Food Contaminants in Tea Products from Kratom Leaves

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Abstract. One of the plants that has been used for generations in Borneo is kratom or *Mitragyna speciosa.* The leaves are used as analgesics and antidepressants. The plant is grown for export to countries other than Indonesia where consumption of this plant as a beverage is still legal, generally in tea from leaves brewed with hot water or tea bags. Some researches found that tea products from kratom leaves contained potentially dangerous levels of toxic metals and microbes. This study aims to examine the food contaminants in tea products from three types of kratom tea leaves used by the people of Borneo: Red, white, and green kratom variants.Bacterial colony tests for red kratom samples produced 2.9 x 10^{-3} colony forming unit (CFU)/gram. In comparison, white kratom samples produced 9.9 x 10^{-3} CFU/gram and green kratom samples produced 2.9 x 10^{-3} CFU/ gram. White kratom samples produced the highest CFU compared to red and green samples. Red kratom samples produced an uncountable number of yeasts at 10^{-2} and 10^{-3} dilution, while at 10^{-4} dilution, it produced a total of 4.8 x 10^{-5} CFU/gram. White kratom samples produced 3.04 x 10^{-4} CFU/gram and green kratom samples produced 1.7 x 10^{-4} CFU/gram. Red kratom samples produced the highest number among the three samples, while green kratom produced the lowest number. Identification with other specific media, namely Eosin Methylene Blue Agar (EMBA) and Salmonella-Shigella Agar (SSA), produced negative results for all samples. The red samples produced the highest of 6.6% b/b sample compared to 6.1% of white and 5.2% of green samples. All samples produced a positive qualitative test of mitragynine alkaloid. White kratom samples showed the highest Cd and Cu contamination, green kratom samples showed the highest Pb contamination and red samples showed the highest Hg contamination. Before preclinical and human clinical trial, it is advised to sterilize herbal simplicias of kratom as they tend to induce bacterial and fungal colonization. In some countries where kratom beverages are still legal, before the leaves were prepared for sale as tea it is better to

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make a ready-to-drink hygiene product and develop a set of "Good Manufacturing Pratices (GMP)" for the kratom industry before exporting it abroad to avoid microbes and heavy metal contaminations.

Keywords: food contaminant · red kratom · white kratom · green kratom

1 Introduction

Kratom (*Mitragyna speciosa*) is an evergreen tree of the family *Rubiaceae* (*Rubiaceae*) that grows naturally in Southeast Asia (SEA) and has a long history of traditional use. Kratom leaves or extracts are usually consumed orally to treat pain and other illnesses and to support agriculture and physical work. Kratom and its alkaloids have been classified as atypical opioids because they are structurally and biologically distinct from the classical opioids (e.g., morphine) derived from the *Papaveraceae* family. Recently, natural products derived from kratom can be purchased online. It has also been identified as a drug of concern by the United States Drug Enforcement Agency. This plant is cultivated for export to countries that still legalize the consumption of this plant, generally in tea from leaves brewed with hot water or tea bags. This plant is planned to be classified by the Indonesian National Narcotics Agency as class I Narcotics in Indonesia. However, Indonesian researchers can still provide input on benefit research results, complementing previous studies and publications abroad.

Kratom contains more than three dozen unique indole alkaloids, one of which is mitragynine. It accounts for about 66% of the total alkaloids in kratom leaves. Preclinical studies on mitragynine show that mitragynine may have the potential to develop new and more effective medicines. Mitragynine is a G-protein-dependent partial agonist of mu-opioid receptor that does not mobilize the β-arrestin signaling pathway like classical opioids. Therefore, it is thought to provide analgesic effects with a lower risk of respiratory depression than classical opioids. Only one human pharmacokinetic study of kratom, including ten healthy male kratom consumers in Thailand, has been reported. Participants were given doses of kratom tea for seven days based on mitragynine levels (6.25–11.5 mg /day). On day 8, the tea was re-administered at different doses (mitragynine content of 6.25–23 mg), and plasma and urine were collected for 0–24 h. The only alkaloid measured, mitragynine, showed a biphasic plasma concentration-time profile. The reported mean (± standard deviation) terminal half-life, 23 ± 16 h, was near or exceeded the sample collection period, raising concern about the robustness of this parameter and subsequently other metrics, including the area under the plasma concentration-time curve (AUC), apparent oral clearance (CL/F), renal clearance (CL_R), and apparent volume of distribution during the terminal phase (Vz/F). As of May 24th, 2018, 199 people from 41 states were reported to be infected with the outbreak of *Salmonella.* The illness began on January 11st, 2017, until May 8th, 2018. However, no deaths have been reported. Epidemiological and laboratory evidence showed that kratom was responsible for this multi-step outbreak, as 76 of 103 people (74%) available for interviews reported consuming kratom tablets, powder, or tea. Most people reported that they consumed kratom powder. People who reported using kratom purchased kratom from retailers in several states and online retailers. Tea products from kratom leaves also

