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CONTROL OF AFLATOXIN-PRODUCING MOULDS IN DRIED COW-SKIN HIDE (PONMO IJEBU) USING CATNIP LEAF EXTRACT

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ABSTRACT

The use of plant extracts as antimicrobial agents has continually gained recognition because of their effectiveness. The study investigated catnip leaf extracts as an antifungal agent against aflatoxin-producing fungi isolated from cow skin (*Ponmo*). A total of 10 samples were purchased from 5 different open market sources in Abeokuta. Fungal and bacterial isolation were carried out using standard microbiological procedures. Aflatoxin quantification was done on *Ponmo* samples using High Performance Liquid Chromatography (HPLC). Methanolic extraction of catnip leaves was stored at room temperature till it was ready for use. Antifungal activities were carried out by the well diffusion method. Catnip leaf extract was introduced into the well-bore into plates of fungal species. *Aspergillus niger* 8.9%, *Aspergillus nomius* 35% and *Aspergillus flavus* 50.6%, were the fungi species found on *Ponmo* Ijebu. *Aspergillus flavus* has the most prevalent occurrence of 50.6%. The bacteria isolates from the sample include: *Staphylococcus, Steptococcus,* and *Micrococcus*. This study revealed that cow-skin hide sold at the Camp area of Abeokuta had the highest total aflatoxin load of 11µg/kg, which poses a great threat to the health of the consumers. The zone of inhibition, which ranged from 10 mm to 14 mm, was measured. This shows the efficacy of catnip to inhibit aflatoxigenic fungi in cow-skin hide during processing and storage, raising concerns about food safety and a need to find a control method. Therefore, it is advisable to educate consumers on proper hygiene practices during the cow skin drying process to mitigate fungal growth.

Keywords: Aflatoxin, Cow skin hide, Antimicrobial agent

INTRODUCTION

Cow skin hide (*Ponmo* ijebu) is a meat product obtained from the tenderization of the hide of cattle, goat, e.t.c, in fire, followed by scraping with sharp objects like a knife or metal sponges (Okiei *et al.*, 2009). It is enjoyed as a local delicacy and used in preparing indigenous foods such as egusi, vegetable, gbegiri, and many others in the southwestern region of Nigeria and several parts of Africa (Adaku, 2019). According to Adaku (2019), cow skin hide is a rich source of dietary fibre, making it a potential alternative to red meat. Its consumption may help reduce the health risks commonly associated with red meat intake. Microbial contamination of fresh and dried cowhides by microorganisms has been reported. According to Keta *et al.* (2020), *Staphylococcus aureus*, *Salmonella* spp, *Shigella* dysenteria, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella* spp, and *Pseudomonas aeruginosa*, were detected in processed cowhide meat examined in Birnin Kebbi, while *Enterobacteriaceae* bacteria were mostly found on the dried hides.

According to Saheed *et al.*, (2022), *Ponmo* contains nutrients such as potassium, calcium, iron, magnesium, phosphorus, and zinc. It is also rich in fibre and collagen, a protein mostly found in the skin, bones, and connective tissues that help keep the skin healthy and smooth. During the singeing of cow's hair (slight burning with flame), the cow's skin hide is exposed to many harmful chemicals, such as tyres, engine oil, metals, and other unclean substances. A previous study by Ariyo *et al.* (2022) has shown that *Ponmo* Ijebu is usually contaminated with microorganisms as a result of handlers' unhygienic practices.

Aflatoxins are naturally occurring compounds that are produced by the moulds; *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are known to be potent human carcinogens (Tirmenstein *et al.*, 2014). Aflatoxin can also be found in animal-derived products from animals fed contaminated feed (Khan *et al.*, 2021).

Aspergillus species cause Aspergillosis, which is a lung infection in immune deficiency. Liver cancer may also occur by consuming low levels of aflatoxin for a long time (Pitt, 2014). Chronic aflatoxicosis includes liver cancer, hepatic cell carcinoma, cirrhosis, and stunted growth in malnourished children, and reduced immunity, whereas acute aflatoxicosis includes vomiting, liver failure, high fever, and foot oedema with a higher mortality rate than chronic aflatoxicosis. Although it is not very well established, consumption of 1000 μg kg^{-1} of aflatoxin concentration may cause aflatoxicosis in humans (Shabeer *et al.*, 2022). Many abiotic and biotic factors influence the production of aflatoxins; however, it is mainly reported to occur in high relative humidity and temperature. The occurrence and extent of aflatoxin contamination might also be associated with unsuitable processing, storage, and transport conditions.

