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COMPARATIVE STUDY ON THE FUNGI DETERMINANTS IN LOCUST BEANS (IRU) PRODUCED FROM *PARKIA BIGLOBOSA* AND *PROSOPIS AFRICANA*

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ABSTRACT

Iru, a traditional fermented condiment widely consumed in West Africa, is produced from the seeds of *Parkia biglobosa* (African locust bean) and *Prosopis Africana* (Mesquite/Okpehe). The quality and safety of Iru can be significantly affected by fungal contaminants during processing and storage. This study aims to compare the fungal contaminants present in Iru produced from *Parkia biglobosa* and *Prosopis Africana*, examining their impact on the nutritional and microbial quality of the final product. Standard mycological techniques were used to isolate and identify fungal species using Potato dextrose agar (PDA). The microbial load, proximate composition, and physicochemical properties of the samples were analysed to assess the quality and safety of the fermented products. Results showed that *Parkia biglobosa* Iru exhibited a higher diversity of fungal species compared to *Prosopis Africana* Iru. *Aspergillus niger* and *Sacharomyces cerevisiae* were the most prevalent with (23.81%) each while *Penicillium chrysogenum* has the least prevalence (1.0%). This diversity poses a greater risk of mycotoxin contamination in Iru from *Parkia biglobosa*. Both *Prosopis Africana* and *Parkia bioglobosa* offers significant nutritional benefits but Parkia generally has higher moisture content, protein and fat content while Prosopis contains higher fibre, ash content and carbohydrates which aids metabolic process overall nutritional value and energy source respectively.

Keywords: Locust beans, *Parkia bioglobosa*, *Prosopis Africana*, Fungi determinants, Nutritional benefits

INTRODUCTION

Fermented locust beans are integral to the sensory appeal of African cuisine, contributing a distinctive taste and aroma to soups, stews, sauces, and condiments (Akpor *et al.,* 2021). They are prepared primarily from seeds of the African locust bean tree (*Parkia biglobosa*) and, increasingly, from *Prosopis africana*, an alternative legume species. *Parkia biglobosa* (African locust bean) is a perennial, deciduous tree of the Fabaceae family. Widely cultivated in tropical Africa, especially in Nigeria, Ghana, Senegal, and Cameroon, it is valued for its pods, which contain sweet pulp and nutrient-rich seeds (Okolo, 2023). *Prosopis africana*, a forest tree of the *Fabaceae* family, is well-adapted to the Guinea Savannah environment of West Africa. Though traditionally underutilized, its seeds show potential as an alternative raw material for fermented locust bean production.

These beans, known as "ogiri" in Igbo, "iru" in Yoruba, and "dawadawa" in Hausa, hold both nutritional and cultural significance across Nigeria (Fagbohun and Lawal, 2011). With a composition of approximately 29% lipids, 35% protein, and 16% carbohydrates, locust beans are highly valued for their nutritional richness, serving as a vital source of fat and calcium, particularly for rural populations. Within the pods, two primary seed types—reddish-dark and dark black—are commonly utilized in fermentation processes (Akpor *et al.,* 2021).

The fermentation of locust beans is a complex biochemical process driven by microbial activities, predominantly involving bacteria and fungi. Fungi play a critical role in breaking down the seed's dense structure, releasing nutrients, and enhancing the product's digestibility. However, not all fungi associated with fermentation are beneficial (Sakai *et al.,* 2017). While some enhance flavor and nutrient availability, others may produce mycotoxins, posing risks to consumer health. Understanding the fungal determinants of fermented products is thus essential for improving food safety and quality. The traditional process of fermenting locust beans involves multiple steps, including depulping, boiling, dehulling, fermentation, and occasionally drying. These steps not only enhance the beans' nutritional value but also enrich their flavor, solidifying their role as a dietary staple in West Africa (Sakai *et al.,* 2017).

Fermented locust beans are also rich in essential nutrients and bioactive compounds. They provide significant amounts of protein, dietary fiber, vitamins such as riboflavin and thiamine, and minerals like calcium, iron, and magnesium (Termote *et al.,* 2022). Additionally, these beans possess bioactive properties, including antibacterial and antioxidant activities (Eboma *et al.,* 2020). Fermentation enhances these attributes by fostering the growth of beneficial lactic acid bacteria, such as *Lactobacillus* species, which contribute to improved gut health, nutrient absorption, and immune system support (Muhammad and Araoye, 2016).

The health benefits of fermented locust beans are widely documented. They exhibit strong antioxidant activity, which helps mitigate oxidative stress and reduces the risk of chronic diseases (Saleh *et al.,* 2021). Other noted benefits include blood sugar regulation (Nasrallah *et al.,* 2023), wound healing through dermal fibroblast proliferation (Kuma *et al.,* 2022), antimalarial effects (Builders *et al.,* 2012), and antimicrobial properties against bacterial infections (Olukunle *et al.,* 2019)

Despite these advantages, safety concerns regarding fermented locust beans persist due to microbial contamination. Poor handling during processing, exposure to environmental contaminants, and inadequate storage conditions create favorable environments for fungal proliferation (Awuchi *et al.,* 2021). Fungi such as *Aspergillus* spp. and *Fusarium* spp. are common contaminants, capable of producing harmful mycotoxins like aflatoxins, ochratoxins, and fumonisins (Xu *et al.,* 2023). These toxins pose serious health risks, including carcinogenic, hepatotoxic, nephrotoxic, and immunosuppressive effects (Zain, 2011).

