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Extraction of 2-Acetyl-1-Pyrroline (2AP) in Pandan Leaves (*Pandanus Amaryllifolius* Roxb.) Via Solvent Extraction Method: Effect of Solvent

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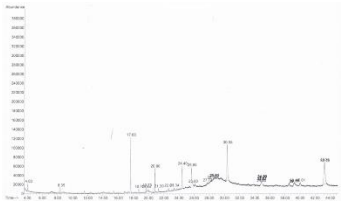
Graphical abstract



Abstract

Pandan (Pandanus amaryllifolius Roxb.) leaves are widely used in Malaysia as a source of natural flavoring. The major compound contributing to the characteristic flavour of Pandan is 2-acetyl-1-pyrroline (2AP). As the consumer requirement for use of natural flavours, extraction of components from natural sources has been sought. In this study, solvent extraction of 2AP from Pandan leaves was performed. The effect of solvent used during extraction process (i.e. methanol, ethanol, propanol) towards the yield of 2AP was investigated. The presence of 2AP was determined using GCMS. The results obtained showed that ethanol was the best solvent to extract 2AP from Pandan leaves compared to methanol as higher 2AP peak arises from ethanol chromatogram. However there is no 2AP detected when propanol was used as solvent. It is believed that polarity of the solvent plays an important role in the extraction of 2AP.

Keywords: Extraction; 2-acetyl-1-pyrroline; pandan leaves; solvent

1.0 INTRODUCTION

Pandanus amaryllifolius Roxb. is a plant with an excellent aroma and is locally known as 'Pandan Wangi or Pandan' [1]. It is from Pandanus genus [1, 2] or also recognizes as screw pine genus [3]. It is commonly found in South East Asia, such as Thailand, Malaysia, Indonesia and India [4]. Pandan leaves has widely used as flavouring ingredients since it gives tremendous imparting aromatic rice flavour to food or modified compositions in foods [5,6]. The major compound contributing to the flavour characteristic Pandan is 2-acetyl-1-pyrroline (2AP) [3].

2-Acetyl-1-pyrroline (2AP) has an IUPAC name of 1-(3,4-dihydro-2H-pyrrol-5-yl) ethanone. The structural homolog of this compound is 6-acetyl-2,3,4,5 tetrahydropyridine. 2AP is a substituted pyrroline and a cyclic imine as well as a ketone. The formula of this compound is CH_9NO. Whereas the molecular weight of 2AP is 111.14 g/mol. It has quite low melting point and high boiling point value which is 19°C at 760 mm Hg and 182 to 183°C at 760 mm Hg. Vapor Pressure of 2AP is 0.793 mm/Hg at 25°C and the flash point is 151°F. The appearance of 2AP is colourless to yellow solid with an assay from 95 to 100%. This compound is categorized as flavour and fragrance chemical compounds.

Laohakunjit *et al*. [7] stated that, Pandan is one of the best natural sources of 2AP. Buttery *et al*. [8] and Lin *et al*. [9] identified 2AP as an important compound contributing to popcorn-like aroma in several Asian aromatic rice varieties. 2AP is a chemical that emits a buttered-popcorn-like aroma at specific concentrations [10]. When the grain is cooked, the characteristic of sweet aroma found in Basmati rice is released [11].

The compound is synthesized chemically and being added to nonaromatic rice varieties and rice-containing dishes including many pilaf, pudding and the like. Moreover, this compound can be used as flavouring ingredients of meat and vegetable products or blended with other flavour enhancing sauces or compositions. This compound has being justified to improve the quality of odour in products such rice [12]. Currently, this type of compound is trying to move in biomedical and other industrial sectors. This can be seen when 2AP has been tested as cockroach repellent an achieved about 65 to 93 percent of repellence [2]. Routray and Rayaguru [2] also found that 2AP in has been reported to recover heavy metals such as lead and copper ions. Besides that, this compound has been applied to recover those metal ions in wastewater [13].

