



PUBLIC HEALTH AND MICROBIOLOGICAL ANALYSIS OF HERBAL MIXTURES SOLD AT VARIOUS LOCATIONS OF OWERRI AND ITS METROPOLIS: A CASE STUDY OF COMMON PRACTICE AMONG THE POPULACE IN THE STUDY AREA

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ABSTRACT: *The use of herbal drugs has become a common practice among the populace in the study area. Its consumption rate is quite alarming in recent times; therefore, the knowledge of its public health importance and microbiological safety should be prioritized in all ramifications. This research was focused on assessing the microbial quality of different popular herbal preparations registered by the National Agency for Food and Drug Administration Control (NAFDAC) sold within the South-West and South-South regions of Nigeria. The microbial diversity of some herbal drugs were examined by aerobic plate count method and microbial isolates were identified based on their morphological, cultural and biochemical characteristics. A total of five (5) herbal samples from six (6) herbal products (Goko cleanser, Gogorigo, Boom, 7 keys) were analyzed and all were found to be free from pathogenic bacteria and indicator organisms of fecal contamination. However, "the presence of microbial contaminants in non-sterile pharmaceutical products in some herbal mixtures (Y-herbal mixtures) can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients taking the medicine." The possible contaminated organisms isolated from these products are *E. coli*, *Staphylococcus aureus*, *Bacillus sp.*, *Aspergillus sp.*, *Proteus sp.*, *Rhizopus sp.*, penicillin. And the organisms isolated are the same with the results from other researchers that work on isolating microorganisms from herbal mixtures.*

KEYWORDS: Public health, microbiological analysis, herbal mixtures.



INTRODUCTION

Since ancient times, herbal medicines have been used by many different countries throughout the world to treat illnesses. The use of herbal medicines in human health care has developed substantially in both developed and developing countries and it is continually expanding. About 60-80 % of the population of every country of the developing world rely on herbal or indigenous forms of medicine. The reasons for the patronage of herbal medicine are the high cost of very effective antibiotics and the problem of antibiotic resistance which is very common in developing countries (Hack et al., 2005). The World Health Organization (WHO, 1998) survey indicated that about 70-80 % of the world's population, particularly in developing countries, rely on non-conventional medicines mainly of herbal origins for their primary health care.

The use of herbal medicine has always been part of human culture, as some plants possess important therapeutic properties, which can be used to cure human and other animal diseases (Oyetayo, 2008). The use of these herbal remedies have increased significantly in the last two to three decades in Nigeria. This has led to the production of herbal products with bogus claims that it can cure all forms of ailments. With the ever increasing use of herbal medicines and the global expansion of the herbal medicines market, safety has become a concern for both health authorities and the public at large.

efficacy and quality of these medicines have been an important concern for health authorities and health professionals (Lau et al., 2003)..

Herbal product purchased was analyzed by isolating and identifying contaminants.

Regarding the microbial properties of some liquid herbal infective drugs produced and marketed in Owerri, Imo State, Nigeria, the level of contamination was estimated and also identified, while isolation of pure culture was done based on morphological difference where elevation forms, pigmentation and size were the major distinguishing factors for the bacterial contamination. The aim of this study is to culture some selected herbal mixtures sold by supermarkets, clinics, chemist, supermarket and streets in Owerri Metropolis for microbial contamination.

LITERATURE REVIEW

MICROORGANISMS ISOLATED FROM HERBAL PREPARATIONS

Herbal medicine is an integral part of 'traditional medicine,' and traditional medicine has a broad range of characteristics and elements which earned it the working definition from the World Health Organization (Oreagba et al., 2011). Traditional medicine includes diverse health practices, approaches, knowledge and benefits that incorporate plant, animal or mineral based medicines, spiritual therapies, manual techniques, and exercises which are applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (WHO,2008). Traditional herbalists use various herbal preparations to treat various types of ailments including diarrhea, urinary tract infection, typhoid fever and skin diseases (Sofawora, 1993). Alternative medicine, such as herbal medicine, is now gaining popularity especially



because of the typically low side effects (Wilt et al., 2000), low cost (Vander Hoof, 2001), and high level of acceptance by patients (Ujam et al., 2013).

