MICROBIAL EVALUATION OF DIFFERENT MULTIVITAMIN SYRUP SOLD AROUND UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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CERTIFICATION

This is to certify that this research work 'MICROBIAL EVALUATION OF MULTIVITAMIN SYRUPS SOLD AROUND UNIVERSITY OF BENIN, BENIN CITY, NIGERIA' was carried out by Chinaka, Ijeoma Purity, in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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DEDICATION

This work is dedicated to the God Almighty who is my source of being and strength.

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I humbly acknowledge the divine guidance and grace of God throughout this project. I want to extend my deepest appreciation to my parents Mr and Mrs Albert Chinaka for their unwavering love, encouragement, and sacrifices throughout this journey.

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ABSTRACT

This investigation was done to evaluate the microbial and physicochemical qualities of 10 samples of different brands of multivitamin syrups sold within university of Benin ugbowo environment . These brands were investigated according to the in-vitro compendial requirements, which include physicochemical properties such as color and taste, pH, total viable aerobic count and type of isolated microorganisms. These tests were performed by standard methods and techniques. Drop plate method was used . The physiochemical qualities such as organoleptic test results had brown and yellow clear and viscous liquid with sweet taste in some multivitamin syrups but one sample have a bitter taste that not suitable for pediatric patients. The pH values were ranged from 2.9-6.8. These findings explained that the eight out of ten different brands of multivitamin syrups sold within the university of Benin ugbowo are comply with Pharmacopeia specifications regarding microbial and physicochemical characteristics.

The prevalence of these microorganisms in pharmaceutical products such as syrups samples may indicate the unhygienic condition, defect in production, poor adoption of Good Manufacturing practice, ineffective preservatives and inadequate quality control. Though these products fall under non-sterile pharmaceutical products, so they need not require sterility but these drugs must conform to the microbiological purity criteria set in the appropriate pharmacopeial standard.

CHAPTER ONE

1.1 INTRODUCTION

Microorganisms are abundant in food, beverages, and even pharmaceuticals (Nahata, 1999; Ogbulie et al., 1998). When contaminated with pathogenic microorganisms, pharmaceutical products become ineffective and impede the diagnosis of diseases (Eka et al., 1987).

Pharmaceutical preparations can be classified into sterile and non-sterile products. While sterile products are expected to be absolutely free of all microbes, non-sterile products are not to be free of all forms of microbes although there are limits as to the permissible levels of these contaminants in such pharmaceutical products.

In the earlier of the 21st century, microbial contamination of non-sterile drugs is one of the main problems for product recalls and production slowdowns (Jumenez 2004) The presence of microbial contaminants was not only found to cause physicochemical changes that led to the spoilage of numerous products but was also proved to be a potential health hazard to the consumer. Non-sterile dosage forms are not required to be sterile, as recommended by most pharmacopeias, but are required to pass microbial bioburden tests for the absence of certain specified indicator pathogens (Escherichia coli, Salmonella sp., Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans) to ensure their efficacy and safety (El-Housseiny et al…,2013;4: 736-742.).

Not only the presence of pathogenic microorganisms but the presence of relatively high number non-pathogenic microorganisms is also objectionable in pharmaceutical products. The presence of the large number of nonpathogenic microorganisms in pharmaceuticals is objectionable for two reasons: firstly, these microorganisms can deteriorate active ingredients and can interfere with the desired activity of the product; and secondly, they can produce some metabolites that may toxic to the consumer (Gad et al., 2011; 10: 437-445.) (Kabir et al.,2013).

1.2 Multivitamin

Vitamins are a group of organic nutrients required in small quantities for a variety of biochemical functions that generally cannot be synthesized by the body, and must therefore be supplied in the diet (Bender et al.., 2009).Vitamin syrups are a non-sterile liquid dosage form mostly prepared for oral administration in children (Muhammed et al.., 2009).Different companies use various preservatives that have antimicrobial activity and these protect syrups from microbial contamination by inhibiting their growth (Hugo et al,1980).

1.2.2 Definition of multivitamin

In the United States, a multivitamin/mineral supplement is defined as a supplement containing three or more vitamins and minerals that does not include herbs, hormones, or drugs, where each vitamin and mineral is included at a dose below the tolerable upper intake level as determined by the Food and Drug Board, and does not present a risk of adverse health effects (National Institutes of Health State-of-the-Science Panel. National Institutes of Health State-of-the-Science Conference Statement: multivitamin/mineral)supplements and chronic disease prevention"(*Am J Clin 20*07)

Composition of multivitamin

Each 5 ml Contains:

Vitamin A (Palmitate) BP................................	3600 IU
Thiamine Hydrochloride BP.............................	2.0 mg
Riboflavin BP..	1.2mg
Pyridoxine Hydrochloride BP................................	0.2mg
Cyanocobalamin BP.......................................	2.0 mg
Nicotinamide BP...	10 mg
Ascorbic Acid BP..	50 mg
Vitamin D3 (Cholecalciferol) BP......................	1200 IU

Appropriate Overages added to compensate for loss on Storage

1.2.4 Indication

Multivitamin Syrup is indicated for loss of appetite and poor eating habits. Anorexia after a brief illness.