contained potentially dangerous levels of toxic metals. The US FDA was the first agency to raise concerns about the contamination of kratom products with potentially toxic levels of toxic metals. Hence, this study was aimed at examining the food contaminants in tea products from three types of kratom tea leaves used by the people of Borneo: Red, white, and green kratom variants.

2 Materials and Methods

The research was performed by pharmaceutical testing at Agrinas Co. Ltd. And the laboratory at the University of Indonesia from September 2019 to September 2020. Research teams bought the kratom products from the farmers. The red, white, and green-kratom leaves were farmed and harvested by the farmers in Borneo, East Kalimantan. Then kratom leaves were then dried, mashed, and the best was selected. The processed kratom leaves were put in a package of tea bags (Rilexa™). The leaves are then diluted in sterile water until the suspension is obtained. The suspension is then diluted several times until mixtures of different dilution levels are obtained. A microbial contamination test is done by mixing Nutrient Agar (NA) and Potato Dextrose Agar (PDA) into each suspension until homogenized suspensions are obtained.

The study uses specific agar media, namely Eosin Methylene Blue Agar (EMBA) and Salmonella-Shigella Agar (SSA). The EMBA is used to detect the presence of *Escherichia coli,* while the SSA is used to confirm the existence of *Salmonella sp.* Any growing microbial colonies obtained from the suspensions were inoculated into a dish containing EMBA and SSA and then incubated. Metallic green microbial colonies confirmed the presence of *E. coli* in the EMBA, and pink colonies with a central black dot affirm the existence of *Salmonella sp.* Colony Forming Units (CFU) were then calculated at the dilutions of 10^{-2}, 10^{-3}, and 10^{-4}. Heavy metal contamination testing was carried out at the Central Laboratory of Padjadjaran University using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP OES).

The kratom extract was also mixed and eluted to observe the presence of alkaloid substances. The observation was done qualitatively under 254 nm and 366 nm ultraviolet light lamps. After being observed, the extract was mixed with Dragendorff reagent to confirm the presence of alkaloids in the extract. Then, quantitative measurement of total alkaloids in the extract was done by mixing Broom Cresol Green (BCG) solution with a phosphate buffer with a pH of 4, 7. The mixture was then extracted using chloroform and homogenized, and its absorbance was measured under a wavelength of 422 nm. A linear regression equation measured the total alkaloid levels in quinine equivalents.

3 Results

Bacterial colony tests for red kratom produced 2.9 x 10^{-3} CFU/gram (Fig. 1). White kratom samples produced 9.9 x 10^{-3} CFU/gram (Fig. 2), and green kratom samples produced 2.9 x 10^{-3} CFU/gram (Fig. 3). White kratom samples produced the highest CFU compared to red and green kratom samples.

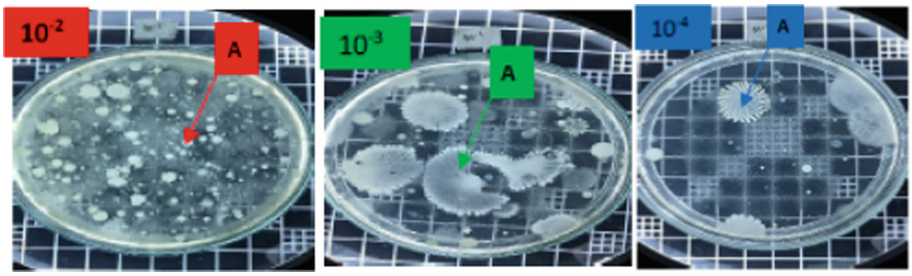


Fig. 1. Bacterial contaminations in red kratom (2,9x10^{-3} CFU/gram).