The growing, harvesting and manipulation methods usually applied cannot avoid microbial contamination of the plant materials which therefore reflects the environmental conditions as well as the specific hygiene during the diverse treatment (Kneifel et al., 2012). Raw materials are most often degraded by microorganisms before harvesting, during handling and after prolonged storage (Matthew, 1995; Kenneth, 1989). The presence of large numbers of microbes can be harmful for human consumption. Herbal medications are likely to be contaminated with a wide variety of other potentially pathogenic bacteria. In the scientific study conducted in Dares, Salam Tanzania, it was observed that liquid and powdered herbal drugs had high levels of bacterial contamination (Justin et al., 1998). Also, in a study evaluated, the bacterial contamination of herbal medicinal preparation sourced from identified herbal remedies were contaminated with *Salmonella typhi* and *Shigella* sp., besides *Escherichia coli* and *Staphylococcus aureus* (Abba et al., 2009). Isolation of a gram negative *P. aeruginosa* from herbal materials raises deep health concerns. Edaphic factors are the probable source of the isolate as the bacteria is primarily a soil bacterium, reflecting poor harvesting and cleaning of herbal materials. *Salmonella* species detected in some samples are causative agents of various infections like salmonella food poisoning which is a major problem in the world (Greenwood et al., 1997). The presence of the fungal contaminant shows the possibility of poor storage conditions. This is a serious contaminant since some common species of fungi produce toxins like aflatoxins. According to the WHO (Greenwood et al., 1997), aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amounts. The limits of microbial contamination are total aerobic bacteria 10⁵ CFU/g, Yeast and Mould are 10³ CFU/g (WHO 2000). However, none of the herbal suspensions exceeded the recommended total aerobic counts. The absence of contaminants may be due to hygienic packing or presence of bactericidal or bacteriostatic substances that would have killed possible microbial contaminants. Investigation of possible antimicrobial adulterants in the herbal suspensions is suggested.

sanitary conditions from the point of collection of the herbs to processing and finally to the point of preparation (Kasilo & Trapsida, 2011) Therefore, the quality and safety of herbal preparations are of great concern because quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of utmost importance and must therefore be investigated (Zhang, 2003).

In developing countries, herbal extracts are recognized as pharmaceutical medicine and are used as complementary medicine, and without adequate supervision, they are released directly into the market. Hence, high microbial contamination may occur in these products (Ali et al., 2005; Sharafati et al., 2011).

In Nigeria, there appears to be an overwhelming increase in public awareness and usage of herbal medicinal products in the treatment and prevention of diseases. This may not be unconnected to the active mass media advertisements embarked upon by the producers and marketers of the herbal medicinal products who have taken advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of the people, particularly in the rural areas, and the reservations by the public due to prevalence of fake, substandard or counterfeit drugs in the market. These have



placed the herbal medicinal products as a ready alternative to conventional dosage forms in the treatment of infections and diseases. .

world has been reported. Microbial loads of some medicinal plants sold in local markets of Benin, Nigeria reported the presence of *P. aeruginosa* and *B. subtilis* among others (Idu et al., 2011). In Kaduna, Nigeria, studies indicated the presence of pathogenic *Salmonella typhi* in 65.7% of herbal products analyzed and *E. coli* in 58.7% of the samples analyzed (Abbha et al., 2009). The evaluation of microbial quality of plant materials in Belgrade indicated the presence of *E. coli*, *Bacillus* and *Clostridia* species (Stevic et al., 2012). When the evaluation of microbial and fungal contaminations of herbal products was carried out in Ghana (Ahene et al., 2011), aflatoxins, nitrobenzene and *P. aeruginosa* among others were found to be present. In South Africa, studies have shown that herbal products are heavily contaminated with bacteria (Adeleye et al., 2005). Also, in a research conducted in Bangladesh to assess the pathogenic proliferation in the locally available commercial herbal medicine, the pathogenic load was compared with the microbiological standard given by the British pharmacopoeia. Out of 85 oral liquid samples, 2 were found to be highly contaminated with total aerobic bacteria and fungi, and some of the samples showed coliforms but none of the samples was contaminated with *Salmonella spp* and *Shigella spp* but with bacteria and fungi in some of the samples, and this suggests the fact that aseptic handling is necessary during the processing of oral herbal medicines. The increasing widespread use of traditional medicine has prompted the W.H.O to promote the integration of traditional medicine and complementary or alternative medicine into the national healthcare system of some countries, and to encourage the development of national policy and regulation as essential indicators of the level of

herbal medicine in the 1800's. The national association of medicinal herbalists was founded to help promote and defend the practice of herbal medicine. In 1941, the Pharmacy and Medicine Act was passed, stripping herbal practitioners of the right to dispense medicinal herbs. In 1968, the Pharmacy Act was passed and restored practitioners' right to dispense medicinal herbs. The British Herbal Medicine Association was also founded and published the British Herbal Pharmacopoeia

In 2000, the British government decreed that herbal medicine should undergo the same testing as conventional drugs. These herbal medicines would then be licensed.

