

## Preliminary study of steroids in *Sericocalyx schomburgkii* (Craib) Bremek by GC-MS

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### Abstract

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*Sericocalyx schomburgkii* (Craib) Bremek is a traditional Thai herb used for osteoarthritis pain relief. A preliminary examination for the steroid components from three different parts of this plant, i.e. wood, leaf and root, was performed using gas chromatography-mass spectrometry (GC-MS) with electron impact (EI). Dried samples of the three plant parts were ground to fine powder and soaked in hexane for the extraction of their steroid components. Thirteen steroids were identified, 10 of them in the wood, 11 in the leaf and 8 in the root extracts.

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**Key words :** *Sericocalyx schomburgkii*, steroids, osteoarthritis, GC-MS

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Steroids are fat-soluble organic compounds found naturally in living organisms. They play a crucial role as essential hormones in both plants and animals. Plants produce numerous steroids and sterols (steroid alcohols), some of which are recognized as hormones in animals (Geuns, 1978, Jones and Roddick, 1988). Plant steroids and sterols are found in wood pulp, leaves, nuts, vegetable oils, corn, rice, and other plant sources.

The cholesterol-lowering potential of dietary plant sterols has been known for over 50 years (Pollack, 1953). Although people consume plant sterols every day in food, the amounts are often not high enough to have significant blood cholesterol-lowering effect. The major plant sterol is sitosterol (approx. 80%). Others include campesterol and stigmasterol. Dietary intake of plant sterols in a traditional Asian diet is 350-400 mg/day, and 600-800 mg/day in a strict vegetarian diet (National heart foundation of Australia, 2003). Previous studies have established that only one of the sterols in ester form can result in a statistically significant reduction in cholesterol (Hendriks *et al.*, 1999; Cater, 2000). Recent studies of plant sterols and esters in humans have shown that maximum cholesterol-lowering benefits are achieved at the doses of 2-3 g per day (Miettinen *et al.*, 1995; Gylling and Miettinen, 1999; Hendriks *et al.*, 1999; Jones *et al.*, 1999; Hallikainen *et al.*, 2000; Jones *et al.*, 2000; Maki *et al.*, 2001).

Plant sterols are similar in structure to cholesterol. The difference is the presence of a methyl or ethyl group in their side chains. The structural similarity of plant sterols and stanols to cholesterol enables them to compete with cholesterol for incorporation into micelles, the particles that transport lipids and cholesterol into the intestinal mucosa. This competition reduces dietary and biliary cholesterol absorption in the gastrointestinal tract (Clifton, 2002; Lichtenstein, 2002). Decreased cholesterol absorption up-regulates LDL-receptor concentration (Ostlund, 2002) and therefore decreases LDL serum levels. In response to decreased absorption, the liver and other tissues increase cholesterol synthesis, but

this effect is less significant than that of inhibited absorption (Clifton, 2002; Lichtenstein, 2002; Vanstone *et al.*, 2002). The overall effect is a reduction in LDL and total cholesterol levels without effects on HDL and triglyceride levels (Anonymous, 2000; Law, 2000; Ostlund, 2002).

There are many kinds of herb in the northern areas of Thailand, one of them is *Sericocalyx schomburgkii* (Craib) Bremek, known in Thai as Kha-Gai, a traditional herb used for relieving osteoarthritic pain (Watcharadul, 1986; Pootakham, 1996). Many researchers have suggested that active constituents found in the herbs are steroids, but no information on pharmaceutical compounds of *Sericocalyx schomburgkii* is available. High-performance liquid chromatography (HPLC) and gas chromatography (GC) methods have been used to analyze the steroids from the non-saponifiable portion of liquid extracts from biological samples and foodstuffs (Volin, 2001). Gas chromatography-mass spectrometry (GC-MS) system has also been used for steroid analysis (Hertmann, 1973; Gaillard *et al.*, 1999; Schroepfer, 2000). The aim of this research was to examine steroid components in three different parts of *Sericocalyx schomburgkii* i.e. wood, leaves, and root, by gas chromatography-mass spectrometry (GC-MS).

## Materials and Methods

### Samples and sample preparation

*Sericocalyx schomburgkii* samples were collected from Doi Intanon National park during October 2003 and February 2004. The samples were oven dried and ground to fine power. Five grams each of dried samples (wood, leaves, and root) were soaked in 100 mL hexane for 1 h to extract the steroid components using the maceration method (Pootakham, 1996). The extracts were filtered through filter paper and evaporated under vacuum to remove the solvent. The evaporated extracts were diluted to 5 mL with chloroform and filtered through cellulose acetate syringe membrane filters before chromatographic analysis.