Consumption of contaminated locust beans underscores the need for stringent processing and storage practices to ensure safety.

Traditionally, *Parkia biglobosa* has been the primary raw material for *Iru* production due to its widespread availability and established use. However, increasing interest in *Prosopis africana* as an alternative has emerged due to its comparable nutritional properties and its adaptation to harsher environments. Despite these advantages, the fungal communities associated with the fermentation of *P. africana* seeds remain underexplored. The potential variations in fungal species between *P. biglobosa* and *P. africana* could have significant implications for fermentation outcomes, sensory properties, and safety (Akpor *et al.,* 2021). This study focuses on the comparative analysis of fungal contaminants in fermented locust beans (*iru*) produced from two leguminous plants: *Parkia biglobosa* and *Prosopis africana*. Findings from this study will contribute to improving fermentation practices, ensuring the safety of this vital food product, and supporting public health initiatives in communities reliant on fermented locust beans. Existing studies on locust bean fermentation have largely focused on bacterial diversity and general microbial contributions to product quality. However, a comparative analysis of the fungal profiles in locust beans produced from these two plant species is limited.

MATERIALS AND METHODS

Sampling Area

Samples of *Parkia biglobosa* (Iruworo and Irupete) and *Prosopis africana* popular known as Okpehe were purchased from two retailers each in three different markets in Ilorin which includes Ipata market, Oja-oba market and Kulende market. The samples were packed in sterile nylon and transported to Microbiology department laboratory in University of Ilorin where the samples were preserved in the refrigerator before their laboratory analysis.

Materials

The materials used in this research work include fermented samples of *Parkia biglobosa* (Iruworo and Irupete) and *Prosopis africana* popular known as Okpehe , Sterile sampling containers, Petri dishes, Agar plates (Potato dextrose agar), Lactophenol cotton blue stain, Microscope, Bunsen burner, mortar and pestle, auto clave, test tubes, Microscope slides and coverslips, Distilled water, inoculating needles, Incubator set to appropriate temperature for fungal growth (usually 25-30°C), Pipettes, Sterile loops, Ethanol (for sterilization), Disinfectants (e.g., soap solution) for decontamination.

Sterilization of Materials

Materials were properly sterilized before and after use to avoid contamination. Glass wares were washed thoroughly after which they were dried, wrapped with aluminium foil and sterilized in thehot air oven at 160ºC for an hour. The culture media used according to the manufacturer's instruction were sterilized in the autoclave at 121ºC for 15 minutes. Sterilization of inoculating loops and needles were done by making sure they were red-hot when flamed. The work-bench was disinfected by swabbing with 70% ethanol-soaked cotton wool.

Media Preparation

Potato Dextrose Agar (PDA) was prepared by adding 39 g in 1 litre of Sterile Distilled Water in a conical flask and the flask was autoclaved at 121^0C for 15 minutes. Several test tubes containing 9mls of distilled water and corked with cotton wool was autoclaved alongside the media for proper sterilization. After autoclaving, the media was allowed to cool to about 40oC and Streptomycin Sulphate was added at the rate of 0.2g/l.

Inoculation of Samples

Each of the samples were labelled appropriately and transferred into a sterile porcelain, a sterile pestle was used to break the lump of the samples into even consistency. One gram of each condiment sample from each market was serially diluted up to (10^3). 1ml inoculum each of 10^1and 10^3was plated with PDA (potato dextrose agar) using pour plate method. The plates were incubated at room temperature and observed after 48 and 72 hours progressively for fungal growth. While two plates of inoculated PDA set plates were kept as controls.

Isolation of Pure Cultures

Most plates were observed to have multiple fungal colonies growing on them. Such plate colonies were separated into different plates. A sterile inoculating loop was used to transfer cells from previous culture into a fresh medium by using the inoculating loop to touch the colony to be separated such that some spores or mycelia stick on to the loop. This was then carefully transferred to an appropriately labelled sterile PDA plate. This was repeated until all the colonies have been transferred into separate PDA plates. The newly inoculated plates were then incubated at room temperature and observed for fungal growth for another 48hours with an uninoculated PDA plate as control. The plates that still had multiple colonies growing on it were further separated until pure culture were obtained using the same method (Mailafia *et al.* 2017). The pure cultures obtained were taken to the central research laboratory University of Ilorin for characterization and identification.

Identification of Pure Isolate

The fungal isolates were identified using morphological features such as colony growth pattern, conidial morphology, and pigmentation, cellular morphology. The technique of Mailafia *et al.* (2017). was also adopted for the identification of the isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Klich *et al.,* (2002).