Patients experience loss of appetite during convalescence following surgery or after any major

1.2.5 Dosage & administration

1 Year to 6 Year: 1/2 teaspoonful (2.5 ml),

7 Years to12 Years - 1 teaspoonful (5 ml)

These dosages may be repeated 3 to 4 times daily as required.

1.3 Contamination of multivitamin syrup

Micro organisms are natural part of our environment. They are present in the air we breathe, the food we eat, and the water we drink. In large numbers, some micro-organisms native to the body exist; they account for about the third of the body's dry weight. It is apparent that raw materials and final products (multivitamin syrups) will contain micro - organisms unless specific measures are adopted to exclude them. the preparation of sterile products requires advanced equipment, skilled staff, and a controlled working environment.

Multivitamin syrups contain nutrient rich ingredients which act as substrate for the growth of microorganisms(Baird and Reid 2004). These microorganisms can deteriorate active ingredients and can interfere with the desired activity of-the product and may produce some metabolites that are toxic to the consumer. Such toxic metabolites can cause illnesses including abdominal discomfort, diarrhoea and acute gastroenteritis (Gad et, al 2011, Kabir and Hossain 2013).

1.4 Pharmaceutical ingredients that are liable to microbial contamination

Pharmaceutical products used in the prevention, treatment and diagnosis of disease contain a wide variety of ingredients, often in quite complex physicochemical states. Such products must not only meet current pharmaceutical Good Manufacturing Practice (GMP) requirements for quality, safety and efficacy, but also must be stable and sufficiently elegant to be acceptable to patients.

These ingredients include:

Sweetening, flavouring and colouring agent, Many of the sugars and other sweetening agents used in pharmacy are ready substrates for microbial growth.Sugars are commonly added for sweetness and as a vehicle for administration. While enhancing palatability,

sugars can potentially serve as a carbon source for microorganisms. Formulators must strike a balance between taste preferences and minimizing microbial growth potential.

Flavoring agents enhance the taste of the syrup, contributing to patient compliance. Natural or artificial flavors should be chosen judiciously, considering their impact on microbial stability and potential interactions with other ingredients.

Water is a primary component in multivitamin syrups, serving as a solvent for water-soluble vitamins and other ingredients. Controlling water quality and purity during formulation is crucial to prevent microbial contamination.

Vitamins (vitamin C, B- complex vitamins) **a**re essential components, contributing to the nutritional value of the syrup. However, some vitamins are susceptible to degradation, especially in the presence of factors like light and oxygen. Proper storage conditions and packaging are crucial to maintain vitamin stability.

Preservatives (e.g., benzoates, sorbates) play a critical role in preventing microbial contamination. Benzoates and sorbates, for instance, are common antimicrobial agents. Formulators carefully choose preservatives based on their effectiveness against specific microorganisms and compatibility with other ingredients.

1.5 Sources of microbial contamination In manufacturing

Microbial contamination in pharmaceutical manufacturing can arise from various sources. Quality must be built into the product at all stages of the processes and not simply assessed at the end of manufacture; the following are sources of contamination during manufacturing :

I.51 Water

water employed in manufacture has been described as the most likely source of gram-negative contaminant in pharmaceutical non sterile products. Water is one of the fundamental requirements of life and any undesired addition of chemical substances leads to its contamination and makes it unfit for human utility (Alo et al., 2012) .The major sources of contamination of pharmaceuticals have always been water, the production environment, personnel and packaging material (Olutimeyin and Onaolapo 1997). It is a common knowledge that water supplies in Nigeria and other third world countries are frequently contaminated with E.coli and other coli forms. . This is due to poor sanitation as well as higher temperatures, which increase the chances that coli forms will be found in water supplies. (Le charallier et al 1996).

1.52 Air of the manufacturing area

There are billions of suspended particles and bacteria in the air. Fungus spores such as penicillium, mucor, aspergillus, and ete. Bacterial spores, such as Bacillius sp., are also detected. These spores and microorganisms have the potential to contaminate pharmaceutical goods. This form of contamination is reduced by production in a clean room or an aseptic environment with a continuous flow of sterile air via a HEPA filter.

1.53 Raw materials

Raw materials such as plant extracts or their derivatives can also deteriorate due to poor harvesting, transportation and storage practices that make them susceptible to microbial attack (Dubey *et al*., 2014).

The basic ingredients used in pharmaceutical products vary greatly. Clays and earth minerals such as bentonite, kaolin can harbour anaerobia spores such as Clostridium sp. Coliform bacteria, such as E. Coli, may be found in starch.

1.54 Equipment

Manufacturing equipment may include germs if it is not sufficiently sterile. Non-specific and local populations of microorganisms may be found in grinders, blenders and filters.

1.55 Personnel

Pharmaceutical items may potentially be contaminated by manufacturing workers. Personnel may become contaminated with coliform bacteria, staphylococci, streptococci, actino bacteria, and Candida. Contamination of this sort can be reduced by regular health checks, vaccinations, and personnel cleanliness. Personnel protection and training may also help to reduce contamination.

