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Secondary Metabolites and Bioactivities of *Aspergillus spp*. in Bir Pletok Herbal Ingredients: A Systematic Review of Potential Risks

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Abstract

Bir Pletok is a traditional Indonesian herbal beverage prepared from various spices containing bioactive compounds with potential applications in functional beverages. However, the use of plant-based raw materials may introduce fungal contamination, particularly by Aspergillus spp., which can produce diverse secondary metabolites, including mycotoxins. This systematic review aimed to characterize the secondary metabolites and biological activities of Aspergillus spp., evaluate potential mycotoxin-related food safety risks, and identify contamination control strategies relevant to the herbal ingredients used in Bir Pletok. Original research articles published between 2020 and 2026 were retrieved from Scopus, PubMed, ScienceDirect, SpringerLink, Google Scholar, and SINTA. Fifty eligible articles were included and synthesized descriptively. The reviewed literature identified A. flavus, A. niger, A. ochraceus, A. fumigatus, A. parasiticus, and A. chevalieri, with reported metabolites including aflatoxins, ochratoxin A, citrinin, sterigmatocystin, cyclopiazonic acid, polyketides, and other bioactive compounds. Their reported biological activities ranged from toxic effects to antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer effects. Nevertheless, the presence of toxigenic Aspergillus species does not establish that mycotoxins occur at detectable levels in Bir Pletok, because the included studies primarily examined spices, medicinal plants, and other plant-based products rather than the beverage itself. These findings highlight a potential food safety concern associated with Bir Pletok ingredients, particularly when cultivation, drying, handling, storage, and distribution practices are inadequate. Risk reduction requires appropriate raw-material selection, good manufacturing practices, moisture and storage management, and systematic fungal and mycotoxin monitoring. Natural antifungal compounds may also warrant further investigation as complementary control measures. Direct studies measuring Aspergillus contamination and mycotoxin concentrations in Bir Pletok are needed to establish the actual exposure risk and determine whether the findings from other plant-based products apply to the finished beverage.

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1. INTRODUCTION

Indonesia is one of the countries with the greatest biodiversity in the world, providing substantial potential for the development of traditional medicines and spice-based herbal beverages (Mandra, 2025). The increasing use of spices as functional food ingredients is associated with their bioactive compounds, which exhibit antioxidant, anti-inflammatory, antibacterial, and immunomodulatory properties (Pallarés et al., 2022). One traditional beverage that remains an important part of Indonesia's cultural heritage is Bir Pletok, a specialty of the Betawi community prepared from ginger (*Zingiber officinale*), sappan wood (*Caesalpinia sappan*), cinnamon (*Cinnamomum burmannii*), cloves (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), cardamom (*Amomum compactum*), nutmeg (*Myristica fragrans*), and black pepper (*Piper nigrum*). These ingredients contain bioactive compounds, including phenolics, flavonoids, gingerol, eugenol, brazilin, and cinnamaldehyde, which contribute to the beverage's antioxidant, antibacterial, and anti-inflammatory potential. The biological properties and quality of Bir Pletok are influenced by the composition of its ingredients and the processing methods employed (Putra et al., 2023). However, its potential as a functional beverage must be accompanied by appropriate food-safety controls, particularly because plant-based raw materials may be exposed to microbial contamination throughout the supply chain.

Food safety in Bir Pletok production depends on both processing conditions and the microbiological quality of its raw materials. Bir Pletok has been described as a low-acid beverage with a pH above 4.6, making appropriate process control important for maintaining product quality and safety (Abna et al., 2022). Thermal processing, production-environment sanitation, packaging, and storage conditions are essential factors in controlling microbial growth (Pallarés et al., 2022). Hasan et al. (2021) demonstrated that pasteurization or hot-filling under appropriate time and temperature conditions could result in undetectable microbial, mold, and yeast counts, thereby extending the beverage's shelf life. Nevertheless, contamination may occur before processing during cultivation, harvesting, drying, handling, storage, and distribution. Fungal contamination is particularly relevant to spices and medicinal plants because these materials may provide suitable substrates for fungal growth under favorable environmental conditions. Chen et al. (2020) reported fungal contamination in 83.3% of the medicinal plant samples examined, with *Aspergillus spp.* accounting for 26.1% of the fungal isolates or occurrences reported in their study. These findings indicate that *Aspergillus* spp. may occur in plant-based materials used in herbal products, although their presence does not necessarily demonstrate that the materials contain mycotoxins.

The food-safety significance of *Aspergillus spp.* contamination depends partly on the capacity of particular species or strains to produce toxic secondary metabolites. Chen et al. (2020) reported that several *A. flavus* isolates carried aflatoxin biosynthesis genes and were capable of producing aflatoxins B1 and B2, while other *Aspergillus spp.* species have been associated with ochratoxin A and citrinin production. However, the detection of a potentially toxigenic species or the identification of toxin-producing genes does not establish that a specific mycotoxin is present at a measurable concentration in a particular food or beverage. Mycotoxin production depends on fungal strain characteristics, substrate composition, moisture, temperature, and storage conditions; therefore, direct detection and quantification are necessary to establish contamination levels. Evidence from other plant-based products demonstrates the relevance of this distinction. Pallarés et al. identified various mycotoxins, including ochratoxin A, zearalenone, beauvericin, and enniatins, in commercially available herbal supplements, illustrating that fungal occurrence and confirmed mycotoxin contamination are related but distinct dimensions of food safety (Pallarés et al., 2022). From a consumer-risk perspective, the potential presence of

mycotoxins is important because certain compounds may cause hepatotoxic, nephrotoxic, carcinogenic, immunotoxic, and other adverse effects, depending on the compound and level of exposure. Nevertheless, actual consumer exposure through Bir Pletok cannot be established without direct evidence of mycotoxin occurrence and concentration in the relevant ingredients or finished beverage, together with information on consumption patterns.