Use of Staining (Lactophenol Blue) Microscopic Examination for Fungi Identification

Staining with Lactophenol Cotton Blue

A small sample of the fungal colony was collected from the Petri dish containing a general purpose culture media (Potato dextrose agar). A drop of LPCB was placed on a clean microscope slide. Using a sterile loop or needle, a small amount of the fungal sample is transferred to the LPCB drop after which the fungal material is gently teased apart to ensure even distribution and avoid clumping which is covered with a coverslip, taking care to avoid air bubbles.

Microscopic Examination
Low Power Magnification (10x): Examine the slide under low power to locate areas ofinterest.
High Power Magnification (40x to 100x): Switch to higher magnification for detailedobservation of fungal structures.

Analytical Procedures for Samples Collection
The following procedures were employed to analyze the composition of each sample:
Moisture Content: Determined using the method of SS Nielsen & SS Nielsen (2017).
Organic and Ash Content: Analyzed following the procedure of Matthiessen *et al.,* (2005).
Lipid Content (Fats and Oils): Determined using the method of Negoiță *et al.,* (2018).
Crude Protein: Assessed using the Micro Kjeldahl Method (Yuen & Pollard, 1953), including preparation of the boric acid indicator.
Carbohydrate Content: Analyzed using the A.O.A.C procedure.
Minerals: Determined following the A.O.A.C procedure.

Phytochemical Screening
Alkaloids
In screening for alkaloids, 1ml of each of the fermented locust beans (*Parkia biglobosa* and *Prosopis Africana*) was heated with 5% hydrochloric acid in a steam bath for 10minutes until the acid is mixed with sample. Remove filter and allow to cool for the following test; Drangendroff, Hagers and Mayer. The presence of a yellow precipitate in Hagers indicates a positive result. A brown precipitate in drangendroff indicates a positive result, a cream precipitate in Mayer also indicates a positive result.

Flavonoids
Equal volume of distilled water was boiled with the sample for 5minutes. The oily part was collected from the measuring cylinder and transferred into a test tube. A piece of magnesium metal and 2mL of concentrated sulphuric acid was added to it reaching with the metal to give a pink colour. This observation indicates a pink result. Selivanoff: the sample was treated with selivanoff to form white precipitate.
Tannins
Five (5) drops of ferric chloride is heated with sample to observe the presence of blue-black colour indicating the presence of tannin. Bromine water was also treated with the samples; a decolouration of bromine water at sample layer shows a positive result.

Saponnins
Frothing test, just a preliminary test. The samples were well shaken in a test tube to obtain froth at the upper layer of the sample tube. After two vigorous shakes, the tubes were left to stand for 10- 15 minutes. If the froth persists, it indicates the presence of saponin. Emulsion test; after the observation of froth test, add 5mL of olive oil and shake vigorously. An observation of cloudy precipitation by the side of the test tube shows emulsion.

Steroids
Liebermann-Burchard Test (Finar, 1986): Add 2 mL of extract to a test tube. Add 1 mL of acetic anhydride and 2 mL of concentrated sulfuric acid. Formation of a blue-green ring indicates the presence of steroids.

Phenols
Ferric Chloride Test (Harborne, 1998). Add 2 mL of extract to a test tube. Add a few drops of ferric chloride solution. Formation of a deep blue or black colour indicates the presence of phenols.

RESULTS AND DISCUSSION
Results Interpretation
Results in Table 1 showed the distribution of isolated fungi from samples across different locations, the isolated fungi included *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium oxysporium, Penicillium chrysogenium* and *Saccharomyces cerevisiae*. The sample *Prosopis* Iru from Ipata (PSI), *Prosopis* Iru from Oja Oba (PSO), Parkia Iru from Ipata (PKI), and *Parkia* Iru (Pete) (PKO [PT]) from Oja Oba accounted for the highest presence of fungal species i.e. *A. fumigatus, A.niger, F. oxysporium* and *S. cerevisiae ; A. flavus, A. niger, F. oxysporium* and *S. cerevisiae ; A. flavus, A. fumigatus, A.niger* and *S. cerevisiae ; A. flavus, A. fumigatus, A.niger* and *P. chrysogenium* respectively while Prosopsis from Kulende Market accounted for the lowest presence of fungal species (only *S. cerevisiae* was present).