SOME HERBAL MEDICINES IN USE IN NIGERIA AND THEIR APPLICATIONS

Few herbal remedies have conclusively demonstrated any positive effect on humans, possibly due to inadequate testing (Ernst, 2007). Many of the studies cited refer to animal model investigations or in-vitro assays and therefore cannot provide more than weak supportive evidence. However, examples of medicinal plants in use in other parts of the world and in Nigeria in particular that have demonstrable some interesting pharmacological results include Aloe Vera. It is traditionally used for the healing of burns and wounds (Maenthaisong et al., 2007). A systematic review (from 1999) states that the efficacy of aloe vera in promoting wound healing is unclear, while a later review (from 2007) concludes that the cumulative evidence supports the use of aloe vera for the healing of first to second degree burns (Ernst 2007; Vogler & Ernst, 1999). Boophone (*Boophone disticha*)—this highly toxic



plant has been used in South African traditional medicine for treatment of mental illness (Stafford et al., 2008). Research demonstrated in vitro and in vivo effects against depression (Pedersen et al., 2008; Sandager et al., 2005, Neergaard et al., 2009). Alligator pepper, Aframomism melegueta. K. Schum (Zingiberaceae) (Local names: Yoruba - oburo ata. Ata ire; Urhobo - Erhie; Hausa - chitta, gyan'dammar yaji): The fruits, seeds, leaves are used as stimulants, and as remedy against cold. Calendula (*Calendula officinalis*) is used traditionally for abdominal cramps and constipation (G. Gordon, 1998). In animal research, an aqueous-ethanol extract of *Calendula officinalis* flowers was shown to have both spasmolytic and spasmogenic effects, thus providing a scientific rationale for this traditional use (Bashir et al., 2006). Goat weed (*Ageratum conyzoides* L (Compositae) (Local names: Yoruba - ime-esu, imi-ewure; Ibo - akwukwo-nwa osi nake, Urhobo - Ikpamak). The whole plant leaves and seeds are used in herbal formula. The juice from fresh plants is used for dressing wounds, ulcers, and craw craw and as a remedy for inflammation. A decoction of the root is a remedy for abdominal pains and the raw root is chewed for digestive disorders. Garlic (*Allium sativum*) L. Liliaceae, Hausa - Tafarnwa, the bulbs and leaves parts are used in ethno medicine. It has diuretic properties and is given in fevers, coughs, flatulence, disorders of the nervous system. It has been used as a remedy for asthma and hoarseness of the chest. The bulb juice is used as a broad spectrum antibiotic against fungi and bacteria. It may also lower total cholesterol levels (Ackerman et al., 2001).

Honey may reduce cholesterol and wound healing (Al Walili, 2004). Lemon grass (*Cymbopogon citratus*) (Local name: Isoko - eghu): When administered daily, the aqueous extract of the fresh leaf lowers total cholesterol and fasting plasma glucose levels in rats, as well as increasing HDL cholesterol levels. Lemongrass administration had no effect on triglyceride levels (Adeneye & Agbaje, 2007). Morinda citrifolia (noni) is used in the Pacific and Caribbean islands for the treatment of inflammation and pain (Pande et al., 2005). Human studies indicate potential cancer preventive effects (Wang et al., 2009). Black cumin (*Nigella sativa*) has demonstrated analgesic properties in mice. The mechanism for this effect, however, is unclear. In vitro studies support antibacterial, antifungal, anticancer, anti-inflammatory and immune modulating effects (Hajhashemi et al., 2004). Pawpaw (*Carica papaya* L. Caricaceae) (Local name: Hausa - gwanda, Ibo - aokwulu ezi, Yoruba - Ibepe, sigun, gbegbere) is used as insecticide, use for wound dressing (Regnault et al., 2004). Peppermint oil is used in Nigerian ethno medicine as a remedy against irritable bowel syndrome (Capello *et al.*, 2007). Pomegranate contains the highest percentage of ellagitannins of any commonly consumed juice. Punicalagin, an ellagitannin unique to pomegranate, is the highest molecular weight polyphenol known. Ellagitannins are metabolized into urolithins by gut flora, and have been shown to inhibit cancer cell growth in mice (Heber, 2008). Rauwolfia serpentina predisposes to a high risk of toxicity if improperly used extensively for sleeplessness, anxiety and high blood pressure, and has been widely used in Nigeria in the management of psychiatric problems.

