World Academy of Science, Engineering and Technology
International Journal of Pharmacological and Pharmaceutical Sciences
Vol:6, No:9, 2012

Agrowaste: Phytosterol from Durian Seed

D. Mohd Nazrul Hisham, J. Mohd Lip, R. Suri, H. Mohamed Shafit, Z.Kharis, K. Shazlin, A. Normah, M.F. Nurul Nabilah

***Abstract*—**Presence of phytosterol compound in Durian seed (Durio zibethinus) or known as King of fruits has been discovered from screening work using reagent test. Further analysis work has been carried out using mass spectrometer in order to support the priliminary finding. Isolation and purification of the major phytosterol has been carried out using an open column chromatography. The separation was monitored using thin layer chromatography (TLC). Major isolated compounds and purified phytosterol were identified using mass spectrometer and nuclear magnetic resonance (NMR). This novel finding could promote utilization of durian seeds as a functional ingredient in food products through production of standardized extract based on phytosterol content.

***Keywords*—**Agrowaste, durian, seed, phytosterol

I. Introduction

DURIAN (*Durio zibethinus Murr*.) is a seasonal fruit grown in South East Asia. It belongs to Bombaceae family, is one of the most well-known tropical fruits in Southeast Asia [1]. Complete morphological details have been given elsewhere [2,3]. Durian cultivars are derived from *D. zibet*hinus, originating in the Malay Peninsula [4]. The fruit can grow as large as 30 cm long and 15 cm in diameter and it typically weighs 1 to 3 kg. Its shape ranges from oblong to round, the color of its husk is green to brown, and its flesh pale is yellow to red. The availability of durian is from the middle of May to the end of July.

D. Mohd Nazrul Hisham. Author is with the Food Technology Research Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89437505; fax: 03-89422906; e-mail: nazrul@mardi.gov.my).

J. Mohd Lip. Author is with Laboratory of Agro Chemistry & Food, Technical Service Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89438068; fax: 03-89451408 ; e-mail: alip@mardi.gov.my).

R. Suri. Author is with the Food Technology Research Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89437743; fax: 03-89422906; e-mail: suri@mardi.gov.my)

H. Mohamed Shafit. Author is with Food Technology Research Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89437382; fax: 03-89422906; e-mail: shafit@mardi.gov.my)

Z. Kharis. Author is with Food Technology Research Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89436909; fax: 03-89422906; e-mail: hariss@mardi.gov.my)

K. Shazlin. Author is with Food Technology Research Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89437743; fax: 03-89422906; e-mail: shazlin@mardi.gov.my)

A. Normah. Author is with Promotion & Technology Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89437802 fax: 03-89429677; e-mail: noraha@mardi.gov.my).

M.F. Nurul Nabilah. Author is with Food Technology Research Centre, Malaysian Agricultural Research & Development Institute, 43400 Serdang Selangor. Malaysia. (phone: 03-89437022 fax: 03-89422906;e-mail: nabilah@mardi.gov.my).

Durian is normally eaten fresh. Only one-third of durian is edible, whereas the seeds (20–25%) and the shell are usually thrown away. The durian consisted of 45% skin, 32% flesh and 23% seed [5,6]. In Malaysia, from 2008 till 2010 the average waste generated from the durian industry is about 90141 metric tones annually [7]. This larger number of agro-waste could create disposal problems in nationwide. Exploitation of nutraceuticals and health benefits especially on durian seed needs to be carried out seriously. The phytochemical aspect need to be emphasized in order to developed usefulness or utilizing it from waste to wealth.

In 2003, the FDA approved that "foods containing at least 0.4 gram per serving of plant sterols, eaten twice a day with meals for a daily total intake of at least 0.8 gram, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease." The beneficial effects of plant sterols have been demonstrated in numerous studies. The FDA allowed companies to add phytosterols to processed foods and to label it as healthy food for the heart. Phytosterols are natural components of the human diet found as minor components (0.1±0.5%, w/w) in edible vegetable oils [8]. Dietary intake of phytosterols in northern Europe has been estimated to be 200±300 mg/day [9], with higher intakes (300±450 mg/day) in vegetarians and Japanese [10, 11]. Phytosterols have been shown to reduce plasma cholesterol by blocking the absorption of cholesterol from the gut [12,13,14] and thus can have a beneficial effect on health by reducing the risk of developing artherosclerosis and coronary heart disease [14].

