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ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM HERBAL MIXTURES SOLD AT OWERRI METROPOLIS

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Abstract

The safety, efficacy and quality of herbal mixtures have been an important concern for health authorities and health professional, especially now there is increase in the use of herbal mixtures. This study was aimed at isolation and identification of microorganisms from some liquid herbal mixtures sold in Owerri metropolis, Imo State, South East of Nigeria. A total of forty-five samples of herbal mixture were selected at random, three samples of same mixtures from three different locations respectively, from herbal shops in Enugu metropolis and were analysed at Orange Research and Diagnostic Laboratory in Owerri, samples were inoculated into, MacConkey agar, CLED and Chocolate agar plates, and incubated at 37°C for 24 hrs. Sabroaud dextrose agar slants were also inoculated for the isolation of fungi for 7 days. Viable bacterial counts of the samples were also performed using nutrient agar. The organisms isolated were identified using biochemical tests, and the following organisms were identified, *Escherichia coli* spp, *Enterobacter* spp, *Aspergillus* spp, *Rhizopus* spp, *Bacillus* spp, *Mucor* spp. Some of the herbal medicine were highly contaminated and most of the organisms, isolated were gotten through poor handling, poor manufacturing of this herbal mixtures and raw materials used in preparing this herbal mixtures.

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Introduction:-

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history [14]. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or inactive, and also which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias [5-8]. The use of the Locally made herbal medicinal products (LHMPs) has been increasing worldwide due to the readily availability of raw materials and low cost compared to the synthetic industrial preparations. It is sometimes referred to as complementary and alternative medicine (CAM) [9-10].

It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing diseases. The World Health Organization (WHO) estimates that four billion people (about 80 percent of the world population) use herbal medicine for some aspect of primary health care [12-14]. The term "herbal drugs" denotes plants and plant parts that have been converted into phytopharmaceuticals by means of some simple processes involving harvesting, drying, processing and storage [15-16]. Herbal drugs are crude preparations of various kinds of medicinal plants or any part thereof, such as leaf, stem, root, flower or seed [17-18].

The World Health Organization also defined herbal medicine as finished labeled medicinal products that contain as active ingredients aerial or underground parts of plants or other plant materials or combinations thereof whether in the crude state or as plant preparations [19]. The widespread use and availability of herbal medicines has been reported to be due to perceived efficacy, safety and absence of side effects from herbal products when compared to orthodox medicines. The use of herbal medicines is also likely to have increased because of emerging infections such as AIDS and drug resistant malaria [20]. The high cost of hospital consultation and orthodox drugs is an additional reason why herbal therapy may be attracting greater patronage [21].

Materials And Methods:-

Study area

The study was carried out in Owerri municipal council of Imo state, Nigeria between in 2021.

Sample collection

A total number of Forty-five (45) different mixtures fifteen(15) NAFDAC registered and Thirty(30) non-registered were purchased from different parts of Owerri metropolis from Chemist, Hawkers, Streetshops and Clinics. The Forty-five(45) samples was made up of 3 samples for each of the fifteen(15) different selected medicines and Information concerning the uses and dosage of each preparation was obtained from the sellers and documented. The samples were purchased as the packaged herbal mixtures and the unpackaged (such as those herbal preparations sold by local retailers and herbalist) were collected in sterile sampling bottles. The samples were stored in the refrigerator at 4°C before the analysis. They were analysed in Orange diagnostic and Research Laboratory in Owerri, Imo State.

Identification of fungi isolates

The growth pattern, pigmentation and size of colonies were recorded at the incubation period to aid identification of the organisms. A smear was made by using wire-loop to pick a colony of the isolate and emulsified on a clean, grease-free slide with lactophenol and viewed under the microscope. It was first focused using X10 objective lens then viewed using X40 objective lens to get a clearer view.

Identification of bacterial isolates

Examination of culture plates

After the incubation, the plates were read and the colonial morphology of the isolates were described. The tubes for fungal cultures were also examined for growth and description of the fungal isolates. e and as a result, they pick up the colour of the counterstain.

Staining procedure

With the aid of a wire loop, the isolate was smeared onto a clean clear grease free slide and allowed to air dry. The primary stain, crystal violet was poured on the smear and allowed to stain for 60 seconds. It was washed in gentle running tap water for 5 seconds, covered with lugol's iodine and rinsed in distilled water. The stain was decolourised using 95% alcohol, rinsed with tap water and then counterstained using neutral red for 60 seconds. The smear was finally rinsed in clean tap water, air dried and then a drop of immersion oil was placed on the stained slide. It was viewed under the microscope using 100x objective lens.

