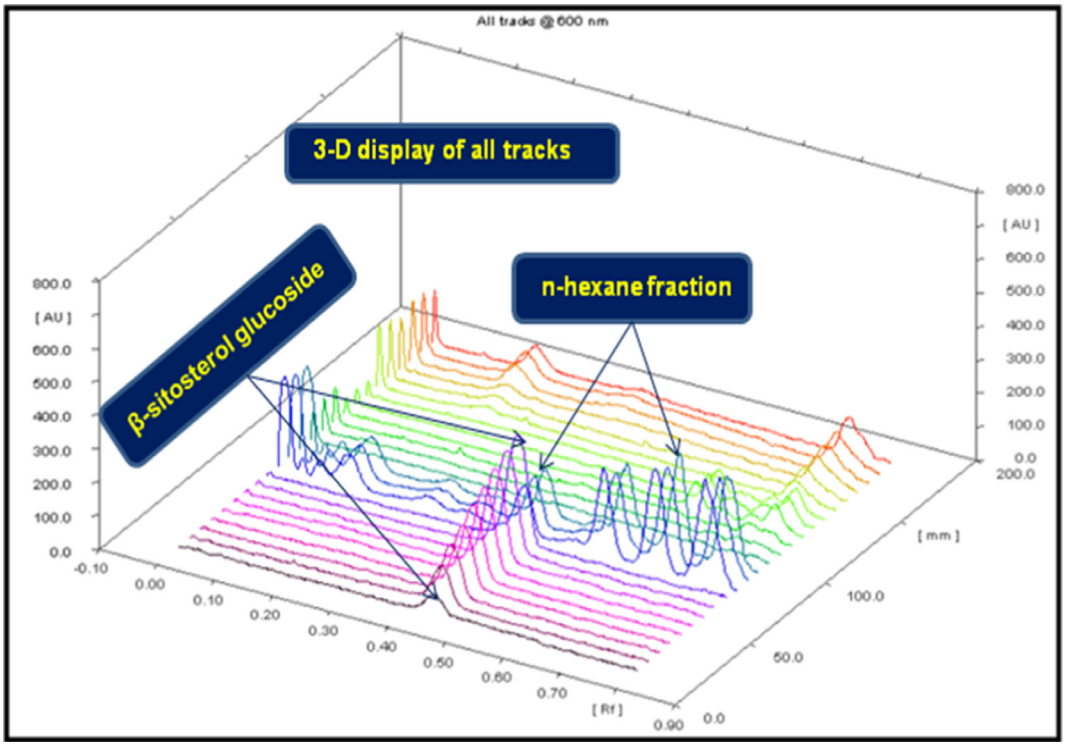


Figure 7 3D display of all tracks at 600 nm; mobile phase:chloroform–methanol (16:4).

3.3. Identification of β-sitosterol glucoside in different fractions of S. irio by validated HPTLC method

3.3.1. HPTLC method development and validation

The developed method was found to be very effective in clearly separating the biomarker β-sitosterol glucoside and different constituents of all the fractions of *S. irio* (Fig. 5). It was found to furnish an intense, compact and sharp peak of β-sitosterol glucoside at R_f 0.43 ± 0.002 (Fig. 6). The optimized saturation time and mobile phase volume for saturation were found to be 20 min and 20 mL, respectively. The developed method was thus, found to be selective with high resolution baseline. The regression equation and correlation co-efficient (r^2) for β-sitosterol glucoside were found to be $Y = 9.518x + 885.57$ and 0.9966 ± 0.0004 in the 100–900 ng/spot linearity range. The Limit of detection (LOD) and limit of quantification (LOQ) for β-sitosterol glucoside were found to be 21.84 and 66.18 ng/band, respectively (Table 3). The % recovery of β-sitosterol glucoside was found to be 98.57–99.64% (Table 4). The %RSD for Intra-day and inter-day precisions for β-sitosterol glucoside was found to be 0.465–0.587% and 0.425–0.548%, respectively. The standard error (SEM) for Intra-day and inter-day precisions was found to be 0.465–0.489 and 0.425–0.459, respectively (Table 5). The SD and % RSD were found to be very less after deliberately modifying the mobile phase which confirmed that the method was highly robust (Table 6).

