Policosanol extraction from beeswax and improvement of the purity

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Abstract. Policosanol is a mixture of high molecular weight aliphatic long chain alcohols (20-36 carbon atoms). It has been use in pharmaceutical composition and food supplements. This research aimed to isolate and improve the purity of policosanol extracted from beeswax. Triglycerides and other impurities were eliminated from beeswax by refluxing with hexane followed by isopropanol. The purified beeswax was hydrolyzed by refluxing with 1 M ethanolic NaOH for 2 hours. Purification of policosanol was performed by extracting the hydrolyzed product with acetone at 50-60 °C for 3 hours and it was stored at 4 °C for precipitation. The precipitate was refluxed with heptanes followed by washing with hot water. The heptanes layer was kept for policosanol precipitation at 4 °C. The purity of policosanol was confirmed by TLC and high performance liquid chromatography (HPLC). The yield of purified policosanol was 13.23-13.89 %.

1 Introduction

Policosanols (PC) are a mixture of high molecular weight of primary aliphatic long chain alcohol (20-36 carbon atoms) containing mainly docosanol, tetracosanol, hexacosanol, octacosanol and triacontanol [1]. Policosanol have effect potential to reducing LDL peroxidation [2-5], plated aggregation, prevention and treatment of cardiovascular disease [2-8] and proliferation of smooth muscle cell. Nowadays, it is used to nutraceuticals and food supplementary in the world market.

Policosanol was originally isolated from plant wax and animal wax. Beeswax is mainly complex mixture of fatty acids and aliphatic long chain alcohols. The monoester of carbon atom 24 to 34 atoms alcohols are about 40% of the composition of beeswax [9]. Thereby, Miguel and Fernando, 2013 [10] reported the carbon length 18 to 34 atoms of aliphatic alcohol which extracted from Portuguese beeswax. Irmak et al., 2006 [1] reported the main component in policosanols of beeswax from USA showing more than 40% is triacontanol (C30-OH). However, the content of beeswax composition including to another substance are various with origin, extraction and purification technique [1, 10-12].

Policosanol can be extracted by hydrolysis or saponification reaction with NaOH or KOH base in various conditions [1, 13-19]. The researchers have investigated on the use of enzyme catalyst methanolysis in supercritical carbon dioxide [20], using High ultrasound (HIU) as catalyst [21] and using pressurised solvent extraction [22]. The purification step of policosanol has to use of organic solvents for purifying [1, 4, 11, 13-19, 22] and following by crystallization and using a silica column to purify [23]. The most commonly technique for policosanol analyzation and characterization can be done by Thin layer chromatography (TLC), Gas chromatography (GC) and High performance liquid chromatography (HPLC) or combining these technique [4, 16-17, 24-26].

The suitable solvent selection is the most important step in policosanol purification for optimising recovery of aspiring to the main components from a complex mixture. Solvent–solute interactions relate to dispersion and and/or multi-polar interactions. The dielectric constant identifies the polarity of a solvent and a key parameter to determine solute–solvent interactions. Moreover, a decrease in dielectric constant of a solvent with increasing temperature, accordingly lowering the polarity of the solvent [27]. Thus, temperature can be used for guide line to match the solvent properties to a recovery of the solute of the interest compound.

In this study, the policosanol was extracted from beeswax by hydrolysis reaction and improvement on the purity of extracted policosanol was done by considering the effect of solvent and temperature collective on purity and recovery of policosanol. The purity of policosanol was confirmed by TLC and HPL.

2 Materials and Methods

2.1 Materials and regents

Beeswax as policosanol source was obtained from bees' farm at a local farm located in Chiang Mai Province, Thailand. Standard aliphatic long chain alcohols and Stearic acid were supplied by Sigma Aldrich, Thailand. Chemical reagents in analytical grade were provided

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from LabScan (Bangkok, Thailand). Commercial rice bran oil (as triglyceride) in food grade was purchased from local supermarket in Chiangmai, Thailand.