Preliminary phytochemical screening on methanol extracts of *D. zibethinus* seed using spray reagents has showed present of phytosterol. The aim of this study is to isolate, purify and analyse the isolated phytosterol using modern spectroscopy such as nuclear magnetic resonance and mass analyser.

II. Procedure

A. Sample preparation

Sample *Durio zibethinus* was collected from Malaysian local market. One kg of fresh *D. zibethinus* seeds were ground to 2.0 mm particle size.

B. Preparation Methanol Extract

The fresh *D. zibethinus* seeds that have been ground to 2.0 mm particle size were soaked in methanol (MeOH) for 4 days at room temperature after which the extract was decanted. The material was added with fresh methanol and the same extraction procedure was repeated twice. The extracts collected from each soaking were pooled and concentrated to dryness using rotary evaporator, yielded 80g of crude extract.

C. Isolation of phytosterol

The crude methanolic extract of *D. zibethinus* seeds was then resuspended in distilled water and solvent partitioned into

hexane, dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc). Hexane fraction (10.0g) was first subjected to normal phase column chromatography (CC) according to previous study by [15] with modification of solvent system. Elution started with hexane followed by mixtures of hexane and chloroform of increasing polarity to yield a total of ten fractions. Based on similar TLC patterns, fractions 5 to 8 (7.0g) were combined and subjected to normal phase open column chromatography using hexane as mobile phase, followed by mixtures of hexane and chloroform of increasing polarity to yield 300 mg of compound 1 and 60 mg of compound 2.

D. Mass analyser

Mass Spectra were recorded on a Shimadzu GC-17A, Gas Chromatography mass Spectrometer (GCMS) with a single quadrupole mass spectrometer. The GCMS was equipped with direct injection probe to run pure samples without pass through the capillary column. Pure isolated compound was subjected to mass spectroscopy analysis and the mass spectrum will be elucidated to support the NMR analysis data for determination of chemical structure.

E. Nuclear Magnetic Resonance (NMR) analyser

All ^{1}H and ^{13}C NMR as well as the two dimensional experiments Heteronuclear Multiple Bond Correlation (HMBC) were recorded on a Varian Unity Inova (500 MHz) equipped with pulsed field gradients (PFG), using an indirect detection probe. Deuterated chloroform was used and chemical shifts (δ and δC) were given in ppm.

III. Results and Discussion

A. The important of phytosterol

In year of 2003, FDA (Food and Drug Administration) allowed companies to add phytosterols to the processed foods and label them as healthy for the heart. To date the plant sterols have widely used for food applications in order to add value of their products. Food product such as margarine, mayonnaise, yogurt, milk, cheese, chocolate and many more has been incorporated with natural phytosterols.

B. Characterisation of compound isolated as phytosterol

Compound 1 was isolated from repeated normal phase column chromatography as white crystal. This compound was subjected to mass analyser (GCMS DI-probe) and NMR spectroscopy for confirmation of the chemical structure.

The mass spectrum of the compound 1 in Figure 3a showed a molecular ion peak [M+] at m/z 414 which corresponded to molecular formula $C_{29}H_{50}O$. Mass spectrum of compound 1, indicated the presence of several characteristic fragments at m/z 399, 396, 381, 329 and 303 which commonly found in mass spectrum of β-Sitosterol [16]. The two later fragments, m/z 329 and 303 were known as characteristic of sterol with Δ5 unsaturation [17,18,19]. The presence of fragment at m/z 399 is due to the loss of a methyl group from molecular ion and further hydrolysis reaction could lead to the formation of fragment at m/z 381.

Hydrolysis reaction from fragment at m/z 414 were resulted the presence of fragment at m/z 396 and further losses of methyl group yielded the fragment at m/z 381.

The ^{1}H spectrum (Figure 1) showed the presence of six methyl groups of a stigmastane carbon skeleton, identical to β-sitosterol which appeared at δH 0.68 (3H, s, C-18), 0.81(3H,m, H-27),.0.83 (3H,m, H-26), 0.84(3H, m, H-29), 0.92 (3H, d , J = 6.5, C-21) and 1.01(3H, s , C-19) ppm. Olefinic proton at δH 5.35 (1H, m, H-6). The deshielding effect of protons at δH 3.53 ppm (1H, m) (H-3) indicated, they were bonded to oxygenated tertiary carbon. Several signals from methylene and methane groups were found each other overlapping from δH1.10 to δH 2.40 in Figure 1.