Rose hips - Small scale studies indicate that hips from *Rosa canina* may provide benefits in the treatment of osteoarthritis. Saw Palmetto can be used for (high blood pressure) BPH. The fatsoluble extract of this berry has become a leading natural treatment for BPH. This extract when used regularly has been shown to help keep symptoms of BPH in check (Schneider et al., 1995). Shiitake mushrooms (*Lentinus edodes*) are edible mushrooms that have been reported to have health benefits, including cancer preventing properties (Fang et al., 2006). In laboratory research, Shiitake extracts inhibit the growth of tumor cells through induction of apoptosis. Both a water extract and fresh juice of Shiitake have demonstrated antimicrobial activity



against pathogenic bacteria and fungi (Hearst et al., 2009). St. John's wort has yielded positive results, proving more effective than a placebo for the treatment of mild to moderate depression in some clinical trials (Kuznetsov et al., 2005). A subsequent, large, controlled trial, however, found St. John's wort to be no better than a placebo in treating depression (Gaster & Holroyd, 2000). However, more recent trials have shown positive results (Davidson or positive trends that failed significance). A 2004 meta-analysis concluded that the positive results can be explained by publication bias but later analyses have been more favorable. The Cochrane Database cautions that the data on St. John's wort for depression are conflicting and ambiguous. Stinging nettle in some clinical studies is effective for benign prostatic hyperplasia and the pain associated with osteoarthritis. In-vitro tests show anti-inflammatory action. In a rodent model, stinging nettle reduced LDL cholesterol and total cholesterol. In another rodent study it reduced platelet aggregation. Umckaloabo (*Pelargonium sidoides*): an extract of this plant showed efficacy in the treatment of acute bronchitis in a controlled trial and is approved for this use in Germany. Willow bark (*Salix alba*) can be used for a variety of anti-inflammatory and antimicrobial purposes due to the presence of salicylic acid and tannins. It has been in use for approximately 6000 yrs and was described in the 1st century AD by Dioscorides (Mahdi et al., 2006). Cam wood *Baphianilida Latal* (*Papilionaceae*) (Local name: Yoruba - owiwi, irosun, Hausa - majiga, Urhobo - orhua). In Urhobo land, a paste of its leaves is applied to the lower portion of the abdomen of pregnant women to prevent miscarriage. Bitter leaf (*Vernonia amygdalina*, L (*Compositae*) (Local name: Yoruba - ewuro, Hausa - Shiwaka, Urhobo - Olugbo). A decoction of the leaves is used for stomach pains, and also used for skin infections as an antipyretic, laxative and anti diabetic.

Ginkgo (*Ginkgo biloba*) has been used in traditional medicine to treat circulatory disorders and enhance memory. Although not all studies agree, ginkgo may be especially effective in treating dementia (including Alzheimer's disease) and intermittent claudication (poor circulation in the legs). It also shows promise for enhancing memory in older adults. Laboratory studies have shown that ginkgo improves blood circulation by dilating blood vessels and reducing the stickiness of increasing availability and affordability of traditional medicines.

Integrating relevant traditional medicine with the national health care system by developing and implementing national medicine policies and programmes.

- Promoting the safety, efficacy of traditional medicine therapy as providing guidance and regulating and quality assurance standards.
- Promoting sound traditional medicine by providers and consumers.
- Documenting traditional medicine and remedies.

COMMON MICROBIAL CONTAMINANTS ASSOCIATED WITH MEDICINAL PLANTS USED IN PRODUCING HERBAL MEDICINE

From studies conducted, it is evident that herbal medicine without control or regulations can be contaminated with microorganisms, which are potential pathogens and hence pose a threat to patients (Gansanjo, 2011). Herbal preparations in developing countries are produced through unhygienic conditions. Many contaminants and residues that may cause harm have been reported. Many are natural such as naturally occurring radioxides, toxic metals, bacteria and fungi. The growing, harvesting and manipulation methods usually applied cannot avoid microbial contamination of the plant material which therefore reflects the environmental



