

Chemical Constituents of *Corchorus olitorius* L.

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ABSTRACT

Chemical investigation of the dichloromethane extract of the stems of *Corchorus olitorius* L. led to the isolation of oleanolic acid (**1**), 2-hydroxyethyl benzoate (**2**), chlorophyll a (**3**), mixtures of phytol fatty acid esters (**4a**) and β -sitosterol fatty acid esters (**4b**) and β -sitosterol (**5a**) and stigmasterol (**5b**). The structures of **1-5b** were identified by comparison of their NMR data with literature data.

Keywords: *Corchorus olitorius*, Malvaceae, oleanolic acid, 2-hydroxyethyl benzoate, chlorophyll a, phytol fatty acid esters, β -sitosterol fatty acid esters, β -sitosterol, stigmasterol

INTRODUCTION

Corchorus olitorius commonly known as jute and locally known as “saluyot” is a popular vegetable in the Philippines. It grows on rice-paddy banks, in fallow paddies, in and near settlements throughout the Philippines¹. A number of studies were conducted on the chemical constituents of *C. olitorius*. The different parts of *C. olitorius* afforded cardiac glycosides: strophanthidin trioside², coroloside², deglycocoroloside³, chorchoroside⁴, chorchoroside B³, olitoriside⁵, cannogenol-3-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-digitoxopyranoside⁶⁻⁷, periplagerin-3-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-digitoxopyranoside⁶⁻⁷, digitogenin-3-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-digitoxopyranoside⁶⁻⁷, canarigenin⁶⁻⁷, and chorchoroside A-E⁸ from the seeds; triterpenes: corosin⁹, ursolic acid¹⁰, corosolic acid¹⁰, oxo-orocin¹⁰ from the seeds, and oleanolic acid¹¹ from the leaves; ionones: corchoionoside A-C¹², (6*S*,9*R*)-roseoside¹², and betulabuside A¹² from the leaves; phenolics: astragalol¹², isoquercetin¹², quercetin-3-galactoside^{11,13}, quercetin-3-(6-malonylgalactoside)^{11,13}, quercetin-3-(6-malonylgalactoside)^{11,13}, circhorone¹², scopolin¹², chlorogenic acid¹²⁻¹³, 3,5-dicaffeoylquinic acid¹²⁻¹³ from the leaves, and 4,7-dihydroxycoumarin¹⁴ from the seeds. Another study reported that the lignin content of a mature *C. olitorius* stem was around 29%¹⁵. Furthermore, the different parts of *C. olitorius* were found to exhibit diverse biological activities. The leaves of *C. olitorius* were reported to exhibit antioxidant¹⁶, antitumor¹⁷, gastroprotective¹⁸, antibacterial and antifungal¹⁹, anti-inflammatory and analgesic²⁰ activities. In addition, the leaves are used as demulcent and febrifuge²¹.

We now report the isolation of oleanolic acid (**1**), 2-hydroxyethyl benzoate (**2**), chlorophyll a (**3**), phytol fatty acid esters (**4a**), β -sitosterol fatty acid esters (**4b**), β -sitosterol (**5a**) and stigmasterol (**5b**) from the stems of *C. olitorius*. To the best of our knowledge this is the first report on the chemical constituents of the dichloromethane extract of the stems of *C. olitorius*. The structures of **1-5a** are presented in Fig. 1.

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl₃ at 600 MHz for ¹H NMR and 150 MHz for ¹³C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

Plant material

Corchorus olitorius L. was bought from a supermarket in Metro Manila in October 2015 and authenticated at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude extract from the leaves were fractionated by silica gel chromatography using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were

