cek jurnal terbit tahun 2019-29

by I Ketut Budaraga

Submission date: 18-Aug-2020 11:07AM (UTC-0500)
Submission ID: 1367844633
File name: 01._08-14_V12N5CT-toksisitas,_terbit,15-6-2019.pdf (378.51K)
Word count: 3452
Character count: 17692

International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.12 No.05, pp 08-14, 2019

Study of Toxicity of Cacao Skin Liquid (Theobroma cacao, L) Using BSLT Method (Brine Shrimp Lethality Test)

I Ketut Budaraga[1]*, Eva Susanti[2]

[1,2]Ekasakti University, Agricultural Technology Department, Faculty of Agriculture, Jalan Veteran Dalam No.26B Padang, Indonesia,
Telp/Fax 0751)28859; (0751)32694

Abstract : Cocoa skin has great potential to be processed into liquid smoke. So far, it has only been processed into animal feed and the rest is disposed of. The aim of the study was to determine the toxicity of the liquid smoke water of cocoa peels. This research was carried out in the Agricultural Technology Technology Laboratory of Ekasakti University, laboratory of the Faculty of Agricultural Technology, Andalas University and LL region X dikti Laboratory in Padang. Analysis of cocoa skin liquid smoke data using descriptive experimental data. The treatment in the study was the water content of cocoa skin, namely 10%, 15%, 20% and 25%. The results showed that the water content of different cocoa peel raw materials gave different toxicity values. Toxicity activities in liquid smoke of cocoa peels with different moisture content met the toxicity standard. The water content of 25 percent has the most toxic value of 59,020 ppm.
Key words: liquid smoke of cacao bean skin, water content.

1. Introduction

Indonesia is one of the largest producers of agricultural products in the world, one of which is cocoa. Cocoa is an agricultural product that has the potential to be developed. The largest cocoa producing area in Indonesia is one of them in the Province of West Sumatra. In 2016 cocoa production in West Sumatra was 62,623 tons with an area reaching 152,885 ha, consisting of 60,254 tons of smallholder plantations with an area of 151,123 ha while private plantation production was 2,369 tons with an area of 1,762 ha. In Padang Pariaman Regency, the total area of cocoa plantations is 9.526 tons with an area of 725 ha with a total of 12,641 farmers. [1]

In the cocoa fruit processing industry, cocoa skin is a very large amount of waste. The results of processing cocoa from plantation land produce biomass waste in the form of cocoa pods, cocoa leaves and cocoa wood. One of the wastes originating from plantations is that cocoa fruit skins have only been discarded and burned [2]. There are around 70% of cacao fruit skin produced from 1 kg of cacao fruit [3]. The handling of agricultural and plantation waste is still an obstacle in the waste management program at the farm level. This

DOI= http://dx.doi.org/10.20902/IJCTR.2019.120502

problem is caused by several factors including limited time, labor, and limited disposal area. Agricultural and plantation wastes, especially cocoa plants, have not been widely used, although in some conditions they have potential as animal feed ingredients and compost raw materials, so observations are needed to support potential waste utilization programs, especially potential waste produced by cocoa, namely cocoa skin waste.

Cocoa peel is a lignocellulose waste containing the main components in the form of lignin, cellulose and hemicellulose. Cocoa fruit skin contains cellulose 36.23%, hemicellulose 1.14% and lignin 20-27.95% [4]. Cellulose and hemicellulose are polymerized from monosaccharides which can be converted into sugar under certain conditions. Lignin is an aromatic polymer that can be converted into phenolic compounds [5]. Lignin decomposition on cocoa skin can use the pyrolysis method [6].

Pyrolysis is the process of heating a substance with limited oxygen so that there is a decomposition of the components of a heated sample [7]. The pyrolysis method is carried out by heating at high temperatures and without using organic solvents. In the pyrolysis process heat energy encourages oxidation so that complex carbon molecules decompose mostly into carbon or charcoal and the remainder in the form of liquid smoke (bio oil) [8].

