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Preparation and Characterization of Liquid Smoke Derived from *Piper betle* L

Ai Emalia Sukmawati, Dodi Irwandi*, Latirah
Pharmaceutical and Food Analysis, Jakarta II Health Polytechnic Ministry of Health, Jakarta, Indonesia
*Corresponding Author: Dodi Irwandi Email: dodiirwandi@poltekkesjkt2.ac.id

***Abstract*—*Liquid smoke made from green betel leaf Piper betle L has been made by pyrolysis using a simple reactor. Furthermore, the liquid smoke was distilled once to obtain grade 2 liquid smoke and then carried out physical, chemical, and microbiological characterizations, namely pH, specific gravity, Total acid, total phenol, and the inhibition of Streptococcus mutans. The test results obtained were 2.81, 1.0071, 26.44%, 735.89 mg GAE/0.5mL and 18.4 mm. It can be concluded that grade 2 liquid smoke of green betel leaf has potential as a raw material for the manufacture of mouthwash.*

***Keywords*—** *Liquid smoke; Piper Betle L.; Streptococcus mutans*

I. INTRODUCTION

Green betel, also known as Piper betle L, is a plant that possesses medicinal properties and has been used for generations by Indonesian people (1). Numerous studies have been conducted on the antibacterial activity of green betel leaf extract (2). Betel leaf extract has been proven to have the ability to inhibit the growth of pathogenic bacteria (3), Streptococcus mutans (4), Staphylococcus epidermidis (5), Escherichia coli (6) and Staphylococcus aureus (7). No research has been found on the activity of betel leaf liquid smoke as an antibacterial for Streptococcus mutans.

The author of this research is interested in conducting physical, chemical, and microbiological tests on liquid smoke extracted from green betel leaves (Piper betle L). The physical tests will include pH, viscosity, and specific gravity tests. The chemical tests will examine the total acid and phenol content, while the microbiological tests will focus on the antibacterial activity of Streptococcus mutans. The objective of this research is to investigate the potential of betel leaf liquid smoke as a viable ingredient in mouthwash formulations.

II. MATERIALS AND METHODS

A. Materials

The set of tools used for research include distillation tools, pH meter, UV-Vis spectrophotometer, Oswald viscometer, pycnometer, analytical balance, autoclave, incubator, oven, microscope, vortex, cork borer, vernier caliper, Laminar Air Flow, heating mantle, and glassware. The ingredients used in the research are betel leaves (Piper betle L.), Amoxicillin antibiotics, TSA, NaCl, Streptococcus mutans.

B. Methods

Preparation of betel leaf liquid smoke

Preparation of liquid smoke according to the method used by Dodi (8). The liquid smoke obtained is then distilled at a temperature of 90ºC to obtain grade 2 liquid smoke.

pH measurement

The pH value of liquid smoke is determined using a calibrated pH meter.

Specific Gravity Measurement

The specific gravity of liquid smoke is determined using a pycnometer. Specific gravity is calculated based on the comparison of sample weight and water weight.

Determination of Total Acid contents

The total acid content was determined using the acid-base titration method using 0.1 N NaOH solution as titrant and phenolphthalein as indicator.

Determination of total phenol content

Phenol content was determined using the equivalence method for gallic acid.

Testing the Antibacterial Activity of Streptococcus mutans

To test the antibacterial activity of *Streptococcus mutans*, follow these steps:

a) Prepare a 24-hour-old microbial culture of Streptococcus mutans.
b) Take 1 dose of the culture and add diluent (sterile TSB), then homogenize.
c) Measure %T with a UV Vis spectrophotometer at a wavelength of 580 nm until it reaches 25%T (108 CFU/mL).
d) Take 0.2 mL of each microbial suspension into a petri dish. 5. Add 20-25 mL of TSA media, homogenize, and allow it to solidify.
e) Absorb the sample, negative control (K-), and positive control (K+) with a disc.
f) Place a disc containing the sample, K-, and K+ on the surface of the solidified media.
g) Leave it for 1 hour to allow the test solution to absorb.
h) Incubate at 30-35°C for 18-24 hours aerobically. 10. Observe the clear zone/inhibition zone.

III. RESULT AND DISCUSSION

Green betel leaves (Piper betle L.) were collected from the Research Institute for Spices and Medicinal Plants (Balitro) and analyzed at the LIPI Bogor Biology Research Center. To make liquid smoke, a simple liquid smoke maker (shown in Fig 1) was used. The final product of liquid smoke can be seen in Fig 2. Figure 2A shows the liquid smoke before filtration, displaying two layers with different color densities. The liquid

smoke after filtration is shown in Fig 2B, revealing that the black part can be separated through filter paper. It is believed that the black part is tar produced from the pyrolysis of betel leaves (9).

Fig. 1. Production of green betel leaf liquid smoke (Grade 3)

After making and filtering, the liquid smoke undergoes a purification process through distillation at a temperature of 90°C. This process is essential to remove carcinogenic tar. The outcome of the first distillation produces grade 2 liquid smoke, which has a brighter color compared to the undistilled state (Fig 3A). The darker color appears in the residue of the distillation process

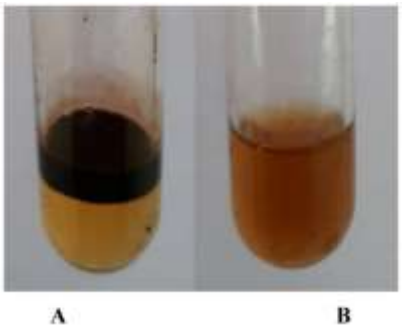

Fig. 2. Grade 3 liquid smoke product (A) before filtering (B) after filtering

After the distillation process, the liquid smoke is thoroughly tested through physical, chemical, and microbiological methods. The physical tests include the measurement of pH, viscosity, and specific gravity. Chemical tests comprise total acid and total phenol. Lastly, the microbiological test determines the diameter of the inhibitory force of Streptococcus mutans bacteria, as shown in Fig 4. Results (Table 1) indicated that the green betel leaf liquid smoke exhibited a strong inhibitory power against Steptococcus mutans bacteria, with a diameter of inhibitory force measuring 18.4 mm (10). The summarized data for the entire test can be found in Table 2.

Fig. 3. Liquid smoke in conditions (A) before distillation, (B) distillation results, (C) undistilled part

Fig. 4. Steptococcus mutans antibacterial test results

TABLE I. Steptococcus mutans inhibitory diameter

Code	Inhibitory diameter *(mm)*		
	Simplo	***Duplo***	***Average***
2 (Liquid smoke)	19.7	17.2	18.4
K+ (Amoxicillin 1000 ppm)	31.0	31.2	31.1
K – (Blank disc)	6	6	6
Disc diameter	6	6	6

TABLE III. Physical, chemical, and microbiological test results for grade 2 liquid smoke

Test	Results
pH	2.81
Specific gravity	1.0071
Total acid (%)	26.44
Total fenol (mg GAE / 0,5 mL)	739.89
Inhibitory diameter (mm)	18.4

IV. Conclusion

Liquid smoke from green betel leaves (Piper betle L.) has strong antibacterial activity against Steptococcus mutans, so it has the potential to be used as an active ingredient in mouthwash solutions.

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