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From Leaf to Lip: Tracing Contaminants in Aceh's Traditional Chewing Tobacco (*Bakông Asóê*)

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Abstract

Bakông Asóê is a typical Acehnese snack that older people in Aceh, Indonesia have loved since ancient times. This snack is made from wet tobacco which is used as chewing tobacco. The open-air drying process of *Bakông Asóê* exposes it to heavy metal contamination from air pollution caused by motor vehicle emissions. The wet storage process also promotes microbial growth of bacteria and fungi due to the aqueous environment. The purpose of this study was to determine the levels of heavy metal contamination (Pb, Cd, Se, Cu and Hg) and microbiological contamination from bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans* and *Aspergillus niger*) in *Bakông Asóê* sold along roadsides without adequate hygiene controls. The research utilized a qualitative approach with a descriptive design. This study showed that heavy metal exposure to Pb, Cu and Se in the three samples was still within safe limits for consumption. However, Cd and Hg levels in all samples exceeded consumption safety standards. Microbiological analysis of the three *Bakông Asóê* samples detected bacterial and fungal contamination, with cocci-shaped bacteria being most prevalent and Aspergillus sp the dominant fungus. In conclusion, this study found evidence of chemical and microbiological contamination in Bakông Asóê sold at markets in Aceh, Ulee Kareng and Lambaro.

Introduction

Indonesia is globally recognized for its vast tropical forests and biodiversity [1–3], including tobacco (Nicotiana tabacum L.) [4]. In 2019, Indonesia had a total national tobacco production of 197.40 thousand tons, making it one of the most widely cultivated crops in the country. Aceh is a major tobacco-producing province, with its annual output steadily increasing. Aceh produced 1.90 thousand tons of tobacco that year. According to the Indonesian Tobacco Society Alliance (AMTI), Aceh has enormous potential for expanding tobacco cultivation, given the suitable climate and soil conditions, which could significantly boost the local economy and livelihoods of the Acehnese people if properly tapped [5,6].

Some Acehnese, particularly senior citizens (older adults) in rural regions, like a tobacco-based snack known as *Bakông Asóê*. Some Acehnese people have consumed *Bakông Asóê* for years since it has numerous characteristics and purposes, including reducing bad breath and as an anti-inflammatory treatment, particularly for teeth and gums. This is most likely owing to the

presence of alkaloid compounds in *Bakông Asóê*, which have been shown to serve as pain relievers (anti-inflammatory) and to inhibit the growth of halitosis bacteria, which produce bad breath [7]. The alkaloid compounds such as piperine and nicotine are known to have pharmacological effects as antioxidants [8]. *Bakông Asóê* can be used as one of the natural sources of antioxidants. The primary function of antioxidants is to balance the adverse effects of free radicals produced during metabolic processes, which is done by donating one electron to the oxidant, thus becoming more stable [9].

Since ancient times, this snack has been a favorite of the senior citizens in Aceh. Until now, *Bakông Asóê*, however, remains in demand among senior citizens, particularly older adults, for its ability to prevent tooth and gum irritation. The *Bakông Asóe* is a wet tobacco variety used in chewing tobacco that is ingested by chewing between the teeth, which then sucks and swallows the tobacco water produced. Usually, the tobacco is utilized after drying to clean the tooth gaps and discard the waste [10].

Bakông Asóê is produced once a year in Aceh, and the annual demand exceeds 100 kilograms, which may be stored and conserved for the next two years. To make *Bakông Asóê*, harvested tobacco leaves (four months old) are incubated in a container for four days until they become yellow, then chopped till smooth and rolled to form *Bakông Asóê* [11]. This processing takes 7-8 days, ready to be consumed and marketed in traditional markets in the open (Figure 1).

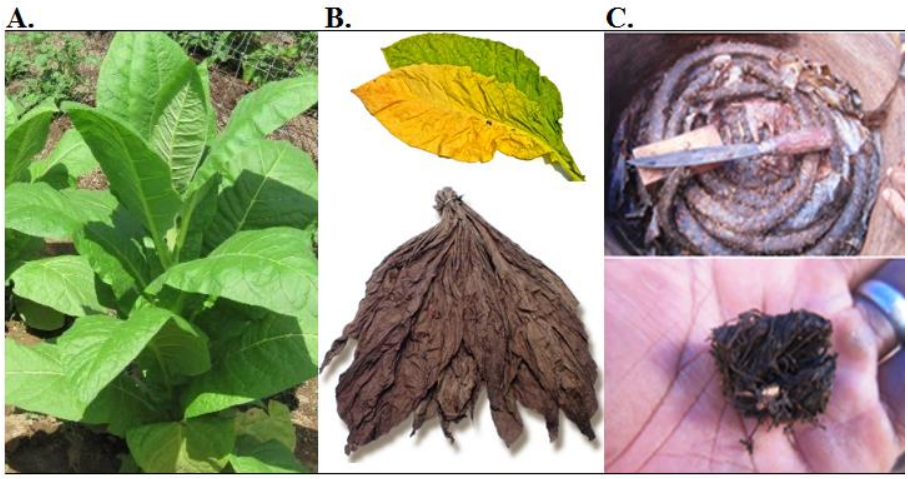

Figure 1. *Bakông Asóê* from leaf to lip: A. Plant of tobacco *Nicotiana tabacum* L; B. dried o leaves tobacco; and C. Aceh's Traditional Chewing Tobacco (*Bakông Asóê*).

