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Assessment of Microbial Contamination of Solid Herbal Medicine sold in Makurdi Metropolis

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Abstract

The consumption of herbal medicines is a common practice in many parts of the world, including Nigeria. However, the safety and quality of these products have been a concern due to the potential contamination with pathogenic microorganisms. This study aimed to isolate and identify microorganisms from powdered herbal medicines sold in Makurdi and Adikpo towns in Nigeria. A total of 30 samples were collected from various herbal medicine vendors and subjected to microbiological analysis. The samples were processed using standard microbiological techniques, including culture, microscopy, mycological identification and biochemical identification. The results showed that samples from both towns were contaminated with microorganisms, including bacteria and fungi. Bacteria species isolated include Salmonella spp., Escherichia coli, Klebsiella spp., Proteus spp. and Staphylococcus spp. the most occurring bacterium was Escherichia coli with the occurrence of 19 representing 25 %, while the most common fungal species include Aspergillus niger which had the occurrence of 143(29.61 %) respectively. This was followed by Trichosporon mucoides 116(24.02 %), Aspergillus ochraceus 92(19.05 %), Aspergillus flavus 70(14.49 %) and Rhizopus stolonifer 16(12.84 %) which had the least occurrence. The study also revealed that the contamination levels were higher in samples from Adikpo town compared to Makurdi town. These findings suggest that there is a need for improved quality control measures in the production and sale of herbal medicines to minimize the risk of microbial contamination and ensure consumer safety.

Keywords: Herbal medicines; Contamination; Microorganisms; Quality control measures; Consumer safety

1. Introduction

Plants have been used for medicinal purposes for thousands of years in almost every culture. Complementary and alternative therapies such as Ayuverdic and Unani medicine in Indian and Chinese traditional medicine are embraced by some cultures as a central part of their medical system. The use of herbal medicines is generally on the increase worldwide. In African countries, up to 80% of the populace rely on herbal medicine as a major source of therapy, with evidence of widespread use of herbal medicines [5]. Also, in developed countries there is increasing evidence for the use of herbal medicines and phytomedicines [5]. Reasons for the rapid growth in the use of herbal medicines include a growing fascination with alternative medicines, desire for self-care, safety concerns over the potential toxicity of

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allopathic medicines, a long history of the safe use of herbs across cultures, unequal distribution of medical facilities, failure of allopathic medicines to address most health concerns of the citizens and the escalating costs of allopathic medicines [5,7].

Hygienic safety of herbal preparations is a major issue in the developing world and there are concerns that such preparations may be produced under poor hygienic conditions and pose risk to consumers. The major sources of microbial contamination of these herbal preparations are from raw materials used for the preparation, handling and processing, their storage and transportation. Some of the common microbial pathogens found in herbal preparations *are Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus spp, Enterobacter spp, Klebsiella pneumoniae and Proteus mirabilis* [6].

As the case with other herbal drugs, raw material and powdered ingredients are subject to operations of contamination by microorganisms during growth (while the fruits are on tree), after harvesting (when fruits are dried), processing and during storage. Post-harvest spoilage by filamentous fungi is one of the most common threats associated with processed and stored herbal products [1]. Fungal contamination of stored herbal drugs not only linked to discoloration, quality deterioration, reduction in commercial values as well as in therapeutic potential but the mycotoxin produced by them in these herbal drugs can also cause several ailments of liver, kidney, nervous system, muscular, skin, respiratory organs, digestive tract, genital organs [2].Herbal medicine is effective against different arrays of microbes but most of these drugs are not prepared under aseptic conditions giving rooms for microbial contamination. This study will evaluate microbial contamination of powered herbal medicines.

1.1. Statement of the Problem

Herbal medicines are widely used in Nigeria and other parts of the world for the treatment of various diseases and health conditions. However, there are concerns about the safety and quality of these products, particularly with regard to microbial contamination. Many herbal medicines are sold in powdered form, which can be easily contaminated by microorganisms during processing, packaging, and storage. The presence of pathogenic microorganisms such as bacteria, fungi, and yeasts in powdered herbal medicines can pose a serious health risk to consumers, especially those with weakened immune systems.

