Chemical Profile of *Ficus Religiosa* (Linn.) Stem

M. S. Manorenjitha[*1], A. K. Norita[2], A. K. Adillah[3], M. Z. Asmawi[4]

[*1]Integrative Medicine Cluster, Medical and Dental Institute, University Sains Malaysia, Penang, Malaysia
[2.3]Animal Research Centre, Medical and Dental Institute, University Sains Malaysia, Penang, Malaysia
[4]School of Pharmaceutical Sciences, University Sains Malaysia, Penang, Malaysia

[*]manorenjitha@amdi.usm.edu.my

***Abstract- Ficus religiosa* is one of the medically important plants belonging to the family Moraceae. It has been used extensively by ayurvedic practitioner in India, Bangladesh and Nepal to treat various ailments such as dysentery, snakebite, skin diseases and constipation. The aim of the study is to identify the phytochemical constituents of petroleum-ether, chloroform and methanol stem extract of *F. religiosa* using GC-MS technique. Identification of chemical composition was conducted using the GC-MS equipped with mass detector supplied with helium. The major chemical compounds with highest yield from petroleum-ether, chloroform and methanol extracts were lupenol (12.41%), gamma-sitosterol (8.56%) and 1,2-Benzenediol (9.85%), respectively.**

Keywords- Ficus Religiosa; GC-MS; Lupenol; Gamma-Sitosterol; 1,2-Benzenediol

I. INTRODUCTION

In many parts of the world, the population depends on plants as their source of medicines which involves the use of plant extract or their active principles [1]. Though many herbal plants have been described for the treatment of various diseases, only a few plants have been scientifically explored.

Ficus religiosa belongs to the family *Moraceae* which consist of more than 700 species. *F. religiosa* is also commonly known as bodhi tree, sacred fig tree (English) or Pokok Ara Suci (Malay). It is found grown in shrines and buildings [2]. *F. religiosa* is a large semi-evergreen tree which could grow up to 5,000 ft [3].

All parts of *F. religiosa* are known to have medicinal properties. It has been used extensively by ayurvedic practitioner in India, Bangladesh and Nepal. Traditionally, *F. religiosa* leaves were used to treat ulcers and wounds while its root is used for stomatitis, to clean ulcers and to promote granulation. The stem bark is also used as astringent and tonic and to treat various skin diseases, ulcers, glandular swelling of the neck and scabies [4]. In Bangladesh, *F. religiosa* is reportedly used to treat various diseases such as inflammation and infectious disease [5]. *Ficus religiosa*'s uses in treating diabetes mellitus has been described by Thapa [6], who documented traditional uses of *F. religiosa* by Tharu community in Nepal. Thus, the aim of the present study is to identify the phytochemical constituents of petroleum-ether, chloroform and methanol stem extract of *F. religiosa* using GC-MS technique.

II. MATERIALS AND METHODS

A. Plant Collections

F. religiosa's stems were collected from Penang, Malaysia. The plant was identified by Tropical Forest Biodiversity Centre, Forest Research Institute Malaysia (FRIM). A voucher specimen (PID 200710-08) has been kept in the Hebarium Unit, School of Biological Sciences, University Sains Malaysia.

B. Preparation of Powder

Upon arrival at research laboratory, F. religiosa's stems were washed under running water to remove dirt. The stem was cut into small pieces before oven dried at 40 ℃. Dried samples were pulverized into powder using mechanical grinder. The powder was kept in air-tight containers and labeled accordingly.

C. Extraction of Samples

The extraction was carried out using following solvents in increasing order of polarity: petroleum-ether (40-60), chloroform and methanol. One kilogram of powdered plant material was soaked in 2 liters of petroleum ether for 3 days. The mixture was frequently stirred. On the 3rd day, the mixture was filtered and the solvent was kept in refrigerator. The remnants were re-soaked again into petroleum-ether for a second time for 3 days. Solvents obtained after the first and second filtration was combined and concentrated in rotary evaporator (Eyela, USA) under reduced pressure. The remaining remnant was soaked again using chloroform and above procedure was repeated.