Beyond their potential role in food contamination, *Aspergillus spp.*, produce secondary metabolites with diverse chemical structures and biological activities (Abrar et al., 2025). Hu et al. (2020) reported that *Aspergillus ochraceopetaliformis* produced at least 17 secondary metabolites, including five novel polyketide compounds that were isolated and characterized. Some of these metabolites exhibited anti-inflammatory activities through the inhibition of superoxide release and neutrophil elastase activity. More broadly, *Aspergillus spp.* species produce diverse metabolites, including alkaloids, polyketides, terpenoids, steroids, peptides, and glycosides, with reported antibacterial, antifungal, antioxidant, cytotoxic, and anticancer activities (Hu et al., 2020). These findings highlight the dual significance of *Aspergillus spp.* secondary metabolism: certain compounds may have pharmacological or biotechnological potential, whereas others, particularly mycotoxins, pose potential food-safety hazards. In the context of Bir Pletok, however, evidence concerning the biological activities or toxigenic potential of metabolites produced by *Aspergillus spp.* in other substrates cannot be directly interpreted as evidence of their presence in the beverage. Although pasteurization and hot-filling may improve microbial safety when properly implemented, the effectiveness of these processes against specific fungal hazards and preformed mycotoxins requires careful consideration (Hasan et al., 2021). Accordingly, evaluating the safety of Bir Pletok requires distinguishing fungal occurrence in raw materials, the toxigenic potential of fungal isolates, measurable mycotoxin contamination, and the possibility of consumer exposure.

Despite existing research on the bioactive properties of Bir Pletok and the secondary metabolites produced by *Aspergillus spp.*, evidence integrating fungal occurrence, metabolite diversity, biological activities, and food-safety implications for the herbal ingredients used in Bir Pletok remains limited. In particular, the available literature primarily concerns spices, medicinal plants, herbal supplements, and other plant-based products rather than direct measurements of *Aspergillus spp.* or mycotoxin concentrations in Bir Pletok itself. This evidence gap limits the extent to which findings from related materials can be generalized to the finished beverage or used to estimate actual consumer exposure. Therefore, this systematic review aims to synthesize research on the secondary metabolites and biological activities of *Aspergillus spp.,* evaluate their potential relevance to the contamination risks associated with Bir Pletok ingredients, and examine the implications for food safety. It also identifies potential control strategies involving raw-material selection, good manufacturing practices, moisture management, appropriate drying and storage, and fungal and mycotoxin monitoring throughout the supply chain. By differentiating established findings from potential hazards and unverified exposure pathways, this review seeks to provide a more rigorous scientific basis for the quality control and safety assessment of Bir Pletok and to identify priorities for future research, particularly the direct detection and quantification of *Aspergillus spp.* and their mycotoxins in the raw ingredients and finished beverage.

2. METHODS

This study employed a systematic review design to identify, select, critically assess, and synthesize research findings in a systematic, transparent, and reproducible manner. The review focused on original research investigating the secondary metabolites and

bioactivities of Aspergillus spp. as fungal contaminants in herbal materials and products relevant to Bir Pletok. To provide an up-to-date overview of the topic, the search was limited to publications published between 2020 and 2026.

The literature search was conducted using national and international scientific databases, including Scopus, PubMed, ScienceDirect, SpringerLink, Google Scholar, and SINTA. Relevant publications were identified using combinations of English and Indonesian keywords with the Boolean operators AND and OR. The search terms included “Aspergillus spp.” OR “Aspergillus” AND “secondary metabolites” OR “fungal metabolites” AND “bioactivity” OR “biological activity” AND “contamination” OR “contaminant fungi” AND “medicinal herbs” OR “herbal medicine” OR “traditional herbal drink” OR “Bir Pletok”. Retrieved records were compiled, and duplicate records were removed before screening. The study selection and reporting process followed the PRISMA 2020 guidelines.

Study selection involved four stages: identification, title and abstract screening, full-text eligibility assessment, and final inclusion. The inclusion criteria were: (1) original research articles published between 2020 and 2026; (2) full-text availability; (3) publication in Indonesian or English; and (4) relevance to the secondary metabolites, bioactivities, or contamination of herbal materials and products by Aspergillus spp. Review articles, meta-analyses, conference proceedings, undergraduate theses, master's theses, doctoral dissertations, duplicate records, and publications that did not address the review objectives were excluded from the primary-study dataset.

To prevent double counting and inappropriate reuse of evidence, each eligible publication was assessed for its relevance, study design, and potential overlap with other publications reporting the same research. Publications based on the same underlying study were checked to identify overlapping samples, isolates, experimental procedures, or findings. Where multiple publications reported the same study, the most comprehensive eligible original research article was retained for the primary synthesis, while overlapping publications were not counted as independent studies. Review articles, if consulted to provide supplementary background or contextual information, were considered separately and were not included in the number of primary studies or in the primary-study synthesis tables.

Data were extracted from the eligible primary studies, with the final number of included studies determined by the screening and eligibility assessment. The extracted information included author names, publication year, country of study, Aspergillus species, source of isolates, types of secondary metabolites, analytical methods, investigated bioactivities, and principal findings. The extracted data were organized into synthesis tables, with each independent primary study represented only once for the relevant evidence. The findings were synthesized descriptively to summarize the characteristics of secondary metabolites and the reported bioactivities of Aspergillus spp. associated with herbal materials and products relevant to Bir Pletok. Any review articles used for additional context were kept separate from the primary-study dataset and synthesis tables.

3. RESULTS AND DISCUSSION

The literature summarized in the preliminary synthesis table addressed three main aspects of Aspergillus spp. and herbal products: mycotoxin contamination, the production of bioactive secondary metabolites, and strategies for controlling fungal growth and mycotoxin formation. The reported evidence covered medicinal plants, dried herbs, spices, tea products, herbal supplements, and plant-derived compounds. These sources provide a broader scientific context for assessing potential fungal contamination in herbal beverages such as Bir Pletok. Several entries focused on mycotoxins associated with Aspergillus spp., particularly aflatoxins and ochratoxin A (OTA). Other entries described

secondary metabolites with potentially beneficial biological activities, including anti-inflammatory, antioxidant, antibacterial, and antifungal effects. One entry also reported the potential of neem leaf extract to inhibit fungal growth and aflatoxin biosynthesis. Together, these findings illustrate the diverse roles of fungal metabolites and plant-derived compounds in the context of herbal product quality and safety.

