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Microbial Assessment of Locally Compounded Herbals Tea Vended in Selected Markets Within Kaduna Metropolis

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ABSTRACT

The study aims to evaluate microbial assessment of locally compounded herbal teas sold within Kaduna metropolis. A total of twenty samples of herbal teas were collected from various sellers situated at Bakin dogo and Kawo markets. The bacterial and fungal counts were conducted following the standard microbiological. Identification of the bacteria and fungi present in the samples were also performed using biochemical test and fungal stain. The bacterial count was found at a ranged from $9.0x10^4$±6.3 cfu/g to $2.6x10^4$±4.2 cfu/g. Aerobic spore-forming bacteria were recovered from the herbal teas. The fungal count ranged from $7.9x10^3$±9.8 cfu/g to $6.9x10^4$±3.5 cfu/g. The most common fungi and bacteria found in the herbal teas were Aspergillus spp. and Bacillus subtilis which were present in all herbal teas. The study unveiled that locally compounded herbal teas sold within Kaduna metropolis were contaminated with both bacteria and fungi at various levels indicating a potential risk for the regular consumer arising public health issues. Therefore, proper quality control should be constituted for herbal tea safety.

Keywords: herbal tea, *Bacillus subtilis*, Kaduna metropolis, microbial assessment

INTRODUCTION

The herb sample can be considered a whole plant or its part such as root and branches. These parts are traditionally used in Africa to treat various diseases or illnesses. Thus, herbal teas can combine roots, leaves, or seeds. They can be prepared or brewed by infusion in hot water so that the fragrance and the nutritional purposes for which they are ready can be achieved. Herbal teas can either be administered individually or blended with other herbs in the tea (Aigbodion, 2013). However, despite herbal teas' numerous benefits, they are reported to be a source of fungal and bacterial contamination. The unhygienic environment where these teas are prepared is prone to various contaminants that, if consumed, will cause illness to the consumer. Other factors that breed these contaminants are improper storage of herbal teas and improper handling. These conditions accelerate the infections of contaminants and cause illness (Aigbodion, 2013). According to (Adukwu, 2019) , previous studies have pointed out how herbal tea samples were analyzed, and the results contained fungal and bacterial species. This contaminant can result in neurological, cardiovascular, and hematological hazards (Aigbodion, 2013).

The presence of a significant quantity of microorganisms in herbal teas can be traced back to the soil and can attach themselves to different components of the plants, including leaves, stems, flowers, seeds, berries, barks, and roots. In addition, it is important to consider the potential for contamination when handling and preparing medicinal plant materials for herbal tea. This risk arises from the current practices employed by various vendors, which typically involve only basic washing with water and lack thorough

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decontamination measures according to Ferdinand *et.al.,* (2017).

Similarly, many herbal tea vendors located in the metropolitan part of Kaduna prepare tea locally for consumers. There is a problem associated with improper processing by herbal tea vendors and this causes various illnesses due to improper processing of the herbal tea is alarming. This problem is serious in Kaduna metropolitan and needs immediate intervention. It is worth noting that locally compounded herbal tea must be free of pathogenic microorganisms and safe for drinking. The presence of contaminants does not add to the medicinal value of the locally compounded herbal teas.

Therefore, the study focused on assessing the microbial contaminants in local herbal teas vended within the metropolitan of Kaduna state.

MATERIALS AND METHODS

Sampling Area

The study was carried out within Kaduna metropolis; which is located in the Northwest latitude and longitude coordinates. Samples of locally compounded herbal teas were collected from Bakin Dogo and Kawo markets, all within the Kaduna Metropolis.

Samples Collection

A total of twenty (20) samples of locally compounded herbal teas were purchased from the two (2) selected markets, ten samples each. A sterile and labeled polythene bag was used for the collection of the samples and transported to the laboratory for mycological and bacterial analyses.

Serial Dilution of the Sample

A quantity of 5 grams of each powdered sample was introduced into 45mL of Peptone water, and subsequently subjected to soaking with intermittent agitation using a sterile glass rod. A serial dilution was performed by transferring 1mL of the homogenized sample into a test tube containing 9mL of peptone water, resulting in a dilution factor of 10^{-1}. A volume of 1mL was extracted from the initial tube and subsequently transferred into the second tube, where it was thoroughly mixed to achieve a dilution factor of 10^{-2}. This procedure was iteratively performed until a dilution factor of 10^{-5} was attained (Apha, 2015).

Isolation and Identification of Bacterial and Fungal Contaminants

From the appropriate dilution, 0.1mL was plated onto the different media using the pour plate Method. Nutrient agar and MacConkey agar were used for bacterial enumeration and identification while sabourad dextrose agar (SDA) was used for fungi. Each inoculated sample was in duplicate, the plates were incubated for 24hrs to 48hrs at 37^{o}C for bacterial growth. While 3-7days at room temperature for fungi growth. Therefore, the plates were examined for growth, and the colonies were counted using a colony counter and recorded in cfu/g. The morphological and microscopic features of the colonies were also recorded.