Catnip (*Nepeta cataria*) is an erect perennial herb from the family Lamiaceae, with its leaves commonly used in teas, infusions, or as flavouring agents (Small, 2009). Traditionally, it has been used as a remedy for conditions such as colds, fever, cough, stomach disorders, and diarrhea (Ajay, 2019). Porkony (2007) opined that the presence of antioxidant compounds, including phenols and flavonoids, in catnip. Pharmacological studies further suggest that catnip possesses antimicrobial and antifungal properties, indicating its potential use in controlling fungal growth and reducing aflatoxin production (Damanjit, 2019)

During the production and storage process, *Ponmo* is contaminated with microorganisms such as fungi, which causes deterioration by the production of spores. Recently, there has been an outbreak of Anthrax disease, a zoonotic disease caused by *Bacillus anthracis* through the consumption of contaminated animal skin hide with *Bacillus anthracis* spores (Adewumi *et al.*, 2021).

This research aimed to explore the microbial contamination of cow skin hide (*Ponmo*

Ijebu), focusing on the control of fungal growth and aflatoxin production. Despite being a popular local delicacy and a source of essential nutrients, *Ponmo* is often contaminated with harmful microorganisms due to improper processing and storage conditions. Given its exposure to hazardous chemicals and the potential for contamination by aflatoxin-producing fungi, investigating natural antifungal agents like catnip (*Nepeta cataria*) could offer a sustainable solution to improve food safety and reduce health risks associated with *Ponmo* consumption.

MATERIALS AND METHODS

Study Area

Ten samples each of dried cow skin hide (*Ponmo* Ijebu) were obtained from various open markets in Ogun State, including Osiele, Ijebu, Odoeran, Camp, and Kuto. Each sample was placed in a sterile ziplock bag, appropriately labeled, and transported to the laboratory for analysis. Fresh catnip leaves (*Nepeta cataria*) were harvested and identified at the Botany Department Garden of the Federal University of Agriculture, Abeokuta, and collected in sterile ziplock bags.

Preparation of Samples

Each *Ponmo* Ijebu sample was scraped and filled into small bits using a sterile knife and stored in sterile air-tight containers.

Preparation of Catnip Leaves

The leaves of *Nepeta cataria* were air-dried for two weeks. The dried leaves were blended into a fine powder using a sterile electric blender and kept in sterile containers until it was ready for use.

Preparation of Media

The media used were Nutrient Agar, Malt Extract Agar, Dichloran Rose Bengal Chloramphenicol Agar, Mueller Hinton Agar, and Yeast Extract Agar. They were prepared according to the manufacturers' instructions and sterilized in an autoclave at 121°C for 15 minutes and then poured aseptically into a petri dish.

Bacteriological Analysis of Cow Skin Hide

One gram of each sample was weighed and introduced aseptically into 9 mL of sterile distilled water and properly homogenized, and a two-fold serial dilution was done. One ml from 10^{-5} and 10^{-3} dilution was pipetted into sterile petri dishes, and 20 ml of freshly prepared nutrient Agar was dispensed into the petri dishes, it was properly mixed and allowed to solidify. The plates were incubated at 37°C for 24 hours.

The gram staining procedure was carried out using the method of Li *et al.*, (2020).

Fungal Analysis of Cow Skin Hide

One gram of each sample was aseptically introduced into 9 mL of sterile distilled water and properly mixed, and serial dilution was done till a 10^{-2} dilution was achieved. One ml of 10^{-5} and 10^{-3} dilutions was pipetted into sterile petri dishes, and 20 ml of freshly prepared Dichloran Rose Bengal Chloramphenicol Agar was dispensed into the petri dishes, mixed well, and allowed to solidify. The plates were incubated at room temperature for 1 week. Developed colonies were counted to obtain the total viable count.

Fungal Isolation of Cow Skin Hide

One ml of 10^{-5} and 10^{-3} dilution of each sample was pipetted into sterile petri dishes, and 20 ml of freshly prepared Malt Extract Agar was dispensed into the petri dishes, mixed well, and allowed to solidify. The plates were incubated at room temperature for 3 days. Developed discrete colonies were counted.

Discrete colonies were subcultured onto fresh agar plates for proper identification.

Macroscopic examination of fungi isolated from cow skin hide

The fungal isolates were examined macroscopically for growth, colour, surface, etc.

