

Full Length Research Paper

Extraction and analysis of β -sitosterol in Herbal Medicines

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***Knoxia valerianoides* is a herbal medicine and β -sitosterol is one of its main components. β -sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health and enhance immunity in the human body. β -sitosterol can also be found in vegetable oils such as: wheat germ oil, cotton seed oil and so on. The amounts of β -sitosterol in herbal medicines and vegetable oils have not been reported in the literature since an analytical method has not yet been well established. This paper shows that high performance liquid chromatography (HPLC) is a suitable analytical method for determining β -sitosterol levels in *Knoxia valerianoids* Thorel and pitard (Hun Da Ji), Jing Da Ji and several kinds of vegetable oils.**

Key words: β -sitosterol, *Knoxia valerianoids*, soxhlet extraction, HPLC.

INTRODUCTION

Natural products have been considered anecdotal to the effective maintenance of good health. Examples include: *Pekinensis Radix* which contains the dried root of *Euphorbia perkinensis* Rupr, *E. soongarica* Borss, *E. pontica* porkh, and *Knoxia valerianoides* Thorel and Pitard = *K. corymbosa* Will (*K. congesta* petard) (Lin and Lin, 1996). *K. valerianoides* Thorel and Pitard is commonly used as drugs in herbal medicine stores in Asia. The major (active) ingredients are considered to be β -sitosterol, Ursolic acid and biotine among others (Lin and Lin, 1996). β -sitosterol is a known plant sterol. The sterol in plant is called phytosterols. It is a waxy substance which is white in color. β -sitosterol has also been reported to be abundant in wheat germ oil, cotton seed oil, corn oil, and soybean oil (Chen, 1991). Its efficacy of is reported as follows in the literature review. The structures of β -sitosterol and cholesterol are quite similar. It is reasonable that β -sitosterol can inhibit the absorbing of

cholesterol in the body (Tatu et al., 2002) and thus reduce the cholesterol levels in the plasma (MacLatchy et al., 1995).

The liver function activity (GDP, GOP) can be improved with β -sitosterol (Zak et al., 2005), and this can reduce prostate cancer and colon-cancer cell growth (Awad and Fink, 2000), too. β -sitosterol can also be found in vegetables and fruits. The presence of β -sitosterol in soybean foods has been reported to suppress carcinogenesis. It can also be the factor used to form the lympho cells and NK in the immunity process circulation (Bouic et al., 1996).

β -sitosterol can be found in vegetables such as peanut oil. It is used in experiments for treating breast cancer and prostate cancer (Awad et al., 2000). β -sitosterol in soybean oil has been reported to lower cholesterol levels (Cicero et al., 2002). β -sitosterol in corn oil, rice bran oil and other vegetables oil can affect the cholesterol level in the plasma (Frank et al., 2005).

β -sitosterol and ursolic acid can be extracted from Hun Da Ji by utilizing a soxhlet apparatus (Das and Bhattacharya, 1969). Sterol and stanol in plants have been analyzed using high performance liquid chromatography-

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atmospheric pressure chemical ionization mass spectroscopy (HPLC-APCI-MS) (Mezine, 2003; Burkhardt, 2005a, b). Kalo et al. have used thin layer chromatography (TLC) to analyze triacylglycerol (Kalo and Kuuranne, 2001). Kuksis et al. used gas chromatography (GC) to analyze sterol in plasma (Kuksis et al., 1986).

Kamm analyzed sterol in the cocoa cream with GC method in 2001 (Kamm et al., 2001). Xin Zhang used capillary gas chromatography-mass spectrometry (GC-MS) method to analyze β -sitosterol oxides in vegetable oils (Zhang et al., 2005). Billheimer used reversed-phase liquid chromatography to separate sterol esters (Billheimer et al., 1983). Parcerisa analyzed olive oil using HPLC (Parcerisa et al., 2000) and Kuksis applied the method of HPLC to analyze the plasma lipids too (Kuksis et al., 1991). High-performance liquid chromatography (HPLC) methods have been widely used in the literature given above, but it has not been exploited for analyzing β -sitosterol in herbal medicines and vegetable oils. *K. valerianoides* has been called Hun Da Ji in herbal medicine stores, but Jin Da Ji is ineffective in clinical studies. However they are easily mixed in commercial products available in the market. This paper reports an effective extracting process and the pertinent conditions in high performance liquid chromatography (HPLC) used to analyze the β -sitosterol in Hun Da Ji, and vegetable oils. We also report a method used to determine the difference between the β -sitosterol in Hun Da Ji and Jin Da Ji.