The ^{13}C spectrum (Figure 2) displayed 29 carbon signals including six methyls. Through inspection of HSQC spectrum of compound 1, led to the assignments of proton signal of the methyl groups to their carbon signals in Table 1. Similarity of the above ^{13}C and ^{1}H spectral data to those of published data [20,21] identified compound 1 as β-sitosterol.

The mass spectrum of the compound 2 in Figure 3b showed a molecular ion peak [M+] at m/z 412 which corresponded to molecular formula $C_{29}H_{48}O$. The ^{1}HNMR spectrum showed the similar proton spectrum except the present of olefinic protons at δH 5.01 ppm (1H each, dd, J = 15.5 Hz, 8.5 Hz) and 5.15 ppm (1H each, dd, J = 15 Hz, 8.5 Hz) represented for H-22 and H-23 side chain. In the carbon ^{13}C spectrum displayed 29 carbon signals including six methyls. The present of C-22 and C-23 were clearly observed in the carbon spectrum.

The ^{13}C and ^{1}H spectral data in Table 2 were compared to those of published data [20,21] and compound 2 was found identical to stigmasterol.

TABLE I
^{1}HNMR AND ^{13}C NMR DATA OF B-SITOSTEROL (**1**)

β-Sitosterol (**1**)					
Carbon No.	δC	δH	Carbon No.	δC	δH
5	140.75		8	31.90	
6	121.73	5.35 (1H, *m*)	2	31.66	
3	71.81	3.50(1H, *m*)	25	29.14	
14	56.76		16	28.26	
17	56.04		23	26.05	
9	50.12		15	24.31	
24	45.83		28	23.06	
13	42.30		11	21.08	
4	42.21		26	19.83	0.83, *m*, 3H
12	39.77		19	19.40	1.01, s, 3H
1	37.25		27	19.03	0.81, *m*, 3H
10	36.50		21	18.78	0.92, 3H. *d*, *J* = 6.5 Hz
20	36.15		29	11.98	0.84, *m*, 3H
22	33.94		18	11.86	0.68, 3H, *s*,
7	31.90				

Measured in $CDCl_3$(500 MHz)

TABLE II
^{1}HNMR AND ^{13}C NMR DATA OF STIGMASTEROL (**2**)

Stigmasterol (**2**)					
Carbon No.	δC	δH	Carbon No.	δC	δH
5	140.75		7	31.90	
22	138.33	5.01(1H, *dd*, *J* = 15.5 Hz, 8.5 Hz)	8	31.90	
23	129.26	5.15 (1H, *dd*, *J* = 15 Hz, 8.5 Hz)	25	31.90	
6	121.73	5.35 (1H, *m*)	2	31.66	
3	71.81	3.50(1H, *m*)	16	28.93	
14	56.86		28	25.42	
17	55.94		15	24.37	
24	51.24		26	21.22	0.83 (3H, *m*)
9	50.12		11	21.08	
13	42.30		21	21.08	0.92, 3H. *d*, *J* = 6.5 Hz
4	42.30		19	19.40	1.01(3H, *s*)
20	40.51		27	18.98	0.81 (3H, *m*)
12	39.68		29	12.26	0.84 (3H, *m*)
1	37.25		18	12.05	0.70 (1H, *s*)
10	36.50				

Measured in $CDCl_3$(500 MHz)