However, the reviewed evidence does not establish that all the reported fungi or metabolites occur in Bir Pletok itself. Findings from other herbal products and dried plant materials should therefore be interpreted as contextual evidence rather than direct confirmation of contamination in this traditional beverage.

Table 1. Characteristics of the studies analyzed.

No.	Author (Year)	Aspergillus Species	Secondary Metabolites	Bioactivity	Key Findings
1	Chen et al. (2020)	A. flavus, A. chevalieri	Aflatoxins (AFB1, AFB2), ochratoxin A (OTA), citrinin	Mycotoxicity	Aspergillus was a predominant genus contaminating medicinal plants. The production of AFB1, AFB2, OTA, and citrinin indicates that spices used as raw materials for Bir Pletok may be susceptible to mycotoxin contamination if storage and handling practices do not meet appropriate standards.
2	Wei et al. (2023)	A. flavus, A. ochraceus, A. niger	Aflatoxins (AFs), ochratoxin A (OTA)	Mycotoxicity	Aspergillus was the predominant fungal genus found in herbal medicines. The presence of aflatoxin biosynthesis genes (aflR) and OTA biosynthesis genes (aoksl) highlights the importance of early detection of contamination in spices used in Bir

					Pletok to prevent mycotoxin formation during storage.
3	Abdul-Kareem et al. (2021)	A. niger, A. ochraceus, A. fumigatus	Ochratoxin A (OTA)	Nephrotoxicity and hepatotoxicity	Aspergillus contamination in tea products indicates that beverages made from dried plant materials are susceptible to OTA contamination. This finding suggests that the raw spices used in Bir Pletok also require quality control to minimize the risk of mycotoxin contamination.
4	Hu et al. (2020)	A. ochraceopetaliformis	Polyketides, including asperochrapyran, asperochralactones A–D, asperlactone, aspyrone, and others	Anti-inflammatory	Isolates of A. ochraceopetaliformis produced various bioactive secondary metabolites, including five novel polyketides. These findings demonstrate the importance of characterizing Aspergillus metabolites when the fungus contaminates Bir Pletok, as not all metabolites produced are toxic.
5	Ali et al. (2026)	A. flavus, A. parasiticus	Neem-leaf phytochemicals, including 3-hydroxyhexadecanoic acid, methyl	Antifungal and anti-aflatoxigenic	Neem leaf extract inhibited Aspergillus growth and aflatoxin biosynthesis.

			octadecanoate, methyl 9,12-octadecadienoate, 4H-pyran-4-one derivatives, and others		These findings indicate the potential use of natural compounds as an alternative strategy for controlling Aspergillus contamination in Bir Pletok raw materials and products during storage.
6	Pallarés et al. (2022)	Specific species not identified; Aspergillus spp. considered mycotoxin producers	Ochratoxin A (OTA), zearalenone (ZEA), enniatins (ENNs), beauvericin (BEA); aflatoxins were not detected	Mycotoxicity	Herbal supplements may contain various mycotoxins due to fungal contamination during storage and distribution. This finding indicates that the spices used in Bir Pletok also require quality control to prevent mycotoxin contamination.
7	Evangelista et al. (2026)	Specific species not identified; Aspergillus spp. identified as one group of mycotoxin-producing fungi	Aflatoxins B1, B2, G1, and G2; OTA; fumonisins FB1–FB3; zearalenone (ZEN), α-ZEL, DON, 3-AcDON, 15-AcDON, D3G, and citreoviridin (CTV)	Hepatotoxicity, nephrotoxicity, estrogenic effects, and immunotoxicity	Analysis of 91 samples representing 33 types of dried herbs showed that 25.3% contained at least one mycotoxin, with ZEN, FB2, AFB1, and OTA being the most frequently detected contaminants. These findings indicate that dried spices used in herbal beverages,

					including Bir Pletok, may be contaminated with mycotoxins if quality control and storage practices are inadequate.
8	Putra et al. (2023)	Specific species not identified; Aspergillus spp.	Spice-derived bioactive compounds, including gingerol, phenolic compounds, and flavonoids	Antioxidant, antibacterial, and anti-inflammatory	Bir Pletok contains various spices rich in bioactive compounds and has potential as a functional beverage. However, spice raw materials require quality control to prevent fungal contamination that may compromise product safety.
9	Navale et al. (2021)	Aspergillus spp.	Aflatoxins, OTA, patulin, citrinin, cyclopiazonic acid, sterigmatocystin, gliotoxin, terrein, secalonic acids, and aflatrem	Hepatotoxicity, nephrotoxicity, carcinogenicity, and neurotoxicity	Various Aspergillus species can produce a wide range of mycotoxins that contaminate food and pose health risks. These findings underscore the importance of monitoring spice raw materials, production processes, and storage conditions to prevent the formation of toxic metabolites in Bir Pletok.

The synthesis of ten research articles indicates that Aspergillus spp. are among the fungal genera most frequently detected as contaminants in herbal materials, medicinal

plants, spices, and plant-based food products. The most frequently reported species include A. flavus, A. niger, A. ochraceus, A. fumigatus, A. parasiticus, and A. chevalieri. Chen et al. (2020) reported that A. flavus and A. chevalieri were prominent contaminants of medicinal plants and were associated with the production of aflatoxins and citrinin. These findings are supported by Wei et al. (2023), who identified A. flavus, A. ochraceus, and A. niger as predominant species in herbal medicines with the capacity to produce aflatoxins and ochratoxin A (OTA). Furthermore, the presence of aflatoxin biosynthesis genes (aflR) and OTA biosynthesis genes (aoksl) may serve as preliminary indicators for detecting the risk of mycotoxin formation in herbal materials during storage.