MATERIALS AND METHODS

Apparatus and reagents

The chromatographic system includes a HITACHI D-6500 MODEL gradient pump (Japan), a stainless steel injector (5 μ L loop), and a UV-VIS detector (Jasco, Tokyo, Japan) operated at 198 nm for detecting β -sitosterol extracted from wheat germ oil, cotton seed oil, peanut oil, soybean oil, corn oil, Jing Da Ji and *K. valerianoids*. A Chromolith RP-18 column (Inertsil 7 ODS-3 4.6 mm i.d., 250 mm, Merck) was used as the analytical column. The optimal composition of the mobile phase is 15% Ethanol and 85% Acetonitrile. The flow rate of the mobile phase was 1 ml/min and the column temperature was kept at 25°C. The sample solution and reagent solution were degassed before each run. A Soxhlet extractor apparatus was used for extracting the β -sitosterol from the desired samples. UV-Visible Spectra were taken using a DU-800 spectrometer (Beckman Coulter, U.S.A). Unless otherwise specified, all reagents were HPLC grade (Merck, Darmstadt, Germany) and these include: methanol, ethanol, acetonitrile, and potassium hydroxide. Petroleum ether was gotten from BDH (Poole, UK). Reagents were degassed in an ultrasonic bath as required before injecting into the HPLC.

Sample preparation

K. valerianoids was placed in the oven for 2-3 days in order to dry the plant body. This dried plant was crushed into pieces to form a coffee color powder. A sample typically contained 100 grams dried powder which was placed in the Soxhlet extraction setup for 48

hours to extract β -sitosterol. A dark green oil was obtained after storing the extracting oil for 7 days. This green oil was separated and 5 ml of aqueous KOH solution (concentration = 10 Molar) was added. An upper layer of liquid was obtained as the HPLC sample. The sample of Jing Da Ji was obtained using the same process described as above. Wheat germ oil, cotton seed oil, peanut oil, soy bean oil, and corn oil were obtained from a supermarket in the Taichung area of Taiwan. The HPLC samples were obtained as above with extracts from solids by adding KOH. All samples were filtered as required before injecting into HPLC. An authentic chemical sample of β -sitosterol was purchased from Sigma-Aldrich Co. (U.S.A) and a concentration of 1mg/1ml was prepared by dissolving it in chloroform.

RESULT AND DISCUSSION

Figure 1a is the HPLC chromatogram of β -sitosterol in the mobile phase and 100% Acetonitrile at pH 6.5. The UV detector was set at 196 nm; it is optimized by the UV-Visible spectrometer. The retention time of β -sitosterol in *K. valerianoides* is shown at 67.89 minutes (Figure 1b) as confirmed by the standard solution in the chromatogram at the same conditions in figure 1a. The selectivity factor and the retention time can be adjusted by varying the compositions of the mobile phase. Thus, 5% ethanol and 95% acetonitrile at mobile phase were adopted to run the standard solutions in figure 2. 15% ethanol and 85% acetonitrile as mobile phase were adopted to run the samples in figure 3a. The retention time of β -sitosterol is shown at 55.75 and 36.23 min (Figures 2 and 3a) respectively. The retention time of β -sitosterol is reduced from 67.25 to 36.23 min. (Figures 1a and 3a) apparently. However, it could not be adjusted further to reduce the retention time by the volume ratio between ethanol and acetonitrile due to the solubility problem. Therefore, the optimizing conditions for analyzing β -sitosterol in *K. valerianoides* by HPLC are 15% ethanol and 85% acetonitrile at mobile phase with other parameters described in the experimental section. The chromatogram of the sample solutions is shown in Figure 3b and its retention time is 36.81 min. This HPLC method was also applied to analyze the samples obtained from Jing Da Ji but no peak was found for β -sitosterol in its chromatogram. Thus, it can be seen that the HPLC method is a good tool to identify the difference between Jing Da Ji and Hung Da Ji scientifically.

Figure 4 is a linear plot of the β -sitosterol between 0.25 and 2.0 mg/ml. Table 1 shows the concentrations of β -sitosterol in the vegetable oils obtained from the Taichung area of Taiwan. The concentration of β -sitosterol in *K. valerianoids* obtained in Taiwan was 0.32 mg/ml. The concentrations of β -sitosterol in the wheat germ oil, cotton seed oil, peanut oil, soya bean oil, corn oil were 1.62, 1.31, 0.78, 0.64 and 0.3 mg/ml (Figure 5).