Several studies on extraction method of 2AP have been conducted. The extraction methods utilized are the solvent extraction method. One of favourable method is solvent extraction method. However, no study has been reported on the effects of solvents on the solvent extraction method using alcohol of 2AP compound from Pandan leaves has been reported. The quantity of extracted component was depended on its stability in the solvent used. In the purpose of extract 2AP from fresh Pandan leaves, Yahya *et al*. [3] found that the yield obtained was lower when using hexane as a solvent compare to the ethanol. The reason is that, the stability of 2AP was low in hexane when compare to ethanol.

Therefore, the study of the solvent effects is very important for the screening and solvent selection of the extraction,

fractionation and purification steps in the plant processing. By understanding the solvent properties, component (solute) properties and solvent-solute interaction, rapid fractionation and isolation of desired components can be achieved [14]. This paper presents the results on the effects of alcohol solvents with different polarities on the extract yield. The performance of those solvents was presented in terms of the existence of the compound in the GCMS chromatogram.

2.0 EXPERIMENTAL

2.1 Materials

Fresh Pandan leaves were purchased from a local supplier in Taman Universiti, Johor. The solvents used such as methanol, ethanol and propanol are analytical-reagent grade and purchased from Merck, Germany. All the chemicals were used as received, without further purification.

2.2 Moisture Content Determination

The moisture content of fresh Pandan leave was determined by gravimetric analysis. The mass of approximately 5 g leave was measured before and after 105^{0}C, 24 h oven drying. The loss mass was assumed equal to the mass of water in the sample. Three replicates were used for each measurement.

2.3 Pre-treatment

Pandan leaves were grinded using a grinder until the leaves scrap of around 0.5-1.0 mm. The samples then were dried in the oven at 30^{0}C for 48 h.

2.4 Solvent Extraction of 2-acetyl-1-pyrroline (2AP) from Pandan Leaves

Approximately 5 g of leaves mass were bath for 24 h in 100 mL 50% analytical grade of solvent. After that, the Pandan leaves were filtered out from the solution. The 2AP extract was obtained from the solution by evaporating the solvents in the extract solution using a rotary evaporator at temperature of the boiling point of the solvent. For this section, three different type of alcohol solvents i.e. methanol, ethanol and propanol were used to investigate the best solvent performance.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

Pandan leaves extract were also being observed using a gas chromatography/mass spectrometry (GC-MS). GC-MS is uses to detect the existance of the 2AP in term of existence percentage in chromatogram. The column dimensions and the carrier gas conditions are same as in GC-FID analysis. The operation of mass spectrometer was done in electron impact (EI) mode, electron energy 70 eV. The temperature of ion source and quadruple are 230 and 150^{0}C constantly with the mass range (m/z) of 10-350. All the identified compounds were matched with the mass spectra (having match quality of over 80%). Chemstation Wiley Spectral Library of standard compounds was used as a retention catalogue stored in the GC-MS.