1.56 Human resources

During usage, pharmaceutical items may get contaminated. The patient may contaminate his own medication. Contaminants can spread to other patients via physicians, nurses, and so on.

1.57 Manufacture area

Should take place in suitable premises in a clean, tidy work area supplied with filtered air. Moulds are the most common flora of walls and ceiling. They are particularly common in poorly ventilated building with painted walls (Ogbuuabo 2002)

1.6 Source of microbial contamination in use

Pharmaceutical manufacturers may jointly argue that their responsibility ends with the supply of a well- preserved product of high microbiological standard in a suitable pack and that the subsequent use or indeed abuse of the product is of little concern to them. During use, multivitamin syrups can become contaminated due to factors like handling

them without proper hygiene, accidentally introducing foreign substances, or exposure to environmental contaminants after opening. These factors highlight the risk of microbial intrusion while using the syrup.

1.7 Mechanisms of contamination during use of medicinal products

1.71 Self infection

Self-infection involves an individual introducing contaminants from their own body, such as through unclean hands

1.72 Cross infections

cross-infection occurs when contaminants are transferred from one person to another. Example from the guardian, Nurses.

1.73 Environmental sources

Air borne contaminants may settle out in products left open to atmosphere. Some of these die during storage, with the rest probably remaining at a static level of about 10^2 - 10^3 colony units (CFU) g^{-1} or ml^{-1} .

1.8 Factors affecting microbial spoilage of pharmaceutical products

1.81 Types and size of contaminant inoculum

Low levels of contaminants may not lead to noticeable spoilage if they are unable to replicate in the product, However an abrupt increase in the contaminant bioburden could pose a significant challenge to the intended formulation. This surge might occur due to various factors, such as unusually contaminated raw materials or the product had been grossly misused during administration. The size of the contaminant inoculum alone may not reliably indicate the potential for spoilage. For example, a weakly preserved syrup with low levels of aggressive pseudomonas could pose a greater risk of spoilage than syrups containing higher numbers of less aggressive fungal and bacterial spores. This highlights the necessity of considering not only the quantity but also the

nature and aggressiveness of contaminants when evaluating the likelihood of spoilage in pharmaceutical syrup formulations. Nutritional factors: The presence of nutrients in the formulation can impact microbial growth. Some ingredients may serve as a food source for contaminants, potentially accelerating their proliferation.

1.82 Moisture content

Water activity (Aw), Microorganisms require readily accessible water in appreciable quantities for growth to occur. By measuring a product's water activity *(Aw),* it is possible to obtain an estimate of the proportion of uncomplexed water that is available in the formulation to support microbial growth, using the formula: *(Aw)* = vapour pressure of formulation/vapour pressure of water under similar conditions.High moisture content can create an environment conducive to microbial proliferation, potentially leading to spoilage.

1.83 Storage temperature

Maintaining the recommended storage temperature is essential for preserving the integrity of pharmaceutical products. Deviations from the specified temperature range can accelerate chemical reactions, compromise the efficacy of preservatives, and provide a conducive environment for microbial growth. In the case of multivitamin syrup ,Multivitamin syrups may contain components sensitive to temperature variations. Deviations from recommended storage temperatures can impact the chemical stability of the vitamins and other ingredients, potentially reducing their efficacy.

1.84 PH

Extremes of pH prevent microbial attack. Around neutrality bacterial spoilage is more likely.The pH level is pivotal for maintaining the chemical stability of active ingredients. It directly impacts the integrity and efficacy of components within the formulation, ensuring that they remain effective over time. In products with low pH levels (e.g. fruit juice-flavoured syrups with a pH 3-4), mould or yeast attack is more likely.

1.85 Packaging design

Packaging can have a major influence on microbial stability of some formulations in controlling the entry of contaminants during both storage and use.Packaging should safeguard from external factors such as light, moisture, and air, which can compromise its stability.For syrup A well-designed seal is crucial to prevent microbial contamination. It ensures that the syrup remains aseptic and uncontaminated throughout its use, safeguarding both product efficacy and consumer health. (Bained 1981).

1.9 Consequences of microbial contamination

microorganisms can deteriorate active ingredients and can interfere with the desired activity of the product and may produce some metabolites that are toxic to the consumer (Gad et al.,2011) (Kabir and Hossain 2013). Such toxic metabolites can cause illnesses including abdominal discomfort, diarrhea and acute gastroenteritis. It is well recognised that a contaminated pharmaceutical product may also present a potential health hazard to the user. Once recognised there is of course a moral obligation to withdraw the offending product and subsequent investigation of the incidence therefore becoming retrospective.

The outcome of using a contaminated product may vary from patient to patient depending on the type and degree of contamination. Other consequence are general spoilage of the product through chemical, physical changes.

Finally spoilage and subsequent wastage of a product have economic implications for manufacturer.

1.10 Quality assurance and control of microbial contamination of multivitamin syrup preparation

Quality assurance of a pharmaceutical product is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the object of ensuring that pharmaceutical products are of quality required for their intended use. It includes design and development, good manufacturing practice for pharmaceutical products.