2.2 High Purity Beeswax Preparation

The compositions of beeswax were studied by TLC. The spot on TLC plate were compost of beeswax, rice bran oil (as triglyceride; TG), standard free fatty acid (steric acid; C18:0) and standard ester (steric acid methyl ester derivative).

High Purity Beeswax were prepared by dissolving crude beeswax in hexane (30 g wax/300 ml) and it was refluxed at 65 °C for 3 hrs with continuous stirring. The refluxed beeswax was washed with hot hexane in twice times. The washed beeswax was refluxed again with isopropanol at 80 °C for 3 hrs and washed again with isopropanol. The purity of beeswax was checked by TLC.

The developing solvent for TLC was a mixture of hexane, ethyl acetate and acetic acid (90: 9: 1, v/v/v) and bands developed by iodine resublimed.

2.3 Extraction of Policosanol by Hydrolyzation

The high purity beeswax was hydrolyzed with 1 M ethanolic NaOH by refluxing with continuous stirring at 80-85 °C for 2 hrs. The sample mixtures of complete hydrolyzed beeswax was tasted by TLC with chloroform: hexane: acetic acid (70: 30: 1, v/v/v) as developing solvents.

2.4 Purification of Policosanol

Approximately 15 g of complete hydrolyzed beeswax and 250 ml of acetone were refluxed for 6 hrs at 50-60 °C and it was stored at 4 °C for precipitation. The precipitate was refluxed with 250 ml of heptanes at 50-60 °C followed by washing with 150 ml of hot water in three times. After that the heptane layer was separated from the solution and it was kept for policosanol precipitation at 4 °C (applied from Gemble et al. (2003)) [23]. The policosanol precipitated were filtered and dried at room temperature. The purification of policosanol test was done by the checking of free fatty acid (FFA) by TLC for primary test. Moreover, the quantification of FFA were studied by HPLC via free fatty acid as external standard. The FFA analysis was carried out by HPLC using acetronitrile/methanol (4:1) solvent system as a mobile phase and C18 (Hewlett Packard) HPLC column (125 mm x 4.0 mm i.d.).

3 Results and Discussion

3.1. High Purity Beeswax Preparation

The compositions of beeswax material are show in Figure 1. It is compost of wax ester, triglyceride and free fatty acid which is closely to the report of Gemble et al. 2003 [23] and Tolloch, 1980, 1971 [24-25]. The triglyceride and free fatty acid were in case of rancidity via lipid oxidation and soap forming via hydrolysis with strong base (saponification). Thus, the removal of the impurities in beeswax is necessary process of wax ester hydrolyzation.

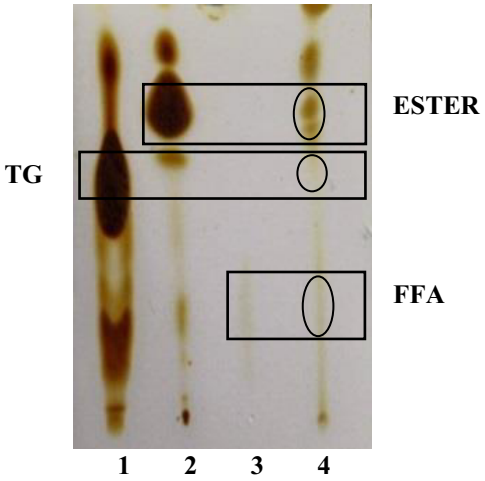

Fig. 1. Thin layer chromatography show crude beeswax composition. (1, rice bran oil (TG); 2, std. fatty acid methyl ester (FAME); 3, std. stearic acid (FFA); 4. Crude beeswax)

The elimination of triglycerides and free fatty acids were made by dissolving beeswax in hexane and reflux for 30 minutes following by washing with hot hexane. TLC plate of the impurities elimination show in Figure 2. Triglycerides and free fatty acids cannot be removed in the first step (refluxing and washing with hexane). However, the remains of the impurities can be removed in second step of refluxing and washing again with isopropanol. We had been success in elimination of the triglyceride and free fatty acids as a resulting in Figure 3. The high purity of wax ester was used as material for policosanol extraction.