Despite the potential risks associated with microbial contamination of herbal medicines, there is limited information available on the types and prevalence of microorganisms present in these products. Therefore, there is a need to conduct a study to isolate and identify the microorganisms present in powdered herbal medicines sold in Makurdi and Adikpo town.

1.2. Justification of the Study

The findings of this study will be useful to various stakeholders, including regulatory authorities, herbal medicine vendors, and consumers. Regulatory authorities can use the results to develop and enforce microbiological standards for the production, processing, and packaging of herbal medicines. Herbal medicine vendors can use the information to improve their production processes and ensure the safety and quality of their products. Consumers can use the information to make informed decisions about the use of herbal medicines and to protect their health.

Aim of the Study

- To determine microbial contamination of powdered herbal medicine in Nigeria

Objectives of the Study

- To isolate and identify microorganisms responsible for powered herbal medicines contamination.
- To evaluate microbial contamination and suggest possible solutions to powdered herbal medicines contamination.

2. Materials and methods

2.1. Study area

Makurdi town is located in Makurdi local government area of Benue state, Nigeria. Makurdi is the state capital. Makurdi town is located between latitude 6^0 25′N and 8^0 8′N of the equator and longitude 7^0 45′E and 10^0 0′E of the Greenwich Meridian. It shares boundaries with Guma Local Government North East, Gwer to the South, Gwer-West to the West and

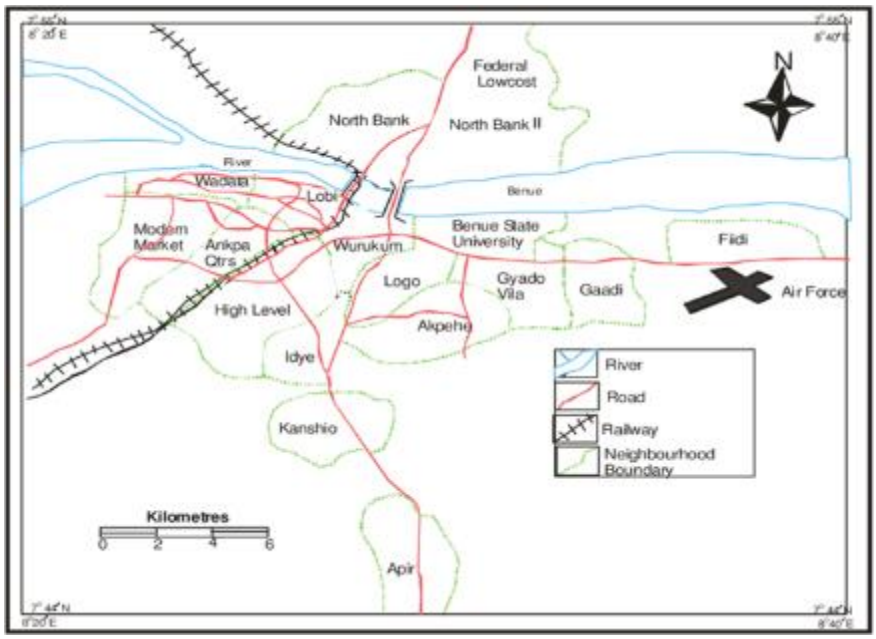
Doma Local Government Area of Nasarawa State to the North-West. The town is divided into two major blocks by River Benue, hence, the North and South banks. It has a population of 300,337 with a landmass of 16Km radius. Makurdi falls within the tropical humid and mega thermal climate. The climate is characterized by wet and dry seasons. The wet season lasts from April to October while the dry season starts in November and ends in March, this variation in season has effect on vegetation.

Makurdi is located almost entirely in the Benue plains. Sandstones of sedimentary formation generally underline the area. The sandstone is divided into micaceous and feldspathic sandstone, Makurdi town itself is situated on the feldspathic sandstone. This feldspathic sandstone is seen near the southern head of old River Benue bridge. Much of the feldspathic sandstone are also ironstone and calcareous materials. The sandstone is overlain by shale unit in other places especially the low-laying areas of Wadata. The soil here ranges from fine sand to the river site silt and clay.