Table 1: Distribution of Isolated Fungi from Samples

Sample code	*A. flavus*	*A. fumigatus*	*A.niger*	*F. oxysporium*	*P. chrysogenium*	*S. cerevisiae*
PSI	-	+	+	+	-	+
PSO	+	-	+	+	-	+
PS K	-	-	-	-	-	+
PKI	+	+	+	-	-	+
PKO (WR)	-	-	+	-	-	+
PKO (PT)	+	+	+	-	+	-
PKK (PT)	+	+	-	-	-	-
PKK (WR)	-	-	-	-	-	-

KEY: PSI = Prosopis Ipata ; PSO = Prosopis Oja Oba; PS K = Prosopis Kulende ; PKI: Parkia Ipata ; PKO (WR) = Parkia Oja Oba (Woro); PKO (PT) = Parkia Oja Oba (Pete); PKK (PT) = Parkia Kulende (Pete) ; PKK (WR) = Parkia Kulende (Woro) + = Present - = Absent

The data presented in Table 2 illustrate the percentage prevalence of fungal isolates from samples of fermented locust beans. The identified fungi include *A. flavus, A. fumigatus, A.niger, F. oxysporium, P. chrysogenium,* and *S. cerevisiae. Saccharomyces cerevisiae* and *Aspergillus niger* were the fungus with the highest prevalence, accounting for 23.81% of the total isolates (5 occurrences). *Aspergillus flavus* and *Aspergillus fumigatus* showed moderate prevalence, with 19.05% prevalence (4 occurrences) each. The lowest prevalence was observed for *Fusarium oxysporium* and *Penicillium chrysogenum* with 9.52% prevalevace (2 occurrence) and 4.76% prevalence (1 occurrence) respectively

Table 2: Percentage Prevalence of Fungi Isolated from Fermented Beverages Sample

Fungi isolates	No of occurrences	% prevalence
Aspergillus flavus	4.0	19.05
Aspergillus fumigatus	4.0	19.05
Aspergillus niger	5.0	23.81
Fusarium oxysporium	2.0	9.52
Penicillium chrysogenum	1.0	4.76
Saccharomyces cerevisiae	5.0	23.81
Total	21.0	100.0

The results in table 3 revealed the mean total fungal counts isolated from the samples. The mean total count ranged from 1.00 ± 0.05 cfu/ml – 7.00 ± 0.57 cfu/ml with Prosopis from sampling location Ojaoba accounting for the highest total fungal count (7.00 ± 0.57 cfu/ml) while PSO Oba2, PK Ipa2 WR, PK Ipa 1 pk, PK Ipa 2 pk recorded the least total fungal count (1.00 ± 0.05 cfu/ml). There was a significant difference ($p < 0.05$) in the total fungal count across the different samples with the dilution factor ×10-1.

Considering dilution factor $\times 10^{-2}$, there was no significant difference ($p < 0.05$) in the total fungal count across the different samples with the dilution factor $\times 10^{-2}$.

Table 3: Microbial Enumeration Results at 48 Hours

Sample	Total fungal count (CFU/mL) $x10^{-1}$	Total fungal count (CFU/mL) $x10^{-2}$
PSIPa 1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PSIPa2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PSO Oba1	7.00 ± 0.57^{e}	2.00 ± 0.15^{b}
PSO Oba2	1.00 ± 0.05^{b}	0.00 ± 0.00^{a}
PK Ipa1 WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK Ipa2 WR	1.00 ± 0.05^{b}	0.00 ± 0.00^{a}
PKO Oba1 WR	3.00 ± 0.15^{d}	0.00 ± 0.00^{a}
PKO Oba2 WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK KI WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK K2 WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK Ipa WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK Ipa 1 pk	1.00 ± 0.05^{b}	0.00 ± 0.00^{a}
PK Ipa 2 pk	1.00 ± 0.05^{b}	0.00 ± 0.00^{a}
PKO Oba1	2.00 ± 0.12^{c}	0.00 ± 0.00^{a}
PKO Oba2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK K1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK K2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are expressed as mean ± standard error of the mean (SEM) for triplicate reading. Values with the same superscript letter in the same column are not significantly different by one – way ANOVA followed by Duncan test.
Key: PK Ipa = Prosopsis Ipata PSO Oba = Prosopis ojaoba PS. K = Prosopsis Kulende

The results in table 4 revealed the mean total fungal counts isolated from the samples. The mean total count ranged from 1.00±0.07 cfu/ml – 48.00±7.21 cfu/ml with the PSO Oba1 accounting for the highest total fungal count (48.00±7.21 cfu/ml) while PSIPa2 recorded the least total fungal count (1.00±0.07 cfu/ml). There was a significant difference ($p < 0.05$) in the total fungal count across the different samples with the dilution factor $\times 10^{-1}$. Considering dilution factor ×10-2 , the mean total fungal count ranged from 1.00 ± 0.03 cfu/ml – 5.00±0.51 cfu/ml with the PK K2 recording the maximum total fungal count (5.00±0.51 cfu/ml) while PSO Oba2 recorded the minimum (1.00 ± 0.03 cfu/ml) total fungal count. There was also a significant difference ($p < 0.05$) in the total fungal count across the different samples with the dilution factor $\times 10^{-1}$.