The chemical components of pyrolysis liquid cacao skin smoke reported by Mashuni [16] and Wijaya [2] are ammonia, hexane, alcohol, ketone, acetic and phenolic acids. Phenolic is known to have antibacterial and antifungal properties against several pathogenic bacteria and carcinogenic bacteria, as well as preservatives and biopesticide applications [16]. Based on previous research by Rachmawati [9]. Phenolic compounds, flavonoids, tannins, and terpenoids in cocoa fruit skin are known to have antimicrobial activity. Phenol active compounds have antifungal activity, as antifungal phenols can damage cell membranes so that cell permeability changes which can result in inhibition of cell growth.

Based on information about the benefits and use of liquid smoke, liquid smoke has the potential to be an alternative preservative, in addition to providing a distinctive aroma, texture and flavor to food products. Therefore, testing of the safety of liquid smoke is needed, so that it can be a safe alternative food preservative. Several studies have reported the mutagenic potential of chemical compounds resulting from pyrolysis. Braun [10] reports that the chemical compounds in wood smoke extract are mutagenic in the human lymph gland, but do not have mutagenic potential in testing using bacteria. Putnam [11] reported that wood smoke is mutagenic to Salmonella. The mutagenic potential of pyrolysis chemical compounds is strongly influenced by the material or type of wood used and the method used to produce the chemical compound. Although the mutagenic potential of wood smoke has been reported, but there has been no study of the toxicity of liquid smoke, especially liquid smoke derived from the results of brown skin waste pyrolysis. Research on the toxicity of liquid smoke is very important considering that liquid smoke is currently used commercially by the food industry [12]; [13]; [14]; [15]

The benefits and use of brown leather liquid smoke has the potential to be an alternative preservative, in addition to providing a distinctive aroma, texture and flavor in food products. Therefore, it is necessary to test the safety of liquid chocolate skin smoke food, so that it can be a safe alternative preservative. The method used is the acute toxicity test of liquid brown smoke to determine the LD_{50} value. One method used is to test the acute toxicity of liquid smoke of cocoa peel is the BLST method. The Brine Shrimp Leathlity Test (BLST) method is aimed at mortality of shrimp larvae Artemiasalina L caused by test extracts. The results obtained were calculated as the lethal concentration (LC_{50}) value of the test extract, ie the number of doses or concentrations of the test extract which could cause 50% mortality of shrimp larvae after a 24-hour incubation period. An extract is declared toxic according to the BSLT method if it has an LC50 of less than 1000 µg / ml [16]. So far there has been no study of the safety of grade 3 liquid smoke from brown leather waste. The purpose of this study was to determine the toxixity of liquid smoke of cocoa peel at different moisture content with the BLST method.

2. Materials and Methods.

This research has been carried out at the Agricultural Technology Laboratory of Ekasakti University, Laboratory of the Faculty of Agricultural Technology Andalas University and LL Dikti Laboratory of Region X Padang. The main ingredients used are cocoa pods obtained in the area of Padang Pariaman Regency and LubukMinturun Kota Padang. In addition, materials used for chemical analysis are also used, namely A. salina

Leach, sea water, methanol, DMSO.The tools used in making liquid cocoa skin smoke are a set of pyrolysis equipment, micropipette (Scilogex), egg salvage container A. salina Leach, incandescent lamp (5 watts), drop pipette (Drpping), 50 ml measuring cup, test tube, petri dish , measuring cup (Pyerex), vertex, magnifying glass, electric scales (Mettler).

2.1. Research Method.

This research is a real experimental study with quantitative descriptive method of different water content in the manufacture of cacao skin liquid smoke as follows: A = Cocoa skin moisture content of 10%, B = Cocoa shell moisture content of 15%, C = Cocoa skin moisture content of 20%, D = Cocoa skin moisture content 25%

2.2. Implementation of Research

2.2.1.Raw Material Preparation

For the preparation of raw materials for liquid smoke, cocoa cacao skin is taken around the Padang Pariaman area. Following is the preparation process for raw materials:

