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Pyrolisis of Salak Seed (*Salacca zallaca)* and Toxicity Test of Its Liquid Smoke to the Mortality of *Artemia salina* Leach Larvae

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Abstract

Pyrolysis of 10 kg of salak seed (*Salacca zalacca*) produces liquid smoke with 10% yield. Fractional distillation of 1 kg of salak seed liquid smoke under the temperature of 150-180 ºC gave percent yield as much as 13% from a fraction named 'F3'. Mortality test for the fraction 'F3' with concentration of 1000 ppm and 100 ppm gave 100% mortality to *Artemia salina* Leach larvae. The fraction 'F3' was then analyzed with Gas Chromatography-Mass Spectrometer, which then showed as much as 18 compounds identified in the chromatogram, those 18 compounds were grouped into three: acetic acids and the derivatives, phenols and the derivatives, ketones and the derivatives. Those three groups of compounds are predicted to possess toxic activity and capable in killing *Artemia salina* Leach larvae with LC_{50} = 23.44 ppm.

Keywords: Pyrolysis, S. zalacca seed, larvicide, brine shrimp larvae, GC-MS

INTRODUCTION

Production of *salak* (*Salacca zalacca*) or snake fruit is ranked fifth in the national fruit production of Indonesia, with 5.65 percent, or as much as 1,118,953 tons of salak production. The main area of salak production in Indonesia is in Java Island which produces 655,707 tons of salak or about 58.60 percent from the total number of national salak production. The next biggest salak producer outside Java Island is North Sumatra with number of production 354,087 tons of salak or about 31.64 percent from the national salak production, while the other province made up the remaining 9.71 percent[1].

Salak is not consumed only as a fresh fruit, it was also processed into varying types of food products such as *salak* chips, '*bakpia salak'*, *salak* sweets, *salak* coffee and '*dodol salak'*. In processing the fruit, the seeds are considered waste by the producer. As the production of salak-based food increases, the waste of its seeds also increases. It is common practice for the producers of salak-based foods to throw the seeds away, and not utilizing them in any other way. The waste of salak reaches 35-44% within processed or consumed salaks. Salak seed has a higher percentage than its skin in case of being wasted. Wasted salak seed is about 25-30%, while the skin is about 10-14% [2].

Research about salak seed has not really improved and there is no publication yet about the pyrolysis of salak seed and the application of its liquid smoke in controlling the brine shrimp larvae. Toxic compounds taken from plants has a good prospect to be developed as larvicide, because the chemicals from plants could be easily decomposed and is eco-friendly. Liquid smoke of salak seed is a liquid material obtained from pyrolysis under high temperature. The liquid smoke is potential to be developed as main ingredients of nature-based pesticide, and is applicable to multiple uses such as insecticide, bactericide, larvicide, acaricide and fungicide. Liquid smoke usually contains many chemical components such as phenols, aldehydes, ketones, organic acids, alcohols and esters. The phenols, acids and alcohols mostly has antioxidant, antibacterial, antimicrobial and antifungal activities[3, 4].

Preceding research showed that the unfractionated liquid smoke of salak seed had killed *Artemia salina* Leach larvae in concentration of 10% (w/v). Generally, whenever a natural product with toxic or poisonous effect research is conducted, the toxicity test always takes place first. The toxicity test method could be done *in vitro*. One of the method is the Brine Shrimp Letality Test (BSLT). The BSLT method is one of many methods that is fast and affordable to predict the toxicity of plant extract or other samples by using oceanic organism such as the *Artemia salina* Leach larvae[5].

The toxicity test with BSLT method has a wide spectrum of toxic activity, along with a simple procedure, it takes less time to implement, does not cost much, and the result is reliable. Based on those considerations, we want to conduct pyrolysis and toxic activity test of salak seed to the fatality of *Artemia salina* Leach larvae.

MATERIALS

Materials used in this research were 10 kg of salak seed waste collected from Desa Pangu, Kecamatan Ratahan, Kabupatan Minahasa Tenggara. The bioindicator for toxicity test was 48-hour-old *Artemia salina* Leach larvae.