Aspergillus contamination has been reported not only in medicinal plants but also in various products made from dried plant materials with characteristics similar to those of the spices used in Bir Pletok. Abdul-Kareem et al. (2021) reported that commercial tea samples were frequently contaminated with A. niger, A. ochraceus, and A. fumigatus, which are associated with OTA production. Pallarés et al. (2022) also identified various mycotoxins in herbal supplements, potentially resulting from contamination during storage and distribution, whereas Evangelista et al. (2026) found that 25.3% of dried herbal samples tested positive for at least one mycotoxin. These findings indicate that the spices used as raw materials for Bir Pletok may be contaminated with molds if drying, storage, and distribution practices fail to meet hygiene and quality control requirements.

The synthesis further demonstrates that Aspergillus spp. produce diverse secondary metabolites with varying bioactivities. Many of the reported metabolites are mycotoxins, including aflatoxins (AFB1, AFB2, AFG1, and AFG2), OTA, citrinin, sterigmatocystin, cyclopiazonic acid, gliotoxin, patulin, and secalonic acids. These compounds have been associated with hepatotoxic, nephrotoxic, carcinogenic, immunotoxic, and neurotoxic effects, as reported by Chen et al. (2020), Navale et al. (2021), and other studies. Nevertheless, not all secondary metabolites produced by Aspergillus are harmful. Hu et al. (2020) reported that A. ochraceopetaliformis produces various polyketides, including five novel compounds exhibiting anti-inflammatory activity. This finding highlights the importance of secondary metabolite characterization in distinguishing toxic compounds from those with potential pharmacological applications.

Bir Pletok is a traditional beverage prepared from various spices, including ginger, cinnamon, cloves, nutmeg, cardamom, and sappan wood. These ingredients contain bioactive compounds such as gingerol, flavonoids, and phenolic compounds, supporting the beverage's potential as a functional drink (Putra et al., 2023). However, the use of natural raw materials also increases the risk of Aspergillus spp. contamination when post-harvest handling, storage, and production processes are inadequate. Therefore, quality control measures involving proper drying, moisture and storage humidity control, environmental sanitation, and monitoring of Aspergillus spp. and their mycotoxins are essential to ensure Bir Pletok safety.

Bioactivities of Aspergillus Metabolites

Secondary metabolites produced by Aspergillus spp. exhibit diverse bioactivities that may provide health benefits or pose potential risks. Certain metabolites have been reported to possess antibacterial, antioxidant, anticancer, and antiparasitic activities, making them potential candidates for pharmaceutical development. However, some Aspergillus species also produce mycotoxins with hepatotoxic, nephrotoxic, and carcinogenic properties, raising concerns regarding food safety. Therefore, synthesizing research findings on the bioactivities of Aspergillus metabolites is important for understanding both their biological potential and their implications for the safety of herbal raw materials, including Bir Pletok.

Table 2. Summary of the bioactivity groups of secondary metabolites produced by *Aspergillus spp.*

Bioactivity Group	Relevant Metabolites	Mechanisms/Biological Effects	References
Antibacterial	Depsidones, depsides, polyketides, xanthones, ethyl acetate-derived metabolites, and aromatic compounds	Inhibit Gram-positive and Gram-negative bacteria through cell membrane damage, disruption of protein synthesis, and inhibition of microbial metabolism. Activity has been demonstrated against Staphylococcus aureus, Escherichia coli, and Aeromonas hydrophila. These findings support the potential development of Aspergillus metabolites as novel antibiotic candidates while also indicating the competitive capabilities of these fungi in their natural environments.	Abrar et al. (2025); Domingos et al. (2022); Safwan et al. (2026); Salasa et al. (2025); Sumilat et al. (2026)
Antioxidant	Phenolic compounds, flavonoids, tannins, alkaloids, and triterpenoids	Act as hydrogen-atom or electron donors, thereby scavenging free radicals, including DPPH radicals, and reducing oxidative stress. These antioxidant properties may protect cells against oxidative damage. However, in the context of food contamination, antioxidant activity does not eliminate the toxicity risks associated with other metabolites produced by Aspergillus.	Chen et al. (2022); Sumilat et al. (2026); Zhu et al. (2023)
Anticancer/Cytotoxic	Depsidones, including nidulin, unguinol, and nornidulin; cyclopeptides; diaryl ethers; pyrones; and sesterterpenoids	Exhibit cytotoxic activity against various cancer cell lines through apoptosis induction, inhibition of cell proliferation, and modulation of cellular metabolic pathways. These findings indicate that some Aspergillus metabolites have considerable pharmacological potential,	Chen et al. (2022); Domingos et al. (2022); Zhu et al. (2023)

		although further purification and safety testing are required.	
Antimalarial and Antiparasitic	Depsidones and other aromatic metabolites	Inhibit parasite growth and exhibit larvicidal and antiparasitic activities. These effects illustrate the diverse biological functions of Aspergillus secondary metabolites, which may serve as defense mechanisms against competing organisms in natural environments.	Domingos et al. (2022)
Biotechnological and Pharmaceutical Potential	Polyketides, non-ribosomal peptides, benzodiazepine alkaloids, diketopiperazines, steroids, and pyrazines	Various metabolites exhibit a broad range of biological activities and may be developed as sources of pharmaceuticals, antibiotics, antioxidants, and other biotechnological products. However, in food-contaminating species such as A. ochraceus, potentially beneficial metabolites must be considered alongside the ability to produce mycotoxins that threaten food safety.	Domingos et al. (2022); Safwan et al. (2026)
Mycotoxicity (Hepatotoxicity, Nephrotoxicity, and Carcinogenicity)	OTA, aflatoxins, sterigmatocystin, and other toxic metabolites	Mycotoxins can damage the liver and kidneys, suppress immune function, and exert hepatotoxic, nephrotoxic, and potentially carcinogenic effects. In the context of Bir Pletok, Aspergillus contamination is a major concern because toxic metabolite production can compromise consumer safety even when the raw materials are rich in plant-derived bioactive compounds.	Edyansyah et al. (2025); Nufus and Rahmayanti (2025)
Anti-inflammatory	Alkaloids, including quinazoline, indole, and	Inhibit inflammatory mediators and reduce inflammatory responses	Chen et al. (2022); Zhu et al. (2023)

	diketopiperazine derivatives, as well as polyketides	through modulation of cellular inflammatory pathways. These activities indicate the potential of Aspergillus metabolites as candidates for anti-inflammatory drug development.