3.0 RESULTS AND DISCUSSION

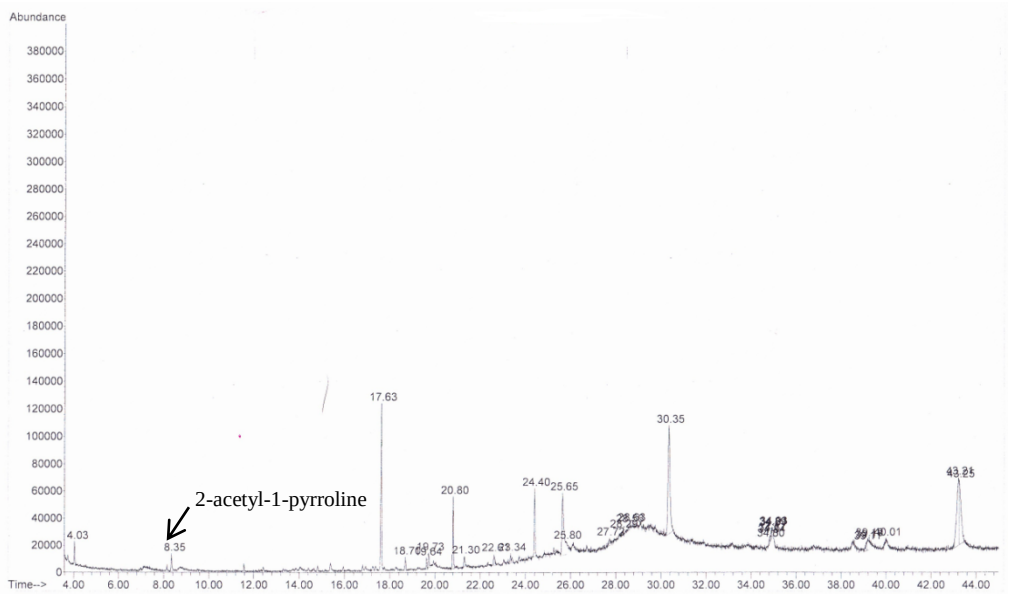
3.1 Moisture Content of Pandan Leaves

Drying pre-treatment was done to determine the moisture content in the yield and 2AP concentration in Pandan leaves extracts. By using oven drying at 105^{0}C for 24 h, the measured moisture content of fresh Pandan leaves was 85.19 ± 0.3%. As mentioned in section 2.2, the drying pre-treatment procedure was oven drying at 30^{0}C for 48 h. As expected, the moisture content of Pandan leaves was decreased dramatically to 16.39 ± 0.2%. This result is distinctive for most plant materials as cited by Yahya *et al*. [3] that about 80% of water is removed. However, the moisture content removed from leaves depend on the biological nature of the plant itself.

3.2 Extract of Pandan Leaves

The performance of solvent extraction on the Pandan leaves extract was investigated using three different solvents i.e. methanol, ethanol and propanol to study their influence on the existence of 2AP in Pandan leaves. From the observation of all the chromatograms in Figure 1 (a), (b) and (c), 2AP was existed in methanol and ethanol solutions at retention time 8.35 and 8.36, respectively. On the other hand, there was no 2AP extracted when propanol was used as the solvent of extraction. The patterns were differed at the peak as appeared at different retention times according to the solvents used. Besides that, a number of components exit in Pandan leaves. This finding was also found by Laohakunjit *et al*. [15], Bhattacharjee *et al*. [16] and Yahya *et al*. [3]. But still, the major focus is on the detection of 2AP.

Comparing both methanol and ethanol performance as a solvent, ethanol seem to has greater yield of 2AP. This can be justified as the existence peak in ethanol chromatogram was higher compared to methanol chromatogram. Ethanol is a very polar molecule. This is due to its hydroxyl (OH) group in ethanol. This kind of alcohol has high electronegativity of oxygen which allowing hydrogen bonding to take place with other molecules. Ethanol therefore attracts polar and ionic molecules. Thus, ethanol can dissolve both polar and non-polar substances. Moreover, ethanol has been claimed as among most important solvent during solvent extraction. Ethanol solvent is also environmentally favourable compared to pure alcohol.