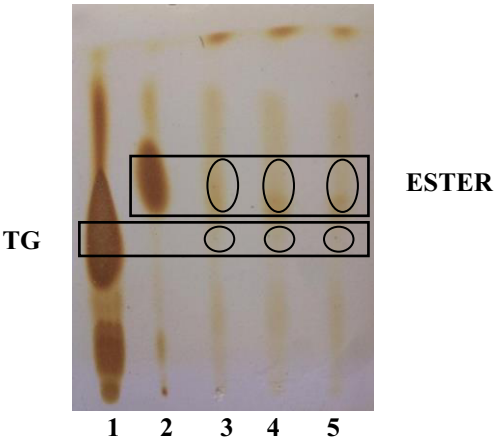

Fig. 2. Thin layer chromatography show purified beeswax composition using hexane in purification. (1, rice bran oil (TG); 2, std. fatty acid methyl ester (FAME); 3, purified beeswax (refluxed with hexane); 4, purified beeswax (refluxed and washed with hexane 1st time); 5, purified beeswax (refluxed and washed with hexane 2nd time)

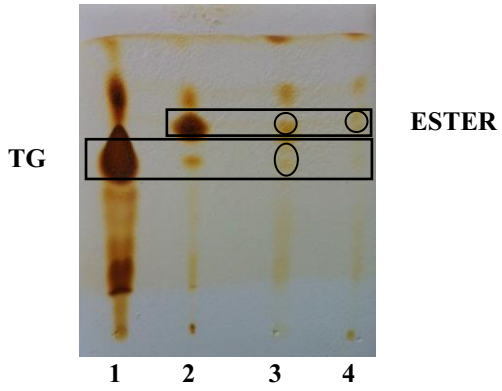

Fig. 3. Thin layer chromatography show purified beeswax composition using isopropanol in purification. (1, rice bran oil (TG); 2, std. fatty acid methyl ester (FAME); 3, purified beeswax (refluxed with isopropanol); 4, purified beeswax (refluxed and washed with isopropanol)

3.2 Extraction of Policosanol by Hydrolyzation

The extraction of beeswax by base catalyzed hydrolysis can be done by refluxing with 1 M ethanolic NaOH at 80-85 °C for 2 hrs as shown in Figure 4. The TLC plate had been present to complete hydrolyzation of the high purity wax ester. Thereby the wax ester did not appear on the TLC plate due to the fact that hydrolyzation of the wax to long chain fatty acids and long chain aliphatic alcohols. The researchers have been using NaOH or KOH base prepared in organic solvent for catalyst the hydrolysis reaction [1, 13-19]. However, the advantage of NaOH solution in ethanol is to protection the possible emulsion formation of the reaction.

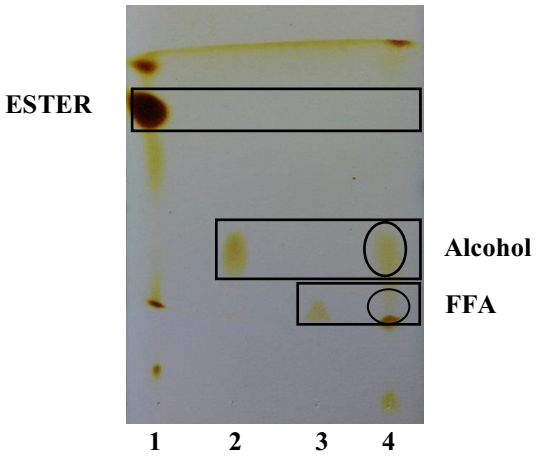

Fig. 4. Thin layer chromatography show hydrolyzed beeswax composition using 1.0 M NaOH in Ethanol. (1, std. fatty acid methyl ester (FAME); 2, std. alcohol (C18-OH); 3, 3, std. stearic acid (FFA); 4, hydrolyzed beeswax)