DOI: 10.5963/LSMR0403001

D. GC-MS Analysis

GC-MS analysis was carried out on an Agilent system equipped with Mass Spectrometer detector and split/splitless injection system. The GC was fitted with a HP-5MS capillary column (30 m X 250 m; film thickness: 0.25 m). The temperature program was as follows: injector temperature 280 ℃, initial oven temperature at 50 ℃, then increased at 25 ℃/min to 300 ℃ and was hold for 10 mins. Helium was used as carrier gas at 17.69 psi pressure with flow 2.1 ml/min. Samples were solved in methanol and 1 µl aliquot were injected automatically. MS spectra of separated components were identified based on WILEY and NIST Libraries for botanical compounds.

III. RESULTS AND DISCUSSION

The GC-MS analysis of petroleum-ether, chloroform and methanol stem extract of *F. religiosa* showed the presence of hundreds of compounds. However, only few compounds were positively identified using WILEY and NIST Libraries with 80-99% matching (Table 1-3). GC-MS analysis of petroleum-ether extract identified 17 compounds of which Lup-20(29)-en-3-ol, acetate (3 beta) showed highest yield (Fig. 1 and Table 1). Similarly, 24 compounds out of hundreds of compounds found presence in chloroform extract were positively identified and gamma-sitosterol showed the highest composition among the identified compounds (Fig. 2 and Table 2). As for methanol extract, among the 13 known compounds, 1,2-Benzenediol were found to be with highest composition (Fig. 3 and Table 3)

Lup-20(29)-en-3-ol, acetate (3 beta) elucidated from petroleum-ether extract is also known as Lupeol or Lupenol. Lupenol is a triterpenes which are secondary metabolites [7]. It is known to exhibits various pharmacological activities such as anti-inflammatory [8, 9], anti-cancer [10] and anti-diabetic [11].

Gamma-sitosterol which was found in chloroform extract is also known as beta sitosterol or sitosterol. It has anti-cancer [12], anti-diabetic [13, 14], antioxidant [14], anti-inflammatory [15] and anti-pyretic [16] properties.

1,2-Benzenediol is an organic phenol extracted from methanol stem extracts of *F. religiosa*. It is also known as catechol or pyrocatechol. Literature survey showed catechol possesses anticancer (breast), antioxidant and pesticides properties [17].

It could be concluded that F. *religiosa* stem contains various bioactive compounds of medicinal importance which justifies the use of stem for various ailments. However, further studies are needed to evaluate its bioactivity and toxicity profile.