The above samples were handled carefully and the analytical method described in the sample section. The data obtained by the above method were repeated to ensure its accuracy. Wheat germ oil was the richest one (1.62 mg/ml) in the β -sitosterol content of all

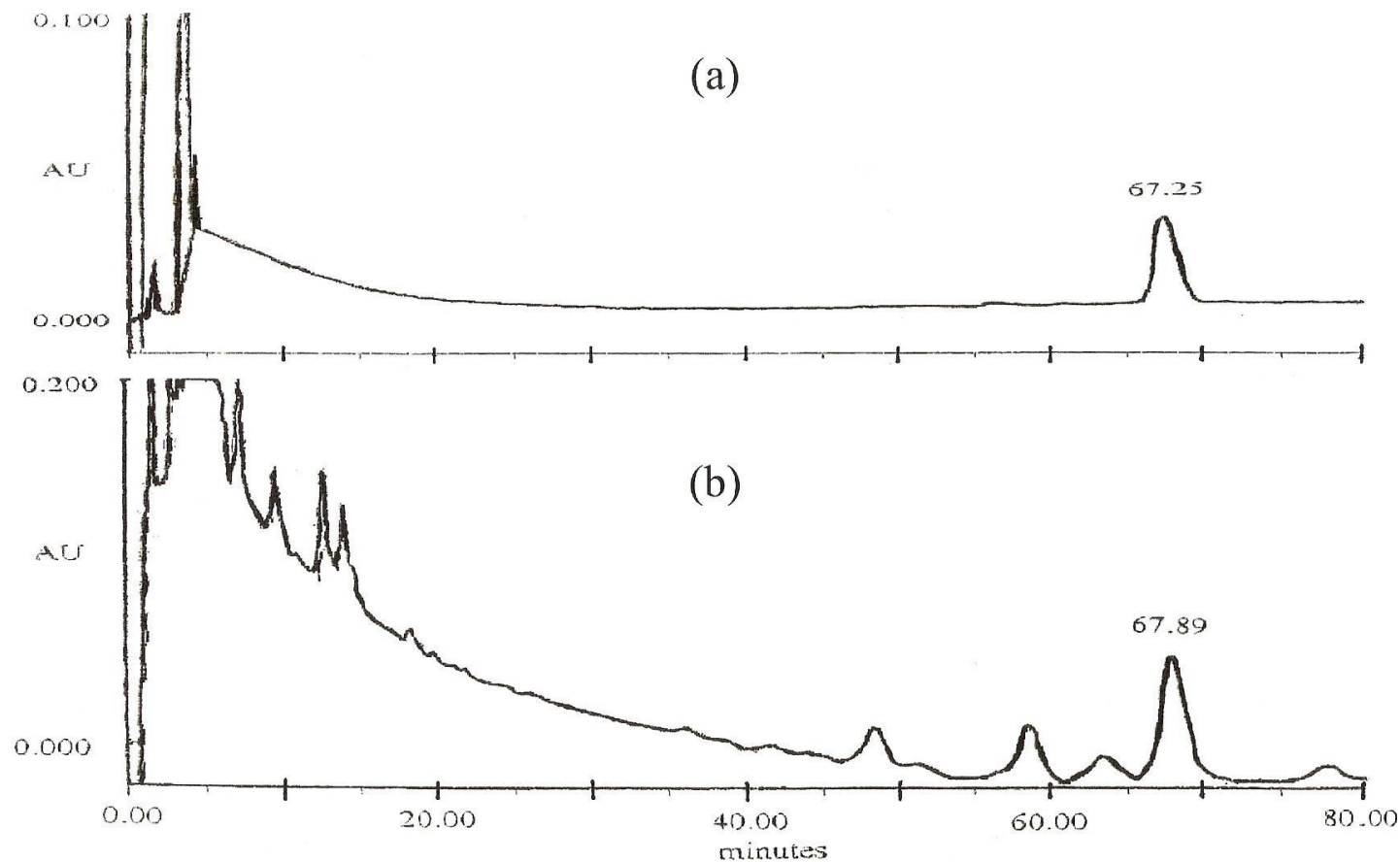


Figure 1. Chromatogram of (a) β -sitosterol and (b) *Knoxia valerianoids*. Mobile phase was 100% Acetonitrile at pH 6.5; analytical column: Inertsil 7 ODS-3 4.6 mm i.d. x 250 mm; flow rate; 1ml/min; 25°C; injection volume: 5 μ L; detection at 196 nm.