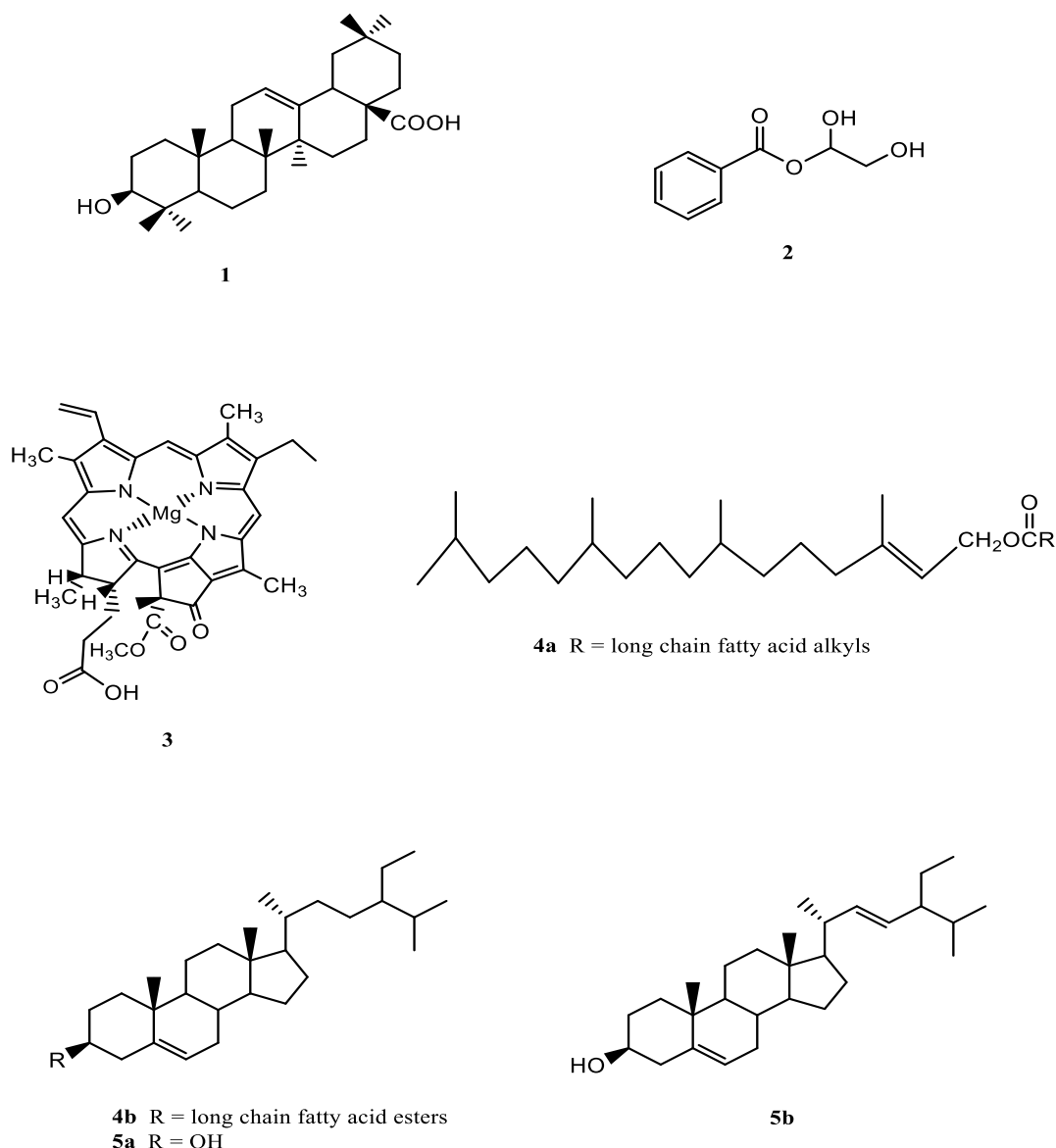


Figure 1: Chemical structures of oleanolic acid (**1**), 2-hydroxyethyl benzoate (**2**), chlorophyll a (**3**), phytyl fatty acid esters (**4a**), β -sitosteryl fatty acid esters (**4b**), β -sitosterol (**5a**) and stigmasterol (**5b**) from the stems of *C. olitorius*.

obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents of *C. corchorus*

The freeze-dried flowers of *C. corchorus* (282.87 g) were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (1.03 g). The extract was chromatographed by gradient elution with using CH_2Cl_2 , followed by increasing amounts of acetone at 10% increment by volume as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 \times) using 5% EtOAc in petroleum ether to afford a mixture of **4a** and **4b** (2 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether

to afford a mixture of **5a** and **5b** (3 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (0.5:0.5:9, v/v) to afford **3** (4 mg) after washing with petroleum ether, followed by Et_2O . The 50% acetone in CH_2Cl_2 fraction was rechromatographed using $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (1:1:8, v/v) to afford **1** (2 mg) after washing with petroleum ether, the 60% acetone in CH_2Cl_2 fraction was rechromatographed using $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (0.5:0.5:9, v/v) to afford **2** (1 mg) after washing with petroleum ether,

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the stems of *Corchorus olitorius* led to the isolation of **1-5b**. The NMR spectra of **1** are in accordance with data reported in the literature for oleanolic acid²²; **2** for 2-hydroxyethyl benzoate²³; **3** for chlorophyll a²⁴; **4a** for

phytyl fatty acid esters²⁵; **4b** for β -sitosteryl fatty acid esters²⁶; **5a** for β -sitosterol²⁷; and **5b** for stigmasterol²⁷.

Literature search revealed that **1**, **3**, **5a** and **5b** exhibited diverse biological activities. Oleanolic acid (**1**) showed anti-inflammatory effects by inhibiting hyper permeability, the expression of CAMs, and the adhesion and migration of leukocytes²⁸. It showed anti-inflammatory activities through the inhibition of the HMGB1 signaling pathway²⁹. It exhibited anti-inflammatory, hepatoprotective, gastroprotective, immunoregulatory and anti-ulcer activities³⁰, and gastroprotective effect on experimentally induced gastric lesions in rats and mice³¹. It was also reported to inhibit mouse skin tumor³², protect against hepatotoxicants and treat hepatitis³³, and showed significant antitumor activity on human colon carcinoma cell line HCT 15³⁴.

Chlorophyll (**3**) and its various derivatives are used in traditional medicine and for therapeutic purposes³⁵. Natural chlorophyll and its derivatives have been studied for wound healing³⁶, anti-inflammatory properties³⁷, control of calcium oxalate crystals³⁸, utilization as effective agents in photodynamic cancer therapy³⁹⁻⁴¹, and chemopreventive effects in humans⁴²⁻⁴³. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided⁴⁴.

β -Sitosterol (**5a**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells⁴⁵. It was shown to be effective for the treatment of benign prostatic hyperplasia⁴⁶. It was also reported to attenuate β -catenin and PCNA expression, as well as quench the radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis⁴⁷. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake⁴⁸. It has also been reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells⁴⁹.

On the other hand, stigmasterol (**5b**) shows therapeutic efficacy against Ehrlich ascites carcinoma in mice while conferring protection against cancer induced altered physiological conditions⁵⁰. It has been reported to lower plasma cholesterol levels, inhibit intestinal cholesterol and plant sterol absorption, and suppress hepatic cholesterol and classic bile acid synthesis in Wistar and WKY rats⁵¹. In other studies, stigmasterol showed cytostatic activity against Hep-2 and McCoy cells⁵², markedly inhibited tumour promotion in two stage carcinogenesis experiments⁵³, and exhibited antimutagenic⁵⁴, topical anti-inflammatory⁵⁵, antiosteoarthritic⁵⁶ and antioxidant⁵⁷ activities.