The indigenes of Makurdi are the Tiv and Jukun people but Makurdi being the State capital has become a cosmopolitan city with people of different tribes as Idoma, Igede, Agatu, Etulo, Igbo,

Hausa and Igala. The major occupation of the indigens are farming and fishing, various occupations and businesses also run in the city. The major mechanic workshops are located at Wadata, North-bank and Apir areas of Makurdi. Several hospitals are situated in different streets of Makurdi [2].

Adikpo, also called “London”, as known for the friendly weather with a high-level of urbanisation. A journey through Kwande links you through to, Cameroun by foot, Cross-River, Akwa-Ibom States, Ushongo town, Ugbema town, Katsina-Ala, Zaki-Biam to Taraba and Adamawa States. The Spiritual and cultural heritage is traced to Jato-Aka town, named after the legendary Tor Jato Aka who was appointed by the colonial masters and custodian of the “Akombo”, the Tiv deity.



Source: Ministry of Lands and survey, Makurdi, 2015

Figure 1 Map of Makurdi

2.2. Collection of samples

Different herbal mixtures were purchased from different markets in Makurdi and Adikpo metropolis. The samples were stored in a refrigerator at 4^{o}C before the analysis. They was analyzed in the Benue State microbiology laboratory, Makurdi, Benue State.

2.3. Preparation of media

Media that was used for this research work include nutrient agar, blood agar, macconkey agar, and Sabouraud dextrose agar, they were used for the enumeration of bacteria and fungi, respectively. All media used were prepared according to the manufacturer’s instructions.

2.4. Bacteriological analysis

Total viable count: the powdered herbal medicines were mixed with distill water and fivefold serial dilution of the samples were carried out. Aliquots of 1 mL of the sample was pipetted from the 10^{-4} dilution into well labelled petri dishes and labelled appropriately. Then 20 mL of molten nutrient agar was added into each plate and swirled gently to allow for proper mixing. The plates were incubated for 24hrs at 37^0C. Then the colonies which developed on the plates were counted using a colony counter and expressed as colony forming unit per millilitre (cfu/mL). The samples from each hawker were analyzed in triplicates and the average will be recorded. The colonies differing in size, shape and colour will be selected from the different plates on nutrient agar and sub-cultured repeatedly to obtain pure isolates. The pure isolates were maintained on agar slant for further characterization and identification.

2.5. Mycological analysis

The fungal count was determined by pipetting 1 mL of the serially diluted herbal infusion on Sabouraud Dextrose Agar (SDA) containing 0.01% chloramphenicol. The plates were incubated for 3 days at ambient temperature.

2.6. Characterization and identification of pure isolates

2.6.1. Biochemical tests for bacterial isolates

Gram's stain

A drop of distilled water was placed on a clean grease free slide. Few colonies of the bacteria were picked and placed in the middle of the slide, emulsified and allowed to air dry. Sample was fixed by passing the slide over flame for 3 seconds. Slide was placed on a staining rack, covered with crystal violet for 60 seconds, and washed with clean water. The smear was covered with Lugo's iodine for 60 seconds and washed with water. The smear was further decolorized rapidly with ethanol and washed with water immediately. It was then covered with safranin stain for 30 seconds and finally washed with water; air dried and observed with microscope (Olympus Model: CX21FS1, Tokyo, Japan) under 100 x objectives using immersion oil. Gram positive bacteria were dark purple while gram negative bacteria were pale to dark red. Typical colonies were observed for color, shape and elevation (Hashen and Abed 2007; Dara, 2000).

Coagulase test

A drop of distilled water was placed on two side of the slides. Colonies of the test organism was then emulsified in each of the drops to make two thick suspensions. A loop full of plasma was then added to one of the suspensions, and mixed gently. Clumping of the organisms within 10 secs was checked. No plasma was added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping. Coagulase test is used to identify *Staphylococcus aureus*, which produces the enzyme coagulase (Mehra and juneja, 2004).