Abundance
2-acetyl-1-pyrroline
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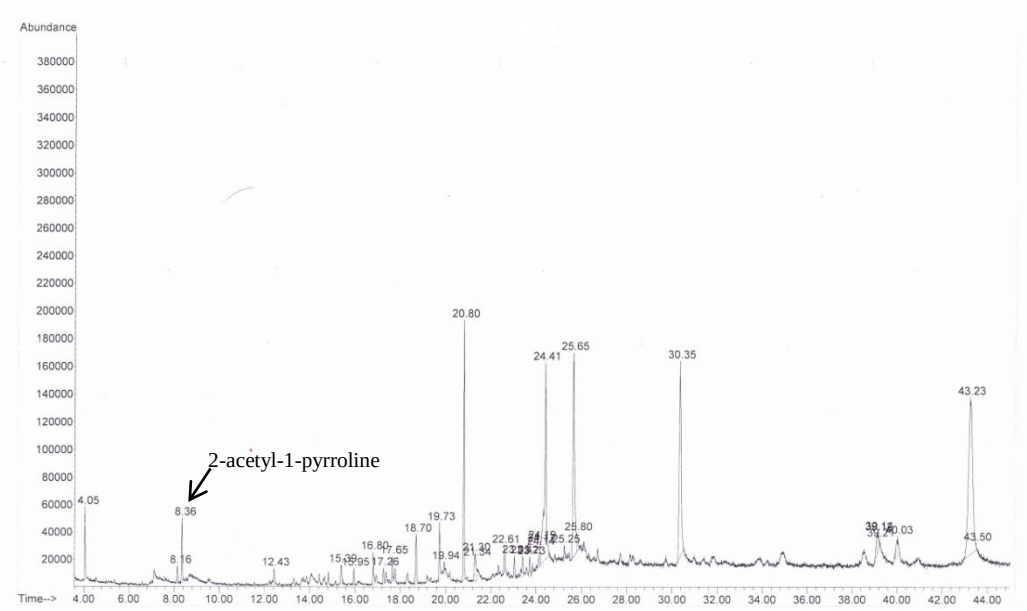
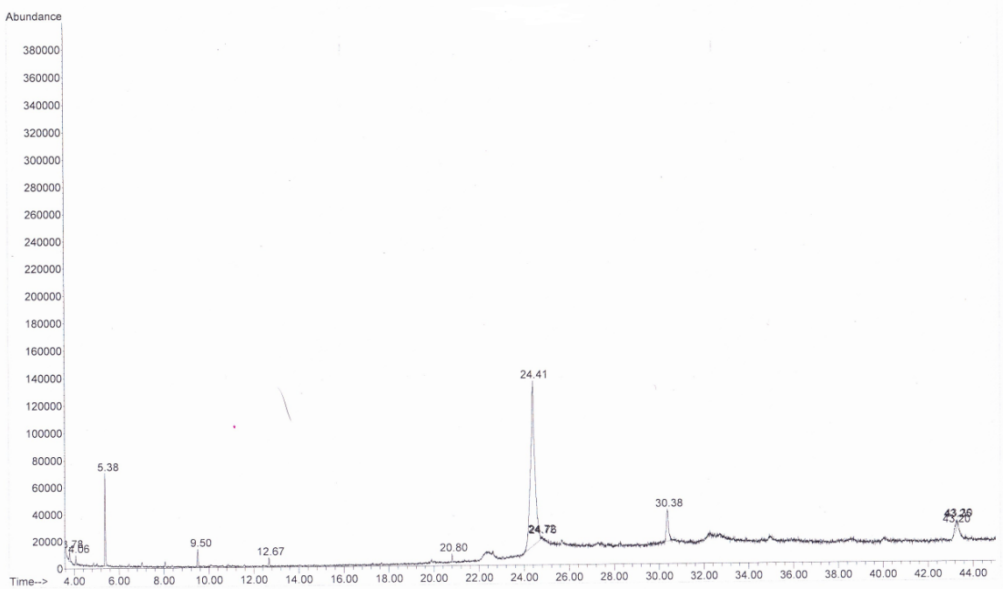
8.35
17.63
20.80
24.40
25.65
25.80
30.35
43.25
Time-->

(a)


Abundance
2-acetyl-1-pyrroline
4.05
8.36
8.16
12.43
18.70
19.73
20.80
24.41
25.65
25.80
30.35
43.23
43.50
Time-->

(b)



(c)

Figure 1 Gas chromatogram of extract 2AP from Pandan leaves using solvent extraction method. (a) methanol (b) ethanol (c) propanol

■4.0 CONCLUSION

The extraction of 2AP compound from Pandan leaves can be achieved using ethanol as solvent in solvent extraction. The existence of 2AP was observed to be greater comparing with methanol solvent. The great result has come from the polarity of the ethanol as compare to methanol. Other parameters such as temperature and extraction time can be further explore to obtain an optimize condition of the extraction.

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