Results:-

The 45 preparations 15 NAFDAC registered and 30 unregistered used in this study were presented by the herbal sellers, stores and hawkers respectively for the treatment of various ailments (Table 1). A total of (96%) of the herbal products were packaged and sold in plastic bottles of which 59.2% were discarded bottles previously used for packaged water and 40.8% were discarded alcoholic drinks containers (figure1).

The mean bacterial load of the samples ranged from zero cfu/ml to 6.4×10^5 cfu/ml. According to the World Health Organization (2007) and the European Pharmacopoeia (2007), for herbal medicinal products to which boiling water is not added before use, the limits specified for total viable aerobic count are 10^5 bacteria and 10^3 fungi per gram or per millilitre. Only 2% of the samples were therefore of unacceptable quality in terms of microbial loads

Table 1:- Sample Code and Indication for drug usage.

SAMPLE CODE	DRUG USAGE
A	INCREASING LIBIDO
B	ANTI-DIARRHOEA
C	TEETH PAIN
D	INCREASE SEXUAL PERFORMANCE
E	ANTI-MALARIA
F	ANTI-VOMITTING
G	ANTI- TYPHOID AND MALARIA
H	TREATMENT OF CANDIDA
I	TREATMENT OF ULCER
J	TREATMENT OF MENSTRUAL PAINS
K	TREATMENT OF ARTRITIS
L	GENERAL BODY PAINS
M	TREATMENT OF PROSTRATE
N	TREATMENT OF SYPHILIS
O	TREATMENT OF RANDOM INFECTIONS

Table 2:- Bacterial (Load) count of some selected herbal medicines.

SAMPLES CODE	No of samples	BACTERIA COUNT(cfu)
A	3	4.0×10^3
B	3	NBG
C	3	1.2×10^3
D	3	3.1×10^3
E	3	NBG
F	3	NBG
G	3	6.4×10^5
H	3	NBG
I	3	3.4×10^3
J	3	3.4×10^3
K	3	1.0×10^3
L	3	NBG
M	3	1.2×10^3
N	3	NBG
O	3	NBG

A total of eight(8) bacterial isolates were recovered from samples and included 5 Gram-positive samples and Three(3) Gram-negative organism .The most frequently isolated contaminants in the tested sample were Bacillus species(70%),followed by Enterobacter species(20%) as shown in table 4 Other Contaminant includes Escherichia coli. Seven(7) samples showed no bacterial growth.

A total of three(3) fungal isolates were recovered from six(6) samples and included Aspergillus species(50%),mucor species (40%) and Rhizopus species(10%) as shown in Table4.3.

Table 3:- Morphological features of fungal organism isolated on the study.

SAMPL E NO	COLOUR ON SLANT	TYPE OF HYPHAE	TYPE OF SPORE	APPEARANCE OF SPORE HEAD	PROBABLE ORGANISM
A	GREY	ASEPTATE	SPORANGIOSPHOR E	SMOOTH SPORANGIU M	MUCOR SPP

B	-----	-----	-----	-----	NFG
C	-----	-----	-----	-----	NFG
D	WHITE	SEPTATE BRANCHING	CONIDIOSPHERE	CONIDIA	ASPERGILLUS SPP
E	WHITE	SEPTATE BRANCHING	CONIDIOSPHERE	CONIDIA	ASPERGILLUS SPP
F	GREY	ASEPTATE	SPORANGIOSPHORE	SMOOTH SPORANGIUM	MUCOR SPP
G	-----	-----	-----	-----	NFG
H	-----	-----	-----	-----	NFG
I	WHITE&FLUFFY	ASEPTATE	SPORANGIOSPHORE	LARGE SPORANGIUM	RHIZOPUS SPP
J	-----	-----	-----	-----	NFG
K	-----	-	-----	-----	NFG
L	-----	-----	-----	-----	NFG
M	-----	-----	-----	-----	NFG
N	WHITE TO YELLOW	ASEPTATE	SPORANGIOSPHORE	SMOOTH & REGULAR SPORANGIUM	MUCOR SPP
O	-----	-----	-----	-----	NFG

Table 4:- Gram Reaction And Biochemical Features Of Bacetrial Isolates On The Study.