Bakong Asoê is highly vulnerable to both heavy metal contamination (such as Pb, Cd, Fe, Hg) and microbiological contamination during the processing, storage & sale process (*Coliform* bacteria, such as members of the *Escherichia* genus). Intoxication caused by the Coliform bacteria group has several symptoms in human digestive tract disorders such as diarrhea, vomiting, and fever. Therefore, this study aimed to analyze metal and microbial contamination *in Aceh's Traditional Chewing Tobacco (Bakông Asóê)*.

Bakong Asoê is highly susceptible to heavy metal contamination (such as Pb, Cd, Fe, and Hg) and microbiological contamination from *Coliform* bacteria like *Escherichia* during processing, storage, and sale [12]. Intoxication caused by *Coliform* bacteria can result in human digestive tract disorders such as diarrhea, vomiting, and fever [13–15]. Therefore, this comprehensive study aimed to thoroughly analyze and quantify the levels of heavy metal contamination and identify and enumerate the microbial contaminants present in Aceh's traditional chewing tobacco (*Bakông Asóê*). The results will provide important insights into the safety and quality control measures needed to ensure *Bakong Asoê* is free from hazardous chemical and microbiological contaminants that could pose a health risk to consumers.

Materials and Methods

Materials

The materials used were *Bakông Asóê* samples, Nutrient Agar (NA), Potato Dextrose Agar (PDA), chloramphenicol, distilled water, 70% alcohol and spirits.

Sample

The samples of *Bakông Asóê* (BA) were obtained from the Aceh traditional market from three different locations. BA1, BA2, and BA3 samples were obtained from Pasar Aceh, Ulee Kareng, and Lambaro.

Metal Contamination Analysis

The metal contamination was analyzed using the Atomic Absorption Spectrophotometry, AAS (Agilent) instrument. The parameters observed in this study were heavy metals Pb, Cd, Hg, Cu and Se in the *Bakông Asóê* sample [16,17].

Preparation and Dilution

All glass utensils are sterilized before using an autoclave at 21^{o}C and a pressure of 2 atm for 15 minutes. Two grams of Bakông Asóê were soaked in distilled water, homogenized with a vortex, and left for 15 minutes. The immersion result is 1 mL and is placed in a test tube containing 9 mL of sterile distilled water, the serial dilution until 10^{-8} dilution is obtained [18,19].

Microbial contamination analysis

Bakông Asóê microbiological contamination analysis was performed on bacterial and fungal contamination. Nutrient Agar (NA) is used for bacterial growth, while Potato Dextrose Agar (PDA) is used for mushroom growth. The dilution results were taken as much as 1 ml, put into a sterile petri dish. PDA medium (which had been added with chloramphenicol) was poured into a petri dish for observing fungal growth and NA media for observing bacteria, then homogenized and incubated. After that, a mixed culture will be obtained so that purification is carried out by transferring one colony of bacteria and fungi to sterile NA and PDA media, then incubating again [20].

Macroscopic and microscopic observations of bacterial isolates

Macroscopic observations were made by observing the properties of bacterial colonies based on the margins of the colonies and their color. Gram staining was used to make microscopic observations on all isolates. This procedure is carried out by pouring distilled water over the slide, then taking a little bit of the bacterial isolate and rubbing it onto the slide with the loop needle, and finally fixing it. After that, one drop of methylene blue was applied and left for 60 seconds before being rinsed with distilled water. One drop of methylene blue was added and allowed to stand for 60 seconds before washing with distilled water. One drop of Lugol's iodine (disinfectant solution) was added and allowed to stand for 60 seconds before washing with pure water. Added one drop of 70% alcohol and allowed to stand for 60 seconds, then washed with distilled water. Added one drop of safranin and allowed to stand for 30 seconds, then washed with distilled water. The next stage is to dry the glass preparations resulting from Gram staining and observe using a microscope [21].

Macroscopic and microscopic observations of fungal isolates

The macroscopic properties of the colonies, such as color, edges, and surface of the colony, were used to identify pure cultures of fungal isolates [22]. Pure fungal cultures were collected using a needle preparation and deposited on the surface of the object glass, then distilled water was added for microscopic observations. The preparations were then covered with a cover glass and examined under a microscope. The microscopic characteristics studied were hyphae, conidiophores, and the conidia color of the isolates [23].