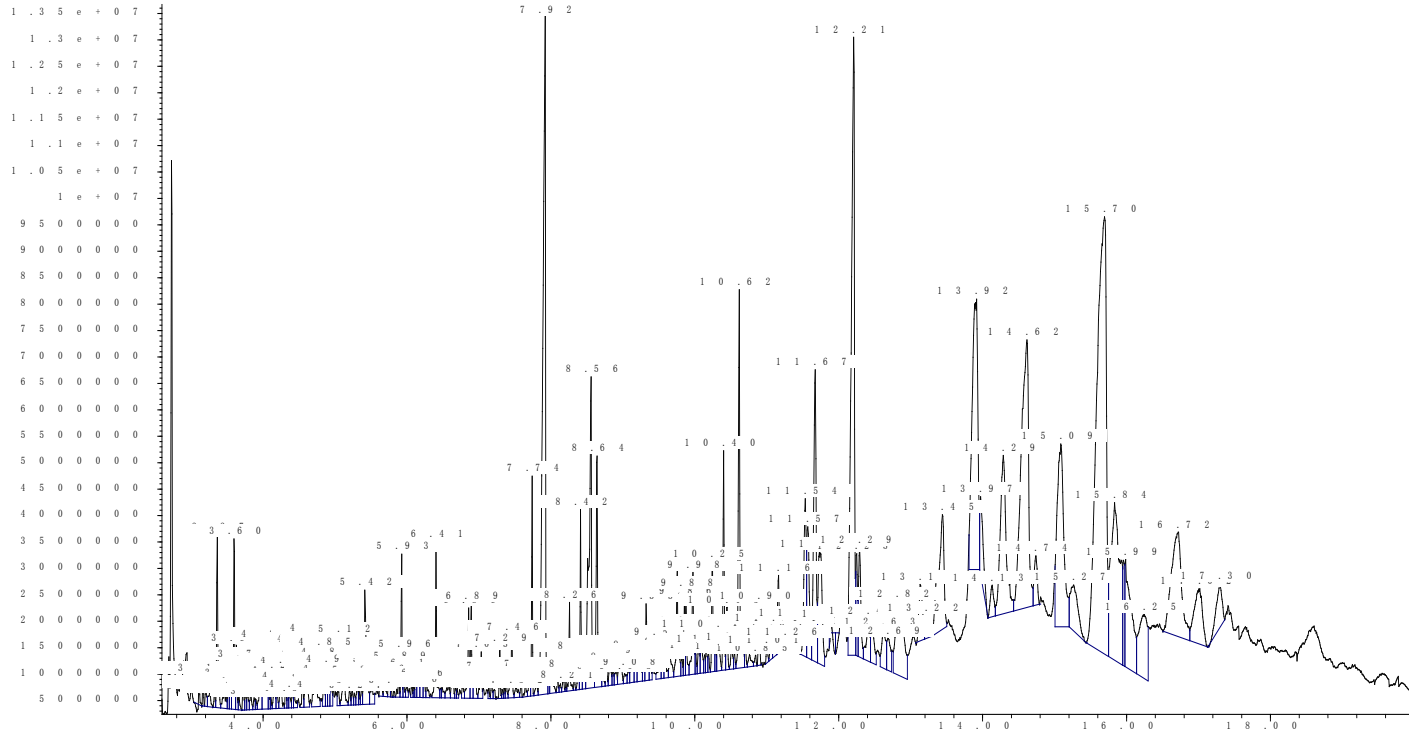

Fig. 1 GC-MS Chromatogram of petroleum-ether stem extract of *F. religiosa*

DOI: 10.5963/LSMR0403001

TABLE 1 GC-MS ANALYSIS OF PETROLEUM-ETHER STEM EXTRACT OF *F. RELIGIOSA*

No	RT (min)	Name of the Compounds	Molecular Formula	Molecular Weight	Composition (%)
1	4.085	Octanoic acid	$CH_3(CH_2)_6COOH$	144	0.14
2	4.617	Nonanoic acid	$C_9H_{18}O_2$	158	0.30
3	4.848	2,4-Decadienal (E,E)	$C_{10}H_{16}O$	152	0.21
4	4.883	Tridecane	$C_{13}H_{28}$	184	0.16
5	5.752	Hexadecane	$C_{16}H_{34}$	226	0.14
6	5.892	Phenol,2,4-bis(1,1-dimethy;ethyl)	$C_{14}H_{22}O$	206	0.12
7	5.927	Pentadecane	$C_{15}H_{32}$	212	0.40
8	7.138	Octadecane	$C_{18}H_{38}$	254	0.04
9	7.922	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.32
10	8.56	9-Octadecanoic acid(z) or oleic acid	$C_{18}H_{34}O_2$	282	2.37
11	10.898	Squalene	$C_{30}H_{50}$	410	0.26
12	11.164	Cyclotetracosane	$C_{24}H_{48}$	336	0.49
13	13.139	Campesterol	$C_{28}H_{48}O$	400	0.60
14	13.447	Lanosta-8,24-dien-3-ol(3 beta)or Lanosterol	$C_{29}H_{50}O$	426	2.01
15	13.916	Gamma sitosterol	$C_{30}H_{50}O$	414	4.67
16	14.616	Lup-20(29)-en-3-ol, acetate (3 beta) or Lupeol or Lupenol	$C_{32}H_{52}O_2$	468	5.96
17	15.695	Lup-20(29)-en-3-ol, acetate (3 beta)	$C_{32}H_{52}O_2$	468	12.41