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REFERENCES

1. Pasau / Pasau-na-haba / *Corchorus olitorius* / Jew's ... - StuartXc. Downloaded from www.stuartxchange.org/Pasau on September 21, 2016.
2. Rao EV, Rao KN, Rao DV, Polar glycosides of the seeds of *Corchorus olitorius*. Ind J Pharm 1972; 34:168.
3. Rao DV, Rao EV. Constitution of a new polar glycoside from the seeds of *Corchorus capsularin*. Ind J Chem 1972; 10:479-481.
4. Frejacque M, Durgeat M. Digitalis like poisons of jute seed. Compt Rend 1954; 238:507509.
5. Sanilova RD, Lagodich TA. Olitoriside - A glycoside from the seeds of *Corchorus olitorius*. Urech Delo 1977; 1:2731.
6. Yukihiko G, Takatoshi N, Shinobu S, Hiroshi M, Kuzunari K, Hiroshi A, Maratake T. Identification and analysis of cardiac glycosides in *Corchorus olitorius* and their acute oral toxicity to mice. Tennen Yuki Kagobutsa Taranka Koen Yashishu 1998; 40:371376.
7. Takatoshi N, Yukihiko G, Shinobu S, Kazunari K, Horoshi A, Masatake T. Cardenolide glycosides from the seeds of *Corchorus olitorius*. Phytochem 1998; 49:2097-2101.
8. Yoshikawa M, Murakami T, Shimada H, Fukada N, Matsuda H, Sachida Y, Yamahara J. Corchorosides A, B, C, D, E new cardiotoxic oligosaccharides from the seeds of *Corchorus olitorius* L. Heterocycles 1998; 48:869-873.
9. Manzoor I, Khuda M, Islain A. Chemical constituents of *Corchorus olitorius* and *Corchorus capsularis* (jute) II. Isolation of corosin and sitosterol from roots. Pak J Sci Ind Res 1971; 14:49-56.
10. Manzoor I, Khuda M, Grerhard H. Chemical constituents of *Corchorus capsularis* and *C. olitorius* (jute plant) III. Isolation of corsolic acid, ursolic acid and oxocorsin and correlation of corosin with tormentic acid. Z Naturforsch 1979; 34:1320-1325.
11. Kohda H, Tanaka S, Yamaoka Y, Moringa S, Ohara Y. Constituents of *Corchorus olitorius* L. Nat Med 1994; 48:213-214.
12. Yoshikawa M, Shimada H, Saka M, Yoshizumi S, Yamahara J, Matsuda H. Medicinal Foodstuffs. V Moroheya (1): Absolute stereostructures of corchoionosides A, B and C, histamine inhibitors from the leaves of Vietnamese *Corchorus olitorius* L. (Tiliaceae). Chem Pharm Bull 1997; 45:464469.
13. Azuma K, Nakayama K, Koshioka M, Ippoushi K, Yamaguchi Y, Kohata K, Yamauchi Y, Ito H, Higashi H. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. J Agric Food Chem 1999; 47:3963-3966.
14. Mukherjee KK, Mitra SK, Ganguli SN. A new coumarin from the seeds of jute (*Corchorus olitorius* L.). Nat Prod Sci 1998; 4:51-52.
15. Tanmoy AM, Alum MA, Islami MS, Farzana T, Khan H. Jute (*Corchorus olitorius* var. O-72) stem lignin: variation in content with age. Bangladesh J. Bot. 2014; 43(3):309-314.
16. Oboh G, Raddatz H, Henle T. Characterization of the antioxidant properties of hydrophilic and lipophilic extracts of jute (*Corchorus olitorius*) leaf. Int J Food Sci Nutr 2009; 60(Suppl 2):124-34.
17. Furumoto T, Wang R, Okazaki K, Hasan AFMF, Ali MI, Kondo A, Fukui H. Antitumor promoters in leaves