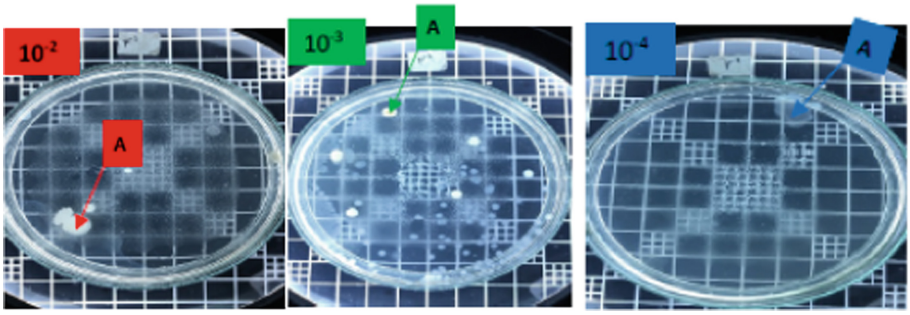


Fig. 2. Bacterial contaminations in white kratom (9,9x10^{-3} CFU/gram).

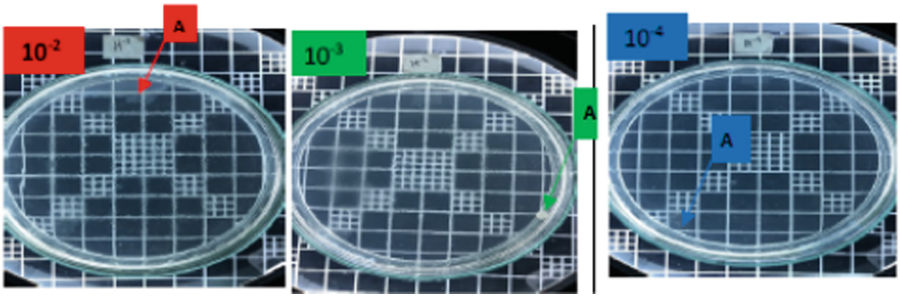


Fig. 3. Bacterial contaminations in green kratom (2,9x10^{-3} CFU/gram).

Red kratom samples produced an uncountable number of yeasts at 10^{-2} and 10^{-3} dilution, while at 10^{-4} dilution, it produced a total of 4.8 x 10^{-5} CFU/gram (Fig. 4). White kratom samples produced 3.04 x 10^{-4} CFU/gram (Fig. 5), while green kratom samples produced 1.7 x 10^{-4} CFU/gram (Fig. 6). Red kratom samples produced the highest number among the three samples, while green kratom produced the lowest number.

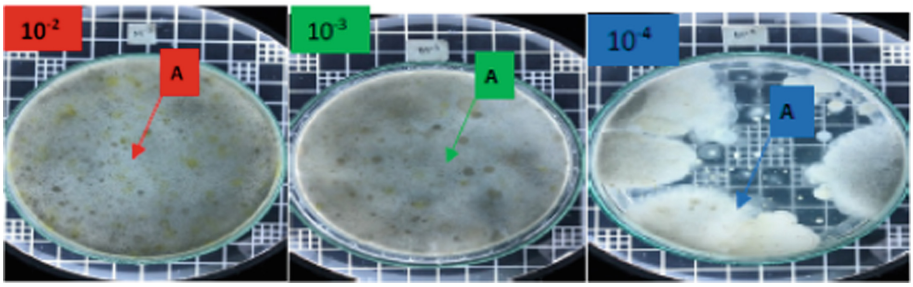


Fig. 4. Yeast contaminations in red kratom ($4{,}8x10^{-5}$ CFU/gram).

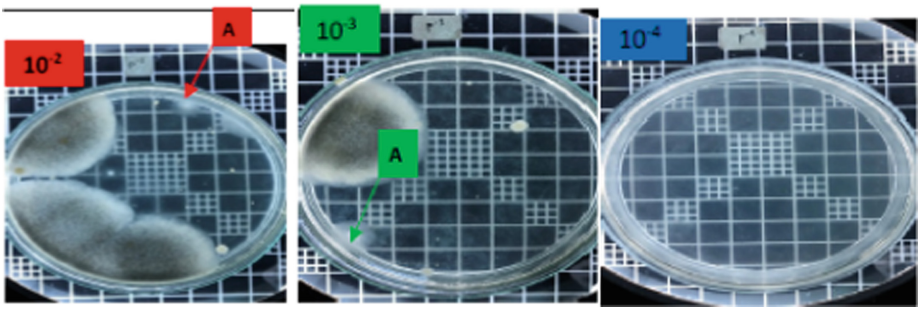


Fig. 5. Yeast contaminations in white kratom ($3{,}04x10^{-4}$ CFU/gram).