Under the Act on Pharmaceutical Law issued on 6 September 2001, GPM refers to practices "ensuring that the medicinal products are manufactured and controlled adequately to their intended use and in compliance with the requirements included in their specifications and documents constituting a basis to issue a permit for marketing authorization of medicinal product" (Act on Pharmaceutical Law, 2001).

Good manufacturing practice for pharmaceutical products requires that the manufacturing process is fully defined before it begins and the necessary facilities are provided.

Since many micro-organisms may be hazardous to patient and / or spoil formulation if they enter and remain active in medicines, it is necessary to perform a contamination risk assessment for each product by examining every stage of its anticipated life from raw materials to administration and develop strategies calculated to reduce the overall risk to acceptably low levels.

Quality control is that part of good manufacturing practice concerned with sampling, specifications, and testing as well as the organization, documentation and release procedures which ensure that the necessary and relevant tests are carried out and that the materials are not released from use nor products release for sake or supply until their quality has be judged satisfactory.

To achieve the quality objective, it is necessary to control all stages of drugs, which covers all matters, which individually or collectively influence the quality of a product, including raw materials, the manufacturing process and the evaluation of finished product. One of control stages is the assessment of microbiological quality of medicinal products.

1.11 Limiting number of viable micro- organism in non- sterile products

Environmental cleanliness and hygiene: microorganisms can be transferred to the product from the working surface, equipment,fixtures. Thus it is essential that all working areas are kept clean, dry and tidy. All floors and ceiling should be easy to clean. All equipment must be easy to dismantle and clean and should be inspected for cleanliness before use. Clean air is a prerequisite during manufacturing processes and the spread of dust during manufacture and packaging must be avoided . Personnel are another source of potential contamination, therefore high standard of personal hygiene are essential. To ensure high standards of personnel hygiene adequate hand washing and hand disinfecting facilities,protective garment must be provided. Also staff should be trained in the principle of Good manufacturing practices.There are government agencies which are responsible for the control in the use of chemicals as preservatives, such are; Food and Drug Administration (FDA) in the United States (lamikanra, 1999)

Quality of starting materials: quality of raw materials account for a high portion of the microorganisms introduced during the manufacture of pharmaceuticals, and the selection of materials of good microbiology quality aids in the control of contamination levels in both product and the environment.

Quality control and documentation: Microbiological standards should be set for raw materials as well as microbial limit for in- process sample and final product. Microbiological quality assurance also covers the validation of cleaning and disinfectant solutions and monitoring of the production environment by microbial count. Documentation is a vital part of quality assurance as details of starting materials, packaging materials and intermediate, bulk and finished products should be recorded so history of each can be traced. Distribution records must be kept. This information is of paramount importance in the event that a defective batch has to be recalled.

Packaging and storage : packaging plays a dual role and acts both to contain the product and to prevent the entry of Micro-organisms and moisture which may result in spoilage.

1.12 Microbial Quality Assurance in Non-Sterile Pharmaceutical Products: Establishing Acceptable Limits

The acceptable limits of microorganisms in non-sterile products, such as multivitamin syrups, are crucial to ensure their safety and efficacy. These limits are typically defined by regulatory authorities and pharmacopeias to mitigate the risk of microbial contamination. Common microbial limits for non-sterile pharmaceutical products like multivitamin syrups include:

- Total Aerobic Microbial Count: This parameter measures the total number of viable aerobic microorganisms present in the product. Acceptable limits are often specified in colony forming units per milliliter (CFU/mL), typically ranging from 10 to 100 CFU/mL.

- Pathogenic Bacteria: Certain pathogens, including Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, and Staphylococcus aureus, should be absent from the product.
- Yeasts and Molds: Limits for yeast and mold counts are also established to ensure product safety and stability. These limits generally fall within the range of 10 to 100 CFU/mL.

1.13 Justification of study

Reports have shown that drug-borne infections are on the rise. Microbial breaking down and contaminating pharmaceuticals is a major cause of product recalls. Regulations in Nigeria are poorly enforced, leading to a disorganized supply system. A large number of fake and counterfeit drugs are entering the market through various channels such as stores, hospitals, wholesalers, and pharmacies. The prevalence of these fake drugs has prompted ongoing research to ensure that all medications meet quality standards.

1.14 Aims and objectives of the study

This study aimed at evaluating the microbial quality of different brands of multivitamin syrup preparations from different manufacturer marketed around the University of Benin Ugbowo environment. This study, therefore,sought to evaluate microbial and physical parameters of multivitamin syrups sold at peripheral drug outlets within Ugbowo.

