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Extraction of 2-Acetyl-1-Pyrroline from Pandan Leaves with Hexane

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ABSTRACT

The extraction of 2-Acetyl-1-Pyrroline (2AP) from pandan leaves with hexane was carried out by cutting pandan leaves into smaller pieces, around 5 mm, grinding with a grinder until the fiber came out. The grinded pandan leaves were mixed with hexane at the ratio of 5 grams of grinded leaves to 40, 60, and 80 ml of hexane. The mixtures were shaken with a shaker at 200 rpm. For 2 hours at ambient temperature and atmospheric pressure. The effect of the volume of hexane on the extraction performance was investigated and compared to that obtained with 60 ml of ethanol. As a common solvent for extraction, ethanol could extract more 2AP than hexane.

With GCMS, the chromatogram of 2AP was found at 8.64 and 8.8 minutes for hexane and ethanol, respectively. The concentration of 2AP obtained in hexane was about 10% less than that in ethanol under the same conditions. By the extraction of 2AP with 40 ml hexane, the concentration became 23% greater than that in ethanol.

Keywords: 2-Acetyl-1-pyrroline, ACE inhibitor, Extraction, Ethanol, Hexane, Pandan leaves

INTRODUCTION

Many countries, including Thailand, have been entering an ageing society. Most of the elder people have had problems with their hearts from various causes, such as atherosclerosis. The demand of the medicines has increased proportionally to the number of elder population. In addition, the number of patients with heart diseases has increased continuously since it might occur in any genders and ages.

Most of the vasodilators medicines have been composed of Angiotensin-Converting-Enzyme (ACE) inhibitors, as shown in Figure 1(a). Since ACE inhibitors have been an important substance for blocking the synthesis of Angiotensin II or hormone that might constrict blood vessels. While, 1-(3,4-Dihydro-2H-pyrrol-5-yl)ethan-1-one (C_6H_9NO), commonly known as 2-Acetyl-1-Pyrroline (2AP), has had similar molecular structure to ACE inhibitors , as shown in Figure 1(b). In addition, 2AP has been a common aromatic substance which could be found in Basmati rice, Jasmine rice, and pandan leaves, which has been planted vastly in the Asian region[1]. Pandan or Pandanus amaryllifolius Roxb Linn.Palm has been one of the well-known plants in Thailand as it could be used in people's daily life[2]. Because of ease of availability, cheap price and non-toxic substances, therefore, people have usually used it for cooking and dessert. Pandan leaves could reduce dirtiness, blood sugar level, cure asthma, and maintain heart functions.

In previous research on the extraction of 2AP, the yield, obtained by hexane, was higher than supercritical carbon dioxide, SC-CO_2[3]. The ranges

of the yield obtained by SC-CO_2 and hexane were 0.18-0.88% and 1.18-5.23%, respectively. Thus, hexane extraction was chosen to study further on the effect of volume on the extraction. In addition, the most common solvent, ethanol, in 2AP extraction, was used to compare their extraction performance. It also reported that the increase in the extraction duration did not improve the yield significantly. However, the increase in the surface area of the pandan leaves by grinding could improve the concentration of 2AP in the extracted solvent since 2AP could dissolve in solvent easily through the rupture of epidermal cells in the leaves structures. Since ethanol was a common solvent used to extract substances from organic matter, it could extract more 2AP, as well as other polar and nonpolar compounds, than hexane.

In addition, the synthesis of ACE inhibitors from 2AP could reduce the steps and complications of the production process. Therefore, solvent extraction processes were carried out batch-wisely in this research. Hexane, which has been a common solvent for the various commercial extraction processes, especially cooking oil industries, was used for the extraction of 2AP from pandan leaves, which contained a large number of organic substances. The extraction performance would be compared with that obtained by ethanol[4].

MATERIALS AND METHODS

1.1 Materials

1. Pandan leaves
2. The solvent used: hexane, ethanol
3. A weight meter with 4 decimal places
4. A grinder
5. A shaker
6. A pipette
7. 5 flasks
8. A scissor
9. 7 sample collectors
10. Filter papers

1.2 Methods

1. Pandan leaves were washed and cut into small pieces, around 5 mm,
2. Grinded the leaves with a grinder until the fiber came out,
3. Put 5 g of grinded pandan leaves in each flask for 2 flasks,
4. Added 60 ml of ethanol and hexane to each flask,
5. Sealed the flasks using rubber stoppers,
6. Weighed the flasks and recorded the mass,
7. Put the flasks into the shaker with the rate of 200 rpm for 2 hours,
8. Weighed the filter papers,
9. Filter the grinded pandan leaves from the mixture,
10. Weighed both filtrate left in the flasks and the pandan leaves on the filter paper and recorded their masses,
11. Collect the solutions for GC/MS analysis,
12. Repeated step 3 to 11 with hexane 40 and 80 ml.