Catalase test

An aliquot of the organism was taken and immersed in 3 ml of hydrogen peroxide solution (SERVICE PHARMACEUTIAL CO. LTD.: HP153, Edo, Benin Republic) in a test tube. It was observed for evolution of immediate bubbles. Active bubbling indicates positive Catalase while no bubbles indicate Negative catalase test (Mehra and juneja, 2004).

Indole test

The test organisms were inoculated in a bijou bottle containing 3ml of sterile peptone water and incubated at 37 °C for 24 hours. Test for indole was afterwards carried out by adding 0.5ml of Kovac's reagent (FERNTEC: 4132845) and shook gently. A red surface layer indicates a positive indole test while no red surface layer indicates a negative result. Indole test is used to test for the presence of *Escherichia coli* (Mehra and Juneja, 2004).

Oxidase test

A piece of filter paper was placed in a clean petri dish and 2 drops of freshly prepared oxidase reagent (TITAN BIOTECH LTD.: C0C0CV01, Rajasthan, India) was added. A sterile stick applicator was used to remove some aliquot of the test organism to prepare a smear on the filter paper. It was then observed for the development of a blue - purple color within few seconds to detect the presence of *Pseudomonas, Neisseria,* and *Vibrio* which produce the enzyme cytochrome oxidase (Mehra and juneja, 2004).

Simmons citrate agar test

Slopes of the Simmons Citrate Agar (TITAN BIOTECH LTD., Rajasthan, India) were prepared in bijou bottles as recommended by the manufacturer (store at 2- 8°C). A sterile straight wire was first streaked on the slope with a saline suspension of the test organism and the butt was stabbed and incubated at 35°C for 48 hours. It was then observed for bright blue color in the medium for the identification of *Enterobacteria sp*. Absence of blue color indicates a negative test result (Andy and Okpo, 2021).

Triple sugar iron (tsi) test

A straight inoculation needle was used to pick a well-isolated colony and inoculated into the Triple Sugar Iron Agar (L: S- BIOTECH: MAW-07, San Diego, USA) by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant.

Incubate the tube at 35°-37°C in ambient air for 18 to 24 hours. Examine the reaction of medium.

A, Acid slant/acid butt with gas, no H2S (A/A). B, Alkaline slant/acid butt, no gas, H2S-positive (K/A H2S+). C, Alkaline slant/alkaline butt, no gas, no H2S (K/K). D, Uninoculated tube (Andy and Okpo, 2021).

Fungal isolation

The mold isolates were characterized based on the color of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spores, presence of foot cell, sporangiophore, conidiophores, and the characteristics of the spore head. A small portion of the mycelia growth will be carefully picked with the aid of a sterile inoculating needle and placed in a drop of lactophenol cotton blue on a microscopic slide and covered with a cover slip. The slide will be examined under the microscope, first with (x10) and then with (x40) objective lens to detect the spores and some special structures of the fungi. The isolates will be identified by comparing their characteristics with those of known taxa using mycology atlas.

2.7. Statistical analysis

Data obtained Will be analyzed using mean ± standard deviation while analysis of chi square (χ^2) will be used to analyze the extent of variation between groups and P values of less than 0.5 will be considered significant while P values greater than 0.5 will be considered non-significant, Fisher's least significant difference (FLSD) will be used to compare several groups.

3. Results

In this study, microbial contaminants of powered herbal medicines sold in selected markets in Makurdi and Adikpo, Benue State were investigated.

Figure 1 shows the bacteria isolated from powdered herbal medicines in Makurdi and Adikpo, Benue State. A total of 5 bacterial isolates were observed which include: *Salmonella* spp., *Escherichia coli, Klebsiella* spp., *Proteus* spp. and *Staphylococcus* spp. the most occurring bacterium was *Escherichia coli* with the occurrence of 19 representing 25 %. This was followed by *Staphylococcus* spp 16(21.05 %), *Proteus* spp and *Staphylococcus* spp with the occurrence of 15(19.74 %) each and *Klebsiella* spp 11(14.47 %) which was the least.