The secondary metabolites produced by Aspergillus spp. exhibit a wide range of bioactivities, indicating considerable potential in pharmaceutical and biotechnological applications. Based on the literature synthesis, metabolites such as polyketides, depsidones, alkaloids, flavonoids, tannins, and triterpenoids have been reported to exhibit antibacterial, antioxidant, anti-inflammatory, anticancer, and antimalarial activities. Antibacterial activity is demonstrated by the ability to inhibit pathogenic bacterial growth, whereas antioxidant activity involves scavenging free radicals and potentially reducing oxidative stress. In addition, certain compounds, including alkaloids, cyclopeptides, and polyketides, exhibit cytotoxic activity against various cancer cell lines and may serve as candidates for the development of new therapeutic compounds. This diversity of biological activities highlights the importance of Aspergillus as a source of secondary metabolites for natural-product drug discovery (Chen et al., 2022; Domingos et al., 2022; Zhu et al., 2023).

Nevertheless, these potential benefits must be balanced against safety concerns because certain Aspergillus species produce mycotoxins, including OTA, aflatoxins, and sterigmatocystin, which may cause hepatotoxicity, nephrotoxicity, and carcinogenic effects. In the context of Bir Pletok, Aspergillus contamination of spice raw materials may compromise product quality and safety through the formation of toxic metabolites during storage. Accordingly, raw material quality control, appropriate storage conditions, and routine fungal contamination monitoring are essential measures for minimizing risks while preserving the functional value of Bir Pletok as a traditional herbal beverage (Chen et al., 2022; Edyansyah et al., 2025).

Mycotoxins and Food Safety Risks Associated with Bir Pletok

Bir Pletok is a traditional Betawi beverage prepared from a mixture of spices, including ginger (Zingiber officinale), sappan wood (Caesalpinia sappan), cinnamon (Cinnamomum burmannii), cloves (Syzygium aromaticum), cardamom (Amomum compactum), lemongrass (Cymbopogon citratus), black pepper (Piper nigrum), and other ingredients that contribute to its flavor, aroma, and potential health benefits. Although these spices are known to be rich in bioactive compounds, their quality and safety require careful attention because they may become contaminated with mycotoxin-producing molds during cultivation, post-harvest handling, storage, and distribution. Mycotoxins are secondary metabolites produced by certain fungi, including Aspergillus, Penicillium, and Fusarium, that may adversely affect human health when consumed at sufficient levels. Understanding the potential for mycotoxin contamination in spice raw materials is therefore essential to ensuring the safety and quality of Bir Pletok as a traditional herbal beverage (Putra et al., 2023).

Table 3. Potential mycotoxin risks in ingredients used to prepare bir pletok.

No.	Author (Year)	Research Subject	Main Findings	Relevance to Bir Pletok
1	Zareshahrabadi et al. (2020)	Spices, including turmeric, red chili, black pepper, and cinnamon	Among 80 spice samples, 40 were contaminated with aflatoxins and 48 with OTA. Red chili had the highest aflatoxin contamination, while all black pepper samples contained OTA above regulatory limits. Aspergillus was the predominant mold.	Highly relevant because turmeric, cinnamon, and black pepper are ingredients used in Bir Pletok and may serve as sources of aflatoxins and OTA when drying and storage practices are inadequate.
2	Su et al. (2025)	Dried red chili from various regions of China	Aflatoxin levels in chili were generally below the maximum limit (≤7.19 µg/kg), although numerous aflatoxin-producing A. flavus isolates were identified. Temperatures of 25–30°C were reported as optimal for aflatoxin formation, highlighting the importance of temperature control during storage.	Demonstrates that dried spices may remain at risk of contamination even when measured toxin levels are low. Appropriate storage temperature management is important for Bir Pletok ingredients.
3	Saeed et al. (2025)	Ginger–turmeric herbal tea	The developed herbal tea exhibited high antioxidant activity, and aflatoxins B1, B2, G1, and G2 were not detected in the final product.	Indicates that ingredients such as ginger and turmeric can be used to produce a safe beverage when high-quality raw materials and appropriate production processes are employed.
4	Falade et al. (2025)	Ginger (Zingiber officinale) across the supply chain in Nigeria	Most ginger samples contained aflatoxins, and some exceeded safety limits. Heavy metals were also detected. The use of probiotics, including Lactiplantibacillus	Highly relevant because ginger is a major component of Bir Pletok. The findings emphasize the need for quality monitoring from harvesting through

			plantarum and Lactobacillus helveticus, reduced aflatoxin levels by approximately 50%.	storage and indicate potential approaches to aflatoxin reduction.
5	Waris (2025)	Tamarind–turmeric herbal drink (jamu kunyit asam)	Mold and yeast counts in all samples were below the maximum limit established by the Indonesian National Agency of Drug and Food Control (BPOM), indicating microbiological compliance.	Although mycotoxins were not directly investigated, the study demonstrates the importance of controlling mold contamination in herbal beverages such as Bir Pletok.
6	Pinandoyo and Masnar (2020)	Bir Pletok small and medium-sized enterprise in Cakung	Good Manufacturing Practices (GMP) training increased workers' knowledge scores from an average of 55.56 to 86.67. Continued mentoring was considered necessary to ensure sustained GMP implementation.	Highly relevant because GMP is a preventive measure for controlling mold growth and reducing mycotoxin contamination during Bir Pletok production.
7	Negedu et al. (2022)	Ginger at different stages of the supply chain in Nigeria	The incidence of aflatoxins, OTA, and fumonisins reached 80.9%, 68.6%, and 90.5%, respectively. Contamination was higher in dried ginger and during storage than in fresh ginger.	Highly relevant because the findings indicate that drying and storage may increase mycotoxin risks in ginger used as a Bir Pletok ingredient.
8	Tang (2025)	Systematic review of 41 food spice types	The review identified spices as potential carriers of various bacteria, molds, and foodborne pathogens. It also developed a framework for risk assessment and critical control points in spice safety.	Provides a scientific basis for microbiological monitoring of all Bir Pletok spices throughout processing and distribution.
9	Nazdar et al. (2024)	Ginger, turmeric, and thyme extracts against aflatoxin B1	A combination of herbal extracts reduced AFB1 toxicity in RAW264.7 cells. Antioxidant activity increased with higher	Suggests that Bir Pletok ingredients may contain compounds with protective effects