Microscopic examination of fungi isolated from cow skin hide

Microscopic examination of fungi isolates was carried out by staining with lactophenol blue. The clean grease grease-free slide was labelled and placed on the staining tray. The smear was made by adding a drop of lactol phenol blue using a sterile micro pipette on the slide, and a small portion of the fungal isolate was picked with a sterile inoculating loop and added to the slide. It was allowed to be air-dried, and a cover slip was placed on the slide and viewed under the microscope.

Aflatoxin Detection of Cow Skin Hide

High-Performance Liquid Chromatography (HPLC) technique was employed for the extraction and quantification of aflatoxin from cow skin hide at the National Agency for Food and Drug Administration and Control (NAFDAC), according to Martins et al. (2008). The *Ponmo* Ijebu samples were mixed with 50 mL of methanol in a 500 mL conical flask to extract aflatoxin, following the standard method (AOAC, 2010). The flask was securely stoppered with masking tape and shaken on a wrist-action shaker for 30 minutes. The mixture was then filtered through fluted filter paper. The filtrate was transferred to a Buchner funnel pre-coated with a 0.45-micrometer micro-syringe filter membrane. When the filtration was slow, it was assisted using a light vacuum. Clean-up was carried out using immunoaffinity columns. The extracted aflatoxin samples were mixed with 100 microliters of trifluoroacetic acid (TFA) and vortexed for 30 seconds, then allowed to stand for 15 minutes. The samples were analyzed using an HPLC system consisting of a Waters 6000A solvent delivery system and a WISP 710B sample processor (Waters Associates, India). The samples were eluted isocratically on a 10-micrometer octadecylsilane cartridge (Waters Associates, India), with a mobile phase placed between the injector and the cartridge. Aflatoxin was detected fluorometrically (excitation wavelength: 365 nm; emission wavelength: 425 nm) using a fluorescence detector (Model 420C, Waters Associates). HPLC chromatograms were recorded using a Waters Data Module (Waters Associates) at a chart speed of 1.0 cm/min. The concentration of aflatoxin in the *Ponmo* Ijebu samples was determined by comparing the peak areas with those of samples containing known concentrations of aflatoxin.

Aflatoxigenicity screening

Screening Fungal Isolates for Aflatoxigenic Potential

Isolates were screened using a Bright Greenish-Yellow Fluorescence experiment (BGYF). For a preliminary screening of aflatoxin production, strains were inoculated at a central point on a 6 cm diameter Petri dish containing 10 ml of Yeast Extract Agar Medium (YEA) supplemented with 0.3% β-cyclodextrin (Fente *et al.*, 2001), and incubated for 5 days in the dark at 28°C. Cultures were tested for 365 nm UV light fluorescence and bright green-yellow colony reverse coloring expression under daylight.

Inoculation and Determination of Media Characteristics

The plates were inoculated with suspected aflatoxigenic moulds and incubated at 30℃ for 48 hours. Thereafter, the plates were examined for some media characteristics: opacity, transparency, and translucency. An uninoculated plate served as a control. The reverse side of each plate, which consist of a large colony, was observed under the long wave (365 nm) Ultra-violet light for blue/ blue green fluorescence and the media were visually assessed for colour and minimal time of pigmentation; intensity, minimal time, peak time and duration of fluorescence every four hours.

Antifungal activity of catnip extract

The antifungal activity of catnip extract was evaluated using the well diffusion method.

Fungal isolates were cultured on Potato Dextrose Agar (PDA) and adjusted to a 0.5 McFarland standard. Inoculated plates were bored with a sterile cork borer to create wells of 6 mm diameter. Each well was filled with 100 μL of the catnip extract. The plates were incubated at 28°C for 72 hours. Zones of inhibition around the wells were measured in millimeters to determine antifungal effectiveness. This method was adopted with slight modifications from the standard procedure described by Valgas *et al.*, (2007).

RESULTS

Morphological characteristics of bacteria isolated from cow skin hide (Ponmo ijebu)

All bacterial isolates from different locations (Ijebu, Kuto, Osiele, Odo-eran, and Camp) shared similar morphological traits — circular shape, creamy-yellow to yellow colour, flat surfaces, smooth edges, and opaque opacity — indicating uniformity in bacterial colony appearance (Table 1).

Viable Bacteria Count of Cow Skin Hide (Ponmo Ijebu)

Bacterial load varied across locations, with Osiele showing the highest bacterial count (6.6 x 10^3 cfu/ml) and Ijebu the lowest (3.0 × 10^3 cfu/ml), suggesting possible differences in hygiene or environmental conditions during processing or storage (Table 2).