SAMPLE CODE	GRAM-REACTION	CAT	COAG	IND	MR	VP	CIT	UREASE	SHAPE	PROBABLE ORGANISM
A	+	+	-	-	-	+	+	-	Irregular	Bacillus spp
B	*	*	*	*	*	*	*	*	*	*
C	-	+	-	-	-	+	+	-	Round raised	Enterobacter spp
D	+	+	-	-	-	+	+	-	Irregular Flat	Bacillus Spp
E	*	*	*	*	*	*	*	*	*	*
F	*	*	*	*	*	*	*	*	*	*
G	+	+	-	-	-	+	+	-	Irregular Flat	Bacillus Spp
H	*	*	*	*	*	*	*	*	*	*
I	+	+	-	-	-	+	+	-	Irregular Flat	Bacillus Spp
	-	+	-	-	+	+	+	-	Round	Enterobacter

J									raised	
K	-	+	-	+	+	-	-	-	Round/ circular	E.coli
L	*	*	*	*	*	*	*	*	*	*
M	+	-	-	-	-	+	+	-	Irregular Flat	Bacillus spp
N	*	*	*	*	*	*	*	*	*	*
O	*	*	*	*	*	*	*	*	*	*

Seven (7) of the herbal samples did not show any bacterial growth. They were further examined for antimicrobial activity against selected bacterial isolates. The sample which did not show any bacterial contamination and a standard antibiotic (Ciprofloxacin) was tested against isolated bacterial strains of *E. coli* and *S. aureus*.

Four (4) of the sample tested showed no zone of inhibition, while Three (3) others showed zones of inhibition of different diameter on the plate as shown in Table 5.

Table 5:- Anti-microbial activity of some herbal medicines without bacteria isolate.

Sample codes	Zone of inhibition on <i>E. coli</i>	Zone of inhibition on <i>Staph aureus</i>
B	6mm±0.2mm	3mm±0.5mm
E	NZI	NZI
F	NZI	NZI
H	2mm±0.5mm	3mm±1.0mm
L	NZI	NZI
N	5mm±1.1mm	1mm±0.3mm
O	NZI	NZI
Ciprofloxacin (10ug/ml)	21mm±0.8mm	23mm±1.0mm

NZI = NO ZONE OF INHIBITION

Discussion:-

This study showed that 98% the herbal medicine was not contaminated as according to W.H.O standard of evaluation or contamination of herbal medicines.

The isolation of some organism like *E. coli* spp and *Enterobacter* spp could be associated with the gastrointestinal tract and indicate the likelihood of faecal contamination. These contaminants could be acquired from the use of water of poor quality for the preparation of the samples and rinsing of containers. Other likely sources are the use of inadequately washed or disinfected plant parts previously exposed to manure.

The practices of transportation and storage may also cause additional contamination and microbial growth. Proliferation of microorganisms also results from failure to control the temperatures of liquid forms and finished herbal products [22]. Other possible sources of contaminants are the environment and utensils in which the preparation was made as well as the containers used for packaging the preparations.

A total of 36 (96%) of the containers of the samples in this study were plastic bottles of packaged water or carbonated drinks that had been discarded after use.

The identity of the contaminants recovered in this study which suggests that possible sources of the contaminants include the environment, raw materials, hands of the producers and the water for production is an indication of a high level of non-adherence to the requirements of Good Manufacturing Practice. This needs to be addressed and producers should be made aware of the benefits of implementing best practice guidelines such as GACP and GMP. In these guidelines, requirements have been described for the raw materials, water for production, preparation utensils and items of equipment, the environment and personnel. The need for hygiene and sanitation of material and environment and the personal hygiene of personnel has been well spelt out to ensure the production of microbiologically safe preparations.

Conclusion:-

This study showed that someherbal medicine showed significant microbial growth and just few significant unacceptable bacterial counts. Regardless there is an urgent need to implement proper herbal medicines monitoring and quality control for producers and the products. Subjection of raw materials for herbal medicines to appropriate processing will reduce the microbial load and potentially the inclusion of preservatives other than toxic alcohol will help keep the microbial load of the products within standard specification, providing safe medicines to the users.