INSTRUMENTS

Instruments used for this research were a set of pyrolysis apparatus which consists of a pyrolysis reactor that has a pipe to direct the smoke, a condenser, and a container to accomodate the liquid smoke. The fractional separation of liquid smoke was done using a set of fractional distillation apparatus which consists heating mantle, thermometer and condenser. A set of petri dishes as a place to hatch the brine shrimp egg and other glassware such as Erlenmeyer flasks, graduated cylinder, volumetric pipettes, Pasteur pipettes, and balance were also utilized. The compound in the fraction 'F3' of the liquid smoke was identified using Gas Chromatography-Mass Spectrometer (GC-MS).

METHOD

1. Pyrolysis and liquid smoke collecting

As much as 10 kg clean salak seed were put inside the pyrolysis apparatus, the apparatus was then closed tightly as to prevent any leak. The initial and final temperature of pyrolysis were carefully recorded. The liquid smoke distillate was contained in a bottle until the pyrolysis process completed. The liquid smoke in a bottle was labeled as B1 and the color was recorded. The B1 distillate was then distillated in a fractional distillation apparatus to obtain the fractions of liquid smoke. The separation was done based on increasing boiling point. The B1 liquid smoke was poured in the distillation flask and was heated with heating mantle. The distillation temperature of fraction 1 (F1) was 90-120 ºC, the fraction 2 (F2) was > 120-150 ºC, the fraction 3 (F3) was > 150-180ºC, the fraction 4 (F4) was > 180-210ºC and fraction 5 (F5) was > 210ºC. The toxicity of fractions F1, F2, F3, F4 and F5 was tested towards the *Artemia salina* Leach larvae. Fraction with the highest toxic activity was analyzed thereafter by GC-MS.

2. Hatching of *Artemia salina* Leach (Purwantini, *et al*., 2002)[6]

The brine shrimp egg was hatched inside a petri dish filled with seawater. A plastic screen with a few 2 mm holes was used to make two compartments, one for a dark compartment and the other for light compartment. The light compartment was lit with a lamp and the dark compartment was covered with a dark paper. Brine shrimp egg as much as 50 mg was placed into the dark compartment, and after 48 hours the larvae in the light compartment was taken for toxicity test.

3. Preparation of testing samples

The toxicity test using *Artemia salina* Leach larvae is based on a method by Parwata[7]. The toxicity of the liquid smoke fractions was tested in four concentrations, 0 ppm, 10 ppm, 100 ppm and 1000 ppm towards the 48-hour-old *Artemia salina* Leach larvae, to get the LC_{50}.

4. Toxic activity of *Artemia salina* Leach larvae

Exactly 10 brine shrimp larvae in 100 mL of seawater were filled inside a test vial, then 100 mL of sample solution was added afterwards. It was repeated three times for each concentration. For the control, a 100 mL blank was mixed with 100 mL of seawater. The vials were observed after 24 hours by counting how many larvae have died and how many larvae are still alive. The mortality was then calculated using percent mortality equation,

$$\%\ \text{Mortality} = \frac{\text{Dead larvae}}{\text{Dead larvae + Living larvae}} \times 100\%$$

A fraction or an extract is considered active if the $LC_{50} \leq 1000$ ppm. For a pure substance, it is considered active if the $LC_{50} \leq 200$ ppm.

5. Analysis of liquid smoke fraction with GC-MS

Fraction of liquid smoke with the highest toxic activity towards *Artemia salina* Leach larvae was selected to be analyzed with GC-MS to identify the chemical compounds which will be known by its GC chromatogram. The molecular mass of compounds will be known from the mass spectra along with the fragment pattern[8].

RESULT AND DISCUSSION

Making of liquid smoke from salak seed

Pyrolysis of 10 kg of salak seed produced 1 kg of its liquid smoke. The percent yield obtained from this research was 10%. Compared with the results reported by Jamilatun[9] that the percent yield of coconut shell liquid smoke without re-distillation is 29.9%. The difference of percent yield could be caused by the specification of pyrolysis apparatus, the type of sample and the duration of pyrolysis. The liquid smoke produced from salak seed was black in color, as depicted in Fig. 1.