Viable Fungal Count of Cow Skin Hide (Ponmo Ijebu)

Ijebu samples had the highest fungal count (3.5 x 10^3 cfu/ml), while Kuto had the lowest (1.35 x 10^3 cfu/ml). Osiele and Odo-eran had closely similar fungal loads, indicating a comparable level of fungal contamination in those areas (Table 3).

Morphological Characteristics of Fungi Isolated from Cow Skin Hide (Ponmo Ijebu

Morphological characteristics of fungi isolated from *Ponmo* samples exhibited septate hyphae with conidia or asci spores. Predominant fungi included *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nomius*, *Byssochlamys fulva*, and *Saccharomyces cerevisiae*, highlighting a diverse fungal population capable of spoilage and toxin production (Table 4).

Frequency of Occurrence of Fungi Isolated from Cow Skin Hide (Ponmo Ijebu)

Fungal species: *Aspergillus flavus* had the highest frequency of occurrence (51%), while *Saccharomyces cerevisiae* had the least frequency of occurrence of 5% (Figure 1).

Aflatoxin Quantification of Cow Skin Hide

There were different levels of four types of aflatoxins (AFB1, AFB2, AFG1, AFG2) and total aflatoxin. Camp samples had the highest total aflatoxin (11.00 μg/kg), while Odo-eran had no detectable aflatoxins (Table 5).

Aflatoxin Potential of Fungal Isolates from Cow Skin Hide (Ponmo Ijebu)

Only *Aspergillus flavus* and *Aspergillus nomius* were confirmed to be aflatoxigenic, capable of producing harmful aflatoxins. In contrast, *Aspergillus niger* and *Saccharomyces cerevisiae* were non-aflatoxigenic (Table 6).

Zone of Inhibition of Catnip (**Nepeta Cataria*****) Leaf Extract Against Aflatoxigenic Fungi Isolated From Cow-Skin Hide (Ponmo* Ijebu*)***

The catnip leaf extract exhibited antifungal activity against all tested fungi, with the highest inhibition observed against *Aspergillus flavus* (14 mm), followed by *Aspergillus nomius* (12 mm), *Saccharomyces cerevisiae* (11 mm), and *Aspergillus niger* (10 mm) – Table 7. This indicates that catnip extract is effective in suppressing the growth of aflatoxigenic fungi, particularly *A. flavus*, which is known to be a major producer of aflatoxins

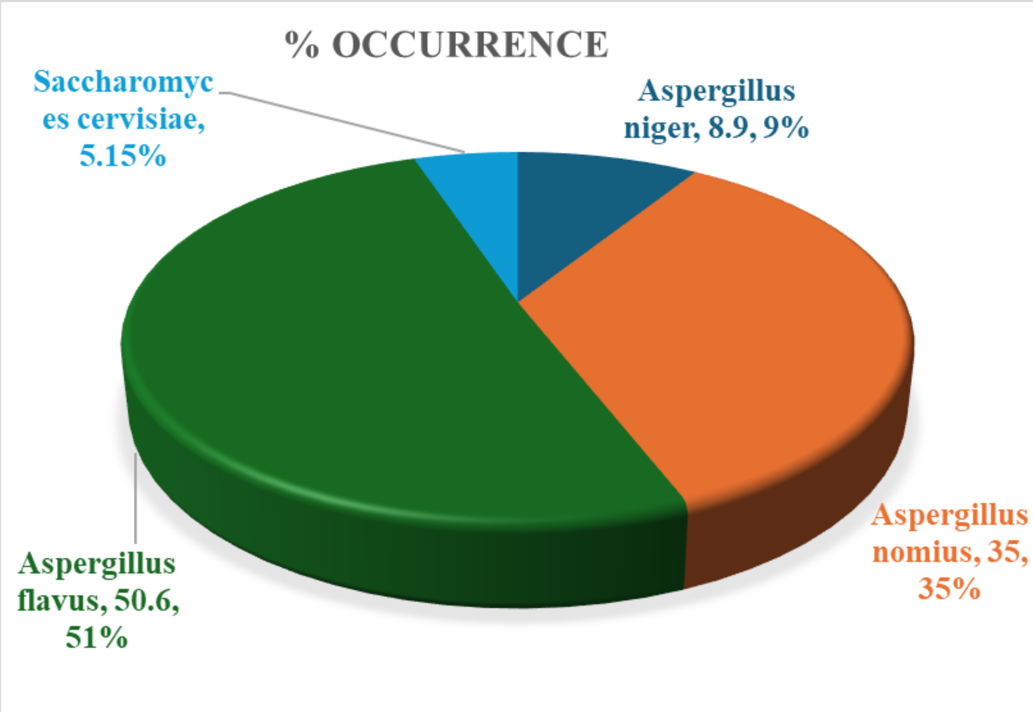

Fig 1: Frequency of Occurrence (%) of Fungi Isolated from Cow Skin Hide (*Ponmo* Ijebu)