3.3.2. HPTLC analysis of β-sitosterol glucoside

The proposed validated HPTLC method was employed for quantitative analysis of β-sitosterol glucoside in *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol fractions and total ethanolic extract of *S. irio* (Fig. 7). The biomarker β-sitosterol glucoside was found to be present in the *n*-hexane fraction (Fig. 8) while it was absent in the remaining fractions as well as the ethanolic extract. The dried *n*-hexane fraction of *S. irio* was found to contain 2.10 µg/mg of β-sitosterol glucoside. This is a maiden report, which demonstrated the development of an economical, precise, accurate and simple HPTLC method for quantitative analysis of β-sitosterol glucoside in different fractions of *S. irio*.

4. Discussion

The obtained compounds **1**–**3** are among the most spread phytosterols in the plant kingdom; β-sitosterol (**1**) and β -sitosterol glucoside (**3**) were isolated previously from *S. irio* (Kahan et al., 1991). On the other hand, stigmasterol (**2**) was reported from many plants belonging to family Brassicaceae, but, to the best of our knowledge, isolated here for the first time from *Sisymbrium*. It is well known that consuming plant-based diet rich in phytosterols can effectively reduce serum low density lipoprotein (LDL), and total cholesterol (Nguyen, 1999). Additionally, β-sitosterol, and plants rich in it, are used as

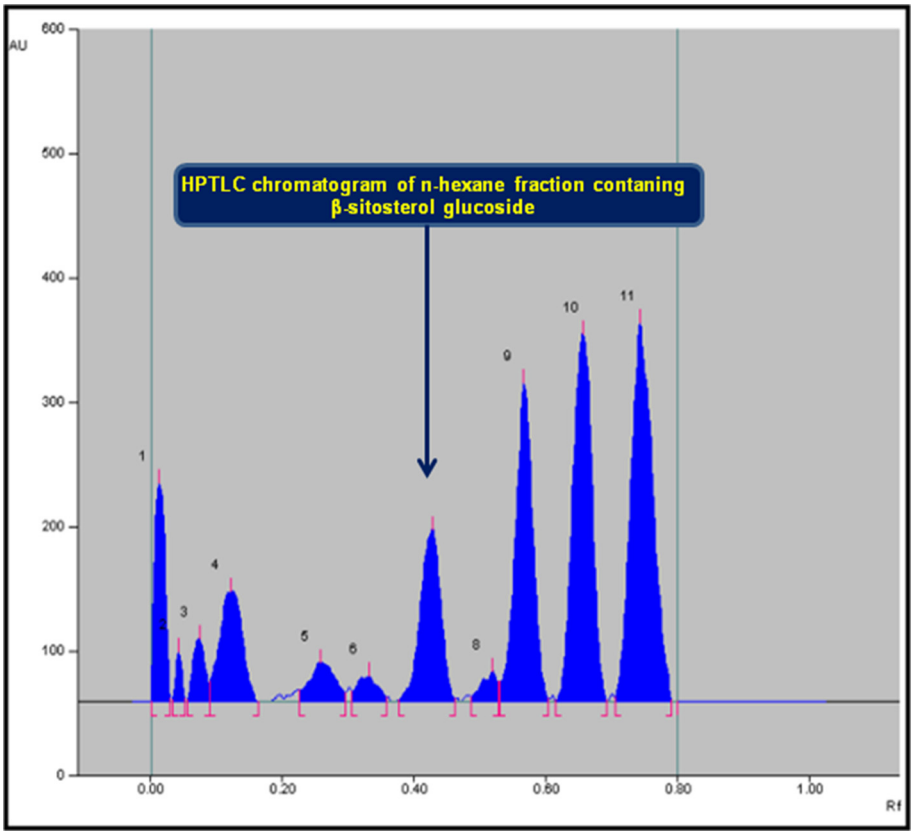


Figure 8 Chromatogram of *n*-hexane fraction scanned at 600 nm (β-sitosterol glucoside; R_f = 0.43); mobile phase:chloroform–methanol (16:4).

dietary supplements for symptomatic treatment of benign prostatic hypertrophy (Wilt et al., 2000).

According to the results shown in Figs. 2–4 and Table 1, all fractions demonstrated a dose-dependent cytotoxic effect against the three tested cancer cell lines. However, the *n*-hexane fraction (A) showed the highest activities (IC_{50} = 11.7, 13.4 and 13.3 μg/mL) against HCT-116, MCF-7 and HepG-2, respectively. The DCM fraction (**B**) displayed remarkable cytotoxic activity against the HCT-116 cell line (IC_{50} = 5.4 μg/mL), while it exhibited less selective activity against the other two cell lines (IC_{50}44.7 and 23.2 μg/mL). The EtOAc fraction was moderately active against all cell lines (IC_{50} = 35.4–46.0 μg/mL). On the other hand, the *n*-butanol fraction had low IC_{50} values (19.8 and 22.3 μg/mL) against HCT-116 and MCF-7 cell lines, respectively, but was totally inactive against HepG-2 cell line (IC_{50} > 50 μg/mL).