Results and Discussion

Metal Contamination Analysis on Bakông Asóê

Table 1 shows the results of heavy metal contamination in each *Bakông Asóê* sample. The table results, *Bakông Asóê* 1 (BA 1) has the most significant Pb contamination of 1.0863 mg/kg. According to the standard reference of the Food and Drug Supervisory Agency Regulation number 5 of 2018, the maximum level of heavy metal contamination in processed food is 2.0 mg/kg, so the three *Bakông Asóê* samples still fulfill the criteria. The content of Cd metal contamination in the three samples was very high, as much as 2.8492 mg/kg in the BA1 sample, 1.7204 mg/kg in the BA2 sample, and 1.4691 mg/kg in the BA3 sample. Based on the previous standard, the maximum Cd contamination is 0.2 mg/kg. This shows that the Cd contamination in the three *Bakông Asóê* samples is unsuitable for consumption [24]. The high level of Cd metal pollution in *Bakông Asóê* is hazardous to the body because elemental Cd has a long biological half-life, owing to its poor elimination rate from the body. As a result of the accumulation of Cd in numerous tissues over time, including the kidneys, liver, central nervous system (CNS), and peripheral nervous system, continuous exposure to Cd will result in toxic effects [25].

Table 1. Results of analysis of heavy metal contamination in *Bakông Asóê* samples.

No.	Test Parameter	Unit	Sample			Indonesian National Standard (SNI) (mg/Kg)
			BA1[a]	BA2[b]	BA3[c]	
1.	Pb	mg/Kg	1.0863	1.0863	1.0863	2.0
2.	Cd	mg/Kg	2.8492	2.8492	2.8492	0.2
3.	Cu	mg/Kg	2.0580	2.0580	2.0580	3.0
4.	Hg	mg/Kg	0.0824	0.0824	0.0824	0.03
5.	Se	mg/Kg	<0.0009	<0.0009	<0.0009	0.55

The BA2 sample has the highest level of Cu contamination compared to the other samples, at 4.9337 mg/kg. Because it does not exceed the maximum standard level of 30 mg/kg, it is still classified as safe. The measurement of Hg contamination in the BA3 sample revealed the highest contamination level of 0.0824 mg/kg, compared to 0.1361 mg/kg in the BA2 sample and 0.6038 mg/kg in the BA3. Based on the previous standard, the levels of Hg contamination in the three samples were high and exceeded the standard limits. This can affect health problems, especially in the kidneys [26]. The result of Se metal contamination in the three samples was <0.009. The National Institutes of Health suggests a daily Se consumption of 0.55 mg/kg to keep the three Bakông Asóê samples within the safe range. Heavy metal contamination in Bakông Asóê can be caused by various circumstances, including agricultural chemicals, unsanitary manufacturing and sales procedures, and direct or indirect exposure to heavy metals, such as motor exhaust emissions and air pollution [27]. Essential heavy metals such as Se and Cu are required in moderate quantities to maintain human metabolism. Non-essential heavy metal contamination, such as Pb, Hg, and Cd, harms the body and can lead to poisoning. Consumption of heavy metals with high levels of pollution in the human body is a major health concern [28]. This contamination can occur not only by direct contact but also through food, water and air [29].

Heavy metals are released into the water and soil environment; these elements can accumulate in food in plants, vegetables and herbs. The main route for heavy metals into the human body is consuming contaminated food. Some toxic heavy metals, such as Pb, Hg, Cr, Cd, and As (As is frequently categorized as a heavy metal due to its chemical similarities), damage health, particularly pregnant women and young children, who are more vulnerable to toxic metal poisoning. In addition, heavy metal contamination is also dangerous for the elderly because they have decreased organ function. Toxic metals can also disrupt many biochemical processes in the human body, posing significant health hazards and eventually leading to an increase in

chronic diseases such as neurological disorders, central nervous system damage, malformations, and cancer [30].

Results of Bacterial contamination results in Bakông Asóê

There are various types of bacterial isolates produced from dilutions samples 10^{-7} and 10^{-8}. Table 2 shows the outcomes of the bacterial isolate acquisition and Gram staining. The table's results were derived from the bacteria's microscopic and macroscopic properties. There were 30 isolates of Gram-negative bacteria in the form of cocci. One bacterial isolate for staining Gram-negative bacteria in the form of bacilli, seven isolates of bacteria for Gram-positive bacteria in the form of cocci, and one bacterial isolate for Gram-positive bacteria in the form of bacillus. Staining of Gram-negative bacteria in cocci was the most common, while Gram-positive bacteria in the form of bacilli and Gram-positive bacteria in the form of bacilli were the least found in bacterial isolates from the three *Bakông Asóê* samples.

Table 2. Observations of bacterial isolates macroscopically and microscopically.