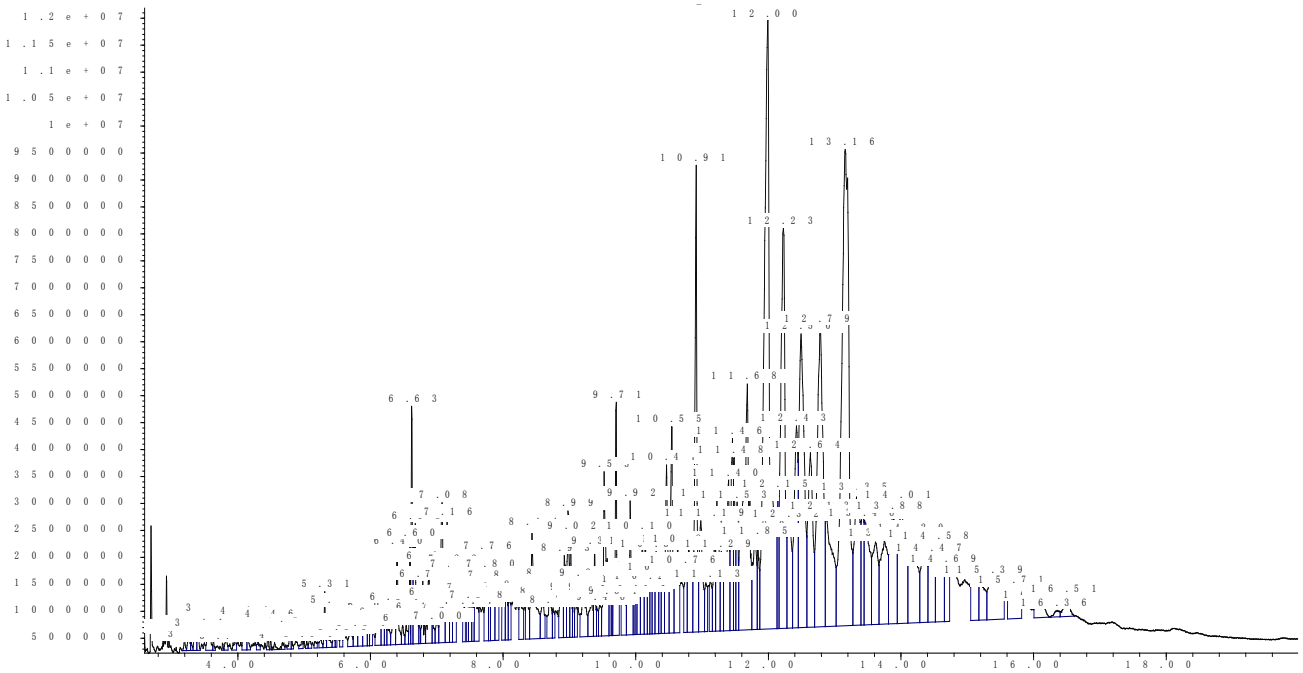

Fig. 2 GC-MS Chromatogram of chloroform stem extract of *F. religiosa*

DOI: 10.5963/LSMR0403001

TABLE 2 GC-MS ANALYSIS OF CHLOROFORM STEM EXTRACT OF *F.RELIGIOSA*

No.	RT min	Name of the Compounds	Molecular Formula	Molecular Weight	Composition (%)
1	3.567	Ethanol, 2-phenoxy	$C_8H_{10}O_2$	138	0.19
2	4.092	2,4-Decadienal (E,E)	$C_{10}H_{16}O$	152	0.20
3	4.456	1-Tetradecene	$C_{14}H_{28}$	196	0.06
4	4.786	2H-1-Benzopyran-2-one	$C_{10}H_8O_2$	160	0.07
5	5.087	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206	0.04
6	5.311	1H-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl	$C_{10}H_{10}O_3$	178	0.15
7	5.451	3-Octadecene(E)	$C_{18}H_{36}$	252	0.11
8	7.082	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.89
9	7.159	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.69
10	7.509	1-Octadecanol	$C_{18}H_{38}O$	270	0.24
11	7.754	9,12-Octadecdienoic acid(Z,Z)	$C_{18}H_{32}O_2$	280	0.53
12	8.048	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	0.26
13	8.609	Hexanedioic acid, bis(2-ethylhexyl)ester	$C_{22}H_{42}O_4$	370	0.30
14	9.064	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	$C_{16}H_{22}O_4$	278	0.14
15	9.344	Octadecanal	$C_{18}H_{36}O$	268	0.34
16	9.981	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl	$C_{30}H_{50}$	410	0.17
17	10.107	1-Hexacosanol	$C_{26}H_{54}O$	382	0.50
18	11.486	Campesterol	$C_{28}H_{48}O$	400	0.74
19	11.682	Lanosterol	$C_{29}H_{50}O$	426	3.62
20	11.998	Gamma sitosterol	$C_{30}H_{50}O$	414	8.56
21	12.229	Lanosta-8,24-dien-3-ol,acetate(3 beta)	$C_{29}H_{50}O$	426	4.00
22	12.432	9,19-Cyclolanost_24-en-3-ol (3 beta)	$C_{30}H_{50}O$	426	1.75
23	12.495	Lupeol	$C_{32}H_{52}O_2$	468	3.73
24	12.789	Lanosta-8,24-dien-3-ol,acetate(3 beta)	$C_{29}H_{50}O$	426	4.17