The high activity observed for the *n*-hexane fraction can be attributed, in part, to its high content of hydrophobic compounds such as steroids, triterpenes, oils and fatty acids and their ability to penetrate cell membrane more easily (Shah et al., 2014). Also, it was reported that β-sitosterol can disrupt the structure of cancer cell membranes and alter the signaling pathways that regulate tumor growth and apoptosis (von Holtz et al., 1998). *In vivo* studies showed that adding β-sitosterol to diets of mice and rats fed with colon carcinogens decreased the proliferative changes of the tumor (Awad et al., 2000).

In vitro antibacterial screening demonstrated various degrees of growth inhibition for the organic solvent fractions. Among the tested fractions, the maximum antibacterial activity was shown by the *n*-hexane and ethyl acetate fractions against the Gram positive bacteria *Streptococcus pyogenes* and the Gram negative bacteria *Salmonella enteritidis* (21.3–21.7 mm and 21.0–22.3 mm, diameter of zone of inhibition, respectively). *S. pyogenes* is the most common bacterial cause of pharyngitis, impetigo and serious skin infections involving deep layers such as erysipelas and cellulitis (Starr and Engleberg, 2006), while *Salmonella* is one of the major diarrhea-causing bacteria (Smith and Bayles, 2007). Furthermore, the *n*-hexane exhibited high activity against the food poisoning bacteria *C. perfringens*, while both *n*-hexane and EtOAc fractions were moderately active against *S. aureus*, the most frequent cause of human skin and soft tissue abscesses (Lowy, 1991). However, DCM and *n*-BuOH fractions showed no growth inhibition for any of the tested Gram-positive bacteria. On the other hand, none of the tested fractions inhibited the growth of the Gram-negative bacteria *N. Gonorrhoeae* at 20 mg/mL.

The standardization of *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol fractions and total ethanolic extract of *S. irio* by validated HPTLC method revealed the presence of anticancer biomarker β-sitosterol glucoside only in *n*-hexane fraction. This result validated the above finding of high cytotoxic and antibacterial activity of *n*-hexane fraction. Moreover, this method may also be applied to study the degradation kinetics of β-sitosterol glucoside and its determination in plasma and other biological fluids.

5. Conclusion

The organic fractions obtained from the edible plant *S. irio* have powerful antibacterial and remarkable cytotoxic activities against the tested bacterial strains and cancer cell lines, respectively. Results revealed that both activities, mainly, reside in the *n*-hexane and ethyl acetate fractions, which can be potential sources of antibiotics and anticancer compounds. The plant is edible and rich in medicinally important bioactive metabolites e.g. flavonoids, phytosterols and unsaturated fatty acids. These facts make it highly nutritious component to integrate more frequently in our meals and also to be used in the production of health-promoting supplements. The applied HPTLC method was found to be very effective in quantification of the biomarker β-sitosterol glucoside present in the *n*-hexane fraction. The method can be used as a rational approach for the quality control of natural products in more scientific and efficacious way.

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References

Al-Jaber, N., 2011. Phytochemical and biological studies of *Sisymbrium irio* L. growing in Saudi Arabia. J. Saudi Chem. Soc. 15, 345–350.

Al-Qudah, M.A., Abu Zarga, M.H., 2010. Chemical constituents of *Sisymbrium irio* L. from Jordan. Nat. Prod. Res. 24, 448–456.

Awad, A., Downie, A., Fink, C., Kim, U., 2000. Dietary phytosterol inhibits the growth and metastasis of MDA-MB-231 human breast cancer cells grown in SCID mice. Anticancer Res. 20, 821–824.

Conrad, A., Biehler, D., Nobis, T., Richter, H., Engels, I., Biehler, K., Frank, U., 2013. Broad spectrum antibacterial activity of a mixture of isothiocyanates from nasturtium (*Tropaeoli majoris* herba) and horseradish (*Armoraciae rusticanae* radix). Drug Res. (Stuttg) 63, 65–68.

Di Sotto, A., Vitalonem, A., Nicoletti, M., Piccin, A., Mazzanti, G., 2010. Pharmacological and phytochemical study on a *Sisymbrium officinale* Scop. extract. J. Ethnopharmacol. 127, 731–736.