No.	Dilution	Isolates	Colony Color	Gram	Cell Forms
1.	P7.1	P7.1 (A)	red	negative	coccus
		P7.1 (B)	red	negative	coccus
		P7.1 (C)	red	negative	coccus
		P7.1 (D)	red	negative	coccus
		P7.1 (E)	red	negative	coccus
		P7.1 (E)(A)	rosy	negative	coccus
		P7.1 (F)	red	negative	coccus
		P7.1 (G)	red	negative	coccus
2.	P7.2	P7.2 (A)	red	negative	coccus
		P7.2 (B)	red	negative	coccus
		P7.2(B)(A)	red	negative	coccus
		P.7.2 (B)(B)	red	negative	bacillus
		P.7.2 (B)(C)	red	negative	coccus
		P7.2 (C)	red	negative	coccus
		P.7.2 (C)(A)	red	negative	coccus
		P7.2 (D)	red	negative	coccus
		P7.2 (E)	red	negative	coccus
		P7.2 (F)	red	negative	coccus
		P7.2 (G)	red	negative	coccus
3.	P7.3	P7.3 (A)	red	negative	coccus
		P7.3 (B)	greenish blue	positive	coccus
		P7.3 (C)	red	negative	coccus
		P7.3 (D)	bluish purple	positive	coccus
4.	P8.1	P8.1 (A)	red	negative	coccus
		P8.1 (B)	bluish purple	positive	coccus
		P8.1 (C)	bluish purple	positive	coccus
5.	P8.2	P8.2 (A)	red	negative	coccus
		P8.2 (B)	red	negative	coccus
		P8.2 (C)	red	negative	coccus
		P8.2 (D)	red	negative	coccus
		P8.2 (E)	red	negative	coccus
6.	P8.3	P8.3 (A)	reddish purple	negative	coccus
		P8.3 (B)	rosy	negative	coccus
		P8.3 (B)(A)	Ungu	positive	coccus
		P8.3 (C)	red	negative	coccus
7.	NA1	NA 1 (A)	red	negative	coccus
		NA 1 (B)	bluish purple	positive	bacillus
		NA 1 (C)	red	negative	coccus

No.	Dilution	Isolates	Colony Color	Gram	Cell Forms
8.	NA2	NA 2 (A)	greenish blue	positive	coccus
		NA 2 (B)	Ungu	positive	coccus
		NA 2 (C)	red	positive	coccus
9.	NA3	NA 3 (A)	red	positive	coccus
		NA 3 (B)	red	positive	coccus
		NA 3 (C)	red	positive	coccus

Notes:
P7,8.1: Bacterial isolation through seventh and eighth dilutions of BA1 samples
P7,8.2: Bacterial isolation through seventh and eighth dilutions of BA2 samples
P7,8.3: Bacterial isolation through seventh and eighth dilutions of BA3 samples
NA 1, 2, 3: Isolation of bacteria on dry samples BA1, BA2 and BA3 without dilution.

The difference in color between Gram-positive and Gram-negative bacteria indicates differences in cell wall structure between the two types of bacteria. Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content, and Gram-negative bacteria have a cell wall structure with a high lipid content [31]. Bacterial growth is thought to be due to unhygienic equipment and materials, and exposure to street dust due to heavy traffic during the processing and drying of *Bakông Asóê* samples. The fact is in line with Sudian [32] which states that contaminated raw materials, inadequate sanitation, conditions (time and or temperature) that are not controlled during the production process or storage or a combination of these conditions will increase the rate of bacterial growth in *Bakông Asóê* samples.

Result of fungal contamination in Bakông Asóê

The isolation of fungus detected in *Bakông Asóê* samples yielded 26 isolates (Table 3). The results of fungal identification revealed that six fungus isolates were successfully isolated from the *Bakông Asóê* 1 (BA1) sample, twelve isolates from the BA2 sample, and eight isolates from the BA3 sample. The isolates from the three types of *Bakông Asóê* samples came from different genera, namely the genus *Aspergillus* (23 isolates), *Trichoderma* (1 isolate), *Trichophyton* (1 isolate), and *Blastomyces* (1 isolate). This is in line with Pauly's (2011) study which reported the presence of the fungus *Aspergillus sp*. in stored chewing tobacco leaves. Determination of the genus of this fungus is based on the macroscopic and microscopic characteristics of each fungal isolate.