- of jute (*Corchorus capsularis* and *Corchorus olitorius*). *Food Sci Technol Res* 2002; 8(3):239-243.
18. Al Batran R, Al-Bayaty F, Abdulla MA, Al-Obaidi MM, Hajrezaei M, Hassandarvish P, Fouad M, Golbabapour S, Talaei S. Gastroprotective effects of *Corchorus olitorius* leaf extract against ethanol-induced gastric mucosal hemorrhagic lesions in rats. *J Gastroenterol Hepatol* 2013; 28(8):1321-1329.
 19. İlhan S, Savaroglu F, Çolak F. Antibacterial and antifungal activity of *Corchorus olitorius* L. (Molokhia) extracts. *Int J Nat Eng Sci* 2007; 1(3):59-61.
 20. Das AK, Sahu R, Dua TK, Bag S, Gangopadhyay M, Sinha MK, Dewanjee S. Arsenic induced myocardial injury: Protective role of *Corchorus olitorius* leaves. *Food Chem Toxicol* 2010; 48:1210-1217.
 21. Nishiumi S, Yabushita Y, Fukuda I, Mukai R, Yoshida K, Ashida H. Molokhia (*Corchorus olitorius* L.) extract suppresses transformation of the aryl hydrocarbon receptor induced by dioxins. *Food Chem Toxicol* 2006; 44:250-260.
 22. Ragasa CY, Ebajo Jr. VD, Lazaro-Llanos N, Brkljača R, Urban S. Chemical Constituents of *Hypnea nidulans* Setchell. *Der Pharma Chemica* 2015; 7(10):473-478.
 23. Sharghi H, Sarvari MH. Highly selective methodology for the direct conversion of aromatic aldehydes to glycol monoesters. *J Org Chem* 2003; 68:4096-4099.
 24. Ragasa CY, de Jesus J. Porphyrins and polyprenols from *Macaranga tanarius*. *Res J Pharm Biol Chem Sci* 2014; 5(3):701-708.
 25. Ragasa CY, Caro JL, Lirio LG, Shen C-C. Chemical Constituents of *Coix lacryma-jobi*. *Res J Pharm Biol Chem Sci* 2014; 5(6):344-348.
 26. Julien-David D, Geoffroy P, Marchioni E, Raul F, Aoud'e-Werner D, Miesch M. Synthesis of highly pure oxyphytosterols and (oxy)phytosterol esters Part II. (Oxy)-sitosterol esters derived from oleic acid and from 9,10-dihydroxystearic acid. *Steroids* 2008; 73:1098-1109.
 27. Ebajo Jr VD, Brkljača R, Urban S, Ragasa CY. Chemical constituents of *Hoya buotii* Kloppenb. *J Appl Pharm Sci* 2015; 5(11):69-72.
 28. Lee W, Yang EJ, Ku SK, Song KS, Bae JS. Anti-inflammatory effects of oleanolic acid on LPS-induced inflammation *in vitro* and *in vivo*. *Inflammation* 2013; 36(1):94-102.
 29. Yang EJ, Lee W, Ku SK, Song KS, Bae JS. Anti-inflammatory activities of oleanolic acid on HMGB1 activated HUVECs. *Food Chem Toxicol* 2012; 50(5):1288-94.
 30. Valchalkova A, Ovessa Z, Hokvathova K. Pentacyclic triterpenic acids: new chemoprotective compounds. *Neoplasma* 2004; 51(55):327-333.
 31. Astudillo L, Schemeda-Hirschmann G, Rodriguez JA. Gastroprotective activity of oleanolic acid derivatives on experimentally induced gastric lesions in rats and mice. *J Pharm Pharmacol* 2002; 54(4):583-588.
 32. Oguro T, Liu J, Klaassen CD, Yoshida T. Inhibitory effect of oleanolic acid on 12-*O*-tetradecanoylphorbol-13-acetate-induced gene expression in mouse skin. *Toxicol Sci* 1998; 45:88-95.
 33. Liu Y, Kreppel H, Liu J, Chaudhuri S, Klaassen CD. Oleanolic acid protects against cadmium hepatotoxicity by inducing metallothionein. *J Pharmacol Exp Therap* 1993; 266(1): 400-406.
 34. Li J, Guo WJ, Yang QY. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. *World J Gastroenterol* 2002; 8(3):493-495.
 35. Edwards BJ. Treatment of chronic leg ulcers with ointment containing soluble chlorophyll. *Physiother* 1954; 40:177-179.
 36. Kephart JC. Chlorophyll derivatives- their chemistry, commercial preparation and uses. *Econ Bot* 1955; 9:3-18.
 37. Larato DC, Pfao FR. Effects of a water-soluble chlorophyllin ointment on gingival inflammation. *N Y State. Dent J* 1970; 36:291-293.
 38. Tawashi R, Cousineau M, Sharkawi M. Effect of sodium copper chlorophyllin on the formation of calcium oxalate crystals in rat kidney. *Invest Urol* 1980; 18:90-92.
 39. Sternberg ED, Dolphin D, Bruckner C. Porphyrin-based photosensitizers for use in photodynamic therapy. *Tetrahedron* 1998; 54:4151-4152.
 40. Nourse WL, Parkhurst RM, Skinner WA, Jordan RT. Photodynamic toxicity of porphyrins and chlorins for a human tumor cell line: combined light and concentration dose responses for the retained fraction. *Biochem Biophys Res Commun* 1988; 151:506-511.
 41. Henderson BW, Bellnier DA, Greco WR, Sharma A, Pandry RK, Vaughan LA. An *in vivo* quantitative structure-activity relationship for a congeneric series of pyropheophorbide derivatives as photosensitizers for photodynamic therapy. *Cancer Res* 1997; 57:4000-4007.
 42. Egner PA, Munoz A, Kensler TW. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. *Mutat Res* 2003; 52(3):209-216.
 43. Egner PA, Wang JB, Zhu YR, Zhang BC, Wu Y, Zhang QN. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. *Proc Natl Acad Sci* 2001; 98(25):1401-1406.
 44. Ferruzzi MG, Blakeslee J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr Res* 2007; 27:1-12.
 45. Awad AB, Chinnman M, Fink CS, Bradford PG. Beta-sitosterol activates Fas signaling in human breast cancer cells. *Phytomed* 2007; 14:747-754.
 46. Jayaprakasha GK, Mandadi KK, Poulouse SM, Jadegoud Y, Gowda GA, Patil BS. Inhibition of colon cancer growth and antioxidant activity of bioactive compounds from *Poncirus trifoliata* (L.). *Raf. Bioorg Med Chem* 2007; 15:4923-4932.
 47. Baskar AA, Ignacimuthu S, Paulraj G, Numair K. Chemopreventive potential of β -Sitosterol in experimental colon cancer model - an *in vitro* and *in vivo* study. *BMC Comp Alt Med* 2010; 10:24.
 48. Jesch ED, Seo JM, Carr TP, Lee JY. Sitosterol reduces messenger RNA and protein expression levels of