1.141 The specific objectives of the study are

1. To determine the physicochemical parameters of multivitamin syrups sold around university of Benin, Benjn city

2. To determine the microbial bioburden present in multivitamin syrups sold around the university of Benin, Benin city.

3. To determine the types and incidence of microbial contaminants present in multivitamin syrups sold around university of Benin, Benin city.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

The following materials were used in this study: Nutrient Agar (Himedia, India) Sabouraud Destrose Agar (Himedia India)

2.1.2 Apparatus/ Equipment

1. Portable autoclave (Gallenkamp, U.K)
2. Binocular Microscope (Olympus)
3. Hot air oven (Gallenkamp, U.K)
4. Incubator (Gallenkamp, U.K)
5. Refrigerator (500-Model, Hair thermocool)
6. Weighing Balance (H80, Mettler, Switzerland)
7. Micro Pipette

2.1.3 Reagents and Chemicals.

All chemicals used were of analytical grades and the include

1. Hydrogen Peroxide
2. Methylated Spirit
3. Distilled Water
4. Normal Saline
5. Gentian Violent (BEMA SCIENTIFIC AND CHEMICAL LTD, UK)
6. Ethanol (AbSolute 99.9% ethanol)
7. Safranine Red (BEMAC SCIENTIFIC AND CHEMICAL LTD, UK)
8. Lugol's iodine (SUZINAL CHEMICAL LID)

Method

2.2.1 Study location

This research was carried out in Benin City, Edo State, Nigeria. It is the capital city of Edo State in South South geopolitical zone, Nigerian, with a population figure of 1,156,000 as at 2006 National Population Census,, It is located roughly 40 kilometer north of the Benin river and 320 kilometer by highway cast of Lagos (Encyclopedia Britannica, 2021).

2.2.2 Sample collection

A total of 10 samples of non sterile pharmaceuticals were collected. No particular sampling procedure was employed other than researcher posing as a customer. The samples comprised of 10 multivitamin syrups locally produced and imported products. Samples were carefuly purchased from 7 Community pharmacies in ugbowo Benin City, Nigeria.

2.3 Laboratory analyses

2.3.1 Preliminary assessment

Following purchase and collection of the drug samples, information on the National Agency for Food and Drug Administration and Control (NAFDAC) number, batch number, date of manufacture, expiry date as well as name and address of manufacturers and country of origin were noted and documented.

2.3.2 Sample preparations

Each liquid samples (syrups), 1.0 ml. of the product was diluted in 9.0 mL normal saline, Usually, ten-fold dilution were prepared as described in United States Pharmacopoeia (2003).

Preparation of media

Sabotraud dextrose agar:

To prepare 20 plates of Sabouraud dextrose agar, 16.25g of the powder was measured and placed in a bottle. 250ml of sterile distilled water was added to dissolve the powder, which was then shaken well to ensure it fully dissolved. The mixture was sterilized at 121°C for 15 minutes Next, 20ml of the solution was added to 10 bottles in a sterile environment and autoclaved at 121°C for 15 minutes. The agar was then poured into petri dishes aseptically before discarding the bottles.

Nutrient agar :

To prepare 20 plates of nutrient agar, 7 g of the powder was measured and placed in a bottle. 250ml of sterile distilled water was added to dissolve the powder, which was then shaken well to ensure it fully dissolved. The mixture was sterilized at 121°C for 15 minutes. Next, 20ml of the solution was added to 10 bottles in a sterile environment and autoclaved for another 15 minutes at 121°C **th**e agar was then poured into petri dishes aseptically before discarding the bottles. On cooling. the plates were then placed into the hot air oven to ensure total removal of moisture.

Drop Plate Method : Procedure and Execution

The drop plate method, also known as the drop count method, is a technique used in microbial dilution to estimate the concentration of microorganisms in a liquid sample. A series of dilutions are prepared from the original sample. Each dilution reduces the concentration of microorganisms in the sample.

0.02 ml of each liquid dilution is then dispensed onto the surface of a solid agar medium in Petri dishes (nutrient agar and sabourand dextrose agar plate) using a micro pipette, drops of the diluted sample are placed onto the agar surface.

The Petri dishes are then incubated under conditions suitable for the growth of the microorganisms present in the sample.Nutrient agar plate were incubation at 37°C for 24 - 48 h for bacterial growth while the SA plates were incubated at room temperature (28°C) for 48-72 h for fungal growth. The resulting microbial colonies were counted.

2.3.3 Total viable aerobic bacteria , yeast and mould count

Viable aerobe mesophilie bacteria, yeast and mould count was evaluated using drop plate method. Enumeration was carried out on Nutrient Agar (NA) for bacteria and on Sabouraud Dextrose Agar (SDA) for fungi. Duplicates of appropriate dilutions were prepared. The NA Plates were incubated at 37°C for 24 - 48 h for bacterial growth while the

SA plates were incubated at room temperature (28°C) for 48-72 h for fungal growth. The resulting microbial colonies were counted.

2.3.4 Detection, isolation and identification of microbial contaminants

An aliquot (0.02 mL) of appropriate dilution of each sample was spread on Nutrient agar and Sabouraud dextrose agar plates. All the plates were incubated at 37°C for 24 h, except the Sabouraud agar plates which were incubated at room temperature (28°C) for 48-72 h. The resultant colonies were examined morphologically and microscopically. Morphological identification was based on size, diameter, elevation, translucency, color, etc. of the colony formed. Microscopic identification was carried out by Gram staining to identify Gram-positive and Gram-negative bacteria. For biochemical identification, a number of biochemical tests including carbohydrate utilization, catalase production, coagulase production, oxidase production, methyl red, Voges-Proskauers, nitrate reduction, starch hydrolysis, tryptophan hydrolysis, hydrogen sulphide production and citrate utilization was performed as described by Bucchannan and Gibbons (1974) in Bergey's Manual of Determinative Bacteriology.