Table 3. Results of Isolation and Microscopic Observation of Fungal Isolates

No	Sample	Isolate	Genus Isolate
1.	BA1[a]	P7.1 A	Aspergillus
		P7.1 B	Aspergillus
		P7.1 C	Aspergillus
		P8.1 A	Aspergillus
		PDA1 A	Aspergillus
		PDA1 B	Aspergillus
2.	BA2[b]	P7.2 A	Aspergillus
		P7.2 B	Aspergillus
		P7.2 C	Aspergillus
		P7.2 D	Aspergillus
		P8.2 A	Aspergillus
		P8.2 B	Aspergillus
		P8.2 C	Aspergillus
		P8.2 D	Aspergillus
		P8.2 E(1)	Aspergillus
		P8.2 E(2)	Trichophyton
		P8.2 E(3)	Trichoderma
		PDA2 A	Aspergillus

No	Sample	Isolate	Genus Isolate
3.	BA3[c]	P7.3 A	Aspergillus
		P7.3 B	Aspergillus
		P7.3 C	Aspergillus
		P8.3 A	Aspergillus
		P8.3 B	Aspergillus
		PDA3 A	Aspergillus
		PDA3 B	Aspergillus
		PDA3 C.	Blastomyces

Notes:
[a]BA1: Bakông Asóê samples from Pasar Aceh; [b]BA2: Bakông Asóê samples from Banda Aceh's Ulee Kareng market;
[c]BA3: Bakông Asóê samples from Lambaro market of Aceh Besar
P7,8.1: Fungal isolation through seventh and eighth dilutions of BA1 sample
P7,8.2: Fungal isolation through seventh and eighth dilutions of BA2 sample
P7,8.3: Fungal isolation through seventh and eighth dilutions of BA3 sample
PDA 1, 2, 3: Isolation of fungi on dry samples BA1, BA2 and BA3 without dilution

Each *Aspergillus sp* isolate displayed distinct macroscopical hues. The isolates of P7.1 A, P7.2 A, P7.3 A, PDA1 B, PDA2 A, and PDA3 A were macroscopically blackish-brown and white to yellow on the colony surface, changing to blackish brown once conidiophores formed. Microscopically, the fungi in this isolate had thin-walled conidiophores, round and semi-round vesicles and brown. Isolates P7.2 B, P8.2 A and P7.3 macroscopically were brown and had an umbonate head shape.

Macroscopic and microscopic observations of bacterial isolates

Figure 2 depicts macroscopic and microscopic observations of bacterial isolates from *Bakông Asóê*. Macroscopic identification was performed by observing colony characteristics such as shape, edge, and color. The macroscopically observed properties of bacterial isolates are often purple and appear like a lobed edge. Gram staining reveals microscopically observed bacterial characteristics. Bacterial isolates' findings are typically Gram-negative bacteria with cocci cell shapes. Gram staining is used to detect bacterial cell shape and identify Gram-positive and Gram-negative bacteria.

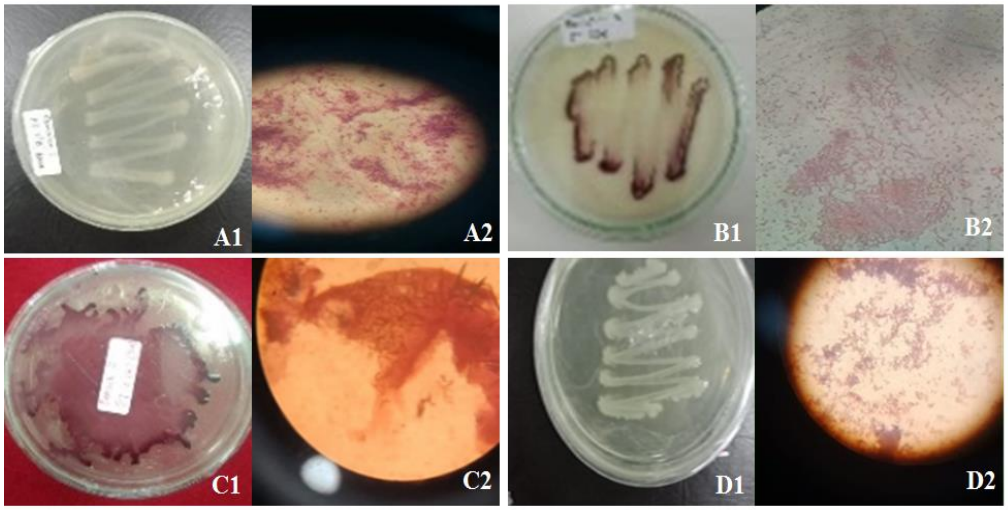

Figure 2. Characteristics of bacterial isolates macroscopically and microscopically. Characteristics of P8.1(B) bacterial isolates macroscopically (A1) and microscopically (A2) Characteristics of P7.3(D) bacterial isolates macroscopically (B1) and microscopically (B2) Characteristics of P7.2(B) bacterial isolates macroscopically (C1) and microscopically (C2) Characteristics of NA.1(B) bacterial isolates macroscopically (D1) and microscopically (D2).

The color difference between Gram-positive and Gram-negative bacteria suggests that the cell wall structure of the two types of bacteria differs. Gram-positive bacteria have a thick peptidoglycan content in their cell walls, whereas Gram-negative bacteria have a high lipid content in their cell walls [31]. The change in the cell wall structure is what generates the color difference when observed under a microscope. Gram-positive bacteria are purple. After all, the methylene blue dye complex is preserved even when exposed to an alcohol acetone solution. In contrast, Gram-negative bacteria are red because the complex is soluble when exposed to the alcohol solution, allowing the red color of safranin to adhere well to the bacteria.