El-Meligy, R.M., Awaad, A.S., Soliman, G.A., Bacha, A.B., Alafeefy, A.M., Kenawy, S.A., 2015. Prophylactic and curative anti-ulcerative colitis activity and the possible mechanisms of action of some desert plants. J. Enzyme Inhib. Med. Chem. 30, 250–258.

Habib, M., Nikkon, F., Rahman, M., Haque, M., Karim, M., 2007. Isolation of stigmasterol and beta-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Pak. J. Biol. Sci. 10, 4174–4176.

Hall, J.C., Sytsma, K.J., Iltis, H.H., 2002. Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. AJB 89, 1826–1842.

International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Harmonized Triplicate Guideline on Validation of Analytical Procedures: Text and Methodology Q2 (R1), Complementary Guideline on Methodology Incorporated in November 2005 by the ICH Steering Committee, IFPMA, Geneva.

Itagaki, H., Hagino, S., Kato, S., Kobayashi, T., Umeda, M., 1991. An in vitro alternative to the Draize eye irritation test: evaluation of the crystal violet staining method. Toxicol. In Vitro 5, 139–143.

Kahan, M., Kalim, S., Kahan, M., 1991. Chemical constituents of the aerial parts of *Sisymbrium irio*. J. Indian Chem. Soc. 68, 532.

Kasahara, Y., Kumaki, K., Katagiri, S., Yasukawa, K., Yamanouchi, S., Takido, M., Akihisa, T., Tamura, T., 1994. *Carthami flos* extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. Phytother. Res. 8, 327–331.

Lev, E., 2003. *Sisymbrium irio* Medicinal Substances in Jerusalem From Early Times to the Present Day *Archaeopress*. Oxford, UK, p. 62.

Lowy, F., 1991. *Staphylococcus aureus* infections. New Engl. J. Med. 339, 520–532.

Nguyen, T., 1999. The cholesterol-lowering action of plant stanol esters. J. Nutr. 129, 2109–2112.

Rahman, S., Mukta, Z., Hossain, M., 2009. Isolation and characterization of β-sitosterol-D-glycoside from petroleum extract of the leaves of *Ocimum sanctum* L. Asian J. Food Agro-Ind. 2, 39–43.

Ray, J., Creamerb, R., Schroederc, J., Murray, L., 2005. Moisture and temperature equirements for London Rocket (*Sisymbrium irio*) emergence. Weed Sci. 53, 187–192.

Saotome, K., Morita, H., Umeda, M., 1989. Cytotoxicity test with simplified crystal violet staining method using microtitre plates and its application to injection drugs. Toxicol. In Vitro 3, 317–321.

Shah, S., Rehmanullah, S., Muhammad, Z., 2014. Pharmacognostic standardization and pharmacological study of *Sisymbrium irio* L. AJRC 1, 241–253.

Smith, J., Bayles, D., 2007. Post infectious irritable bowel syndrome: a long-term consequence of bacterial gastroenteritis. J. Food Prot. 70, 1762–1769.

Song, L., Thornalley, P.J., 2007. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. FCT 45, 216–224.

Starr, C., Engleberg, N., 2006. Role of hyaluronidase in subcutaneous spread and growth of group A Streptococcus. Infect. Immun. 74, 40–48.

Vohora, S.B., Naqvi, S.A., Kumar, I., 1980. Antipyretic, analgesic and antimicrobial studies on *Sisymbrium irio*. Planta Med. 38, 255–259.

von Holtz, R., Fink, C., Awad, A., 1998. Beta-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. Nutr. Cancer 32, 8–12.

Wang, X., Wu, J., Liang, J., Cheng, F., Wang, X., 2015. Brassica Database (BRAD) Version 2.0: Integrating and Mining Brassicaceae Species Genomic Resources. Database (Oxford). pii: bav093.

Wayne, P., 2002. National Committee for Clinical Laboratory Standards (NCCLS) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Approved Standard M27–A2.

Wayne, P., 2004. Performance Standards for Antimicrobial Susceptibility Testing, National Committee for Clinical Laboratory Standards (NCCLS) 16th Informational Supplement, M100–S14, NCCLS.

Wilt, T., Ishani, A., MacDonald, R., Stark, G., Mulrow, C., Lau, J., 2000. Beta-sitosterols for benign prostatic hyperplasia. Cochrane Database Syst. Rev. 2, CD001043.