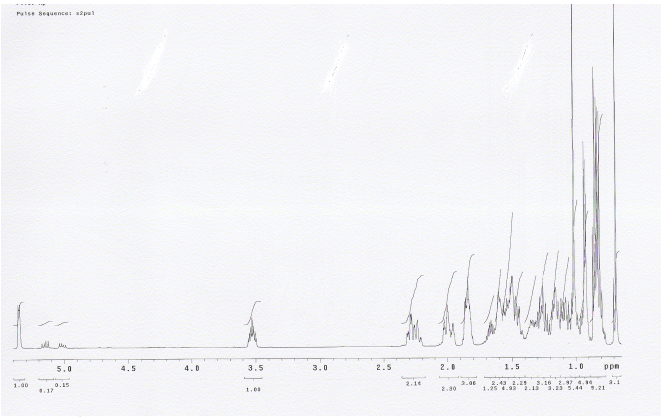

Fig. 1 ^{1}HNMR spectrum of β-Sitosterol (1) with proton integration

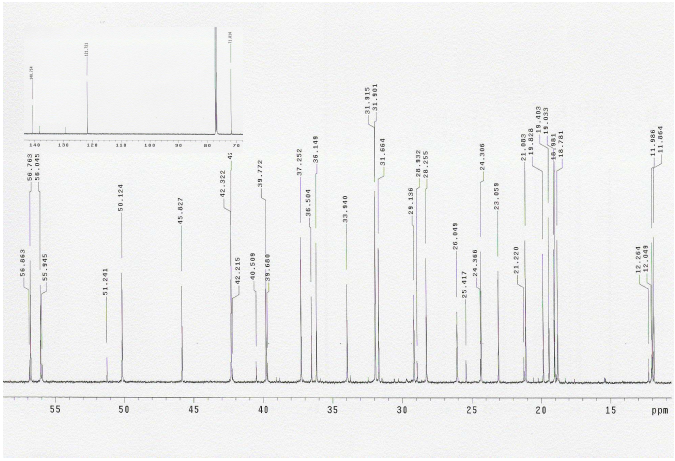

Fig. 2 ^{13}C NMR spectrum of β-Sitosterol (1)

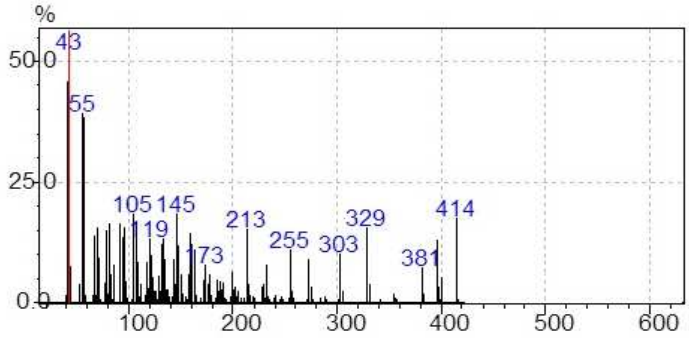

Fig. 3a Mass spectrum of β-sitosterol (1)

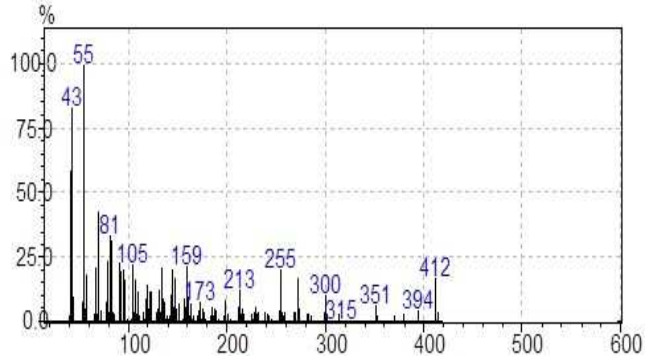

Fig. 3b Mass spectrum of stigmasterol (2)

ACKNOWLEDGMENT

This research was sponsored by the Malaysian Agriculture Research and Development Institute (MARDI).

REFERENCES

[1] Chin, S.T., Nazimah, S.A.H., Quek, S.Y., Che Man, Y.B., Abdul Rahman, R.. and Mat Hashim, D., 2007. Analysis of volatile compounds from Malaysian durians (Durio zibethinus) using headspace SPME coupled to fast GC-MS. Journal of Food Composition and Analysis 20, 31–44

[2] Wong, K.C., Tie, D.Y., 1995. Volatile constituents of durian (Durio zibethinus Murr.). Journal of Flavour and Fragrance 10, 79–83.

[3] Moser, R., Duvel, D., Greve, R., 1980. Volatile constituents and fatty acid composition of lipids in Durio zibethinus. Phytochemistry 19, 79–81.