Macroscopic and microscopic observations of fungal isolates

This fungus has thin conidiophores and spherical conidia under the microscope. The P8.2 B isolate was also macroscopically brown but slightly faded and exhibited spherical vesicles, smooth-walled conidiophores, and globose-shaped conidia microscopically. The P7.2 D, PDA1 A, and PDA 3 B isolates were macroscopically green, with white to yellow colonies on the surface. This fungus possesses conidiophores that radiate and virtually cover the entire vesicle. Unlike isolates P7.3 B, P8.2 E, and PDA3 B, which were macroscopically green and had brown exudate droplets. This fungus had brown conidiophores and spherical conidia, according to microscopic examination.

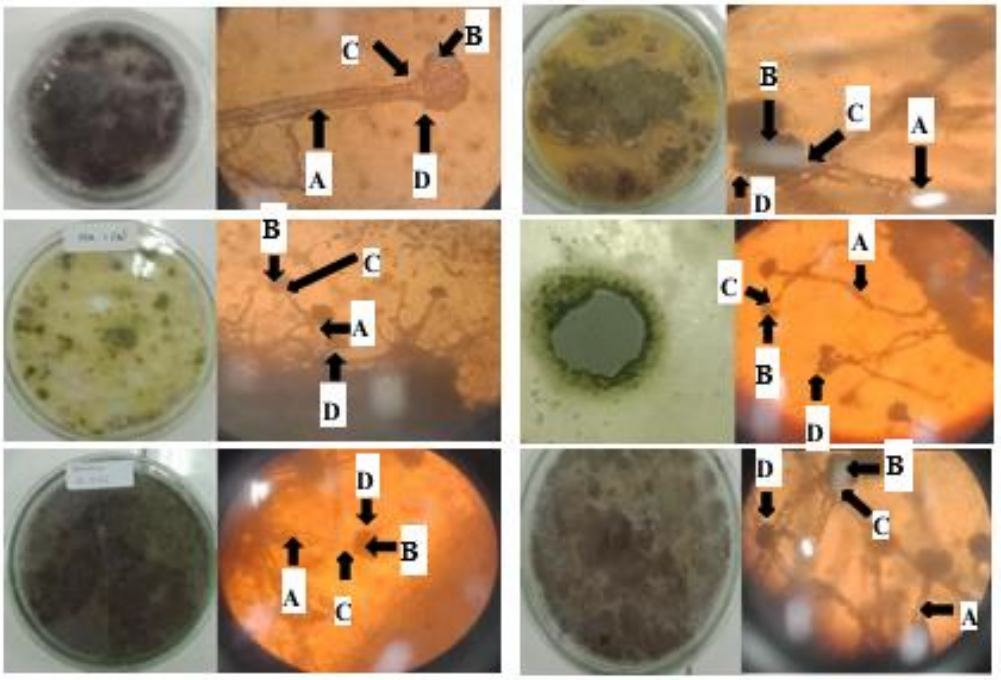

Figure 3. Macroscopic and microscopic characteristics of the genus *Aspergillus sp*. Notes: (A: conidiophores; B: phialides; C: vesicles; D: conidia).

Furthermore, aspergillus colonies were discovered in isolates P7.2 C, P8.1, P8.2 C, and P8.3 B. All of these isolates had the same macroscopic appearance, that is, grey colonies and microscopically, these colonies had radiating conidia, thin-walled conidiophores and spherical vesicles. According to Noerfitryani and Hamzah [33], the macroscopic characteristics of the fungus Aspergillus sp on PDA media were light green to dark green and black, with a flour-like texture while the microscopic characteristics were spherical conidia, with septate and hyaline hyphae. Figure 3 shows the colony morphology of the genus *Aspergillus sp*. based on the identification results.

Aspergillus sp. is a common fungus that plays an essential part in worldwide carbon and nitrogen recycling. Even though their primary habitat is soil or decaying plants, *Aspergillus sp*. generates microscopic hydrophobic conidia that quickly travel into the air and can survive in various environmental circumstances [34]. Conidia can infiltrate host tissues through wounds, ingestion, or inhalation (the most common method) [35]. This allows conidia to enter the upper respiratory tract and reach the alveolus, which binds to surfactant proteins through receptor recognition [36].