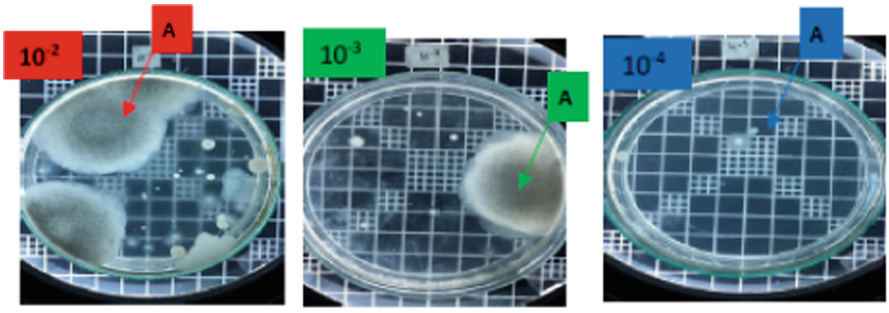


Fig. 6. Yeast contaminations in green kratom ($1{,}7x10^{-4}$ CFU/gram).

Identification with EMBA and SSA produced negative results for all samples.

The red samples produced a 6.6% b/b sample (Table 2). White kratom samples produced 6.1%, and green kratom produced 5.2%. All samples produced a positive qualitative test of mitragynine alkaloid (Fig. 7).

We found a high level of copper (Cu) and lead (Pb) in the green kratom samples. The red kratom samples contained a high level of Cu and mercury (Hg), and white kratom contained a high level of Cu and cadmium (Cd) (Table 3).

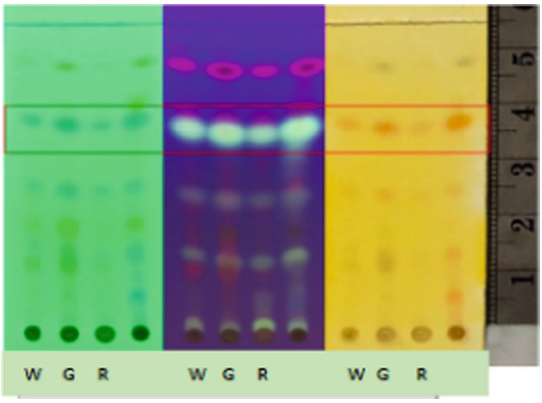


Fig. 7. Alkaloids Qualitative Test. The red rectangle indicates the spot for the mitragynine compound

Table 1. Identification in specific media

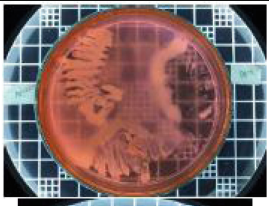
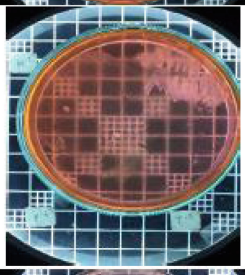
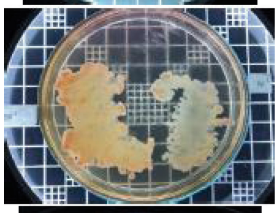
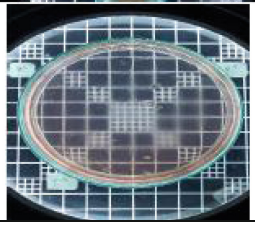
Sample	Media	Observation Photos	Results and Conclusions
Red kratom	EMBA		Negative in one gram sample
Green and white kratom	EMBA		Negative in one gram sample
Red kratom	SSA		Negative in one gram sample
Green and white kratom	SSA		Negative in one gram sample

Table 2. Total alkaloid content in quinine equivalent form (QE) in triplo*

Sample	Alkaloid Level Total (% b/b sample)	Standard Deviation
Green kratom	5.2	0.053
Red kratom	6.6	0.045
White kratom	6.1	0.064

*The results may differ when another standard alkaloid is used.

Table 3. Heavy Metal Contamination in Kratom tea products.