- Niemann-Pick C1-like1 in FHs 74 Int cells. *Nutr Res* 2009; 29(12):859-66.
49. Moon DO, Kyeong JL, Yung HC, Young KG. Beta-sitosterol induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. *Int Immunopharmacol* 2007; 7:1044-1053.
50. Ghosh T, Maity TK, Singh J. Evaluation of antitumor activity of stigmasterol, a constituent isolated from *Bacopa monnieri* Linn aerial parts against Ehrlich Ascites Carcinoma in mice. *Orient Pharm Exp Med* 2011; 11:41-49.
51. 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(3):292-299.
52. Gómez MA, García MD, Sáenz MT. Cytostatic activity of *Achillea ageratum* L. *Phytother Res* 2001; 15(7):633-634.
53. Kasahara Y, Kumaki K, Katagiri S, Yasukawa K, Yamanouchi S, Takido M. Carthami flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytother Res* 1994; 8(6):327-331.
54. Lim J-C, Park JH, Budesinsky M, Kasal A, Han Y-H, Koo B-S, Lee S-I, Lee D-U. Antimutagenic constituents from the thorns of *Gleditsia sinensis*. *Chem Pharm Bull* 2005; 53(5):561-564.
55. García MD, Sáenz MT, Gómez MA, Fernández MA. Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. *Phytother Res* 1999; 13(1):78-80.
56. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthr Cartil* 2010; 18(1):106-116.
57. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoter* 2009; 80(2):123-126.