Table 1: Morphological Characteristics of Bacteria Isolated from Cow Skin Hide (*Ponmo* Ijebu)

ISOLATE CODE	SHAPE	COLOUR	SURFACE	EDGES	OPACITY
IJB	Circular	Creamy Yellow	Flat	Smooth	Opaque
KT	Circular	Yellow	Flat	Smooth	Opaque
OS	Circular	Creamy Yellow	Flat	Smooth	Opaque
OE	Circular	Yellow	Flat	Smooth	Opaque
CP	Circular	Yellow	Flat	Smooth	Opaque

KEY: Ijebu- IJB, Odo-eran- OE, Osiele- OS, Kuto- KT, Camp – CP

Table 2: Bacterial Count of Cow Skin Hide (*Ponmo* Ijebu)

ISOLATE CODE	COLONY FORMING UNITS (cfu/ml)
IJEBU	3.0×10^3
KUTO	6.0×10^3
OSIELE	6.6×10^3
ODO-ERAN	4.5×10^3
CAMP	3.5×10^3

Table 3: Fungal Count of Cow Skin Hide (*Ponmo* Ijebu)

ISOLATE CODE	COLONY FORMING UNITS (cfu/ml)
IJEBU	3.5×10^3
KUTO	1.35×10^3
OSIELE	3.3×10^3
ODO-ERAN	3.2×10^3
CAMP	3.1×10^3

Table 4: Morphological Characteristics of Fungi Isolated from Cow Skin Hide (*Ponmo* Ijebu)

ISOLATE CODE	COLOUR	TYPE OF SPORE	HYPHAE	MICROORGANISM
IJB	Black	Conidia	Septate	*Aspergillus niger*
	Yellow green	Conidia	Septate	*Aspergillus nomius*
KT	White	Asci	Septate	*Byssochlamys fulva*
OS	Cream	Asci	Septate	*Saccharomyces cerevisiae*
				Aspergillus nomius
OE	Brown	Conidia	Septate	*Aspergillus flavus*
CP	Black	Conidia	Septate	*Aspergillus niger*

Table 5: Aflatoxins Quantification of *Ponmo* Ijebu

Sample Code	AFB_1 (µg/kg)	AFB_2 (µg/kg)	AFG_1 (µg/kg)	AFG_2 (µg/kg)	Total Aflatoxin (µg/kg)
IJB	2.00	0.00	2.00	1.00	5.00^b
KT	4.00	0.00	2.00	0.00	6.00^b
OS	1.00	0.00	1.00	0.00	2.00^c
OE	0.00	0.00	0.00	0.00	0.00^d
CP	7.00	0.00	2.00	2.00	11.00^a
Mean ± SD	**2.80 ± 2.59**	**0.00 ± 0.00**	**1.40 ± 0.89**	**0.60 ± 0.89**	**4.80 ± 3.87**

Note: Values with different superscript letters (a, b, c, d) in the same column are significantly different at $p < 0.05$ (One-way ANOVA followed by Tukey's HSD test)

Table 6: Aflatoxigenic Potential of Fungi Isolated from Cow Skin Hide (*Ponmo* Ijebu)

ORGANISM	AFLATOXIGENICITY
Aspergillus niger	Non-aflatoxigenic
Aspergillus flavus	Aflatoxigenic
Aspergillus nomius	Aflatoxigenic
Saccharomyces cerevisiae	Non- aflatoxigenic

Table 7: Zone of Inhibition of Catnip Leaf Extract against Aflatoxigenic Fungi Isolated from Cow-Skin Hide (*Ponmo* Ijebu)

Fungal Isolate	Zone of Inhibition (mm)
Aspergillus flavus	14 mm
Aspergillus nomius	12 mm
Aspergillus niger	10 mm
Saccharomyces cerevisiae	11 mm

DISCUSSION

The current study investigated the microbiological quality and aflatoxin contamination of dried cow-skin hide (*Ponmo* Ijebu) from different locations, as well as the potential for controlling aflatoxigenic fungi using catnip (*Nepeta cataria*) leaf extract. The findings reveal significant variations in microbial load, fungal species distribution, and aflatoxin levels, thereby underscoring the importance of food safety interventions in traditionally processed animal products.