Sample	Concentration (mg/kg)					
	AS	Cd	Cu	Pb	Se	Hg
Green kratom	<0.0001	<0.0001	8.6417	4.6417	<0.0001	<0.0001
Red kratom	<0.0001	<0.0001	12.3113	<0.0001	<0.0001	7.3008
White kratom	<0.0001	0.6978	13.9653	<0.0001	<0.0001	<0.0001

(AS: Arsenic; Cd: cadmium; Cu: copper; Pb: lead; Se: selenium; Hg: Mercury)

4 Discussion

In this study, we discovered that bacteria and fungi-contaminated tea made from kratom leaves. In addition, our study also found the high contamination of several heavy metals in the kratom leaves variants. The worrying biological and chemical pollution of kratom leaves observed in this study is a warning sign of the health hazard of natural products in Indonesia.

A particular study by Prozialeck et al. also found that microbes also contaminated kratom products. All products derived from plant leaves are expected to contain microorganisms under non-sterile conditions. Previously, *Salmonella sp.*, a pathogen associated with kratom products, has been reported. This study did not find any *Salmonella*-contaminated kratom product by specific agar media.

Tea products from kratom leaves also contained potentially dangerous levels of toxic metals. In green kratom, we found a high level of copper (Cu) and lead (Pb), while red kratom contained a high level of Cu and mercury (Hg), and white kratom contained a high level of Cu and cadmium (Cd). Each of these findings has significant ramifications for public health. The US FDA was the first agency to raise concerns about the contamination of kratom products with potentially toxic levels of Nickel (Ni) and Pb, but no other toxic metals were mentioned in the study. A report also showed that seven of the eight commercially available kratom products from a variety of sources examined contained relatively high concentrations of four metals, including Iron (Fe), Pb, Ni, and Cr, followed by As, Cd, and Hg.

Given this fact, Pb is considered to be the most problematic metal detected in contaminated kratom products. It is classified as a Class I contaminant, indicating high

potency for toxicity, especially in chronic use. Pb can cause severe neurological, psychological, cognitive, reproductive, developmental, immunological, cardiovascular, and renal effects. Toxic neurological effects can be particularly severe in children and young adults. The allowed daily oral exposure level of lead in foods and medicines is only 5 μg/day. The Pb levels of some of the tested samples ranged from 0.25 to 1.6 μg/g products, which is an essential finding since kratom consumers consuming 5 to 15 g of kratom leaves per day can easily exceed their permissible levels of daily Pb intake. This increases the likelihood that some of the anomalous toxicity of "kratom" products in the West may be due to, at least in part, lead contamination. The further forensic study is warranted to clarify this suspicion.

Cu is a trace element found in high concentrations in the brain, liver, and kidney tissues. However, because of their size, bone and muscle contain more than half of the copper in the body. Signs of overt acute copper toxicity depend somewhat on the mode of copper overload, with ingestions presenting most commonly with gastrointestinal side effects.

Hg is a toxic heavy metal widely known to cause public health disasters in Minamata Bay, Japan, and Iraq. The clinical impact of low mercury exposure remains controversial. Mercury in all forms is toxic to cellular function by altering the tertiary and quaternary structures of proteins and binding to sulfhydryl and selenohydryl groups. Cd poisoning has been reported in many parts of the world, causing global health problems that affect many organs and can even lead to death annually. Long-term exposure to cadmium through the air, water, soil, and food causes cancer and toxicity to human body.

The solution to reducing contamination of kratom products is to conduct a quality test with the Indonesian National Agency of Drug and Food Control before being disseminated to the broader community. It would be essential that the products be tested to show that they have not been fortified or contaminated with other substances, particularly heavy metals substances. The limitation of this study is mainly the law and regulation because kratom will still be regarded as class I narcotics by the Indonesian National Narcotics Agency if the study on its efficacy is still scarce. Future studies are warranted on the efficacy of kratom on a larger scale. Various local brands should be studied and compared to analyze the contamination profile and show the best efficacy. Current study limitations relate to only one kratom product evaluated. It is unclear whether the findings of significant metal and microbial contamination apply to other kratom products.

5 Conclusion

It is apparent that the local kratom tea product, Rilexa™ is contaminated with metals, namely Pb, Hg, Cd, and Cu, as well as microbes. The biological and chemical contaminations put consumers at potential risk of adverse effects and health problems. Before preclinical and human clinical trial, it is advised to sterilize herbal simplicias of kratom as they tend to induce bacterial and fungal colonization. In some countries where kratom beverages are still legal, before the leaves were prepared for sale as tea it is better to make a ready-to-drink hygiene product and develop a set of "Good Manufacturing Pratices (GMP)" for the kratom industry before exporting it abroad to avoid microbes and heavy metal contaminations.

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