Table 1 shows the distribution of bacterial isolates in selected markets in Makurdi. Wadata market was observed to have the highest occurrence of bacteria with the total of 14(36.84 %). Wurukum and North Bank had equal occurrence of 12(31.58 %) each. There was no significant association between occurrence and location in Makurdi ($X^2 = 0.080$; df = 2; p = 0.781).

Table 2 shows the distribution of bacterial isolates in selected markets in Adikpo, Benue State. The result showed that Jako-Aka market and Mbaniege market had the highest bacterial occurrence of 13(34.21 %) each; while Adikpo market had 12(31.58 %). No significant occurrence in relations to the location was observed in Adikpo. ($X^2 = 0.080$; df = 2; p = 961).

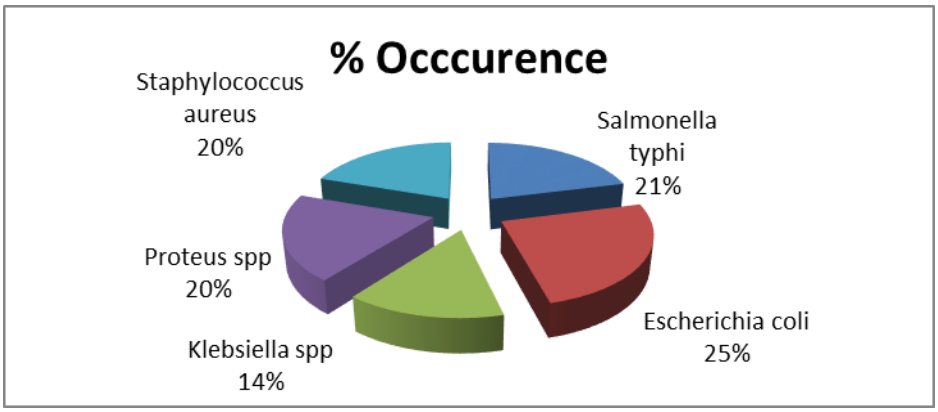
Table 3 shows the comparative bacterial occurrence in powdered medicines in Makurdi and Adikpo, Benue State. Both areas were observed to have equal bacterial occurrence of 38(50 %) each. No significant association between bacterial occurrence and the sampled towns was observed ($X^2 = 0.000$; df = 1; p1.000).

Figure 2 shows the fungi isolated from powdered herbal medicines in Makurdi and Adikpo, Benue State. A total of 5 fungal isolates were observed. They include: *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Rhizopus stolonifer* and *Trichosporon mucoides.* The highest occurring fungus was the *Aspergillus niger* which had the occurrence of 143(29.61 %) respectively. This was followed by *Trichosporon mucoides* 116(24.02 %), *Aspergillus ochraceus* 92(19.05 %), *Aspergillus flavus* 70(14.49 %) and *Rhizopus stolonifer* 16(12.84 %) which had the least occurrence.

Table 4 shows the distribution of fungal isolates in selected markets in Makurdi. Wurukum market had the highest occurrence of 80 representing 35.24 %, North Bank had 75(33.04 %) and Wadata 72(31.72 %). There was no significant relationship between fungal occurrence and location in Makurdi ($X^2 = 0.140$; df = 2; p = 0.932).

Table 5 shows the distribution of fungal isolates from powdered medicines in Adikpo. Mbaniege market was observed to have the highest fungal occurrence of 95(35.69 %) followed by Jato-Aka market 91(35.69 %) and the least which was observed in Adikpo market 69(27.06 %). No significant association between fungal occurrence and location was observed ($X^2 = 1.820$; df = 2; p = 0.403).

Table 6 shows the comparative fungal occurrence in powdered medicines in Makurdi and Adikpo, Benue State. Adikpo was observed to have slightly higher fungal occurrence 255(52.90 %) than Makurdi 227(47.10 %). No significant association was observed ($X^2 = 0.360$; df = 1; p = 0.549)