In addition to the genus *Aspergillus sp*, the Bakông Asóê samples also identified other genera, namely *Trichoderma sp*, *Trichophyton sp*, *Blastomyces sp*. *Trichoderma sp* isolates are saprophytic fungi whose presence can be harmful under certain conditions for immunocompromised hosts and cause infections from fungal spores such as allergies. Trichophyton sp. morphological traits can be differentiated depending on the growth of macroconidia and microconidia. Macroconidia are clavate to fusiform in shape and are generally connected to the side of the hyphae or on short stalks with thin or thick-walled walls. Macroconidia, on the other hand, are uncommon in many species. Macroscope observations revealed a white-green fungus with a cotton-like texture. In addition, the fungus *Blastomyces sp*. This fungus can be found in moist soil as well as easily biodegradable objects like leaves or wood. This mushroom is green on the surface, with white filaments that mimic snow encircling it. Microscopic examination revealed the existence of branching hyphae containing conidia. Blastomycosis can be caused by *Blastomyces sp* contamination.

Conclusions

The analysis revealed the presence of hazardous heavy metal and microbial contaminants in *Bakông Asóê* that could threaten consumer health. Further research is warranted to fully characterize the risks. Improving production and storage controls should be explored to reduce contamination. Ongoing monitoring is advisable to guide evidence-based safety recommendations for this traditional product. The study revealed a dual contamination threat from toxic metals and disease-causing microbes in *Bakông Asóê*, warranting further investigation and risk mitigation efforts to ensure consumer safety.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data is available by request.

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Conflicts of Interest: All the authors declare that there are no conflicts of interest.

References

[1] Abas AH, Tallei TE, Idroes R, Fatimawali F. Ficus minahassae (Teijsm. & de Vriese) Miq.: A Fig Full of Health Benefits from North Sulawesi, Indonesia: A Mini Review. Malacca Pharmaceutics 2023;1:1–7. https://doi.org/https://doi.org/10.60084/mp.v1i1.24.

[2] Indriaty I, Ginting B, Hasballah K, Djufri D. A Comparative Study of Total Tannin Contents and Antimicrobial Activities in Methanol Extracts of Rhizophoraceae Species. Heca Journal of Applied Sciences 2023;1:62–70. https://doi.org/10.60084/hjas.v1i2.89.

[3] Ningsih DS, Celik I, Abas AH, Bachtiar BM, Kemala P, Idroes GM, et al. A Review of the Ethno-dentistry Activities of Calotropis gigantea. Malacca Pharmaceutics 2023;1:8–15.