conditions as well as the specific hygiene during the diverse treatments (Kneifel et al., 2002). Biological contamination refers to impurities in medicinal herbs, their preparations and products, and may involve living microbes such as bacteria and their spores, yeasts and moulds, viruses, protozoa, insects (their eggs and larvae), and other organisms. However, products of microbial metabolism such as toxins and low-molecular-weight metabolites from moulds are important chemical contaminants (Kosalec et al., 2009). The main microbial contaminations of plant materials used to prepare herbal medicine in general are attributed to total aerobic mesophilic Enterobacteria, yeasts and moulds (Kneifel et al., 2002). The presence of higher numbers of spores of bacteria could be explained by the fact that some of these organisms (e.g., *Bacillus* and *Clostridium* sp.) produce spores which are resistant to harsh processing, elevated heat and dry conditions. Therefore, they can survive for a long time on the product in a dormant state. *Bacillus cereus* and *Clostridium perfringens* are recognized as having potential pathogenicity and have been incriminated in food poisoning (Kunene et al., 1999). Although bacterial endospores and fungal spores can be regarded as the two dominating groups of contaminants associated with medicinal plants, a broad diversity of bacterial, fungal cells and viruses can be found either in or on the plant material (Kneifel et al., 2002). *B. cereus* and *C. perfringens* were isolated from chamomile and other herbs (Martins et al., 2001). Although Enterobacteria can be found in nature, this family possesses some indicative value towards faecal contamination.

The presence of Enterobacteria and *Escherichia coli* reflect the situation regarding faecal contamination (Ezeh et al., 2001). Together with the group of coliforms, it can be taken as an indicator for undesirable hygiene conditions, although this conclusion has to be related to the magnitude of viable count measured (Kneifel et al., 2002). *Staphylococcus aureus* is not a common contaminant of this type of plant material and relatively rarely found. However, contamination could provide the amount of enterotoxin produced by *S. aureus*, depending on the specific nature of the individual (Kosalec et al., 2009). Herbal medications are likely to be contaminated with a wide variety of other potentially pathogenic bacteria. In a study which was evaluated, the bacterial contamination of powdered herbal medicinal preparations was sourced from identified herbal retail outlets in different parts of Kaduna, Nigeria. The results showed that a number of herbal remedies were contaminated with *Salmonella typhi* and *Shigella* spp., besides *E. coli* and *S. aureus* (Abba et al., 2009). In addition, the presence of pathogenic bacteria like *Bacillus cereus*, *Aeromonas hydrophila*, *Shigella* sp., *Enterobacter agglomerans*, *E. cloacae*, *Vibrio fluvialis*, *Pasteurella multocida*, *S. epidermidis*, *Acinetobacter lwoffii*, *Klebsiella* sp., *B. subtilis*, *Pseudomonas aeruginosa*, and fungi *Rhizopus stolonifer* were also observed to be present in plant samples analyzed recently (Alwakeel, 2008; Idu et al., 2011). Because they are widespread in the atmosphere, moulds are common natural contaminants of medicinal herbs. It is known that, under favourable conditions, some fungi can synthesize toxic metabolites (mycotoxins). Among the known mycotoxins, the most toxic one is aflatoxin synthesised by species of *A. flavus* and *A. parasiticus*, and a minor number of other fungi (Kulshrestha et al., 2011). Contamination by *A. flavus*, the most famous aflatoxin producer, is common in medicinal plants and herbal tea (Halt, 1998). *Aspergillus flavus* colonization does not necessarily reduce yield, but causes economic losses by contaminating with aflatoxin (Amaike et al., 2011). In a study of 91 medicinal.

DECONTAMINATION OF PLANT MATERIALS USED IN HERBAL MEDICINE

Attempts have always been made to decontaminate and preserve these medicinal plants so as to get safer, natural and potent medicines. The number of methods has been tried for



decontamination, such as heat treatment, UV irradiation and fumigation. However, volatility and heat sensitivity of the delicate flavour and aroma components of the medicinal plants do not permit the use of heat treatment (Gupta et al., 2011). Low penetration power of UV radiations makes this irradiation method unsuitable (Gupta et al., 2011). Fumigation with gaseous ethylene oxide brings down the microbial burden but this method is now prohibited or restricted in many countries due to the carcinogenic nature of one of its residues in treated medicinal plants (Kim et al., 2000; Satomi et al., 2005). Various disinfectant technologies have been suggested, which include electromagnetic radiations, photodynamic pulsing, ultra high pressure and carbon-dioxides (CO₂) treatment (Gupta et al., 2011). Gamma irradiation is now getting recognition throughout the world as a phytosanitary treatment of herbal materials. It improves the hygienic quality of various herbal materials and reduces the losses due to microbial contamination and insect damage (Farkas, 1998). Besides, it is a fast, safe, convenient, eco-friendly method which reduces the reliance on chemical fumigants and preservatives currently used by industries. The chances of recontamination are also reduced as it can be done after packaging (Some studies showed that the exposure of plant samples to different doses of gamma radiation can result in reduction in total bacterial counts, and this also indicated that the microbial load could be decreased by increasing the radiation-absorbed dose. These studies indicate that gamma irradiation is an effective treatment for microbial decontamination of medicinal plants (Cupt et al., 2011; Khattak, 2012; Aquino et al., 2010). Certain plants contain natural barriers and antimicrobial substances which exert typical inhibitory effects on microbial growth and stability. It has been estimated that around 1400 herbs and spices may possess antimicrobial agents of different chemical nature as oils, peptides, liquid and organic extracts (Kneifel et al., 2002). Some medicinal herbs contain essential oils which act as natural antimicrobials and may inhibit mould development and mycotoxin production (Kosalee et al., 2009). Different studies have demonstrated the effectiveness of antimicrobials and their effective compounds to control or inhibit the growth of pathogenic and spoilage microorganisms (Negi, 2012; Wang et al., 2009).