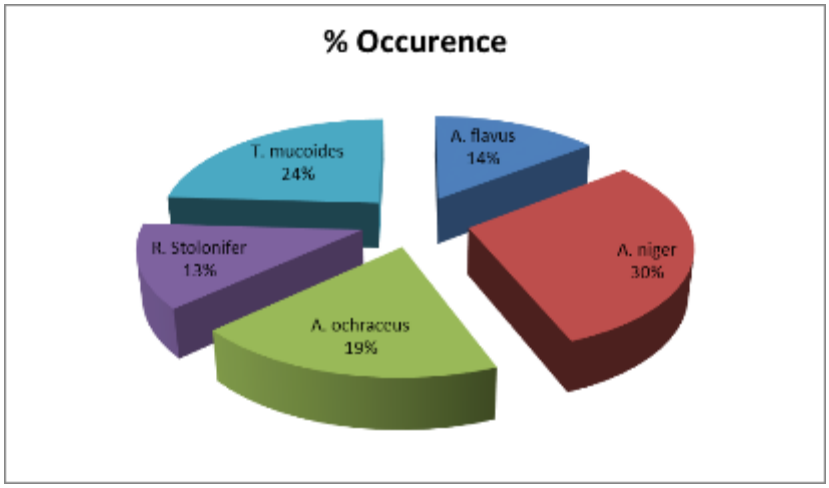


$\chi^2 = 3.100$; df = 4; P = 0.541

Figure 1 Bacteria isolated from powdered herbal medicine in Makurdi and Adikpo, Benue State

Table 1 Distribution of bacterial isolates from powdered medicines in selected markets in Makurdi.

Bacteria Isolates	Wadata	Occurrence in Markets Wurukum	North Bank	Total
Salmonella spp	4	3	2	9
Escherichia coli	1	4	4	9
Klebsiella spp	3	2	2	7
Proteus spp	3	0	2	5
Staphylococcus spp	3	3	2	8
TOTAL (%)	14(36.84)	12(31.58)	12(31.58)	38(100)



χ^2= 10.100; df = 4; P = 0.039

Figure 2 Fungi isolated from powdered herbal medicines in Makurdi and Adikpo, Benue State.

Table 2 Distribution of bacterial isolates from powdered medicines in selected markets in Adikpo.

Bacterial Isolates	Adikpo Market	Jato-Aka Market	Mbaniege Market	Total
Salmonella spp	3	1	3	7
Escherichia coli	3	3	4	10
Klebsiella spp	2	1	1	4
Proteus spp	3	4	3	10
Staphylococcus spp	1	3	2	7
Total	12(31.58)	13(34.21)	13(34.21)	38(100)

X^2 = 0.080; df = 2; p = 0.961

Table 3 Comparative bacteria occurrence in powdered medicines in Makurdi and Adikpo, Benue State.

Bacterial isolate	Makurdi	Adikpo	Total
***Salmonella* spp**	9	7	16
Escherichia coli	9	10	19
***Klebsiella* spp**	7	4	11
***Proteus* spp**	5	10	15
Staphylococcus spp	8	7	15
Total	38(50.00)	38(50.00)	76(100)

X^2 = 0.000; df = 1; p = 1.000

Table 4 Distribution of fungal isolates from powdered medicines in selected markets in Makurdi

Fungal Isolates	Wadata	Occurrence in Markets Wurukum	North Bank	Total
Aspergillus flavus	10	0	16	26
Aspergillus niger	18	28	14	60
Aspergillus ochraceus	17	14	19	50
Rhizopus stolonifer	11	13	5	29
Trichosporon mucoides	16	25	21	62
Total (%)	72(31.72)	80(35.24)	75(33.04)	227

Table 5 Distribution of fungal isolates from powdered medicines in selected markets in Adikpo.

Fungal Isolates	Adikpo Market	Jato-Aka Market	Mbaniege Market	Total
Aspergillus flavus	14	6	24	44
Aspergillus niger	28	35	20	83
Aspergillus ochraceus	8	13	21	42
Rhizopus stolonifer	5	15	12	32
Trichosporon mucoides	14	22	18	54
Total (%)	69(27.06)	91(35.69)	95(37.25)	255

Table 6 Comparative fungal occurrence in Makurdi and Adikpo, Benue State.