Fungal growth was identified using pigmentation, sporulation, mycelia arrangement and microscopically, by lactophenol needle mount as well as using standard manuals, such as Introducing mycology by examples (Bernward and Gabriele, 1980).

2.3.5 Gram staining of the organisms

The wire loop was sterilized by flaming and was allowed to air cool. A glass slide was degreased with cotton wool soaked in acetone. A loop-full of sterile distilled water was positioned on the degreased glass slide.

A pinch of the bacterial colony was taken from the culture of the organism in a nutrient agar slant and mixed with the sterile distilled water to form a smear the size of a coin.

The smeared glass slide was air dried. Thereafter the smeared glass slide was heat fixed.

The smear was then stained with a few drops of Gentian violet (primary dye) and a contact time of sixty (60) seconds was allowed before washing off with gently running tap water and permitted to dry out. The spread was then flooded with Lugol's iodine solution (mordant) and a contact time of sixty (60) seconds was maintained before washing off with water.

A few drops of Acetone (fast decolouriser) was used to decolourise the smear and washed off immediately with gently running tap water. The smear was counter stained with Safranin (secondary dye) and maintained for sixty (60) seconds before washing off with gently running tap water and allowed to air dry. A drop of oil immersion was positioned on the smear and mounted on a microscope and viewed, using the oil immersion objective lens. (Chessbrough, 2003) Organisms were classified as Gram positive or Gram negative based on colour, shape and arrangement.

SLIDE VIEWING

All lens were swabbed with cotton wool soaked in xylene and the light source was turned off to ensure the cells are not destroyed before viewing. After fixing the slide the lens of 40x was used to view the organism, the fine adjustment, stage controls and brightness adjustment and mechanical stage where all adjusted for clear viewing/visibility of the organism cells.

2.3.6 Biochemical Tests for identification of organisms

Catalase Test

A glass slide was degreased with cotton wool soaked in acetone. A wire loop was sterilized by heating red hot and allowed to air cool. A loopful of sterile distilled water was placed on the slide. A pinch of the bacterial colony of the test organism from the nutrient agar slant was taken. An emulsion of the bacterial colony and water was made on the slide and few drops of 3% hydrogen peroxide was added. The results were recorded as effervescence (+) or no effervescence (-)

Coagulase Test

A glass slide was degreased with cotton wool soaked in acetone. A wire loop was sterilized by heating red hot and allowed to air cool. A loopful of sterile distilled water was placed on the slide. A pinch of the bacterial colony of the test organism from the nutrient agar slant was taken. An emulsion of the bacterial colony and water was made on the slide and a few drop of pooled human plasma was added. It a loopful of sterile distilled water was placed on the slide. It was mixed by tilting the slide to and fro. The results were recorded as either Clumping (+) or no clumping (-).

Indole Test

A loopfull of the culture of the organism was inoculated in a test tube containing 3 mL. of sterile peptone water. It was incubated at 37°C for 24hrs and 0.5 mL. was added to the inoculated peptone water and shaken gently. It was observed for a purple colour in the surface layer within 10 minutes. The result was recorded.

Oxidase Test

A piece of paper was placed on a clean grease-free Petri dish and pure cultures were smeared on the filter paper using a sterilized wire loop. A few drops of freshly prepared oxidase reagents was added. A positive oxidase test is indicated by purple coloration within 10 seconds.

Urease Test

A sterilized urease medium was dispensed into test tubes asceptically and the test bacterial isolates were inoculated into the medium and incubated at 37°C for 24 hrs. A change in colour from brown to pink confirmed the presence of urease producing organisms

Gelatin Hydrolysis

This reaction is catalysed by a specialised enzyme produced by certain bacteria species. It is useful in the identification of Bacillus species. A plate of gelatin agar is inoculated with the test organism and incubated at 32° C for 2-14 days. After incubation period, the plate was flooded with 8 mL mercuric chloride solution. Appearance of clear zones indicated gelatin hydrolysis

Lactophenol in Cotton Blue Mount

A drop of lactophenol cotton blue was positioned on a dirt free glass slide degreased with cotton wool soaked in acetone. The wire loop was sterilized by heating red hot and air cooling. A small portion of the spores of the yeast in the inoculated Sabouraud Dextrose Agar slant was placed on a slide. The organism was emulsified. A clan cover slip was placed gently on the preparation, It was viewed on the microscope using X40 objective lens.

CHAPTER THREE

RESULTS

3.0 Label Information on Products

It is pertinent to state that all the samples complied with NAFDAC labeling regulations which states that all registered products must have the full Names of the of the Manufacturers and their location addresses, Ingredients list and weight of each ingredients, Generic names, Brand names, Net weight/fill volume. Strength per mL for liquid preparation and strength per tablet or capsule for solid dosage forms. Direction for use, storage conditions, indication for use, date markings (Batch Number, Manufacturing Date, Expiry Date). Leaflet insert containing all the information about the drugs and NAFDAC registration numbers were also included, as appropriate.