Table 4: Microbial Enumeration Results at 72 Hours

Sample	Total fungal count (CFU/mL) $x10^{-1}$	Total fungal count (CFU/mL) x10-2
PSIPa 1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PSIPa2	1.00 ± 0.07^{b}	0.00 ± 0.00^{a}
PSO Oba1	48.00 ± 7.21^{g}	4.00 ± 0.24^{d}
PSO Oba2	3.00 ± 0.27^{d}	1.00 ± 0.03^{b}
PS K	5.00 ± 0.83^{e}	2.00 ± 0.13^{c}
PK Ipa1 WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK Ipa2 WR	1.00 ± 0.07^{b}	0.00 ± 0.00^{a}
PKO Oba1 WR	3.00 ± 0.27^{d}	0.00 ± 0.00^{a}
PKO Oba2 WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
PK KI WR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

PK K2 WR	0.00±0.00[a]	0.00±0.00[a]
PK Ipa WR	3.00±0.27[d]	0.00±0.00[a]
PK Ipa 1 pk	7.00±1.20[f]	2.00±0.13[c]
PK Ipa 2 pk	3.00±0.27[d]	0.00±0.00[a]
PKO Oba1	3.00±0.27[d]	0.00±0.00[a]
PKO Oba2	2.00±0.12[c]	0.00±0.00[a]
PK K1	2.00±0.12[c]	0.00±0.00[a]
PK K2	13.00±2.05[f]	5.00±0.51[e]

Values are expressed as mean ± standard error of the mean (SEM) for triplicate reading. Values with the same superscript letter in the same column are not significantly different by one – way ANOVA followed by Duncan test.
Key: PK Ipa = Prosopsis Ipata PSO Oba = Prosopis ojaoba PS. K = Prosopsis Kulende

The results presented in Table 5 provide a qualitative phytochemical analysis of the fermented beverages samples. The table indicates the qualitative presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenols, with their presence denoted by either marked as either present (+) or moderately present (++), or highly present (+++) indicating higher abundance. Alkaloids and flavonoids were found to be highly present (+++) in AP, B and C, respectively, while Tannin and Saponin were found to be highly present (+++) in AP while moderately present (++) in B and C. Additionally, terpenoids, steroids, and phenols were present (+) in AP, B and C

Table 5: Qualitative Phytochemical Analysis of Samples

Samples	**Phytochemicals**		
	AP	**B**	**C**
Alkaloids	+++	+++	+++
Flavonoids	+++	+++	+++
Tannin	+++	++	++
Saponin	+++	++	++
Terpenoids	++	++	++
Steroids	+	+	+
Phenol	+	+	+

The results in table 6 revealed the quantitative phytochemical analysis of samples. The mean amount of alkaloids ranged from 21.73±3.17 – 26.45±5.02mg/100g with the Iru Pete sample (CP) accounting for the highest amount of alkaloids (26.45±5.02mg/100g) while Prosopis Iru sample (AP) recorded the least amount of alkaloids (21.73±3.17 mg/100g). The mean amount of flavonoids ranged from 22.03 ± 2.58 – 38.19 ± 5.25mg/100g with the Prosopis Iru sample (AP) accounting for the highest amount of flavonoids (38.19 ± 5.25mg/100g) while Iru Woro sample (BW) recorded the least amount of flavonoids (22.03 ± 2.58 mg/100g). The mean amount of tannin ranged from 9.45±2.03 – 17.54±3.57mg/100g with the Prosopis Iru (AP) sample accounting for the highest amount of tannin (17.54±3.57mg/100g) while Iru Woro (BW) sample recorded the least amount of tannin (9.45±2.03 mg/100g). The mean amount of saponin ranged from 14.28±1.73 – 15.87±2.31mg/100g with the Iru Pete sample (CP) accounting for the highest amount of tannin (15.87±2.31mg/100g) while Prosopis Iru (AP) recorded the least amount of saponin (14.28±1.73 mg/100g). The mean amount of terpenoids ranged from 6.91 ± 0.53 – 8.73±1.20mg/100g with the Iru Pete sample (CP) accounting for the highest amount of terpenoids (8.73±1.20mg/100g) while Prosopsis Iru (AP) recorded the least amount of terpenoids (6.91±0.53 mg/100g). The mean amount of steroids ranged from 0.17±0.03 – 0.35±0.10mg/100g with the Prosopsis Iru (AP) accounting for the highest amount of steroids (0.35±0.10mg/100g) while Iru Pete sample (CP) recorded the least amount of steroids (0.17±0.03 mg/100g). The mean amount of phenol ranged from 0.42±0.05 – 0.85±0.10 mg/100g with the Iru Pete sample (CP) accounting for the highest amount of phenol (0.85±0.10mg/100g) while Iru Woro sample (BW) recorded the least amount of phenol (0.42±0.05 mg/100g). There was a significant difference ($p < 0.05$) in the mean amount of all phytochemicals across the different samples.

Table 6: Quantitative Phytochemical Analysis of Samples

Samples	**Phytochemicals**		
	AP	**BW**	**CP**
Alkaloids (mg/100g)	21.73±3.17a	23.77±3.82b	26.45±5.02c
Flavonoids (mg/100g)	38.19±5.25c	22.03±2.58a	26.18±3.21b
Tannin (mg/100g)	17.54±3.57c	9.45±2.03a	9.69±2.11a
Saponin (mg/100g)	14.28±1.73a	14.67±1.85b	15.87±2.31c
Terpenoids (mg/100g)	6.91±0.53a	7.46±0.71a	8.73±1.20b
Steroids (mg/100g)	0.35±0.10c	0.27±0.07b	0.17±0.03a
Phenol (mg/100g)	0.82±0.08b	0.42±0.05a	0.85±0.10b

Values are expressed as mean ± standard error of the mean (SEM) for triplicate reading. Values with the same superscript letter in the same rows are not significantly different by one – way ANOVA followed by Duncan test.