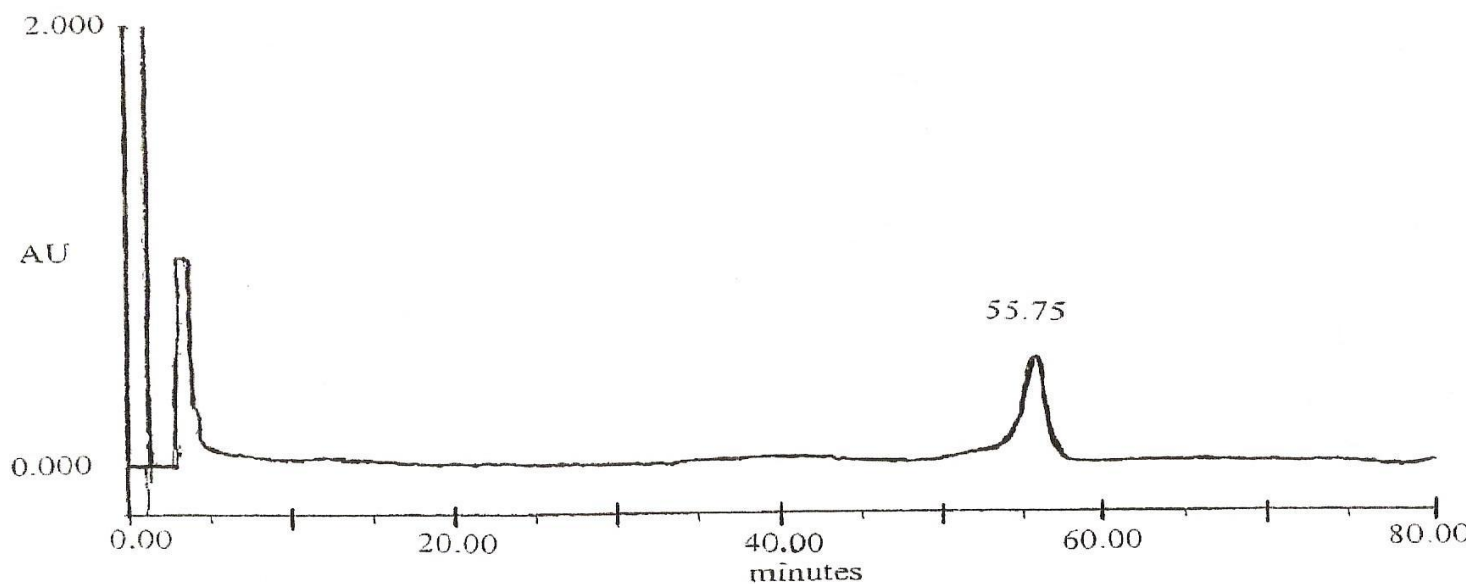


Figure 2. Chromatogram of β -sitosterol. Mobile phase was 95% Acetonitrile and 5% Ethanol at pH 6.5; analytical column: Inertsil 7 ODS-3 4.6 mm i.d. x 250 mm; 1 ml/min; 25°C; injection volume: 5 μ L; detection at 196 nm.