1. Cocoa skin. Cocoa pods were taken from Padang-Pariaman cocoa farmers.
2. Cleaning. Cocoa skin is cleaned from dirt or soil that sticks to the cocoa skin.
3. Reducing the size of 5-9 cm using a machete so that the drying process is faster.
4. weighing. Cocoa bark was weighed using a scale of each weight obtained by the cocoa skin sample of 27 kg.
5. To determine the initial moisture content of the cocoa peel, the water content of the material was measured using a spectrameter with an initial moisture content of 80%.
6. Cocoa skin drying is carried out in the sun, for each drying treatment is done differently. At treatment A (10% cocoa skin moisture content) was dried for seven days, treatment B (15% cocoa peel moisture) was dried for six days, treatment C (20% cocoa peel moisture) was dried for 4 days , and D treatment (25% cocoa peel moisture content) is carried out drying for 3 days.
7. Then the measurements of the dry weight of the ingredients were carried out with each treatment with a spectrameter (water content of ingredients 10, 15, 20 and 25%)
8. Dry cocoa skin.

2.2.2. Pyrolysis Process (Making) of Liquid Cocoa Skin Smoke

The process of making liquid cacao skin smoke uses a 200^{o}C pyrolysis temperature [17]. And here are the stages of the pyrolysis process (making) of cocoa skin liquid smoke:

1. In the preparation of raw materials for cocoa skin liquid smoke dried cocoa skin with different moisture content is 10, 15, 20 and 25%
2. Preparation of tools. Before the preparation of cocoa skin liquid smoke, it is necessary to prepare the tools, namely stove, gas, and a set of pyrolysis devices [17]. And also other supporting tools such as dipper, used aqua tube and bucket.
3. Then the dried cocoa skin was put into a pyrolyzer for 4 hours with a capacity of 4 kg at a temperature of 200^{o}C, for treatment A (10% cocoa peel moisture content), treatment B (15% cocoa peel moisture content), treatment C (skin moisture content) cacao 20%) and at dairy level (25% cocoa peel moisture content).
4. Conditioning burning smoke
5. Observation tools. Observation of the tool is used to check the state of the device such as checking gas, stove and the temperature of the pyrolysis device.
6. Activated charcoal. Cocoa skin activated charcoal is produced from the pyrolysis process at 200^{o}C to produce activated charcoal and liquid smoke.
7. Grade 3 cocoa skin liquid smoke is obtained in the process of pyrolysis of liquid smoke
8. Cocoa skin liquid smoke is allowed to stand for 1 week so that the tar with separate cocoa skin liquid smoke is then filtered using filter paper [18].
9. Observation of the nature of toxicity was carried out by the BLST method.

2.2.3. Toxicity Test Procedure with BSLT Method [16]

BSLT is the first bioasay for the study of natural materials and one method of using materials that are toxic. The advantages of the BSLT test do not take a lot of time, the procedure is simple, fast, does not require cost, does not require aseptic techniques, does not require special equipment and requires only a few test samples. Bioassay is a test that uses living organisms to determine the effectiveness of a living material or organic and inorganic materials. The BSLT method uses Artemiasalina leach larvae as experimental animals and is a toxicity test because of a compound determined in a short time (for 24 hours). The basis of testing with the BSLT method is the ability of compounds to kill shrimp larvae. The toxicity test procedure using the BLST method is as follows: a).1 ml of sample was pressed in a 100 ml flask added with methanol to the boundary mark as the main ocean. b}.From the mother liquor piped 500 μl for 1000 ppm, 50 μl for 100 ppm, 5 μl for 10 ppm applied for 24 hours in a water bath at 45 ^{o}C.c).After 24 hours plus 10 artemiasalina leach larvae add 50 μl of DMSO as much as 0.05 ml then add 5 ml of sea water after 24 hours. Calculates the percent mortality of test larvae after 24 hours of treatment using the formula:

$$\% \text{ Of Death} = \frac{\text{number of larvae of A. salina death}}{\text{number of test larvae}} \times 100\%$$

d).The results of larval deaths were calculated using a formula and analyzed using ecxel 2007 to obtain linear line equations to determine the value of LC_{50}.

3. Result and Discussion

Cocoa Skin Liquid Smoke Toxicity Test The results of a real descriptive experimental analysis showed the concentration of liquid smoke with different toxicity activities produced. The LC 50 value of each of the cacao skin liquid fumes is presented in Table 1.