		toxicity	phenolic content.	against aflatoxin toxicity, although these cannot replace contamination control measures for raw materials.
10	Gialluisi et al. (2025)	Commercial spices and herbs in Italy	Approximately 84% of spice samples and 60% of herbal samples contained one or more mycotoxins. Aflatoxins and OTA were detected in several spices, while fumonisins and zearalenone were also frequently identified.	Highly relevant because commercial spices may contain multiple mycotoxins, emphasizing the need for routine quality testing of Bir Pletok ingredients.
11	Farouk et al. (2022)	Ginger (Zingiber officinale) essential-oil nanoemulsion as an edible coating for nuts	Ginger essential-oil nanoemulsions exhibited antifungal activity against A. flavus, inhibited mold growth by 84.87–92.51%, and reduced aflatoxin production. Sesquiterpenes such as α-bisabolene and ar-curcumene were implicated in inhibiting aflatoxin-producing enzymes.	Highly relevant because ginger is a key Bir Pletok ingredient. The findings indicate that ginger essential oil may help suppress aflatoxin formation and could be investigated as a natural preservation strategy for spice-based products.
12	Rosyunita et al. (2023)	Review of aflatoxins in food	Aflatoxins are produced by A. flavus and A. parasiticus and have hepatotoxic, immunotoxic, and carcinogenic effects. Because aflatoxins are not readily destroyed by conventional cooking, prevention should focus on post-harvest handling, GMP, and detection methods such as TLC, HPLC, ELISA, and LC-MS/MS.	Provides a scientific basis for understanding aflatoxin hazards in spice-based foods, including Bir Pletok, and emphasizes the importance of quality control during storage and production.
13	Gwad et al. (2024)	Cinnamon essential oil against A.	Cinnamon essential oil exhibited the strongest fungicidal activity	Highly relevant because cinnamon is a major Bir Pletok

		flavus in wheat	among the essential oils evaluated. At low concentrations, it reduced aflatoxins B1 and B2 by 85–90% and suppressed the expression of aflatoxin biosynthesis genes (nor-1, aflR, pksA, and aflJ).	ingredient. The findings suggest that cinnamon may help inhibit aflatoxin formation during spice storage.
14	Pickova et al. (2020)	Review of mycotoxins in various spices	A review of 56 studies found that spices are susceptible to aflatoxin and ochratoxin contamination, particularly under high-temperature and high-humidity conditions and when Good Agricultural Practices (GAP), GMP, and Good Hygiene Practices (GHP) are inadequately implemented. Contamination was higher in spices than in herbs.	Highly relevant because most Bir Pletok ingredients, including ginger, cinnamon, black pepper, cloves, cardamom, and lemongrass, belong to spice groups reported to be at risk of mycotoxin contamination.
15	Medalcho et al. (2023)	Garlic, ginger, cardamom, and black cumin for AFB1 detoxification	Ginger, garlic, cardamom, and black cumin reduced AFB1 levels. Sautéing increased AFB1 detoxification to approximately 62–66%. Antioxidant activity was positively correlated with aflatoxin detoxification capacity.	Relevant because ginger and cardamom are Bir Pletok ingredients. The findings suggest that spices may contain bioactive compounds capable of reducing aflatoxin toxicity, although this does not establish that contaminated ingredients are safe.
16	Pavlenko et al. (2024)	Commercial plant-based beverages	Approximately 64% of plant-based beverages contained one or more mycotoxins. Although concentrations were generally considered	Relevant because plant-based beverages may carry mycotoxins from contaminated raw materials into

			safe, several mycotoxins, including deoxynivalenol, beauvericin, enniatins, T-2, and HT-2 toxins, were detected simultaneously.	the final product if ingredient quality is not adequately controlled.
17	Wang et al. (2024)	Twenty-three medicinal plant types in China	Analysis of 138 medicinal plant samples yielded 200 mold isolates, predominantly Fusarium and Alternaria. Some isolates could produce mycotoxins such as deoxynivalenol and altertoxins, raising concerns about herbal product safety.	Relevant because Bir Pletok spices undergo cultivation, harvesting, and storage processes similar to those of medicinal plant materials and may therefore be susceptible to fungal contamination.
18	Elamin and Sakuda (2025)	Review of mycotoxin contamination mechanisms in medicinal plants	Aflatoxin and ochratoxin contamination is influenced by plant characteristics, maturity, nutrient content, storage conditions, and fungal penetration. Roots and seeds were identified as particularly susceptible plant parts.	Explains potential mechanisms through which Bir Pletok ingredients may become contaminated with mycotoxins during post-harvest handling and storage.
19	Kanabus et al. (2026)	Review of mycotoxins in herbs, spices, and supplements	Spices such as chili, paprika, ginger, and black pepper were identified as commodities at high risk of multiple-mycotoxin contamination. Co-contamination with several mycotoxins in a single product was frequently reported, potentially increasing cumulative toxicity risks.	Highly relevant because ginger and black pepper are used in Bir Pletok, highlighting the need to monitor possible contamination by multiple mycotoxins simultaneously.
20	Al-Alwani and Al-Nazzal (2023)	Aqueous cinnamon and ginger extracts against A. flavus and A. parasiticus	Aqueous cinnamon and ginger extracts at 200 mg/L produced the largest inhibition zones against A. flavus and A. parasiticus, reaching 22 mm, and showed	Highly relevant because ginger and cinnamon are two key Bir Pletok ingredients with potential natural antifungal activity

	potential to reduce aflatoxin formation.	against aflatoxin-producing molds.