### Gas chromatography-mass spectrometry procedure

Gas chromatography-mass spectrometry was performed using a gas chromatograph-mass spectrograph (GC-MS) (Agilent 6890/Hewlett-Packard 5975) fitted with electron impact (EI) mode. The analytical column was HP-1MS (Agilent 6890/Hewlett-Packard 5975) capillary column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature was programmed at 80°C for 5 min then increased to 300°C at the rate of 15°C/min. The temperatures of injector and EI detector (70eV) were 280°C and 300°C, respectively. Each plant extract of 2.0 µL was injected with a Hamilton syringe to the GC-MS manually.

### Results and Discussion

The chromatograms of steroidal compounds of the three different parts of *Sericocalyx schomburgkii* are shown in Figure 1. Mass spectra were used to identify the structure of the steroids found, comparing with those in NIST (National Institute of Standards and Technology) library. The plant extracts appeared to have thirteen steroids, as shown in Table 1, ten of them in the wood,

eleven in the leaf and eight in the root extracts, respectively.

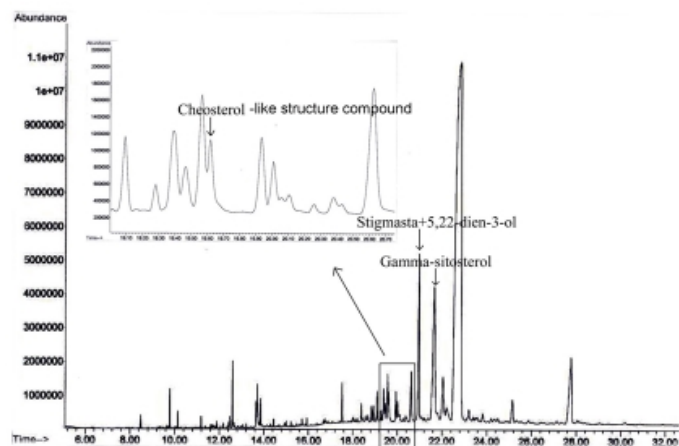
Seven steroids were common to all extracts from this herb, i.e. stigmastan-3-5-dien, campesterol, stigmasta-5, 22-dien-3-ol, sitosterol, stigmasterolen-3-one, beta-amyrin, and a cholesterol-like compound. Three of them, i.e. sitosterol, stigmasterol, and campesterol, are the most common plant sterols. They have been classified as the Generally Recognized As Safe (GRAS) food-grade substances, as a history of intake has not demonstrated any harmful effects to health. The U.S. Food and Drug Administration (FDA) has approved the following health claims for plant stanol (hydrogenated sterol)/sterol esters: Diets low in saturated fat and cholesterol that include at least 1.3 grams of plant sterol esters or 3.4 grams of plant stanol esters, consumed in 2 meals with other foods, may reduce the risk of heart disease. In addition to and in the presence of the health claim, products with plant stanol/sterol esters may also state that they can reduce cholesterol levels. Recently, the FDA has indicated they will consider expanding the use of the health claim for a broader range of eligible food products comprised of free (unesterified) stanols and sterols (FDA, 2000; FDA,