Figure 1. Liquid smoke of salak seed

Separation of chemical component in the liquid smoke was carried out using fractional distillation. The temperature of fraction 1 (F1) was 90-120 ºC, the fraction 2 (F2) was > 120-150ºC, the fraction 3 (F3) was > 150-180ºC, the fraction 4 (F4) was > 180-210ºC and fraction 5 (F5) was > 210ºC. The color of each fraction is shown in Fig. 2.

Figure 2. Fractions of liquid smoke from salak seed

From 1 kg of liquid smoke that was fractionated, the percent yield obtained for each fraction was 60% for F1, 12% for F2, 13% for F3, 9% for F4 and 6% for F5. The result of toxicity test for fractions F1-F5, along with its concentration (ppm) and percent mortality (%) of *Artemia salina* Leach larvae is shown in Table 1.

Table 1. Toxicity test results for fractions of salak seed liquid smoke, its concentrations (ppm) and percent mortality (%) of *Artemia salina* Leach larvae.

Fractions of salak seed liquid smoke	Concentration of the solution (ppm)	percent mortality (%) of *Artemia salina* Leach larvae
F1	1000 100 10	50 50 20
F2	1000 100 10	60 50 30
F3*	1000 100 10	100* 100* 80*
F4	1000 100 10	90 70 50
F5	1000 100 10	60 40 10
Blank	0	0

* selected for further analysis

The results of the toxicity test showed that the fraction F3 has the highest toxic activity, with 100% mortality in 1000 ppm and 100 ppm solutions and 80% mortality in 10 ppm solution. To find out the chemicals which causes the toxic activity, F3 was chosen to be analyzed with GC-MS, while the remaining fractions (F1, F2, F4, F5) will not be investigated in this research.

The result of GC analysis showed that there were 18 peaks in the chromatogram, which means there are 18 compounds in the fraction F3. The chromatogram of fraction F3 can be seen in Figure 3 and the chemical components in the fraction F3 is shown by Table 2.

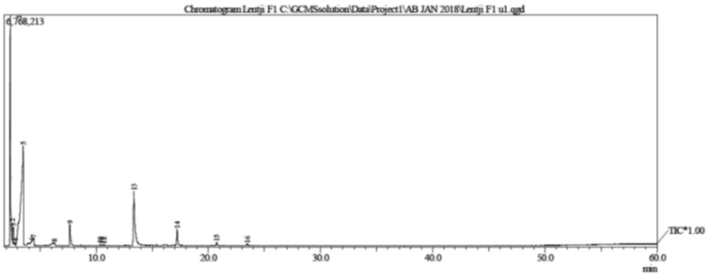

Figure 3. GC chromatogram of fraction F3

Table 2. Data of chemical components in fraction F3

Peak number	Components	Retention time (minute)	Peak area (%)
1	Benzeneethanamine	2.331	0.80
2	Acetone	2.532	1.85
3	Formic acid	2.676	1.19
4*	Acetic acid	3.472	73.80*
5	2-propanone, 1-hydroxy	3.793	1.19
6	Propanoic acid	4.311	1.24
7	1-Hidroxy-2-butanone	5.842	0.66
8	Epoxypropane	6.025	0.84
9	Butyric acid	6.150	0.30
10	2-Cyclopenten-1-one	7.684	0.29
11	2-Butanone	8.549	0.21
12	Butyrolactone	10.581	1.23
13	2,5-Hexanedione	11.235	0.05
14*	Phenol	13.343	14.94*
15	Methacrylic acid, vinyl ester	13.992	0.08
16	1,2-Cyclohexanedione	14.901	0.44
17	Phenol, 2-methoxy	17.210	0.69
18	Benzene, 1,4-domethoxy	20.722	0.19