3.1 Table 1: Label information of different brands of multivitamin syrups investigated.

Brand Code	Manufacturing Date	Expiry Date	Batch Number	Nafdac Number
M1	11 – 25	08 – 25	+	+
M2	06- 23	05 –2025	+	+
M3	04 – 23	03 –2024	+	+
M4	06 – 23	06 – 26	+	+
M5	02 – 22	02 – 25	+	+
M6	02 – 23	01 – 25	+	+
M7	19 – 22	08 – 24	+	+
M8	11 – 22	11 – 24	+	+
M9	08 – 23	07 – 24	+	+
M10	08 – 2023	08 –2025	+	+

3.2 Table 2: Physicochemical parameters of different brands of multivitamin syrup investigated.

Brand Code	Description	Colour	Taste	PH
M1	Viscous Solution	yellow	sweet	4.5
M2	Clear Solution	yellow	sweet	5.2
M3	Clear Solution	brown	sweet	3.2
M4	Clear Solution	yellow	sweet	3.9
M5	Clear Solution	brown	sweet	2.9
M6	Clear Solution	brown	sweet	3.6
M7	Clear Solution	brown	sweet	4.2
M8	Clear Solution	dark brown	bitter	6.8
M9	Clear Solution	brown	sweet	4.4
M10	Clear Solution	yellow	sweet	2.9

3.3 Table 3: Microbial load/counts of the different bands of multivitamin syrups investigaled.

Brand Code	TAB C(cuf/ml)	TYMC(cuf/ ml)
M1	$2x10^1$	$2x10^1$
M2	$2x10^1$	$2x10^1$
M3	$1x10^1$	$2x10^3$
M4	NG	NG
M5	$1x10^1$	NG
M6	NG	NG
M7	$1x10^1$	$1x10^1$
M8	NG	NG
M9	$4x10^1$	$1x10^3$
M10	$1x10^1$	NG

KEY

TABC: Total Aerobic Bacterial Count

TYMC: Total Yeast and Mold Count

NG: No growth

3.4 Table 4: Gram staining results of positive samples

Brand Code	Microscopic View
M1	Gram positive cocci
M2	Gram positive cocci
M3	Gram positive Bacili
M5	Gram positive Bacili
M6	Gram positive Bacili
M9	Gram positive Bacili

3.5 Table 5 : Frequency of microbial isolates obtained from investigated samples

Organism	Number of positive organisms	Percentage %
Bacteria :		
Bacillus subtills	5	50.0
Staphylococcus aureus	2	20.0
Staphylococcus epidermidis	3	30.0
Fungi :		
1. Penicillium notatum	3	27.3
2. Mucor fuscus		
3 Microsporum fulvum	6	54.5
	2	18.2

CHAPTER 4

DISCUSSION:

In the current study, all multivitamin syrup samples collected from different medicine stores had a manu- facturing date, expiry date, batch number and had new unused packs. The variations in manufacturing and expiry dates, batch numbers, and NAFDAC registration among the multivitamin syrup brands provide valuable insights into product traceability and regulatory compliance. These variations may stem from differences in manufacturing schedules and regulatory requirements across manufacturers.

The physico-chemical parameters provide insights into the appearance, taste, and pH levels of the multivitamin syrups. The diverse color, taste, and pH levels observed across different brands indicate variations in formulation, processing methods, and storage conditions. The pH of the samples where taken and according to “pH and titratable acidity of different multivitamin syrups in Nigeria” the pHs of syrups range from 3.06 to 8.4. Thus, from the result obtained, following this research report only 8 out of 10 met up to the range. Brand M5 and M10 exhibit lower pH levels, indicating potential degradation or alteration of ingredients. Moreover, brand M8 stands out with a dark brown color and bitter taste, suggesting potential formulation deviations that may impact consumer acceptance and efficacy.Factors such as oxidation, microbial activity, and ingredient interactions could contribute to color changes and pH fluctuations, underscoring the importance of quality control measures throughout the manufacturing process.

The microbial load/count data provide critical insights into the presence or absence of aerobic bacteria, yeast, and mold in the multivitamin syrup samples. Brands M4, M6, and M8 exhibit no microbial growth, indicating adherence to stringent quality control measures during manufacturing and storage. This is an encouraging finding, suggesting effective sanitation protocols and hygienic practices implemented by these manufacturers.

Brand M1 and M2 total Aerobic Bacterial Count (TABC) 2×10^{1} CFU/ml and Total Yeast and Mold Count (TYMC) 2×10^{1} CFU/ml, these brands exhibit relatively low microbial counts within the acceptable range. The observed counts suggest moderate levels of contamination that may be considered acceptable for pharmaceutical products, as they are below the specified limits set by regulatory authorities. Brand M3 and M9 TABC ranging from 1×10^{1} to 4×10^{1} CFU/ml and TYMC ranging from 2×10^{3} to 1×10^{3} CFU/ml.