1.3 Evaluation

The extraction performance of 2AP from pandan leaves with hexane was evaluated by comparison to that with ethanol. The 2AP in the extracted solvent was analysed by GC/MS, which was suitable for small and volatile substances [5,6]. The concentration of 2AP in the solvent obtained was evaluated from the area of 2AP chromatogram. Subsequently, the percentage difference of 2AP concentration in hexane from that in ethanol was determined.

RESULTS

When 60 ml of ethanol was added into the flask of grinded pandan leaves, the colour of the solution changed from colourless to dark green rapidly. The colour became darker to almost black during the batchwise extraction for 2 hours. By adding hexane, on the contrary, in the flask of grinded pandan leaves, the colour changed slightly from colourless to light green. The colour still appeared as light green for the whole experiment until filtering the grinded pandan leaves. Meanwhile, the aroma of 2AP diffused throughout the laboratory.

After the extraction with ethanol, the grinded pandan leaves, which were separated by filtration, on the

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filter paper were still wet. The mass of the grinded pandan leaves increased by almost 60%, as summarised in Table 1. While, the grinded pandan leaves, filtered from hexane, were slightly wet. The mass of grinded pandan leaves increased about 6%. With 80 ml hexane, the mass of filtered pandan leaves was increased as similar to the extraction with 60 ml hexane. When the grinded pandan leaves were extracted with 40 ml hexane, it decreased almost 1%. Subsequently, the grinded pandan leaves were almost dried as before extraction.

During batchwise extraction for 2 hours, both ethanol and hexane evaporated less than 10%. After filtration in the fume hood for less than 10 min, the mass of ethanol and hexane obtained were 27.75 g and 26.37 g, respectively. In the circumstances, about one third of both ethanol and hexane evaporated to the atmosphere, as summarised in Table 2. For the extraction with 80 ml hexane under the same conditions, the filtrate of hexane decreased to

38.53 g. In other words, almost 40% of hexane evaporated to the atmosphere. With 40 ml hexane, only 14 g of hexane remained in the filtrate. It evaporated to the atmosphere about 44.6% during filtration.

The amount of 2AP extracted was analysed by GC/MS at Scientific and Technological Research Equipment Centre (STREC). For the extraction with ethanol, the 2AP was detected at 8.8 min. on the chromatograms, as shown in Figure 2 (above), which was similar to the results of Norzita Ngadi[4]. For hexane, it was detected at 8.64 minutes, as shown in Figure 2 (below), which was slightly earlier than that of ethanol. These demonstrated that 2AP could be extracted from grinded pandan leaves by both solvents.

Based on the area of 2AP chromatograms, the concentration of 2AP in 60 ml hexane was lower than that in ethanol about 10%, as shown in Figure 3. In the circumstances, 14.6 g ethanol and 13.6 g hexane evaporated to the atmosphere, as summarised in Table 2. With 80 ml hexane, the concentration was less than that in ethanol about 14% although 25.4 g of hexane vaporised during filtration. While the extraction with 40 ml hexane, under which hexane vaporised about 11.3 g, the concentration of 2AP became higher than that in ethanol by 23%. The results indicated that hexane was a suitable solvent for extraction of 2AP from grinded pandan leaves.

DISCUSSION

As the colour of ethanol became dark green rapidly, a large number of compounds in pandan leaves, such as chlorophyll, could be extracted simultaneously, besides 2AP. While the extraction with hexane, a small number of compounds in pandan were extracted simultaneously with 2AP. Consequently, the extraction with ethanol required additional purification steps to separate various extracted compounds from ethanol. The boiling points of hexane and ethanol were 69oC and 78oC, respectively. Both solvents could evaporate easily, especially during the filtration. 2AP in both grinded pandan leaves and solvents, i.e. hexane and ethanol, vaporised together with solvents and dispersed throughout the atmosphere.

Although ethanol was a common solvent for dissolving both hydrophilic and hydrophobic compounds, the molecule contained a chemically active hydroxyl-group, leading to chemical reactions with certain compounds as extracting a large number of compounds from pandan leaves. On the contrary, hexane, which was relatively low chemical reactivity, extracted only hydrophobic compounds, and rarely caused undesired chemical reactions. Consequently, hexane was able to extract more 2AP from pandan leaves than ethanol, leading to the higher concentration of 2AP in the extracted solvent. However, as the volume of solvent increases, the concentration of extracted 2AP decreased due to the amount of 2AP was limited in the pandan leaves.