*peak 4 and 14 have the most dominant peak area

From Table 2, we can see that the component could be classified into three main groups, which are acetic acids and the derivatives, phenols and the derivatives and ketones and the derivatives. Each group works together in causing the toxic activity towards *Artemia salina* Leach larvae. Phenols and the derivatives can act as either bacteriostatic or bactericidal due to its ability to inactivate essential enzymes, and to coagulate SH and NH groups in proteins. The phenols (phenol, methyl phenol and guaiacol) and the acid components (derivative of benzoic acids) are identified as antibacterial agent in liquid smoke[4]. Acids and phenols are the main component of liquid smoke that are effective in influencing the mortality of termites. The acids in liquid smoke also effective in killing and inhibiting microbes in food products by penetrating the cell membrane of microorganism which in turn causing the microorganism experience lysis and die.

Liquid smoke contains active compound which acts as antimicrobial or disinfectant and can also be utilized as insecticide, and as dipping medicine in some animal diseases[10]. Carbonyl compounds contribute to the aroma of food products and can act as insecticide[11]. The antimicrobial and antioxidant activity of liquid smoke could be used as preservatives, antitermite, and antifungal; it could also be used to coagulate gum and as a natural pesticide[11].

Based on the GC chromatogram of fraction F3 of the salak seed liquid smoke, the peak 4 is an acetic acid with peak area 73.80% and peak 14 is a phenol with peak area 14.94%. Both are the most dominant peak in the chromatogram and together gave high toxic activity which caused the mortality of *Artemia salina* Leach larvae. The mass spectra and fragment pattern of peak 4 and 14 will be discussed in the following subsection.

Fragmentation of compound in peak 4

The mass spectrum of peak 4 with retention time of 3.472 minutes and abundance of 73.80% showed similarity in fragment pattern with the mass spectrum of acetic acid according to the data in Wiley229.LIB library. The comparison of both spectra is shown in Fig. 4.

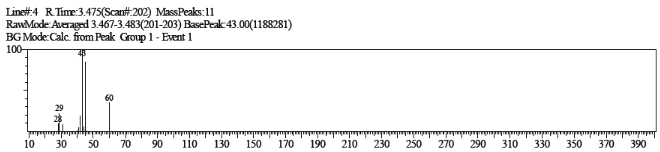

Figure 4a. Mass spectrum of peak 4

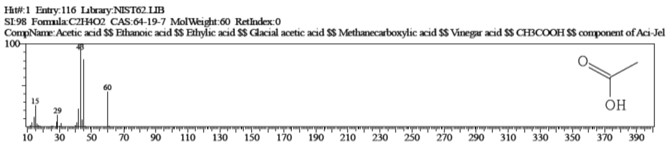

Figure 4. b. Mass spectrum of acetic acid

The compound in peak 4 showed the same base peak with the data in Wiley229.LIB library, it has the m/z = 43. Both spectra showed molecular ion peak at m/z = 60 which indicate the molecular mass of the molecule. Electron discharge in molecule with m/z = 60 produces fragment of molecular ion M^+ = 60. The release of –OH group (17 amu) produces fragment of molecular ion with m/z = 43, subsequently, the release of $–CH_2$ (14 amu) produces molecular ion with m/z = 29.

Grounded on the similarity of base peak at m/z = 43 (100%), the similarity of molecular ion M^+ = 60, and similarity in fragment pattern, it is concluded that the compound in peak 4 is identic to acetic acid with molecular formula $C_2H_4O_2$. The fragment pattern of peak 4 compound can be seen in Fig. 5.

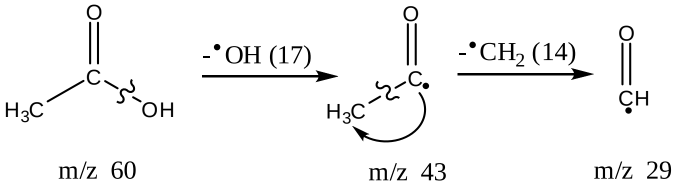

Figure 5. Suggested fragment pattern for compound in peak 4 or acetic acid

Fragmentation of compound in peak 14

The mass spectrum of peak 14 with retention time of 13.343 minutes and abundance of 14.94% showed similarity in fragment pattern with the mass spectrum of phenol according to the data in Wiley229.LIB library. The comparison of both spectra is shown in Fig. 6.