[4] Bella A, Swarnata A, Vulovic V, Nugroho D, Meilissa Y, Usman U, et al. Macroeconomic impact of tobacco taxation in Indonesia. Tobacco Control 2023.
[5] Central Bureau of Statistics. Indonesian farm plant production 2017-2019. Jakarta: 2019.
[6] Putra HA, Fijay AH, Suriani S, Seftarita C, Ringga ES, Wintara H, et al. Understanding Short-Term and Long-Term Price Fluctuations of Main Staple Food Commodities in Aceh Province, Indonesia: An ARDL Investigation. Ekonomikalia Journal of Economics 2023;1:26–32. https://doi.org/10.60084/eje.v1i1.50.
[7] Batoro J, Ekowati G. An ethnobotanical tobacco (Nicotiana tabacum L.) in Indonesia. a review. Advances in Life Sciences 2017;7:26–9.
[8] Xiao D, Wang L, Huang X, Li Y, Dasgupta C, Zhang L. Protective Effect of Antenatal Antioxidant on Nicotine-Induced Heart Ischemia-Sensitive Phenotype in Rat Offspring. PLOS ONE 2016;11:e0150557. https://doi.org/10.1371/journal.pone.0150557.
[9] Alkadi H. A Review on Free Radicals and Antioxidants. Infectious Disorders - Drug Targets 2020;20:16–26. https://doi.org/10.2174/1871526518666180628124323.
[10] Marechause Adventura. Bakong Asoe (Snacks of grandparents in Aceh) 2017. https://steemit.com/culturevulture/@dilimunanzar/bakong-asoe-snacks-of-grandparents-in-aceh (accessed September 2, 2023).
[11] Ru Q-M, Wang L-J, Li W-M, Wang J-L, Ding Y-T. In Vitro Antioxidant Properties of Flavonoids and Polysaccharides Extract from Tobacco (Nicotiana tabacum L.) Leaves. Molecules 2012;17:11281–91. https://doi.org/10.3390/molecules170911281.
[12] Darna MT. Analysis of Coliform Bacterial Residues on Traditional Pangkong Satoong Food in the Merdeka Street of Pontianak City Based on Most Probably Number (MPN). Jurnal Protobiont 2017;6.
[13] Irianto K. Mikrobiologi Medis: Pencegahan Pangan Lingkungan 2013.
[14] Departemen Kesehatan RI. Keputusan Menteri Kesehatan Republik Indonesia No 364/Menkes/SK/V/2006 Tentang Pedoman Pengendalian Demam Tifoid, (about typhoid fever control guidelines). Jakarta: Depkes RI; 2006.
[15] Lala A, Marlina M, Yusuf M, Rivansyah Suhendra, Maulydia NB, Muslem M. Reduction of Microbial Content (Escherichia coli) in Well Water Using Various Processes: Microfiltration Membranes, Aeration and Bentonite Adsorption. Heca Journal of Applied Sciences 2023;1:24–9. https://doi.org/10.60084/hjas.v1i1.17.
[16] Kunsah B, Kartikorini N, Ariana D. Analysis of heavy metal resin (Pb, Cd, Zn) on food and beverage containers using atomic absorption spectrophotometry method (SSA). THE JOURNAL OF MUHAMMADIYAH MEDICAL LABORATORY TECHNOLOGIST 2021;4:100. https://doi.org/10.30651/jmlt.v4i1.7604.
[17] Winarsih A, Idroes R, Zulfiani U, Yusuf M, Mahmudi M, Saiful S, et al. Method Validation for Pesticide Residues on Rice Grain in Aceh Besar District, Indonesia Using Gas Chromatography-Electron Capture Detector (GC-ECD). Leuser Journal of Environmental Studies 2023;1:18–24. https://doi.org/10.60084/ljes.v1i1.37.
[18] Marlina R, Wibowo RS. Identification of Vibrio parahaemolitycus bacteria by biological method and gentoxr detection by PCR. Jurnal Sains Dan Teknologi Farmasi 2008;12:11–7.
[19] Kartika E, Khotimah S, Yanti AH. Bacterial detection of food safety indicators on chicken sauce in the traditional flamboyan pontianak market. Jurnal Protobiont 2014;3.
[20] Sabdaningsih A, Budiharjo A, Kusdiyantini E. Isolation and morphology characterization of red algae (rhodophyta) association bacterial colonies from bali's Kutuh waters. Jurnal Akademika Biologi 2013;2:11–7.
[21] Tyas DE, WIdyorini N, Solichin A. The difference between the number of bacteria in the sediment in the Bermangrove and non-Bermangrove areas in the waters of Bedono Village, Demak. Management of Aquatic Resources Journal (MAQUARES) 2018;7:189–96.
[22] Andriani D, Heriansyah P. Identification of fungus contaminations on various explanations of culture of natural cranberry tissue (Bromheadia finlaysoniana (Lind.) Miq. Agro Bali: Agricultural Journal 2021;4:192–9.
[23] Barnett HL, Hunter BB. Illustrated marga of imperfect fungi. 4th ed. USA: Prentice-Hall; 1998.
[24] BPOM. Badan Pengawas Obat dan Makanan No. 5/2018 tentang batas maksiumum cemaran logam dalam pangan olahan biji-bijian (on the limits of the maximum concentration of metals in processed cereal foods). Jakarta Pusat: Badan Pengawas Obat dan Makanan; 2018.
[25] Wang B, Du Y. Cadmium and its neurotoxic effects. Oxidative Medicine and Cellular Longevity 2013;2013.
[26] Zuluaga Rodríguez J, Gallego Ríos SE, Ramírez Botero CM. Content of Hg, Cd, Pb and As in fish species: a review. Vitae 2015;22:148–9.
[27] Onakpa MM, Njan AA, Kalu OC. A review of heavy metal contamination of food crops in Nigeria. Annals of Global Health 2018;84:488.
[28] Miskiyah M. Indonesian National Standard study of liquid milk in Indonesia. Jurnal Standardisasi 2011;13:1–7.
[29] Adhani RH, Husaini H. Logam berat sekitar manusia. Banjarmasin: Lambung 2017.

[30] Liang G, Gong W, Li B, Zuo J, Pan L, Liu X. Analysis of heavy metals in foodstuffs and an assessment of the health risks to the general public via consumption in Beijing, China. International Journal of Environmental Research and Public Health 2019;16:909.
[31] Nurhidayati S, Faturrahman F, Ghazali M. Detection of patogenic bacteria associated with Kappaphycus alvarezii (Doty) symptom ice disease. Jurnal Sains Teknologi & Lingkungan 2015;1.
[32] Sudian S. Food microbiology testing. Info POM Badan Pengawas Obat Dan Makanan Republik Indonesia 2008;9:1–12.
[33] Noerfitryani N, Hamzah H. Inventory of types of fungi in the Rhizosphere of padi agriculture. Jurnal Galung Tropika 2018;7:11–21.
[34] Dagenais TRT, Keller NP. Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clinical Microbiology Reviews 2009;22:447–65.
[35] Oliveira M, Caramalho R. Aspergillus fumigatus: a mere bioaerosol or a powerful biohazard? NACC: Nova Acta Científica Compostelana Bioloxía 2014:2.
[36] Paulussen C, Hallsworth JE, Álvarez-Pérez S, Nierman WC, Hamill PG, Blain D, et al. Ecology of aspergillosis: insights into the pathogenic potency of Aspergillus fumigatus and some other Aspergillus species. Microbial Biotechnology 2017;10:296–322.