The results in table 7 revealed the proximate composition of samples. The mean percentage of moisture content ranged from 11.625 ± 1.20 – 22.090 ±5.28% with the Iru Pete (CP) sample accounting for the highest amount of percentage moisture content (22.090 ±5.28 %) while Prosopis Iru (AP) recorded the least amount of percentage moisture content (11.625 ± 1.20 %). The mean percentage of ash content ranged from 2.495±0.31 – 3.165±0.85% with the Prosopis Iru (AP) sample accounting for the highest amount of percentage ash content (3.165±0.85 %) while Iru Pete (CP) sample recorded the least amount of percentage ash content (2.495±0.31%). The mean percentage of crude protein ranged from 23.080 ± 4.32 – 26.935±5.38 % with the Iru Pete (CP) accounting for the highest percentage crude protein (26.935 ± 5.38 %) while Iruworo (BW) recorded the least percentage crude protein (23.080±4.32%). The mean percentage of Fat content ranged from 5.915±0.73 – 9.895±2.38 % with the Iru Pete (CP) sample accounting for the highest percentage fat content (9.895±2.38 %) while Proposis Iru (AP) recorded the least percentage fat content (5.915±0.73 %). The mean percentage of crude fibre ranged from 1.590±0.07 – 1.965±0.18 % with the Proposis Iru (AP) accounting for the highest percentage crude fibre (1.965±0.18 %) while Iru Pete (CP) recorded the least percentage crude fibre (1.590±0.07 %). The mean percentage carbohydrate from 36.995±3.82 – 51.655±7.20 % with the Iru Pete (CP) sample accounting for the highest percentage carbohydrate (51.655±7.20%) while Proposis Iru (AP) recorded the least percentage carbohydrate (36.995±3.82%). There was a significant difference ($p < 0.05$) in the mean amount of all phytochemicals across the different samples.

Table 7: Proximate Composition of Samples

Parameters	Samples		
	AP	**BW**	**CP**
Moisture content (%)	11.625± 1.20a	17.290±2.07b	22.090±5.28c
Ash content (%)	3.165±0.85c	2.590±0.72b	2.495±0.31a
Crude protein (%)	25.690±5.20b	23.080±4.32a	26.935±5.38c
Fat content (%)	5.915±0.73a	9.815±2.01b	9.895±2.38c
Crude fibre (%)	1.965±0.18c	1.700±0.10b	1.590±0.07a
Carbohydrate (%)	51.655±7.20c	45.525±5.13b	36.995±3.82a

Values are expressed as mean ± standard error of the mean (SEM) for triplicate reading. Values with the same superscript letter in the same column are not significantly different by one – way ANOVA followed by Duncan test.