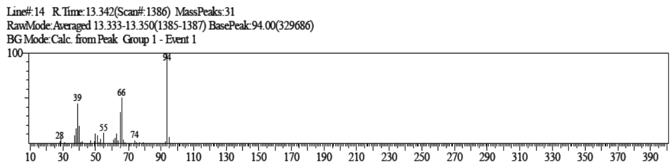

Figure 6a. Mass spectrum of peak 14

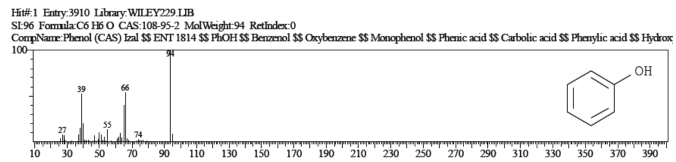

Figure 6b. Mass spectrum of phenol

Compound in peak 14 showed the same base peak with the one showed by the Wiley229.LIB library, for m/z = 94. Both spectra exhibit the same molecular ion peak at m/z = 94 which is the molecular weight of the compound. The fragment was formed by firstly breaking the aromatic ring and rearrangement of molecular ion happened subsequently. The release of –CO group (28 amu) was then caused a peak at m/z = 66, which then followed by the release of $-C_2H_3$ (27 amu) to form molecular ion with m/z = 39.

By the similarity of base peak at m/z = 94 (100%), similarity on molecular ion M^+ = 94, and similarity in fragment pattern of the compound, it can be concluded that compound in peak 14 is identical to phenol with molecular formula of C_6H_6O. The fragment pattern of compound in peak 14 can be seen in Fig. 7.

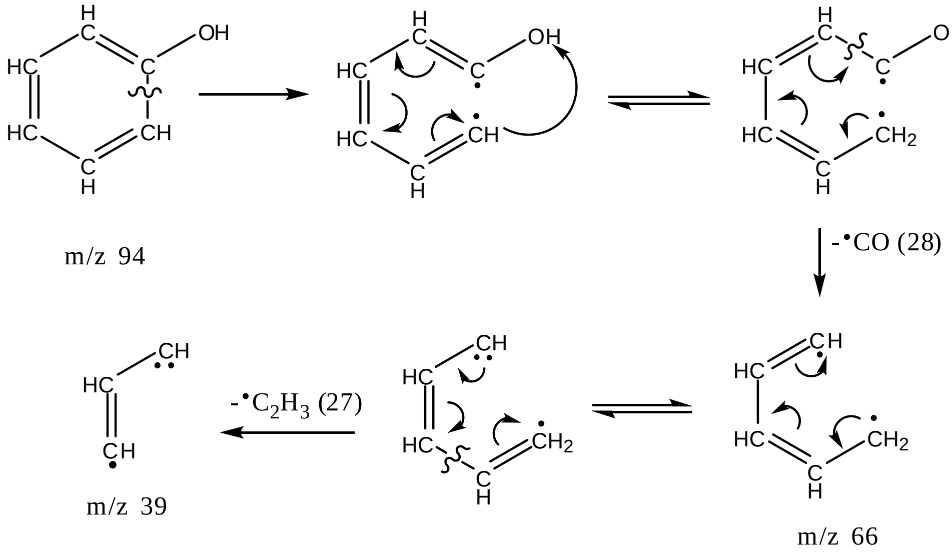

Figure 7. Suggested fragment pattern for compound in peak 14 or phenyl alcohol

CONCLUSION

1. Fractional distillation of 1 kg of salak seed liquid smoke produced 13% yield of fraction F3.
2. Acetic acid and phenol work together to perform high toxical effect that caused the death of *Artemia salina* Leach larvae.
3. The liquid smoke of salak seed showed toxic activity towards *Artemia salina* Leach larvae with LC_{50} = 23.44 ppm.

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