Identification and Biochemical Characterization of Bacterial Isolates

Gram staining and microscopy

This procedure was conducted for every individual isolate. A minute layer of the bacteria was meticulously applied onto a pristine slide devoid of any grease. The smear was subsequently allowed to dry naturally and then subjected to heat fixation. The specimen was immersed in a crystal violet solution for one minute. Subsequently, the stain was subjected to decolorization using a solution of 95% alcohol for 30 seconds, followed by thorough rinsing with water. Subsequently, the specimen underwent counter-staining

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using safranin. The counterstain was incubated for 30 seconds being rinsed and subsequently left to dry naturally. Subsequently, the stained slides were subjected to microscopic examination utilizing oil immersion objectives with a magnification factor of 100x. An examination and documentation of the Gram's reaction, cellular shape, and cellular arrangement was conducted as described by Muhammad *et al.,* (2020).

Biochemical test for bacterial identification

Following the gram reactions of the bacterial isolates, biochemical test, which include catalase, coagulase, Citrate test and Oxidase Tests were carried out to further identify the isolates as described and modified by Muhammad *et al.,* (2020).

Catalase test

Also known as the hydrogen peroxide test, is a laboratory procedure used to determine the presence of the enzyme catalase.

A bacterial culture exhibiting a high density of cells was deposited onto a glass slide, followed by the addition of a droplet of hydrogen peroxide. The researchers to observe the presence of bubbles in the vicinity of the colony. The emergence of bubbles, which were generated as a consequence of oxygen gas production, served as an indicator of a positive catalase reaction. According to Muhammad *et al.,* (2020), the absence of bubbles is indicative of a detrimental impact.

Coagulase test

The coagulase test is a laboratory technique used to determine the presence of coagulase enzyme in a bacterial sample.

Two drops of physiological saline, spaced approximately 2cm apart, were individually dispensed onto a designated area of a pristine glass slide. Two thick suspensions were prepared by emulsifying a colony of the bacterial isolate in each drop. Subsequently, a loopful of citrated human plasma was added to one of the suspensions, which was then gently rocked for 10 seconds. The aggregation of the organism after agitation demonstrated an affirmative outcome. The absence of agglutination in the second sample indicated a negative outcome, as reported by Muhammad *et al.,* (2020).

Citrate test

The isolates were inoculated onto Simmon citrate slants in agar bijou bottles and incubated for 24 hours at a temperature of 37°C. According to Muhammad *et al*. (2020), a positive outcome was indicated by the emergence of a deep blue hue, while a lack of color change signified a negative outcome. The present test is predicated upon the capacity of an organism to utilize citrate as its sole carbon source, and serves as a means of distinguishing between various enterobacteria strains.

Oxidase Test

A small piece of filter paper was immersed in a solution containing 1% Kovacs oxidase reagent and subsequently subjected to a drying process. An aseptic technique was employed to select a thoroughly isolated colony from a recently cultured bacterial plate (18-24 hours) using a sterile loop, which was subsequently transferred onto the treated filter paper. According to Muhammad *et al.,* (2020), the presence of a dark purple color within a time frame of 5 to 10 seconds is indicative of a positive oxidase result. Conversely, an oxidase negative result either exhibits no change in color or requires more than 2 minutes for any observable color change to occur.

The Indole Test, also known as the Kovacs' reagent test, is a biochemical assay used in microbiology to determine the ability

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The organism's culture was introduced into a medium of peptone broth. The incubation process was conducted at a temperature of 37°C for 24 hours. Subsequently, half of the Kovacs reagent was introduced into the broth culture. A positive outcome was indicated by the presence of a pink or red-violet hue on the surface, whereas a negative result was characterized by a yellow color or no observable alteration (Muhammad *et al.,* 2020).

Identification of Fungal Isolates

From the primary plate, a distinct colony was subcultured onto a freshly prepared SDA plate and incubated for 3-5 days at room temperature obtained pure isolate.

The pure isolates obtained were further inoculated onto the freshly prepared SDA slant and kept at 4°c until required.

Staining of fungi

The aforementioned procedure was conducted for each fungal isolate kept in the slant. A microscopic slide devoid of oil or grease was prepared by applying a single droplet of Lactophenol Cotton Blue stain. The fungal specimen was then introduced onto the droplet of Lactophenol Cotton Blue using sterile inoculating needles. Utilizing two sterile dissecting needles, the fungus was carefully separated to ensure a thin dispersion within the Lactophenol solution. A pristine cover slip edge was carefully positioned onto the mixer containing Lactophenol and the fungal specimen, and it was gradually lowered (with caution to prevent the entrapment of air bubbles beneath the cover slip). Subsequently, the periphery of the coverslip was secured by employing either nail polish or paramount Astrid (2019). The slide was viewed under 10x and 40x magnification for fungal identification based on microscopic appearance using an atlas as a guiode.

RESULTS

The results of the bacterial load count revealed high count in the samples collected from Bakin Dogo market with ($9.0x10^4$±6.3) compared to kawo market that accounted the least count of ($6.2x10^3$±11.3). Similarly, the fungal load count of the samples indicated high fungal load from from Bakin Dogo market ($7.9x10^3 \pm 9.8$), while Kawo market was observed with ($6.9x10^4 \pm 3.5$), which is lower than that of Bakin-dogo as presented in Table 1.