PUBLIC HEALTH IMPORTANCE OF HERBAL MEDICINES

Herbal medicine is practiced today in countries around the world. Each government determines the extent to which practitioners, in Europe for example, practice if a medical consultation is given the herb which Herbs are purchased across the countries, and they are considered herbal supplements in the United States in which herbalists cannot prescribe drugs legally unless they also happen to be medical doctors; herbs can only be sold as herbal supplements, and these supplements can be found in stores locally and online. The FDA (Federal drug agency) does not approve most of these herbs because they are not considered as medication. Currently, there is an ongoing dispute between certified medical professionals and herbalists. Some people believe that herbal medicine is outdated and those who practice it should be shut down; other people believe that it may be healthier than synthetic drugs. In either case, there are people trying to meet the two camps in the middle – the doctors of naturopathy. Hopefully there will be a meeting of the treatment program of patients who do not respond well to traditional medical procedures. After all, many drugs used by respected members of the medical community are created from plants. Presently, companies selling herbal supplements are experiencing a boom in sales. People are trying to live healthier lives and eliminate chemicals from their diet. The only question is whether or not herbalists will finally be treated as respected members of their community or forced out of business by conventional medical practitioners (Charlotte, 2009).



Modern medical science certainly comes with a high price tag, and pharmaceuticals are no exception. One reason why herbal medicine is becoming more popular recently is because people simply cannot afford to pay for their medication month after month. A systematic review published in evidence based complementary and alternative medicine evaluated whether or not natural health products provide a cost effective choice in the treatment of diseases. Researchers found that natural health products show evidence of cost effectiveness in other areas of modern medicine, but the preliminary data suggests that herbal products are more affordable than pharmaceuticals. Herbal products, such as herbal extracts, essential oils and herbal teas are available in most healthy foods and even grocery stores, so you do not have to see doctors to get prescriptions before purchasing them. This certainly makes it easier to obtain herbal products and avoid additional health care costs. Herbs are classified as dietary supplements, so they can be produced, sold and marketed without going through the FDA; this makes it easier to purchase and use these beneficial products. It is our job as consumers to choose among the competitors and make sure to read the ingredients and labels carefully before using a reputable and trustworthy company that verifies the product is 100% pure grade.

Herbs are used for the treatment of chronic and acute conditions and various ailments, including major health concerns like cardiovascular diseases, prostate problems, depression, inflammation and weakened immune system. Herbs are used around the world to treat conditions and diseases, and many studies prove their efficacy. Regarding the 177 drugs approved worldwide for the treatment of cancer, more than 70% are based on natural products or chemical limitations of natural products. There are numerous advantages of f herbal medicine; anyone considering using herbal medicine to treat health conditions should speak with qualified health professional. Most herbal medicines are well tolerated by patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects compared to traditional medicine, and may be safer to use over time. Herbal medicine tends to be more effective for long standing health complaints that do not respond well to herbal One example is the herbs and alternative remedies used to treat arthritis (vioxx); a well-known prescription drug used to treat arthritis was recalled due to increased risk of cardiovascular complications. Alternative treatments for arthritis, on the other hand, have few side effects. Such treatment includes dietary changes like adding simple herbs, eliminating vegetables from the nightshade family and reducing white sugar consumption. Another advantage of herbal medicine is cost. Herbs cost much less than prescription medications: research, testing, and marketing and even the cost of prescribing medicines. Herbs tend to be inexpensive compared to drugs. Yet, another advantage of herbal medicine is availability. Herbs are available without a prescription; you can grow some simple herbs, such as peppermint and chamomile, at home in some remote parts of the world; herbs may be the only treatment available to the majority of people (Kathleen, 2016). Herbs are not without disadvantages and herbal medicine is not appropriates in all situations; there are few disadvantages to consider. Modern medicine treats sudden and serious illnesses and accidents much more effectively than herbal or alternative treatments. An herbalist would not be able to treat serious trauma, such as a broken legs nor would he be able to heal appendicitis or a heart attack as effectively as a conventional doctor using modern diagnostic tests, surgery and drugs. Another disadvantage of herbal medicine is the very real risk of doing yourself harm through self-dosing with herbs. While you can argue that the same thing can happen with medication, such as accidentally overdosing on cold remedies, many herbs do not come with instructions or package inserts. There is a very real risk of overdosing. Harvesting herbs in the wild is risky, if not foolhardy; yet some people try to identify and pick wild herbs. They run a very real risk of poisoning themselves if they do