These brands show higher microbial counts, particularly in TYMC for brand M3 and M9. The presence of elevated microbial counts, especially for TYMC, may indicate potential contamination issues . While some levels of microbial contamination may be expected, these counts may exceed acceptable limits for pharmaceutical products, raising concerns about product safety and quality.

In summary, while brands M1 and M2 , M5 , M7 and M10 exhibit microbial counts within acceptable limits, brands M3 and M9 show higher counts that may raise concerns about product safety. Continuous monitoring and improvement of manufacturing practices are essential to minimize microbial risks and ensure the quality and safety of multivitamin syrups. Brands M4, M6, and M8 demonstrate desirable microbial quality, indicating effective quality control measures and adherence to regulatory standards.

The isolated aerobic bacteria were mainly Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis, whereas the fungi isolates were made up of Penicillium notatum, Mucor fuscus and Microsporum fulvum species.This suggests that the contamination of the preparations may have resulted from production personnel due to inappropriate handling, contagion from the tools and or raw materials or poor sanitation

from the industrial unit at some point in production, packaging and distribution. Non-sterile preparations, must not only meet microbial limit tests, but also demonstrate that objectionable microorganisms like P. aeruginosa, E. coli, S. aureus, and Salmonella spp. and candida spp. are not present in them, as a condition for use by patients (USP, 2003). The presence of Staphylococcus aureus and its relative Staphylococcus epidermidis implicated contacts from human sources, like, from the hands, skin, or hair. Specifically, Staphylococcus epidermidis is most often suspected in medical-devise-associated infection. S. aureus is a common cause of boils. Middle ear infection, pneumonias and osteomyelitis.The presence of Bacillus specie in some of the products was an indication of dust contamination. As a result, any change in structure to buildings or unrestrained sweeping may be responsible for the presense of Bacillus subtillis in these preparations.

The presence of microbial contaminants in multivitamin syrups underscores the importance of implementing robust quality control measures throughout the manufacturing process. Effective sanitation practices, environmental monitoring, and microbial testing are essential for identifying and mitigating potential sources of contamination.

Microbial loads in excess of 10-CFU per ml or g is a serious health worry (Carstersen and Rhodes, 2000). Microorganisms may themselves cause infections or they may produce harmful metabolites and or toxins that turn out to be injurious even if they are present in small amount. (Shukla et al, 2004).

CHAPTER 5

CONCLUSION

Analysis of label information revealed variations in manufacturing and expiry dates, batch numbers, and NAFDAC registration among the multivitamin syrup brands. These details are crucial for ensuring regulatory compliance and product traceability.

Physico-chemical parameters such as color, taste, and pH levels varied across different brands, reflecting differences in formulation, processing methods, and storage conditions. The pH of all brands met up with the range of standard specifications except 2 brands with a pH of 2.9 Showing high acidity.

Microbial load/count data revealed varying levels of microbial contamination among the multivitamin syrup samples. While some brands exhibited acceptable microbial counts within regulatory limits, others showed elevated counts that may raise concerns about product safety. Continuous monitoring and improvement of manufacturing practices are essential to minimize microbial risks and ensure product quality.

Microscopic analysis identified the presence of Gram-positive cocci and bacilli in certain brands, indicating potential contamination issues.The isolated aerobic bacteria were mainly Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis, whereas the fungi isolates were made up of Penicillium notatum, Mucor fuscus and Microsporum fulvum species. The occurence of yeasts and staphylococci in some of the product tested was unacceptable.

Robust quality control measures and adherence to Good Manufacturing Practices (GMP) are imperative to mitigate microbial risks and maintain product integrity.

Overall, this study underscores the importance of rigorous quality assurance protocols in pharmaceutical manufacturing to ensure the safety and efficacy of multivitamin syrups.

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Recovery and detection of microbial contaminants in some non-sterile

syrups and antibiotic susceptibility profile of the isolated Escherichia coli.

APPENDIX I

MEDIA/REAGENTS COMPOSITION AND PREPARATION

SAROURAUD DEXTROSE AGAR

Ingredients	g/dL
Mycological peptone	10.00
Agar	15.00
Dextrose	40,00
pH.	5.2 ± 0.2 at 25°C
Distilled water	1000mL

Preparation: 16.25g of powdered Sabouraud dextrose agar was weighed into 250 ml of distilled
water in a conical flask. It was then heated gently for complete dissolution after which it was
dispensed in units of 20ml- into universal bottles and sterilized by autoclaving at I5psi
(121°C) for 15 minutes . It was the transferred aseptically into sterile plate for the experiment

APPENDIX II

NUTRIENTS AGAR

Ingredrient.	g/L
Beef Extract.	3.00
Peptone water	5.00
Sodium chloride	8.00
Agar	15.00
pH	6.8 + 0.2 at 25°C
Distilled water	1000mL

Preparation: 7g of powdered Nutrient agar weighed into 250 ml of distilled water in a conical

flask. It was then heated gently for complete dissolution after which it was dispensed in units

of 20mL. into universal bottles and sterilized by autoclaving at 15psi (121°C) for 15minutes.

it was the transferred aseptically into sterile plate for the experiment