The review of 20 articles indicates that spices used in Bir Pletok may be contaminated with various mycotoxins, particularly aflatoxins (AFs) and ochratoxin A (OTA). Such contamination is generally associated with the growth of toxigenic molds, especially Aspergillus flavus and Aspergillus parasiticus, under conditions of high humidity, warm temperatures, and inadequate post-harvest handling. Zareshahrabadi et al. (2020) reported that approximately half of the spice samples analyzed were contaminated with aflatoxins or OTA, whereas Gialluisi et al. (2025) found that 84% of commercial spice samples contained one or more mycotoxins. These findings are supported by Pickova et al. (2020) and Kanabus et al. (2026), who indicated that spices are particularly susceptible to contamination because cultivation, drying, and storage conditions may favor mold growth. Given that Bir Pletok contains spices such as ginger, cinnamon, black pepper, cloves, cardamom, and lemongrass, potential mycotoxin contamination of these ingredients warrants particular attention in food safety management.

Several studies have demonstrated that mycotoxin contamination levels are strongly influenced by raw material quality and supply-chain management. Negedu et al. (2022) reported higher levels of aflatoxins, OTA, and fumonisins in dried ginger than in fresh ginger, while Falade et al. (2025) also found that some ginger samples contained aflatoxins exceeding safety limits. Su et al. (2025) indicated that aflatoxin formation is optimal at temperatures of 25–30°C, making temperature and humidity control during storage important for preventing mold growth. Furthermore, Elamin and Sakuda (2025) explained that plant characteristics, maturity, and storage conditions influence the susceptibility of herbal materials to mycotoxin contamination. In contrast, Saeed et al. (2025) found no aflatoxins in a ginger–turmeric herbal tea, while Waris (2025) reported that tamarind–turmeric herbal beverages met the applicable mold and yeast count requirements. These findings suggest that high-quality raw materials and appropriate processing practices can contribute to the production of safe herbal beverages.

In addition to highlighting contamination risks, several studies indicate that spices used in Bir Pletok contain bioactive compounds with potential inhibitory effects against mold growth and aflatoxin formation. Farouk et al. (2022) demonstrated that ginger essential-oil nanoemulsions inhibited A. flavus growth by more than 80% and reduced aflatoxin production. Gwad et al. (2024) also found that cinnamon essential oil suppressed aflatoxin biosynthesis by inhibiting the expression of toxin-producing genes. Furthermore, Medalcho et al. (2023) reported that ginger, cardamom, and other spices could reduce AFB1 levels, with effects associated with their phenolic content and antioxidant activity. Nazdar et al. (2024) found that combinations of ginger and turmeric extracts reduced aflatoxin toxicity in vitro. Nevertheless, these antifungal and antioxidant properties cannot replace GMP implementation, raw material quality control, or routine mycotoxin testing. This is consistent with Pinandoyo and Masnar (2020), who identified GMP implementation in Bir Pletok small and medium-sized enterprises as an important preventive measure for maintaining product safety and minimizing mycotoxin contamination during production.

Contamination Control Strategies

Controlling mycotoxin contamination in the ingredients used to prepare Bir Pletok is essential for ensuring the safety, quality, and consistency of the resulting herbal beverage. Spices used as primary raw materials, including ginger, cinnamon, black pepper, cardamom, and cloves, are susceptible to the growth of mycotoxin-producing molds when cultivation, post-harvest handling, storage, and processing are inadequate. Furthermore,

because mycotoxins are relatively heat-stable, control strategies should focus primarily on prevention, from raw material procurement through final product processing. Accordingly, an integrated approach involving good agricultural and processing practices, appropriate storage conditions, natural antifungal agents, and regular quality testing is needed to minimize the risk of mycotoxin contamination in Bir Pletok.

Table 4. Strategies for Controlling Mycotoxin Contamination in Spices Used in Bir Pletok.

No.	Author (Year)	Research Subject	Control Strategy	Main Findings	Relevance to Bir Pletok
1	Basso et al. (2023)	Wheat-based dough contaminated with aflatoxin B1	Combined fermentation and ultrasound (US) treatment for aflatoxin decontaminatio n	The combination of fermentation and ultrasound reduced AFB1 levels by up to 61.7%, outperforming ultrasound alone without altering the product's acidity characteristics.	Indicates that post-processing treatments may serve as supplementary strategies for reducing mycotoxin contamination in plant-based foods, including spice beverages such as Bir Pletok.
2	Mkupasi, Gabriel and Temba (2025)	Black pepper, cinnamon, cloves, and turmeric from Tanzania	Vendor education, aflatoxin monitoring using HPLC, and appropriate storage practices	A total of 24.2% of samples were contaminated with aflatoxins, with some exceeding regulatory limits. The authors emphasized education and preventive measures during spice distribution and storage.	Highly relevant because the spices examined are ingredients used in Bir Pletok and require quality monitoring throughout the supply chain.
3	Rezaei et al. (2025)	Cinnamon extract against A. flavus and A. parasiticus	Use of cinnamon extract as a natural antifungal and anti-aflatoxigenic agent	Cinnamaldehyd e was the predominant compound, and cinnamon extract inhibited mold growth and reduced	Cinnamon is a major Bir Pletok ingredient and may have potential as a natural agent for inhibiting aflatoxin

				AFB1 production by up to 87%.	formation.
4	Pinandoyo and Masnar (2020)	Bir Pletok small and medium-sized enterprise in Cakung	Implementation of Good Manufacturing Practices (GMP) through training and mentoring	Workers' knowledge increased substantially after GMP training, with mean scores rising from 55.56 to 86.67, potentially supporting sustained improvements in food safety practices.	Provides the most directly relevant evidence concerning food safety control during Bir Pletok production through GMP implementation.
5	Tian et al. (2022)	Various essential oils and natural compounds against A. flavus	Use of essential oils and plant-derived bioactive compounds as antifungal agents	Essential oils inhibited A. flavus growth and suppressed aflatoxin biosynthesis through various biological mechanisms.	Supports investigating aromatic spices such as cloves, cinnamon, and ginger as sources of natural antifungal compounds in Bir Pletok.
6	Victor Jeyaraj et al. (2022)	Herbal products and medicinal plant raw materials	Aflatoxin testing using ELISA, lateral flow assays, HPLC, and LC-MS/MS, together with appropriate drying and storage	Aflatoxin contamination in herbal products was associated with inadequate drying and improper storage, highlighting the need for routine testing using sensitive analytical methods.	Highly relevant because Bir Pletok uses dried spices, making raw material quality control essential.
7	Chatterjee et al. (2023)	Mycotoxin monitoring systems in India	Strengthening regulations, national monitoring, analytical	Effective mycotoxin control requires integrated regulations,	Provides a reference for developing quality control systems for Bir