Table 1: Mean values of bacterial and fungal loads of locally compounded herbal tea

Location	Bacteria	Fungi
	Mean count (cfu/g) ±SD	
Bakin dogo	$9.0x10^4$±6.3	$7.9x10^3$±9.8
Kawo	$6.2x10^3$±11.3	$6.9x10^4$±3.5

The result revealed that all of the isolates exhibited positive result of the catalase test, indicating the presence of the catalase enzyme. And tested negative for coagulase and oxidase tests, suggesting the absence of coagulase and oxidase enzymes, respectively. Additionally, the isolates showed positive results for the citrate test, indicating their ability to utilize citrate as a carbon source. Lastly, they tested negative for indole test, indicating the absence of indole production.

Therefore, *Bacillus subtilis* and *Bacillus cereus* were confirmed with percentage occurrence of 60% and 40% respectively as shown in the Table 2.

Table 2: Percentage occurrence of bacteria isolated from locally compounded herbal teas

Bacterial Isolate	Percentage occurrence (%)
Bacillus subtilis	60
Bacillus cereus	30

Table 3, shows the distributions and percentage occurrence of the fungal isolates.

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Five different fungal species were identified and confirmed these include *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Mucor mucedo* and *Saccharomyces* sp. *Aspergillus flavus was observed to be the most* common contaminating fungal sp. in the sample with 40% occurrence. On the other hand, *Aspergillus niger* and *Mucor mucedo* had the lowest occurrence of 10% each.

Table 3: Percentage Occurrence of Fungi Isolated from Locally Compounded Herbal Teas

Fungi Isolate	Percentage occurrence (%)
Aspergillus flavus	40
Aspergillus fumigatus	20
Aspergillus niger	10
Mucor mucedo	10
Saccharomyces sp.	20

DISCUSSION

The study highlighted that locally prepared herbal teas has the potential to transmit harmful microorganisms due to the presence of various microbial contaminants. The high bacterial and fungal load count observed from Bakin-dogo market could be attributed to the open market system, unhygienic packaging and storage system.

Additionally, the presence of *Bacillus subtilis* and *Bacillus cereus* in the samples could be due to the ability of these bacteria to produce spores that exhibit resistance to rigorous processing methods, high temperatures, and dry environments. Consequently, these bacteria can endure for an extended duration within the product while remaining in a state of dormancy. This finding is consistent with the findings reported by Martin (2014), wherein *Bacillus cereus* and *Clostridium perfringens* were isolated from chamomile and other herbs. Additionally, a portion of the bacterial bio burden may be attributed to the individuals responsible for handling the tea materials subsequent to processing, particularly in cases where adherence to rigorous Good Manufacturing Practice (GMP) standards and hygienic conditions was lacking.

Further, the fungal species identified from the herbal teas in this research might be as a result of improper storage facilities that may enhance the proliferation of these mould, particularly the *Aspergillus flavus*. Therefore, *Aspergillus flavus* occurred most frequently than others. The findings of this study provide support for the existence of these fungi in herbal teas, as previously documented by Lee (2016). The authors observed that *Aspergillus niger, A. flavus, Rhizopus sp.,* and *Alternaria* sp. are frequently encountered airborne contaminants that are likely to be found in areas where drying and packaging activities take place. To this note, the presence of these mould has a link with an increase in moisture content of the either the sample or the storage facility, which influence the proliferation of moulds that could lead to product spoilage and potentially the production of mycotoxins (Zhang *et al.,* 2015). The primary focus of this study is the isolation of different strains of *Aspergilli,* with particular emphasis on *Aspergillus flavus.*

Previous study indicated that the presence of these moulds species is of significant concern due to its ability to produce aflatoxin and thrive in environments with low water activities (Riba *et al.,* 2018). Especially, A. flavus detected in the herbal tea it has the ability to generate aflatoxin, a potent toxin associated with a range of health complications including liver cancer, as well as disorders affecting the digestive, urinary, and reproductive systems according to Ancott *et al.,* (2017).

CONCLUSION

The microbial assessment of herbal teas commercialized revealed the presence of

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microbial contaminants in herbal teas. One significant finding was the presence of *Aspergillus species* and *Bacillus* spp,. The samples obtained from Bakin Dogo market has more contaminants than the samples obtained from Kawo market. The percentage occurrence of the bacterial isolates, *Bacillus subtilis* was found to be the most dominant, accounting for 60% of the total isolates on the other hand the percentage occurrence of the fungi isolated from the samples, and the most dominant species was *Aspergillus flavus*, accounting for 40% of the total fungi identified.

Therefore, the local manufacturers should maintain clean and sanitized equipment, utensils, and storage areas to minimize the risk of microbial contamination. The need to encourage checking for proper packaging, labels and expiration dates when purchasing herbal teas. Because the presence of microbial contamination can result in not only the deterioration of the product but also a reduction or complete loss of its effectiveness and pose serious threat to public health.

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