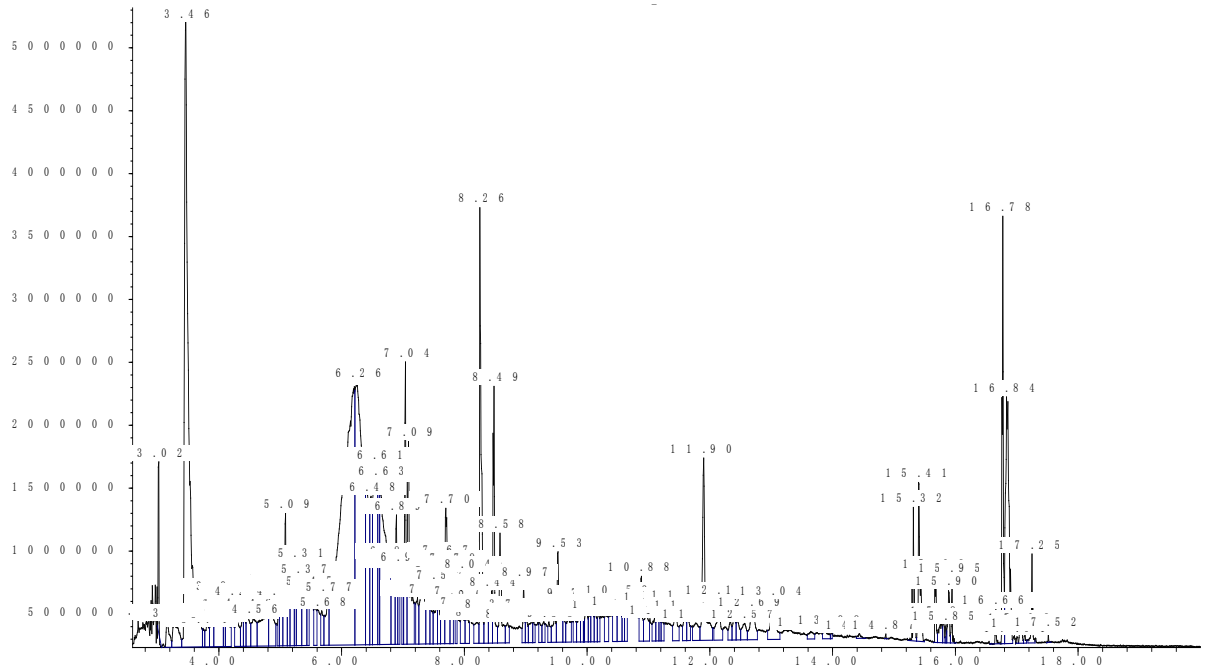

Fig. 3 GC-MS Chromatogram of methanol stem extract of *F. religiosa*

DOI: 10.5963/LSMR0403001

TABLE 3 GC-MS ANALYSIS OF METHANOL STEM EXTRACT OF *F.RELIGIOSA*

No	RT (min)	Name of the Compounds	Molecular Formula	Molecular Weight	Composition (%)
1	3.16	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	0.30
2	3.462	1,2-Benzenediol	$C_6H_6O_2$	110	9.54
3	4.288	Phenol,2,6-dimethoxy	$C_8H_{10}O_3$	154	0.47
4	4.827	Methylparaben	$C_8H_8O_3$	152	0.80
5	5.087	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206	0.95
6	6.634	Caffeine	$C_8H_{10}N_4O_2$	194	4.07
7	6.893	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.73
8	6.991	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.38
9	7.789	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.50
10	11.444	Ergost-5-en-3-ol(3 beta)	$C_{28}H_{48}O$	400	0.45
11	11.598	Stigmasterol	$C_{29}H_{48}O$	412	0.32
12	11.899	Stigmasterol,22,23-dihydro	$C_{29}H_{50}O$	414	1.76
13	12.152	Lanosta-8,24-dien-3-ol, acetate(3 beta)	$C_{29}H_{50}O$	426	0.57

ACKNOWLDEGMENT

This study was financially supported by Universiti Sains Malaysia's USM Short Term Grant (304/CIPPT/6310058). Authors would like to thank Mr. R. Tannimalay and Mr Packirisamy for their kind assistance in completion of this project.

REFERENCES

[1] N. R. Fransworth, O. Akerele, A. S. Bingel, D. D. Soejarta and Z. Guo, "Medicinal plant in therapy," Bulletin of the World Health Organization 63, pp. 965-981, 1985.