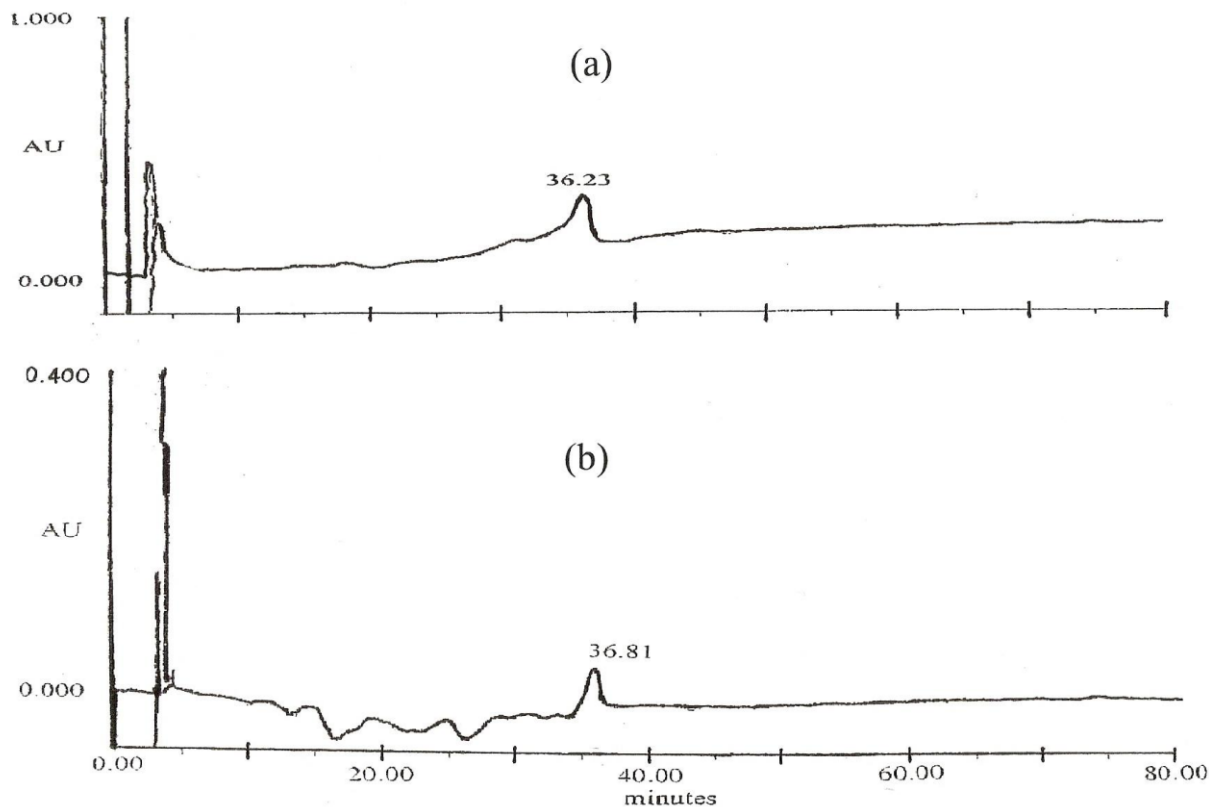


Figure 3. Chromatogram of (a) β -sitosterol and (b) Knoxia valerianoids. Mobile phase was 85% Acetonitrile and 15% Ethanol at pH 6.5; analytical column: Inertsil 7 ODS-3 4.6 mm i.d. x 250 mm; 1 ml/min; 25°C; injection volume: 5 μ L; detection at 196 nm.