Photomicrographs of Identified Fungi Isolates

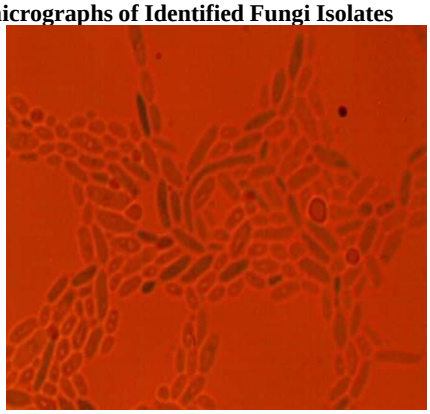

Plate 1: *Saccharomyces cerevisiae*

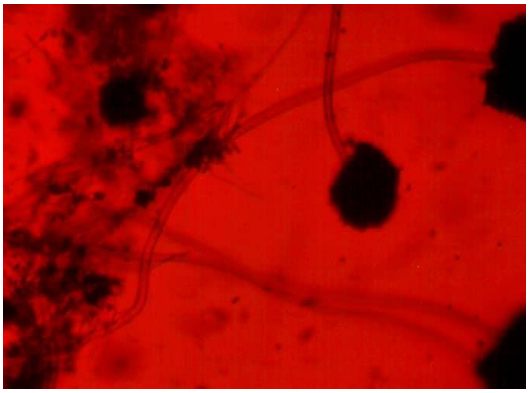

Plate 2: *Aspergillus flavus*

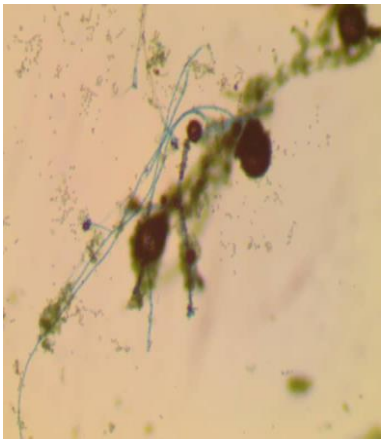

Plate 3: *Aspergillus fumigatus*

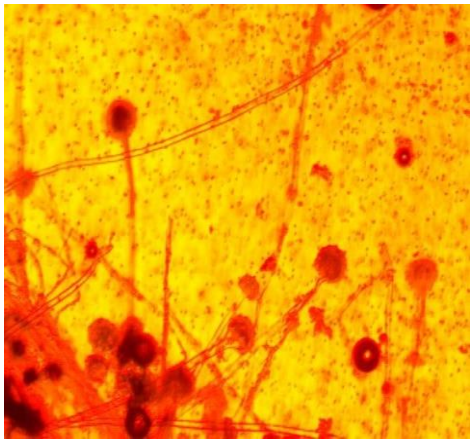

Plate 4: *Aspergillus niger*

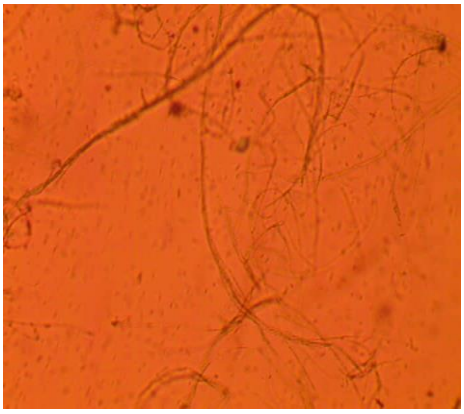

Plate 5: *Penicillium chrysogeneum*

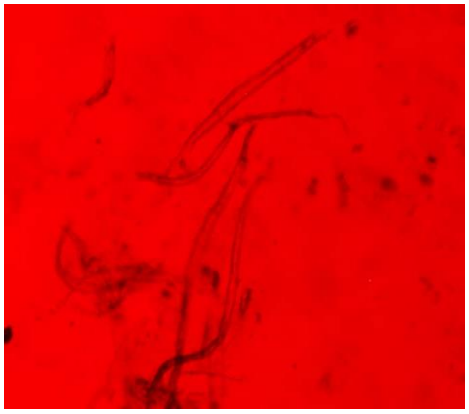

Plate 6: *Fusarium oxysporum*

Discussion

Fungi was isolated from both African mesquite (Okpehe) and African locust beans sold in and around Ilorin markets. Table 1 shows the presence of fungi which could be as a result of contamination or they may be part of fermentation process of the locust beans. Six different fungi were isolated from the samples which include *Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Fusarium oxysporum, Aspergillus fumigatus* and *Saccharomyces cerevisiae* having the highest domination. There was significant difference in fungal population across the sample with Prosopis having the highest fungal count followed by Parkia pete and Parkia woro having the least fungi count.

The prevalence of the fungal species were established in Table 2. The total fungi occurrence was 21 with *Aspergillus niger* and *Saccharomyces cerevisiae* being the most prevalent with (23.81%) each followed by *Aspergillus flavus* and *Aspergillus fumigatus* with (19.05%) each, *Fusarium oxysporum* has a prevalence of (9.52%) and *Penicillium chrysogenum* has the least prevalence of (4.76%).*Aspergillus* species play several important roles in the processing and utilization of locust beams especially in the traditional fermentation practices which include nutritional enhancement through enzymatic activities of Aspergillus that releases essential nutrient from locust beans such as amino acids, vitamins and minerals which is also in consistent with the findings of (Okolo *et al.*, 2023). *Aspergillus* species are also known to produce bioactive compounds with potential health benefits such as antioxidants and other secondary metabolite. *Aspergillus* species produce a variety of enzymes such as protease, amylase and lipase which breaks down protein starch and fats in locust beans of which these enzymatic breakdown is crucial for the fermentation process transforming the beans into softer, more digestible products with enhanced flavor and aroma of the fermented locust beans popularly known as iru or ogiri.

Saccharomyces cerevisiae commonly known as baker's yeast ferments sugar present in locust beans to produce ethanol and carbon dioxide. They also produce B vitamins during fermentation. These vitamins enhance the nutritional profile of the fermented locust beans making them a richer source of essential nutrients.

Penicillium chrysogenum is popularly known for its role in the production of antibiotic. Penicillin can also be involved in the fermentation and processing of locust beans producing enzymes such as protease and lipases which breaks down proteins and fats in locust beans. The enzymatic activities helps in developing complex flavors and aromas of fermented locust beans while certain strains of *Penicillium chrysogenum* can produce mycotoxins such as ocrhatoxin A which are harmful to human healths. The presence of mycotoxins in fermented locust beans can cause serious health risks including kidney damage and immune suppressing. *Penicillium chrysogenum* if not properly controlled can result in off flavor and undesirable texture in fermented locust beans which could reduce the quality and acceptability of the product (Pitt, J. I. and Hocking, A. D. 2009)

*Fusarium oxysporum*is a significant fungal pathogen known to affect various crops. The presence of *Fusarium oxysporum* in fermented locust beans can impact both the safety and quality of the final product thereby necessitating stringent hygiene and storage practices to protect public health. *Fusarium oxysporum* identified in fermented locust beans contributes to mycological contamination which poses public health risk due to the potential production of mycotoxins which can be harmful when consumed which is consistent with the findings of (Okolo *et al.*, 2023). Fermented locust beans effluent also shares bio pesticidal properties effectively causing mortality in insects like bean weevils due to antimicrobial or toxigenic properties of *Fusarium oxysporum* (Olakunle *et al.*, 2018).