not correctly identify the herb, or if they use the wrong part of the plant. Herbal treatment can interact with medication; nearly all herbs come with some warming, such as valerian and St. Johnswort, and can interact with prescription medication like antidepressants. It is important to discuss your medication and herbal supplements with your doctor to avoid dangerous interactions. Because herbal products are not tightly regulated, consumers also run the risk of buying inferior quality herbs; the quality of herbal products may vary among batches, brands or manufacturers. This can make it much more difficult to prescribe the proper dose of an herb (Kathleen, 2016).

Herbal drugs appear relatively safe but there is limited human research or prospective data concerning the adverse effects and herbal drug interactions. They are however less potent than their pure drug equivalent because they contain a mixture of many photochemicals in small quantities even so herbal products are to be totally free of risk and therefore it is necessary to see them with discretion.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING

A total of 5 different herbal preparations was purchased randomly from identified herbal shops and retail outlets in Douglas and Relief Market of Owerri metropolis. Packaged herbal samples were collected and taken to the laboratory, while those that were not packaged (such as herbal preparations sold by local herbalists) were collected in sterile polythene bags. All the samples collected from the sites were analyzed in the laboratories of the Department of Microbiology, Faculty of Biological Science, Imo State University, Owerri, Imo State Nigeria.

PREPARATION OF MEDIA

The media used for the work will be nutrient agar, blood agar, MacConkey agar, and Sabouraud Dextrose agar for the isolation of bacteria and fungi respectively. All the media were prepared according to the manufacturer's instructions.

INOCULATION OF THE MEDIA FOR BACTERIAL ISOLATION

The herbal mixtures were mixed and inoculated into a culture plate, with the help of a loop. Also, 2 ml of the samples will be added to 5 ml of peptone water. They will be incubated at 37°C for 24 hours (overnight). The peptone water culture will be subcultured onto the solid nutrient agar media and incubated at 37°C for 48 hours.

INOCULATION OF THE MEDIA FOR FUNGI ISOLATION

The herbal mixtures were inoculated into tubes containing potato dextrose agar with the help of sterile swab sticks and straight wire loop. The tubes were incubated at room temperature for seven (7) days.

IDENTIFICATION OF FUNGI ISOLATES

The growth pattern, pigmentation and size of colonies were recorded at the incubation period to aid the identification of the organisms.



USE SLIDE CULTURE METHOD

The isolate was identified using cultural characteristics and morphology. With the help of sterile petri dishes, sterile filter paper was placed in each of the petri dishes and 1 ml of distilled water was added into the petri dish as sterile U shape glass rod was placed in each petri dish. With the help of an inoculating needle, a cube like shape of already prepared SDA was cut. Four days to 1 week fungal growth was smeared by the four sides of the SDA using a sterile wire loop, and a sterile cover slip was placed on the inoculums in the petri dish. The plate was covered and kept at room temperature for 4 to 7 days as growth was observed daily before examination.

CELL MORPHOLOGY OF FUNGI ISOLATE

A drop of lactophenol (LP) was placed on a clean microscopic slide. The cover slip from the glass culture was gently removed and placed in the drop of lactophenol (LP), and also a drop of LP was dropped on the slide from the old culture as the media cultured on the slide was gently removed. A sterile cover slip was placed on the slide and observed microscopically. It was first viewed at X10 to focus the lens well, and then X40 to get a clearer view.

EXAMINATION OF THE PLATE CULTURES

After the incubation, the plate was read and the colonial morphology of the isolates was described. The tubes for fungal cultures were also examined for growth and description of the fungal isolates.