Table 1. LC 50 values of each of the cacao skin liquid smoke

Sample	Linear equation	IC_{50} (ppm)
Water content 10 %	y = 0,71x + 3,446 R^2 = 0,993	154,170
Water content 15 %	y = 0,78x + 2,883 R^2 = 0,946	517,606
Water content 20 %	y = 0,71x + 3,446 R^2 = 0,993	138,995
Water content 25 %	y = 0,725x + 3,716 R^2 = 0,980	59,020

The results of linear equations show that the treatment of water content 25 percent has the most toxic activity of 59,020. The higher the water content used, the higher the toxicity as shown in the probit graph 1,2,3,4. This is presumably due to the large amount of water content contained in the raw material making the liquid liquid, which affects the level of toxicity from liquid smoke

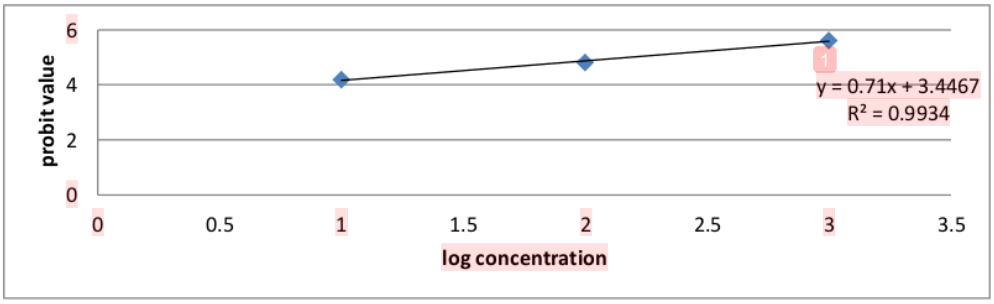

Figure 1. Toxicity of brown skin liquid smoke at 10 percent moisture content

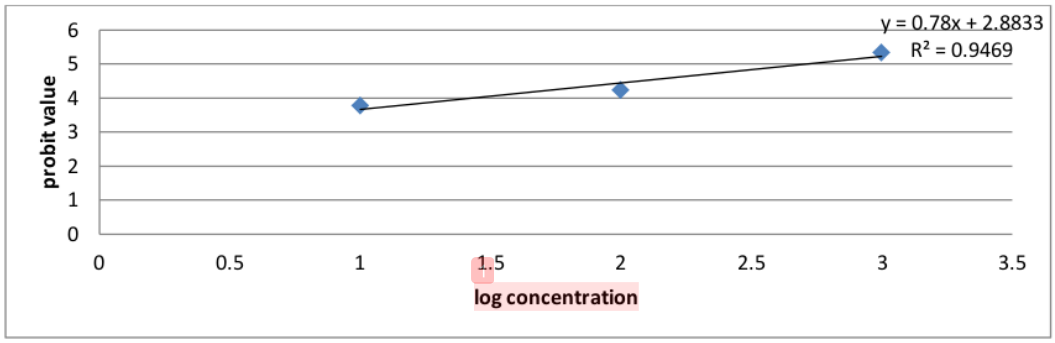

Figure 2. Toxicity of brown skin liquid smoke at 15 percent moisture content

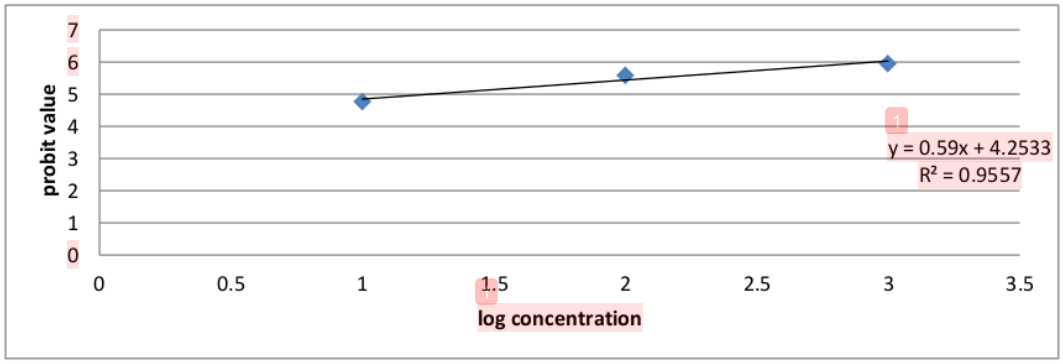

Figure 3. Toxicity of brown skin liquid smoke at 20 percent moisture content

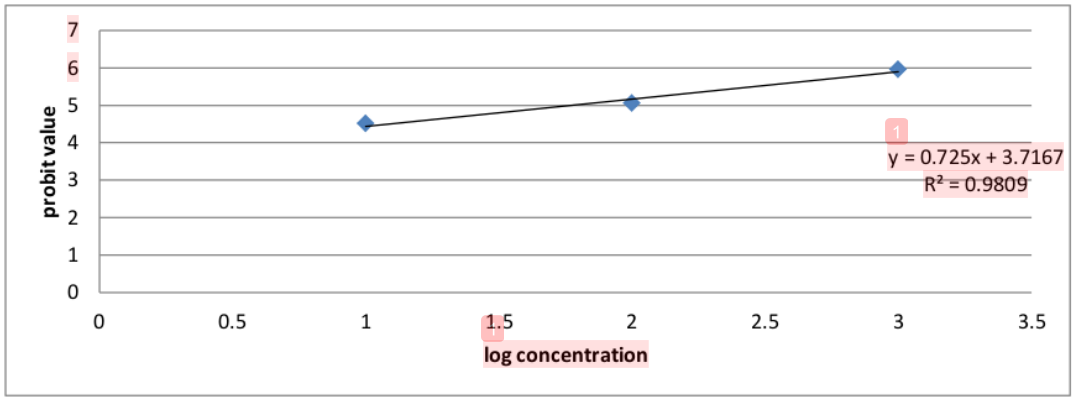

Figure 4. Toxicity of brown skin liquid smoke at 25 percent moisture content

This is related to the fact that liquid smoke contains a lot of tar, phenol which acts as an antioxidant. According to Simamora [19] explained that flavonoids are polyphenol compounds which are one of the classes of antioxidants that can inhibit the oxidation process triggered by free radicals from toxic compounds. The mechanism of flavonoid protection is by inhibiting oxidation reactions caused by compounds that contain toxic compounds containing toxins that are free radicals in the body which then by flavonoids free radicals will be stabilized by being involved in the oxidation process which will bind complex with flavonoid compounds [19].