CONCLUSION

Besides 2AP, a large number of other substances in pandan leaves were extracted by ethanol simultaneously, while hexane could extract less number of substances than ethanol. At least about one third of solvent evaporated during filtration of grinded pandan leaves. With 40 ml of hexane, the concentration of 2AP in hexane was higher than that in ethanol by 23%.

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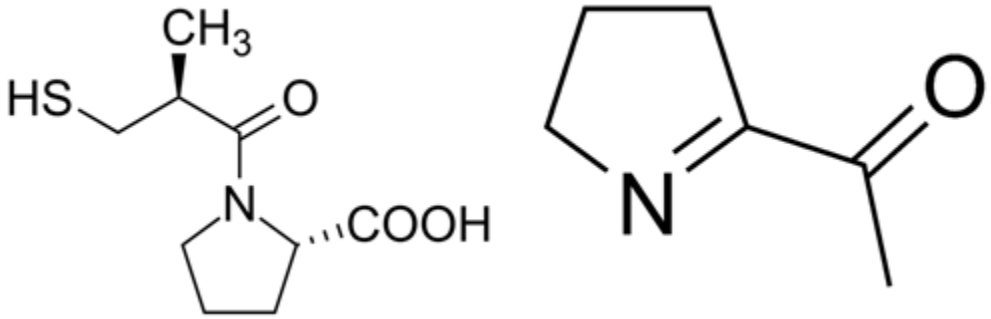
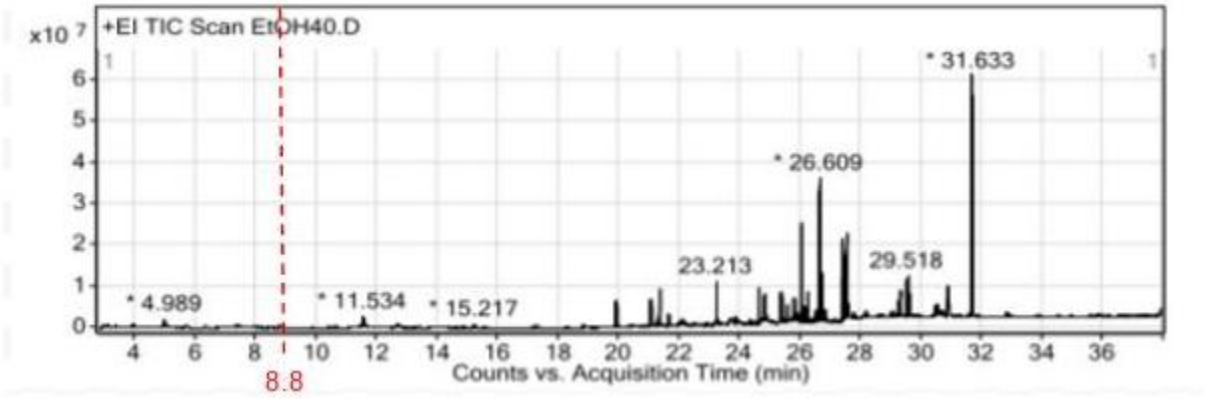
FIGURE LEGENDS



(a) **(b)**

Figure 1: Angiotensin-Converting-Enzyme (ACE) inhibitor molecular structure (a), 2-Acetyl-1-Pyrroline molecular structure (b).



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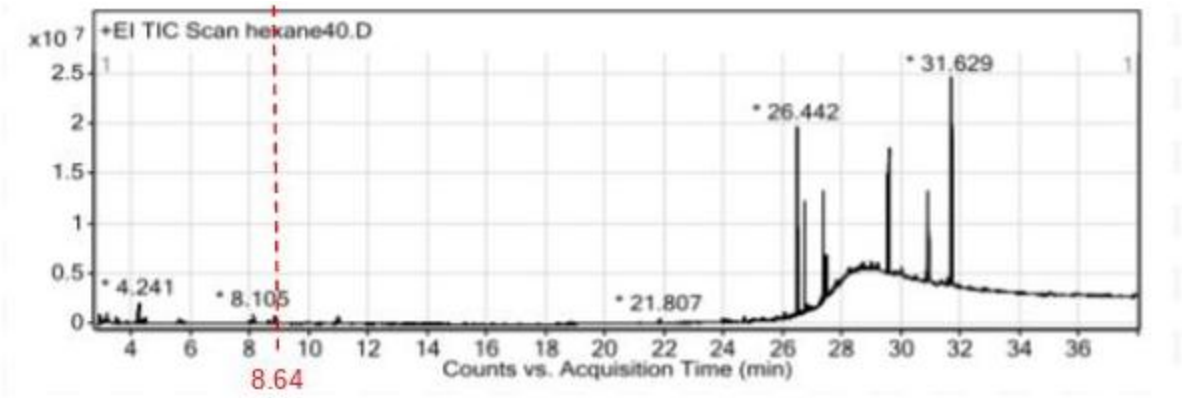