The viable bacterial counts ranged from 3.0 × 10^3 cfu/ml in Ijebu samples to 6.6 × 10^3 cfu/ml in Osiele samples, indicating a substantial microbial burden, likely introduced during post-slaughter processing and handling. The bacterial morphology was predominantly circular, with smooth surfaces and opaque appearance, consistent with typical mesophilic bacterial colonies often associated with poor hygiene or environmental exposure (Ray and Bhunia, 2013).

Similarly, fungal counts ranged from 1.35 × 10^3 cfu/ml in Kuto to 3.5 × 10^3 cfu/ml in Ijebu, with isolates displaying septate hyphae and diverse spore types (conidia and asci). These fungal features are indicative of mould species such as *Aspergillus* and *Byssochlamys*, known for their resilience and mycotoxin-producing capability (Pitt and Hocking, 2009).

The most frequently occurring fungal species was *Aspergillus flavus,* a well-known aflatoxin B_1 (AFB_1) producer. This aligns with previous reports identifying *A. flavus* as the primary agent in aflatoxin contamination of both plant-based and animal-derived foods (Cotty *et al.*, 2008). Other isolates such as *Aspergillus nomius* were also aflatoxigenic, while *Aspergillus niger* and *Saccharomyces cerevisiae* were non-aflatoxigenic. The presence of *Byssochlamys fulva*, although not traditionally aflatoxigenic, points to the possibility of other spoilage and heat-resistant fungi colonizing the hides.

Quantification revealed that total aflatoxin levels varied significantly across the sampled locations. Camp (CP) samples recorded the highest level at 11.00 µg/kg, which, although below the maximum permissible limit for aflatoxins in foods set by the European Union (4 µg/kg for total aflatoxins in ready-to-eat food), may still pose chronic health risks through long-term exposure (EFSA, 2020). The complete absence of aflatoxins in the Odo-eran sample may suggest better hygienic handling or less favourable conditions for fungal growth. Compared to other studies on aflatoxin contamination in animal-derived foods, this research finds that the contamination levels in *Ponmo* are comparable to those observed in dried meat products, particularly in regions with inadequate food safety practices (Bankole *et al.*, 2021). Studies by Akande *et al.* (2021) on meat products in Nigeria reported similar bacterial and fungal profiles, with Aspergillus species being the most prevalent. The frequent occurrence of *Aspergillus niger* and *Aspergillus flavus* in this study also aligns with previous reports on the dominance of these fungi in animal-derived products (Bankole *et al.*, 2020).

The zones of inhibition observed highlight the antifungal potential of catnip (*Nepeta cataria*) against aflatoxigenic fungi in cow-skin hide. This finding supports previous studies that reported the effectiveness of plant-based extracts in controlling *Aspergillus* species. For instance, Kowalska *et al.* (2020) demonstrated the antifungal activity of catnip essential oil against toxigenic fungi, while Razzaghi-Abyaneh *et al.* (2009) emphasized the potential of medicinal plant extracts as natural inhibitors of aflatoxin-producing fungi. These results suggest that catnip could serve as a natural preservative, enhancing food safety during *Ponmo* processing and storage.

CONCLUSION

This study confirms microbial contamination of cow-skin hides (*Ponmo* Ijebu), particularly by aflatoxigenic fungi like *Aspergillus flavus* and *A. nomius*, due to poor hygiene during processing and storage. Aflatoxin detection highlights potential public health risks. The antifungal efficacy of catnip extract suggests it may serve as a natural, eco-friendly control method. Further research is needed to explore its concentration-dependent effects and possible integration into post-processing treatments to enhance food safety in traditional meat products.

RECOMMENDATIONS

Conduct further studies to optimize catnip extract concentrations and application methods. Explore the economic and environmental feasibility of incorporating catnip-based solutions into food processing practices. Educate stakeholders in the food industry about the benefits of using natural alternatives like catnip for mould control in food products. Therefore, it is recommended that proper handling, storage, and processing practices be implemented to reduce microbial and aflatoxin contamination. Future research should focus on developing cost-effective methods for reducing aflatoxin levels in traditional food products like *Ponmo*.

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