[4] Voon, Y. Y., Hamid, A. N. S., Rusul, G., Osman, A., and Quek, S. Y., 2007. Characterization of Malaysian durian (Durio zibethinus Murr.) cultivars: Relationship of physicochemical and flavour properties with sensory properties. Journal of Food Chemistry, 103, 1217−1227.

[5] Michael J Brown 1997. Durio- Bibliography Review. p84

[6] Ng, K.S. and S. Thamboo. 1967. Nutrient removal studies on Malayan fruits. Durian and rambutan. Malaysian Agricultural Journal 46: 164-182..

[7] Anuar Abdullah Mohd Fakhrul Zaman Omar, Zainuddin Zakaria, Hong Ding Long and Abdul Ghani Abdullah. Durian Skin Machine. 6th MARDI Science & Technology Exhibition 2011, p75

[8] S.P. Kochhar Influence of processing on sterols of vegetable oils. Progress in Lipid Research, 22 (1983), pp. 161–188

[9] Morton, G.M., Lee, S.M., Buss, D.H., Lawrance, P., 1995. Intakes and major dietary sources of cholesterol and phytosterols in the British diet. J. Hum. Nutr. Diet. 8, 429–440.

[10] Nair, K.R., Manji, F. and Gitonga, J.N. 1984 The occurrence and distribution of fluoride in groundwaters in Kenya. In: Challenges in African Hydrology and Water Resources, Proceedings of Harare Symposium, IAHS Publ. 144, 75-86.

[11] Nakashima, I. Ikeda, K. Fuchigami, Y. Shiroishi, M. Sugano (1981).The compositions of regular meals in university refectory—with special reference to the content of sterols and dietary fiber Journal of Clinical Nutrition (Rinsho Eiyo), 58 (1981), pp. 261–268

[12] Mattson, 1977F.H. Mattson, F.A. Volpenheim, B.A. Erickson Effect of plant sterol esters on the absorption of dietary cholesterol. Journal of Nutrition, 107 (1977), pp. 1139–1146.
[13] Sugano, Morioka, & Ikeda, 1977M. Sugano, H. Morioka, I. Ikeda. A comparison of the hypocholesteremic activity of β-sitosterol and β-sitostanol in rats. Journal of Nutrition, 107 (1977), pp. 2011–2019
[14] Martin, Hulley, Browner, Kuller, & Wentworth, 1986M.J. Martin, S.B. Hulley, W.S. Browner, L.H. Kuller, D. Wentworth. Serum cholesterol, blood pressure and mortality: implications from a cohort of 361,662 men.Lancet, ii (1986), pp. 933–936
[15] Mohd Nazrul Hisham Daud, Mohd Lip Jabit, Arif Zaidi Jusoh and Normah Ahamad 2010. Main non-polar chemical constituent from Morinda citrifolia fruits. Journal of Tropical Agriculture and Food Science 38(1) 97-102
[16] Pateh, U. U., Haruna A. K., Garba, M., Iliya, I., Sule, I. M., Abubakar, M. S. and Ambi A.A., 2009. Isolation of stigmasterol, β-sitosterol and 2-hydroxyhexadecanoic acid methyl ester from the rhizomes of Stylochiton lancifolius pyer and Kotchy (araceae). Nig. Journ. Pharm. Sci. 8 (1), 19 – 25.
[17] Naqvi, S.H.M., 1973. Chemical synthesis and mass spectrometric characterization of some C-27 steroids. Steroids, 285-292.
[18] Brooks, C. J. W., Henderson, W., and Steel, G., 1973. The use of trimethylsilyl ethers in the characterization of natural sterols and steroids. Characterization of natural sterols and steroid diols by gas chromatography-mass spectrometry. Biochim. Biophys. Acta296, 431-445
[19] Knights, B. A., 1967. Identification of plant sterols using combined GLC/mass spectrometry. J. Gas Chromatogr. 5, 273-282.
[20] De-Eknamkul,W. and Potduang, B., 2003. Biosynthesis of β-sitosterol and stigmasterol in Croton sublyratus proceeds via a mixed origin of isoprene units. Phytochemistry 62, 389–398.
[21] Anjoo Kamboj and Ajay Kumar Saluja 2011. Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae). Journal of Pharmacy and Pharmaceutical Sciences 3 (1) 94-96. 4, pp. 8–16.