Figure 2: Chromatograms of extracted 2AP from grounded pandan leaves by ethanol (above) and hexane (below).

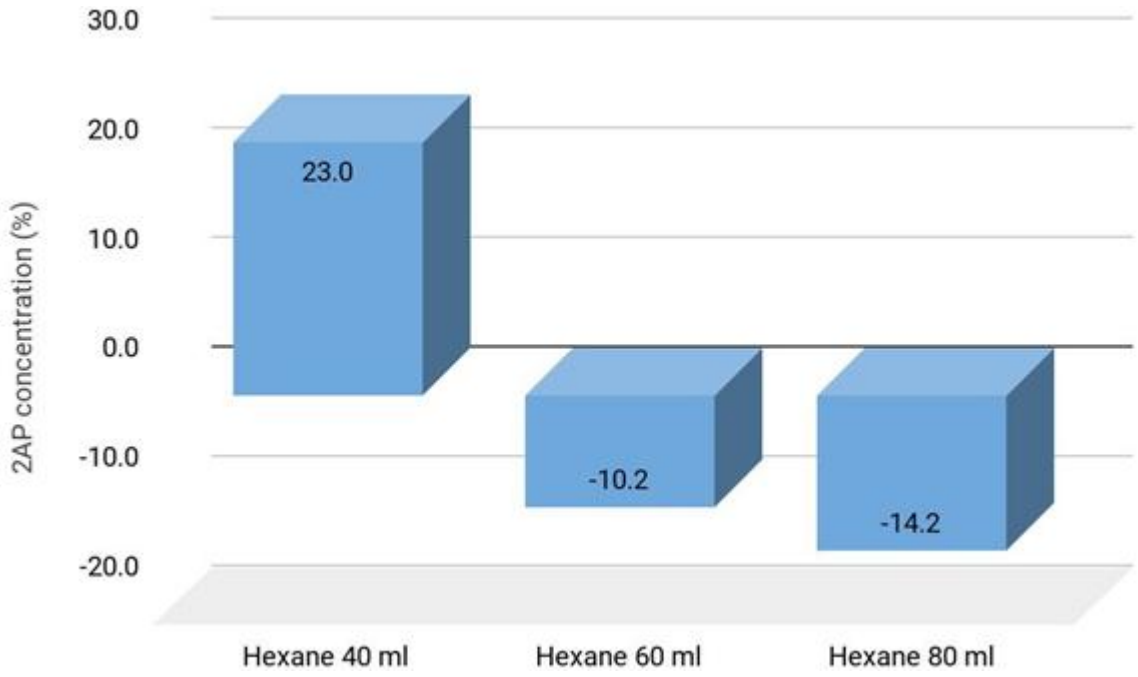

Figure 3: Percentage difference of 2AP concentration between hexane solutions and ethanol 60 ml.

TABLES

Table 1: Summarized the mass of grinded pandan leaves before extraction and after filtration.

Solvent	Mass before extraction, g	Mass after filtration, g	Change in mass of grinded pandan leaves, %
Ethanol 60 ml	4.6352	7.3710	59.02%
Hexane 40 ml	4.6868	4.6469	-0.85%
Hexane 60 ml	4.6333	4.9203	6.19%
Hexane 80 ml	4.6324	4.8962	5.69%

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Table 2: Summarized the mass of solvents before extraction and after filtration

Solvent	Mass before extraction, g	Mass after filtration, g	Mass evaporated, g	Change in mass, %	Evaporated solvents, %
Ethanol 60 ml	45.1258	27.7498	14.6402	-38.51%	32.45%
Hexane 40 ml	25.3312	14.0737	11.2974	-44.44%	44.60%
Hexane 60 ml	40.2949	26.3656	13.6423	-34.57%	33.86%
Hexane 80 ml	64.1593	38.5337	25.3618	-39.94%	39.53%

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