3.3 Purification of Policosanol

Beeswax is a complex mixture of compounds with a large range of long chain alcohol molecular and long chain fatty acid structures so the solubility of these molecules would be different in various organic solvent types. The aim of purification the hydrolyzed wax ester is to get rid of long chain fatty acid. The difference properties of solvent were used in two purification step. The result of policosacol purity was tasted by TLC showing in Figure 5. The high dielectric constant solvent (acetone) using in the first step cannot use to purify the policosanol due to sodium salt of long chain fatty acid can be dissolved in acetone too. However, the policosanol was repeated extraction via low dielectric constant solvents (hepane) in the second step of this study. It is capable of efficiently extracting policosanol thereby the residual sodium salt of long chain fatty acid can be eliminated by washing with hot water. The result of policosanol purity was confirmed by HPLC displaying in Figure 6. Qualification of the free fatty acids were carried out using of stearic acid as an external standard (Figure 6 (A)). The high purity of policosanol extracted was indicated in Figure 6 (B). It was not found free fatty acids residual to show in the chromatograms. The suitable solvent selection is the most important step in policosanol purification for optimizing recovery of aspiring to the main components from a complex mixture. The dielectric constant is a measure of the polarity of a solvent and a key parameter in determining solute–solvent interactions [27]. Moreover, a store of a complex mixture of the extracted substance with solvent at low temperature (4 °C) exhibit in increasing of the extracted substance precipitation. It is similarly to previous report of Gemble et al. 2003 [23] which use cooling technique at 2 to -10℃ for 18 hours to purify the policosanol. The selection of solvent used and precipitation at low temperature is an important role in the complete of policosanol purification process. We had been success in purification of policosanol extracted from beeswax thereby the total yield of high purity policosanol was 13.23-13.89 %.

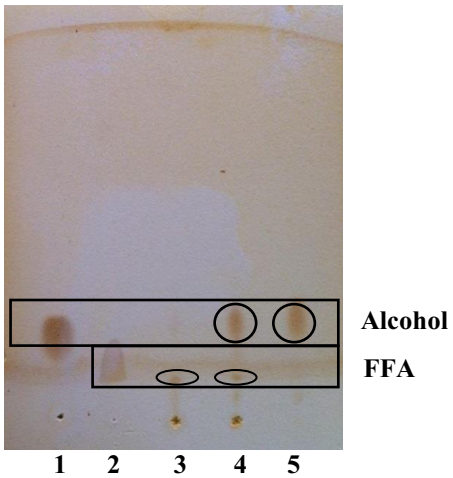

Fig. 5. Thin layer chromatography show purified policosanol. (1, std. alcohol (C18-OH); 2, std. stearic acid (FFA); 3, hot water layer; 4, purified policosanol (refluxed with acetone); 5, purified policosanol (refluxed with heptane and washed with hot water)

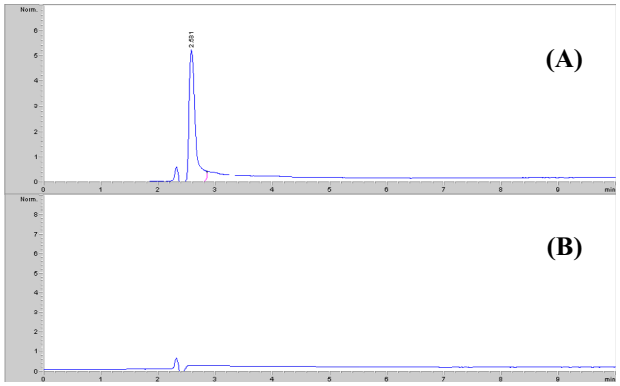

Fig. 6. Chromatogram shows high purity policosanol. (A; Std. stearic acid (FFA); B, purified policosanol)

4 Conclusions

The mixture of long chain alcohols key compounds within the beeswax have been extracted, purified, identified and quantified. All the technique in the process of policosanol extraction had significant effects on purity, recovery yields and policosanol composition. Moreover, the solvent selectivity and temperature collective on exhibit in improving efficiency of purification process. The high purity of policoanol extracted from beeswax have many potential in applications.

The authors wish to thank National Research Council of Thailand and Department of Chemistry, Maejo University, Chiang Mai, Thailand for financial support.

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