**Table 1. Thirteen steroids found in three different parts of *Sericocalyx schomburgkii*.**

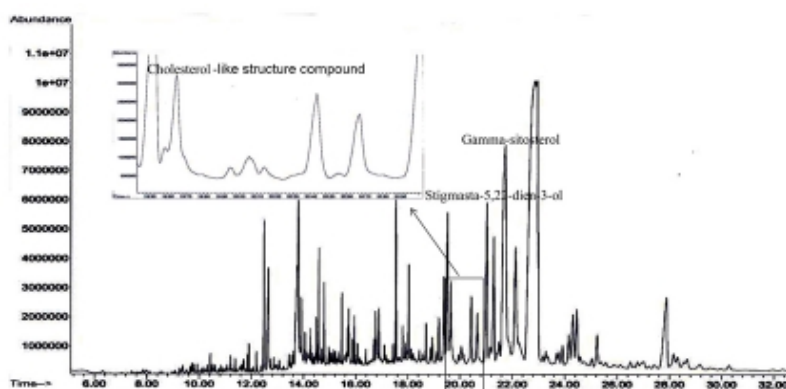
Steroid	MW <sup>a</sup>	Quality <sup>b</sup> (%)	Retention Time (min)		
			Wood	Leaf	Root
1. Stigmastan-3,5,22-trien (C <sub>29</sub> H <sub>46</sub> )	394	90	19.10	-	-
2. Stigmastan-3-5 dien (C <sub>29</sub> H <sub>48</sub> )	396	98	19.57	19.58	19.56
3. Cholesterol-like compound (C <sub>27</sub> H <sub>46</sub> O)	386	99	19.62	19.65	19.62
4. Campesterol (C <sub>28</sub> H <sub>48</sub> O)	400	99	20.63	20.67	20.67
5. Stigmasta-5,22-dien-3-ol (C <sub>29</sub> H <sub>48</sub> O)	412	99	20.98	21.03	21.04
6. Gamma-sitosterol (C <sub>29</sub> H <sub>50</sub> O)	414	99	21.66	21.74	21.73
7. Stigmasterol-4-en-3-one (C <sub>29</sub> H <sub>48</sub> O)	412	94	23.21	23.28	23.68
8. Beta-Amyrin (C <sub>30</sub> H <sub>50</sub> O)	426	96	22.04	22.13	22.08
9. Taraxasterol (C <sub>30</sub> H <sub>50</sub> O)	426	86	22.76	-	-
10. Lupeol (C <sub>30</sub> H <sub>50</sub> O)	426	87	-	22.89	-
11. Fern-7-en.beta-ol (C <sub>30</sub> H <sub>50</sub> O)	426	90	-	23.00	-
12. Friedelin (C <sub>30</sub> H <sub>50</sub> O)	426	99	-	24.46	-
13. Betulin (C <sub>30</sub> H <sub>50</sub> O <sub>2</sub> )	442	97	27.76	27.88	27.67

<sup>a</sup> Molecular weight from GC-MS (EI) data

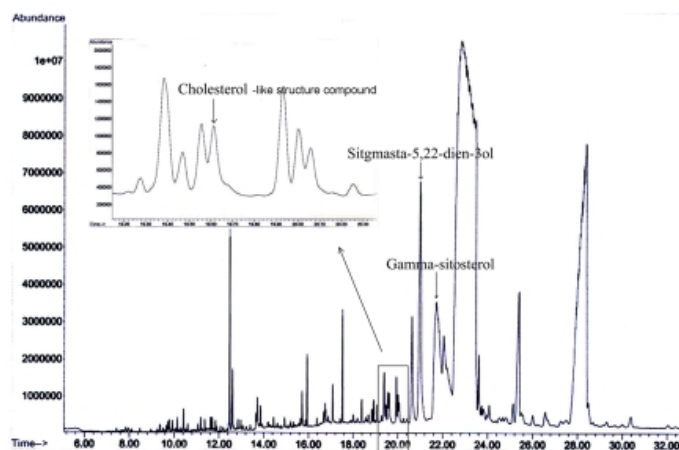
<sup>b</sup> MS quality comparison with database



(a) Wood extract



(b) Leaf extract



(c) Root extract

Figure 1. Chromatograms of steroidal compounds in three different parts of *Sericocalyx schomburgkii*.

2003)

There have been concerns that plant stanols/sterols may reduce absorption of fat-soluble vitamins such as vitamins A, E, and D. However, there are no reports of impaired vitamin A status or related adverse effects after several years of use in Europe and the U.S. Some studies have reported that absorption of  $\beta$ -carotene was affected in some people; however, at no time did its concentration fall outside the normal range for blood levels (Hendriks *et al.*, 1999; Gylling *et al.*, 1999, Nguyen, 1999). Until more is learned, it may be reasonable for people using stanol ester products to make sure to consume carotenoid-rich vegetables (yellow/orange and dark green vegetables) as well (Hallikainen *et al.*, 1999).

It is reasonable to suggest that *S. schomburgkii* (Craib) Bremek is a good source of plant steroids, which may be extracted for use as nutraceutical and functional food ingredients. However, this work was only a preliminary and qualitative study. Though some steroids, especially sitosterol, stigmasterol, and campesterol, have been identified, more work is needed on quantitative and safety analyses, and appropriate extraction and purification methods, in order to reap full benefits from the steroids of this plant without concerns on potential health hazard.

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