References:-

1. Barnes J, Anderson A, Phillipson D. (Herbal medicine. 3rd Edition,Pharmaceutical Press, London, 2007;1-23.
2. Asomugha IC, Uwaegbute AC, Obeagu EI. Food insecurity and nutritional status of mothers in Abia and Imo states, Nigeria. *Int. J. Adv. Res. Biol. Sci.* 2017;4(10):62-77. DOI: 10.22192/ijarbs.2017.04.10.010. [links/5a00974faca2725286dc5869/Food-insecurity-and-nutritional-status-of-mothers-in-Abia-and-Imo-states-Nigeria.pdf](https://www.academia.edu/links/5a00974faca2725286dc5869/Food-insecurity-and-nutritional-status-of-mothers-in-Abia-and-Imo-states-Nigeria.pdf)
3. Obeagu EI, Aloh GS, Emeka OC, Kanu SN, Okpara KE, Chibunna OM, Nnamdi BO. The anti-epileptic/anti-convulsive effects of mustard seed extracts. *World J Pharm Res.* 2015;4(3):219-31.
4. [links/5ab3ccf0a6fdcc1bc0c3ed7d/THE-ANTI-EPILEPTIC-ANTI-CONVULSIVE-EFFECTS-OF-MUSTARD-SEED-EXTRACTS.pdf..](https://www.academia.edu/links/5ab3ccf0a6fdcc1bc0c3ed7d/THE-ANTI-EPILEPTIC-ANTI-CONVULSIVE-EFFECTS-OF-MUSTARD-SEED-EXTRACTS.pdf..)
5. Alum EU, Aja W, Ugwu OP, Obeagu EI, Okon MB. Assessment of Vitamin Composition of Ethanol Leaf and Seed Extracts of Datura Stramonium. *Avicenna J Med Biochem.* 2023;11(1):92-7. [links/64b6b71fb9ed6874a52c91ba/Assessment-of-Vitamin-Composition-of-Ethanol-Leaf-and-Seed-Extracts-of-Datura-Stramonium.pdf](https://www.academia.edu/links/64b6b71fb9ed6874a52c91ba/Assessment-of-Vitamin-Composition-of-Ethanol-Leaf-and-Seed-Extracts-of-Datura-Stramonium.pdf).
6. Nwakuilite A, Nwanjo HU, Nwosu DC, Obeagu EI. Evaluation of some trace elements in streptozocin induced diabetic rats treated with Moringa oleifera leaf powder. *WJPMR.* 2020;6(12):15-8. [links/5fcb587092851c00f8516430/EVALUATION-OF-SOME-TRACE-ELEMENTS-IN-STREPTOZOCIN-INDUCED-DIABETIC-RATS-TREATED-WITH-MORINGA-OLEIFERA-LEAF-POWDER.pdf](https://www.academia.edu/links/5fcb587092851c00f8516430/EVALUATION-OF-SOME-TRACE-ELEMENTS-IN-STREPTOZOCIN-INDUCED-DIABETIC-RATS-TREATED-WITH-MORINGA-OLEIFERA-LEAF-POWDER.pdf).
7. Okorie HM, Obeagu EI, Okpoli HC, Omoviye EO. The Effect of GongronemaLatifolium (Utazi) on Some Haematological Parameters of Rabbit. *J CardiolCardiovas Res.* 2020;1(3):1-9. [links/61eed38edafcdb25fd4b3073/The-Effect-of-Gongronema-Latifolium-Utazi-on-Some-Haematological-Parameters-of-Rabbit.pdf](https://www.academia.edu/links/61eed38edafcdb25fd4b3073/The-Effect-of-Gongronema-Latifolium-Utazi-on-Some-Haematological-Parameters-of-Rabbit.pdf).
8. Nwakuilite A, Nwanjo HU, Nwosu DC, Obeagu EI. EVALUATION OF ENZYME ANTIOXIDANTS IN STREPTOZOCIN INDUCED DIABETIC RATS TREATED WITH MORINGA OLEIFERA LEAF POWDER. *European Journal of Biomedical.* 2020;7(11):285-8. [links/5fb428e6299bf10c36897872/EVALUATION-OF-ENZYME-ANTIOXIDANTS-IN-STREPTOZOCIN-INDUCED-DIABETIC-RATS-TREATED-WITH-MORINGA-OLEIFERA-LEAF-POWDER.pdf](https://www.academia.edu/links/5fb428e6299bf10c36897872/EVALUATION-OF-ENZYME-ANTIOXIDANTS-IN-STREPTOZOCIN-INDUCED-DIABETIC-RATS-TREATED-WITH-MORINGA-OLEIFERA-LEAF-POWDER.pdf)
9. Stanley MC, Ifeanyi OE, Nwakaego CC, Esther IO. Antimicrobial effects of Chromolaena odorata on some human pathogens. *International Journal of current microbiology and applied sciences.* 2014;3(3):1006-12. https://www.academia.edu/download/38320100/Mbajiuka_Chinedu_Stanley1_et_al_1.pdf.
10. Etim EA, Adebayo YA, Ifeanyi OE. Effect of Luffa cylindrica leaf extract on hematological parameters of Swiss Albino mice. *Med Aromat Plants (Los Angeles).* 2018;7(318):2167-0412. [links/5b5893beaca272a2d66840b6/Effect-of-Luffa-Cylindrica-on-Hematological-Parameters-of-Swiss-albino-Mice.pdf?_sg%5B0%5D=started_experiment_milestone&origin=journalDetail..](https://www.academia.edu/links/5b5893beaca272a2d66840b6/Effect-of-Luffa-Cylindrica-on-Hematological-Parameters-of-Swiss-albino-Mice.pdf?_sg%5B0%5D=started_experiment_milestone&origin=journalDetail..)
11. Ampofo A, Tetteh W, Bello M. Microbiological Profile of Some Ghanaian Herbal, 2012; 4:16-25
12. Etim EA, Adebayo YA, Ifeanyi OE. Effect of Achyranthes Aspera leaf extract on hematological parameters of Swiss albino mice. *World.* 2019;4(4):96-101. doi:10.11648/j.wjph.20190404.14 https://www.academia.edu/download/63697072/Effect_Of_Achyranthes_Asp_era_Leaf_Extract_on_Hematological_Parameters_of_Swiss_Albino_Mice20200621-82472-cxlvny.pdf.
13. Eze-Stephen PE, Udeozo IP, Emmanuel O, Farida O. The effects of ethanol extract of Desmodiumvelutinum stem on liver markers of albino wistar rats fed with high fat diet. *World Applied Science Journal.* 2014;31(10):1684-8. https://www.academia.edu/download/38320063/IDOSI_2.pdf.
14. Abel, Busia K. An Exploration Ethnobotanical Study of the Practice of Herbal Medicine by the Akan People of Ghana. *Alternative Medicine Review.*2005;6:70-77.
15. Sunday AG, Ifeanyi OE, Uzor OF. Wound Healing Potential Of Water And Ethanol Extract Of Fresh Leaves And Bark Ofgambia Albidum In Albino Rats. *IOSR J. Dental Med. Sci.* 2014;13:16-22. https://www.academia.edu/download/38320090/IOSR_PAPER6.pdf.