According to Astuti's research [20] the level of ketoxic extract if the LC 50 <30 ppm value is very physical, the LC 50 30-1000 ppm value is toxic and > 1000 ppm is not toxic. The literature study shows several

compounds belonging to the quinone group, triterpenoids, flavonoids such as tannins, flavonoid aglycones and their highly toxic glycosides [21] (Jamilah 2008)

The test results showed that various different moisture content in the liquid smoke of cacao skin had high antioxidant activity and toxicity. Because the effective concentration of cacao skin liquid smoke is capable of capturing DPPH free radicals by 50% (IC50) and 50% LC 50 toxicity.

4. Conclusions and Suggestions

4.1. Conclusion

The toxicity test results of liquid smoke of cocoa peel on raw materials, the water content of 25 percent, yields the most toxic amounting to 59,020 ppm, still classified as toxic.

4.2. Suggestion

It is recommended to conduct further research on the purification and filtration process of liquid cocoa skin smoke to be safer if applied as a food preservative.

Acknowledgment

We would like to thank the Directorate of Research and Community Service, Directorate General of Research and Development Strengthening of the Ministry of Research, Technology and Higher Education according to the 2019 Budget Year Research contract number 005/LPPM-UNES/Contract-Research-J /2019. Chancellor of Ekasakti University, Chair of the Institute for Research and Service to the Community of Ekasakti University, Dean of the Faculty of Agriculture, Ekasakti University, research team and students who assisted this research.

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