Table 3 revealed the mean total of fungal counts isolated from the samples at 48hours with *Prosopis* gotten from Oja-oba having the highest fungal count. These may be as a result of less stringent hygiene practice during production and packaging. Other *Prosopis* samples have moderate fungal population while Parkia pete have fungal growths in the plates inoculated, Parkia woro has least fungal growth or no fungal growths in some of the plates inoculated which could be as a result of inhibitory effects of salt on microbial cells, including fungi which leads to dehydration of the cells and inhibit their growth and reproduction which support the findings of (Madigan *et al.*, 2009). The preservation effect of salt not only lowers fungal counts but also extends the shelf life of locust beans by reducing the water activity in locust beans which is a critical factor for microbial growth. Most fungi requires a certain level of water activity to strive and lowering this parameter can effectively reduce fungi proliferation which also aligns with the finding of (Olakunle *et al.*, 2018).

After 72hours of incubation as shown in Table 4, all the inoculated plates of *Parkia biglobosa* (pete) from the three markets has fungi growth. High moisture content which provides an ideal environment for fungi growth. High moisture content support the metabolic activities of fungi leading to proper proliferation. Factors such as humidity, temperature and hygiene practices also plays a significant roles in fungi contamination. *Parkia biglobosa* (woro) has the least fungi load and *Prosopis Africana* has a moderate microbial load with prospis from Oja-oba having the highest fungi load which may be as a result of poor handling or

environmental contamination during processing and storage condition.

The results presented in Table 5 provides a qualitative analysis of the fermented locust beans sample which indicates the presence of bioactive compounds such as Alkaloids, flavonoids, tannins, saponins, terpenoids, steroids and phenol which have various beneficial effects including antioxidant, antimicrobial and health promoting activities. Fermentation enhances these properties making fermented locust beans a more potential food for health promotion (Oboh *et al.*, 2008).

Table 6 shows the quantitative phytochemical analysis of the fermented products of which *Prosopis Africana* has the highest flavonoids (38.19 ± 5.25mg/100g), tannin (17.54 ± 3.57mg/100mg) and steroids (0.35 ± 0.10mg/100g). *Parkia biglobosa* (pete) having the highest alkaloid content (26.45 ± 5.02mg/100g), saponin (15.87 ± 2.31mg/100g) and phenol (0.85 ± 0.10mg/100g). *Parkia biglobosa* (woro) maintains moderate phytochemical properties.

Table 7 establish the proximate composition of *Prosopis Africana*, *Parkia biglobosa* (woro) and *Parkia biglobosa* (pete). The comparison looks at moisture content, ash content, crude protein, fat content, crude fibre and carbohydrate. With Parkia (pete) having the highest moisture content (22.09 ± 5.28%) while Parkia (woro) exhibit reduced water activity which may be as a result of salting aiding in preservation and Prosopis has the least mean water activity (11.625 ± 1.20%) due to its preservation techniques as compared with (Aremu *et al.*, 2008 and Guissou *et al.*, 2020). Prosopis has least water content compared to *Parkia biglobosa*. *Prosopis Africana* and *Parkia biglobosa* both offers significant nutritional benefits but *Parkia biglobosa* generally has higher moisture content, crude protein and fat content while *Prosopis Africana* has a higher carbohydrate content making it a good source of energy. *Prosopis* also contains higher crude fibre and ash content attributing to different raw materials and fermentation process. The higher level of ash content is an indication of mineral content which contributes to the metabolic process and overall nutritional value of the fermented product.

CONCLUSION

This study revealed significant variation in fungi contaminants between *Prosopis Africana* and *Parkia biglobosa* sold across different markets in Ilorin, Kwara state, Nigeria. The most prevalent fungi specie were *Aspergillus niger* and *Saccharomyces cerevisiae* which has a prevalence of (23.81%) each. *Parkia biglobosa* were observed to have a larger microbial load compared to *Prosopis Africana* except in one of the Prosopis gotten from Oja-oba which has a high fungi load as shown in table 3 and this could be as a result of poor processing or storage condition. These emphasizes the importance implementing hygienic processing and storage practices to minimize fungi growth and mycotoxin production in fermented locust beans.

Both *Prosopis Africana* and *Parkia biglobosa* offers significant nutritional benefits but Parkia generally has higher moisture content, protein and fat content while Prosopis contains higher fibre ash contents and carbohydrate which aids metabolic process, overall nutritional value and energy source respectively.

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