17. Hitokoto S, Morozumi S, Wauke T, Sakai S, Kurata H. Fungal contamination and mycotoxins detection of powdered herbal drugs. *Applied Environmental Microbiology*, 1978; 36, 252–256.
18. Eze-Stephen PE, Udeozo IP, Chidiebere EU, Emmanuel O, Chigozie OE. Anti-Lipidaemic Effect of Kola Pod Extract on Wistar Albino Rats Fed with High Fat Diet. *World Applied Sciences Journal*. 2014;31(9):1614-7. DOI: 10.5829/idosi.wasj.2014.31.09.83177 links/57120e9c08ae4ef74525f8ca/Anti-Lipidaemic-Effect-of-Kola-Pod-Extract-on-Wistar-Albino-Rats-Fed-with-High-Fat-Diet.pdf
19. World health organization. General guide lines for themethodologies on research and evaluation of traditional medicine, Geneva. 2000.
20. GyasiM, Darko T, Mensah M. Use of Traditional Medicine by HIV/AIDS Patients in Kumasi Metropolis, Ghana: A Cross - sectional Survey of *International Journal on ContemporaryResearch*, 2013; 3(4):117.
21. Gyasi M, Mensah M, Osei P, Adjei W, Agyemang S. Public Perceptions of the Role of Traditional Medicine in the Health Care Delivery System in Ghana. *Global Journal on Health Sciences*. 2011; 3(2):40-49.
22. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007; <http://www.who.int/mmedicinedocs/index/assoc/s14878e/s14878e.pdf>.