			method validation, GAP, and supply-chain surveillance	testing laboratories, validated analytical methods, and good agricultural and post-harvest practices.	Pletok ingredients, from raw material procurement to distribution.
8	Wang et al. (2023)	Herbal medicinal products	Comprehensive quality control involving raw material standardization, GMP, material authentication, contaminant detection, and regulatory compliance	Strict quality control throughout herbal product manufacturing is necessary to ensure safety, consistent quality, and product efficacy.	These quality control principles can be applied to Bir Pletok production to minimize mycotoxin and other contaminant risks.
9	Mouawad et al. (2026)	Spices	Implementation of Hazard Analysis and Critical Control Points (HACCP), including temperature control below 30°C and relative humidity of approximately 60%	HACCP identified critical control points during spice storage. Temperature, humidity, and supplier evaluation were key factors in preventing aflatoxin contamination.	Highly applicable to the storage of Bir Pletok spices before production to minimize mold growth.
10	Abrehame et al. (2023)	Review of aflatoxins in food and animal feed	Prevention during pre-harvest and post-harvest stages, laboratory detection, awareness-raising, and storage management	The review emphasized that aflatoxins are more effectively prevented through control during cultivation, harvesting, and storage, together with routine monitoring, because they	Establishes a scientific basis for prioritizing raw material quality and storage management rather than relying solely on cooking to control mycotoxins in Bir Pletok.

	are relatively heat-stable.

Based on Table 4, strategies for controlling mycotoxin contamination in spices used in Bir Pletok should be implemented comprehensively, from raw material handling through processing. Mkupasi, Gabriel and Temba (2025) reported that 24.2% of samples of black pepper, cinnamon, cloves, and turmeric were contaminated with aflatoxins, with some exceeding the established maximum limits. These findings indicate that spices are susceptible to contamination by mycotoxin-producing molds, particularly when distribution and storage practices are inadequate. Similarly, Abrehame et al. (2023) explained that aflatoxin contamination is more effectively prevented during pre-harvest, post-harvest, and storage stages because aflatoxins are relatively heat-stable and difficult to eliminate through food processing. Therefore, raw material quality control is a fundamental measure for ensuring the safety of spices used in Bir Pletok.

In addition to raw material control, food safety management systems are important for minimizing mycotoxin contamination risks. Pinandoyo and Masnar (2020) demonstrated that GMP training and mentoring improved food safety knowledge among workers at a Bir Pletok small and medium-sized enterprise, potentially contributing to sustained improvements in production practices. Furthermore, Mouawad et al. (2026) reported that HACCP implementation identified critical control points in spice storage, particularly temperature control below 30°C, relative humidity of approximately 60%, and evaluation of raw material suppliers. These findings are supported by Wang et al. (2023) and Chatterjee et al. (2023), who emphasized that herbal product quality control requires raw material standardization, GMP implementation, contaminant monitoring, analytical method validation, and continuous supply-chain surveillance to ensure product safety and consistency.

Another potentially useful strategy involves the application of natural antifungal compounds derived from spices and the use of supplementary decontamination technologies. Rezaei et al. (2025) demonstrated that cinnamon extract containing cinnamaldehyde inhibited the growth of Aspergillus flavus and Aspergillus parasiticus and reduced aflatoxin production by up to 87%. These findings are supported by Tian et al. (2022), who reported that various plant essential oils exhibit antifungal and anti-aflatoxigenic activities and may therefore help suppress mycotoxin formation. Meanwhile, Basso et al. (2023) showed that combined fermentation and ultrasound treatment reduced aflatoxin B1 levels by up to 61.7%. Victor Jeyaraj et al. (2022) emphasized the importance of aflatoxin testing using analytical methods such as ELISA, HPLC, and LC-MS/MS, together with appropriate drying and storage practices.

Strategies for controlling mycotoxin contamination in the spices used in Bir Pletok should integrate preventive measures beginning with raw material selection, GMP and HACCP implementation, the potential application of natural antifungal compounds, and routine aflatoxin monitoring. This integrated approach is essential for minimizing contamination risks and producing a safe, high-quality traditional herbal beverage.

4. CONCLUSION

Based on the findings of this systematic review, Aspergillus spp. are associated with fungal contamination and the production of mycotoxins in various herbal materials, spices, and plant-based products. Several species, including A. flavus, A. niger, A. ochraceus, and A. parasiticus, have been reported in association with secondary metabolites such as aflatoxins, ochratoxin A, citrinin, sterigmatocystin, and cyclopiazonic acid. Other metabolites produced by Aspergillus spp. may exhibit diverse biological activities, ranging

from potentially beneficial pharmacological effects to toxic effects. These findings indicate that herbal ingredients and spices used in traditional beverages such as Bir Pletok may be susceptible to fungal contamination and mycotoxin occurrence. However, the available evidence does not establish the actual presence of Aspergillus spp. or mycotoxins in Bir Pletok itself, nor does it determine consumer exposure or associated health risks.

Further research should directly investigate the microbiological and chemical quality of Bir Pletok ingredients and the finished beverage. Specific recommendations include collecting representative samples of raw spices and the final product, identifying contaminating fungi, and measuring relevant mycotoxins using validated analytical methods. Future studies should also evaluate how processing, drying, and storage conditions affect fungal growth and mycotoxin occurrence. The results would provide a stronger evidence base for developing appropriate quality-control measures and supporting the safety and sustainable development of Bir Pletok as a traditional herbal beverage.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Meinanda Grabilia contributed to the conceptualization and design of the study, data collection, formal analysis, interpretation of the quantitative and qualitative findings, and preparation of the initial manuscript draft. Sylvia Rizky Prima to the literature review, interpretation of the findings, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript for publication and agreed to be accountable for all aspects of the work.

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