[2] R. M. Kunwar and R. W. Bussmann, "*Ficus* (Fig) Species in Nepal: A Review of Diversity and Indigenous Uses," Lyonia 11, pp. 85-97, 2009.

[3] F. Starr, K. Starr and L. L. Loope, *Ficus Religiosa*, United States Geological survey- Biological Resources Division, Haleakala Field Station, Maui, Hawai'i. pp. 1-4, 2003.

[4] N. Nayeem, R. M. Rohini, S. M. B. Asdaq and A. K. Das, "Wound healing activity of the hydro alcoholic extract of *Ficus religiosa* leaves in rats," 2009. (available online: 17 Oct 2012, 17:40)
http://www.ispub.com/.../wound_healing_activity_of_the_hydro_alcoholic_extract_officus_religiosa_leaves_in_rats.html

[5] S. J. Uddin, I. D. Grice and E. Tiralongo, "Cytotoxic effects of Bangladeshi medicinal plant extracts," Evidence Based Complement Alternative Medicine, 2011.

[6] S. Thapa, *Documentation of traditional uses of plants by Tharu community around Royal Sukla-Phanta Wildlife Reserve, Far west Nepal*, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, 2001.

[7] M. B. C. Gallo and M. J. Sarachine, "Biological Activities of Lupeol," *International Journal of Biomedicals and Pharmaceutical Sciences*, vol. 3(Special Issue 1), pp. 46-66, 2009.

[8] A. Fernandez, A. Alvarez, M. D. Garcá, M. T. Saenz, "Anti-inflammatory effect of Pimenta racemosa var. ozua and isolation of the triterpene lupeol," *Farmaco*, vol. 56, pp. 335-338, 2001.

[9] J. F. Vasconcelos, M. M. Teixeira, J. M. Barbosa-Filho, A. S. Lúcio, J. R. Almeida, L. P. de Queiroz, "The triterpenoid lupeol attenuates allergic airway inflammation in a murine model," *Int Immunopharmacol*, vol. 8, pp. 1216-1221, 2008.

[10] P. G. Bradford and A. B. Awad, "Phytosterols as anticancer compounds," *Mol Nutr Food Res*, vol. 51, pp. 161-170, 2007.

[11] R. Gupta, A. K. Sharma, M. C. Sharma, M. P. Dobhal and R. S. Gupta, "Evaluation of antidiabetic and antioxidant potential of lupeol in experimental hyperglycaemia," *Natural Product Res*, vol. 26(12), pp. 1125-1129, 2012.

[12] A. A. Baskar, S. Ignacimuthu, G. B. Paulraj and K. S. A. Numair, "Chemopreventive potential of β-Sitosterol in experimental colon cancer model - an In vitro and In vivo study," *BioMedCentral Complementary and Alternative Medicine*, vol. 24, pp. 1-10, 2010. (doi:10.1186/1472-6882-10-24)

[13] M. D. Ivorra, M. Paya and A. Villar, "A review of natural products and plants as potential antidiabetic drugs," *Journal of Ethnopharmacology*, vol. 27, pp. 243-275, 1989.

[14] R. Gupta, A. K. Sharma, M. P. Dobhal, M. C. Sharma and R. S. Gupta, "Antidiabetic and antioxidant potential of β-Sitosterol in streptozotocin-induced experimental hyperglycemia," *Jourmal of Diabetes*, vol. 3(1), pp. 29-37, 2011.

DOI: 10.5963/LSMR0403001

[15] S. Loizou, I. Lekakis, G. P. Chrousos and P. Moutsatsou, "Beto-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells," *Mol Nutr Food Res*., vol. 54(4), pp. 551-558, 2010.

[16] M. B. Gupta, R. Nath, N. Srivastava, K. Shanker, K. Kishor and K. P. Bhargava, "Anti-inflammatory and antipyretic activities of β-Sitosterol," *Planta Medica*, vol. 39(6), pp. 157-163, 1980.

[17] M. S. Manorenjitha, A. K. Norita, S. Norhisham and M. Z. Asmawi, "Gc-ms analysis of bioactive components of *Ficus religiosa* (Linn.) stem," *International Journal of Pharna and Bio Sciences*, vol. 4(2), pp. 99-103, 2012. (available online: 21Oct 2012) http://www.ars-grin.gov/cgi-bin/duke/chem-activities.pl

DOI: 10.5963/LSMR0403001