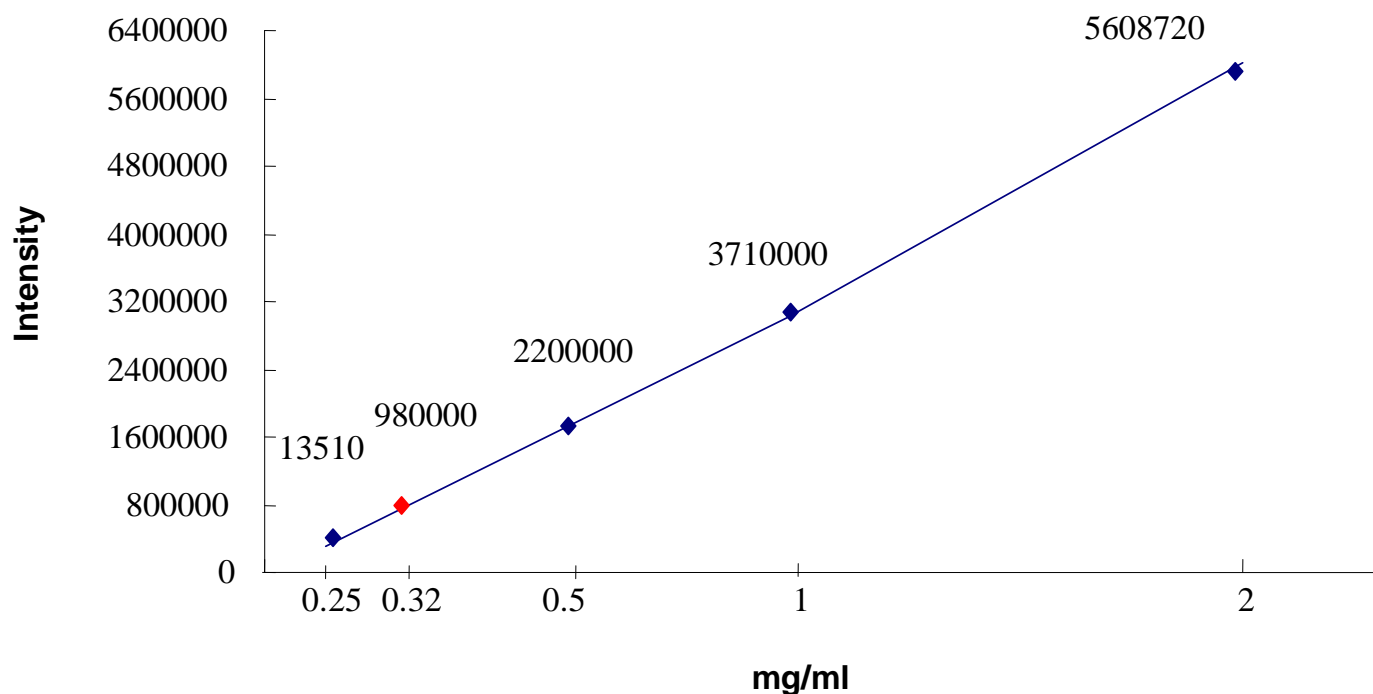
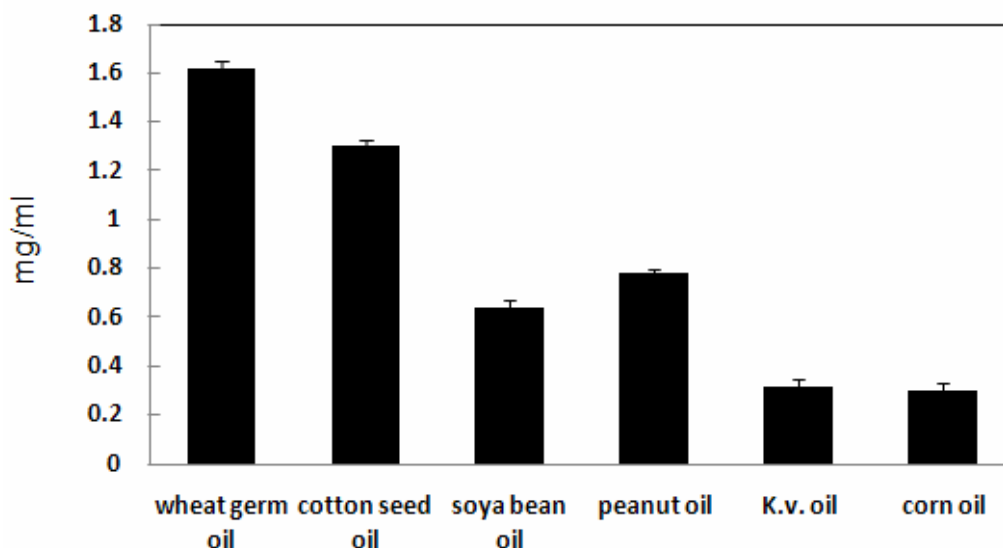


Figure 4. The concentration of β -sitosterol in Knoxia valerianoids carried standard concentration from 0.25 - 2 mg/ml.

Table 1. The amounts of β -sitosterols of the vegetable oils detected by HPLC.

No	Retention time	Concentration (mg/ml)
Wheat germ oil	36.91	1.62
Cotton seed oil	36.21	1.3
Soya been oil	36.47	0.64
Peanut oil	36.12	0.78
K.v.	36.81	0.32
Corn oil	36.08	0.3

**Figure 5.** The chart of the detected concentration of β -sitosterol in *Knoxia valerianoids* and the vegetable oils.

studied samples here. Corn oil had the lowest contents of β -sitosterol (0.3 mg/ml) as can be seen in Table 1. The HPLC method presented here can also be adopted for analyzing β -sitosterol in dairy products. The activity of β -sitosterol from *K. valerianoids* as an active agent to treat cancer cells is under investigation in our laboratory.

Conclusion

This paper gives the pertinent conditions for using high performance liquid chromatography (HPLC) to determine the β -sitosterol levels in Hun Da Ji and its difference from that of Jin Da Ji. This analytical method can also be applied to determine the amounts of β -sitosterol in vegetable oils.

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