Fungal isolates	Makurdi	Adikpo	Total
Aspergillus flavus	26	44	70
Aspergillus niger	60	83	143
Aspergillus ochraceus	50	42	92
Rhizopus stolonifer	29	32	61
Trichosporon mucoides	62	54	116
Total (%)	227(47.10)	255(52.90)	482(100)

$X^2 = 0.360$; df = 1; p = 0.549

4. Discussion

The bacteria isolated from powdered herbal medicines in Makurdi and Adikpo, Benue State comprises of a total of 5 bacterial isolates were observed which include: *Salmonella* spp., *Escherichia coli, Klebsiella* spp., *Proteus* spp. and *Staphylococcus* spp and 5 fungal isolates which include *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Rhizopus stolonifer* and *Trichosporon mucoides.* The highest occurring fungus was the *Aspergillus niger* which had the occurrence of 143(29.61 %) respectively. This was followed by *Trichosporon mucoides* 116(24.02 %), *Aspergillus ochraceus* 92(19.05 %), *Aspergillus flavus* 70(14.49 %) and *Rhizopus stolonifer* 16(12.84 %) which had the least occurrence. The most occurring bacterium was *Escherichia coli* with the occurrence of 19 representing 25 %. This was followed by *Staphylococcus* spp 16(21.05 %), *Proteus* spp and *Staphylococcus* spp with the occurrence of 15(19.74 %) each and *Klebsiella* spp 11(14.47 %) which was the least. These findings agree with Oyewole and Afolayan, [7]., 86% of the herbal medicines tested were found to be contaminated with microorganisms. Fungi were the most common contaminants, with *Aspergillus* spp. being the most frequently isolated, this also agrees with this study and reason being

that the storage condition and the consistency of the powdered herbal medicine is favorable for the growth of fungi compare to bacteria but this disagrees with [9].as 375 % of the isolates were fungi while the rest were bacteria.

Among the sampled markets in Makurdi metropolis, Wadata market was observed to have the highest occurrence of bacteria with the total of 14(36.84 %). Wurukum and North Bank had equal occurrence of 12(31.58 %) each. There was no significant association between occurrence and location in Makurdi ($X^2 = 0.080$; df = 2; p = 0.781). While among the sampled markets in Adikpo, Jako-Aka market and Mbaniege market had the highest bacterial occurrence of 13(34.21 %) each; while Adikpo market had 12(31.58 %). No significant occurrence in relations to the location was observed in Adikpo. ($X^2 = 0.080$; df = 2; p = 961). Both of the two sample locations had equal bacteria occurrence. Similar isolates were isolated from a study by Anibijuwon *et al*. (2018) which include *Escherichia coli* and *Klebsiella* spp.

Adikpo was observed to have slightly higher fungal occurrence 255(52.90 %) than Makurdi 227(47.10 %). No significant association was observed ($X^2 = 0.360$; df = 1; p = 0.549). this agrees with Sule *et al*. (2014) as he had 78 % fungal isolates but still no significant relation among the two locations he sampled. These findings disagree with Ameh *et al.* (2015) as he had low fungal isolates in his study and high bacterial isolates, this could be because of the storage facility of the herbal medicine.

5. **Conclusion**

Powdered herbal medicines sold in Makurdi and Adikpo town are contaminated with various microorganisms including bacteria and fungi. Some of these microorganisms are know to cause diseases in humans, and their presence in the powdered herbal medicines could pose a risk to the health of consumers. Therefore, it is important to ensure that proper hygiene and quality control measures are in place during the production, packaging and storage of herbal medicines to minimize the risk of contamination and ensures the safety of consumers.

Recommendations

- Regulatory bodies should enforce standards and regulations for the production, packaging, and storage of herbal medicines to ensure they meet minimum safety standards.
- Manufacturers of herbal medicines should implement good manufacturing practices (GMP) to ensure the quality and safety of their products.
- There should be regular monitoring and testing of herbal medicines to detect and prevent the presence of harmful microorganisms.
- Consumers should be educated on the potential risks associated with the consumption of contaminated herbal medicines and advised to only purchase products from reputable sources.
- Additional research should be conducted to identify specific sources of contamination and develop effective strategies for preventing microbial contamination of herbal medicines.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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