BIOCHEMICAL TESTS

Identification test was carried out according to the method of cow and steel. The isolates first underwent gram staining and various biochemical tests from the key cited by Aneja, K.R. from the Bergey's Manual of determinative bacteriology; the tests performed were: Oxidase test, Catalase test, Citrate utilization test, Indole test, Coagulase test, Motility test, and Urease test.

METHOD OF GRAM STAINING

The slide was placed with a heat fixed smear on the staining tray. The smear was gently flooded with crystal violet and was left for 1 minute. The slide was slightly tilted and gently rinsed with tap water or distilled water using a wash bottle. The smear was gently flooded with gram's iodine and left standing for 1 minute. The slide was slightly and gently rinsed with tap water or distilled water using a wash bottle. The smear showed a purple circle on the slide. It was decolorized using 95% ethyl alcohol or acetone. The slide was tilted slightly and the alcohol was applied drop by drop for 5-10 seconds until the alcohol ran almost clear. We were careful not to over-decolorize. It was immediately rinsed with water and then gently flooded with safranin to counter-stain and made to stand for 30 seconds. The slide was tilted slightly and gently rinsed with tap water or distilled water using a wash bottle. The slide was blot-dried with paper.

The smear was viewed using a light microscope under oil immersion.



RESULT

Isolation process is a procedure of separating the mixture of colonies to a single colony. This process was done by using streaking method to obtain pure culture according to the morphological presentation of microorganisms on petri dishes containing Nutrient agar, Macconkey Agar and SDA are circular, irregular, punctiform, smooth, raised, the colonies were small and big and creamy in colour. The morphological presentation of microorganisms on petri dishes containing blood agar are circular, irregular, smooth, and raised; colony size, big and small and colonies are pink in colour. The morphological presentations of microorganisms on agar slant containing potatoes dextrose agar are pigmentation, optical characteristics, texture of cultures, amount of growth which is slight, moderate and large and its form which are filiform (thread like), echinulate (pointed out growth), beaded (like bead), effuse (thin spread surface), arborescent (branched tree like growth), rhizoid (root like growth). Gram staining was conducted to identify if these organisms are gram positive or negative; the shapes are bacillus and cocci and arrangements; they are chain, single and pairs.

TABLE 1: Colonies Morphology of the Microorganism Isolated on

Nutrient Agar

ISOLATE	SHAPE	ELEVATION	SURFACE	SIZE	PIGMENTATION
A	CIRCULAR	ENTIRE	RAISED	SMALL	MILKISH
B	CIRCULAR	ENTIRE	RAISED	SMALL	MILKISH
C	IRREGULAR	ENTIRE	RAISED	LARGE	MILKISH
D	CIRCULAR	ENTIRE	RAISED	PINPOINT	MILKISH
E	IRREGULAR	ENTIRE	RAISED	LARGE	MILKISH

TABLE 2: Colonies Morphology of the Organism Isolated on Mackonkey Agar

ISOLATE	SHAPE	ELEVATION	SURFACE	SIZE	PIGMENTATION
A	CIRCULAR	ENTIRE	RAISED	SMALL	MILKY LACTOSE
B	CIRCULAR	ENTIRE	FLAT	PINPOINT	FERMENTER
C	CIRCULAR	ENTIRE	FLAT	SMALL	MILKY
D	CIRCULAR	ENTIRE	FLAT	SMALL	LACTOSE FERMENTER
E	CIRCULAR	ENTIRE	RAISED	SMALL	MILKY

**TABLE 3: Colonies Morphology of the Organism Isolated on Salmonella Shigella Agar**

ISOLATE	SHAPE	ELEVATION	SURFACE	SIZE	PIGMENTATION
A	CIRCULAR	ENTIRE	RAISED	SMALL	COLOURLESS
B	CIRCULAR	ENTIRE	FLAT	PINPOINT	PINK-RED
C	CIRCULAR	ENTIRE	FLAT	SMALL	BLACK-CENTERED
D	CIRCULAR	ENTIRE	FLAT	SMALL	COLOURLESS
E	CIRCULAR	ENTIRE	RAISED	SMALL	COLOURLESS

Note: Gram-negative bacilli ferment lactose produces pink to red colonies, lactose-non fermenters such as *Salmonella and Shigella* form transparent colourless colonies while organisms which produce H₂S form black-centered colonies like some strains of *Salmonella*.

Table 4 shows the total heterotrophic bacteria count obtained from herbal mixtures:

The Total heterotrophic bacteria count obtained ranged from 2.3×10^5 - 2.9×10^5 . Sample G has the highest count of 2.9×10^5 while Sample H has the least count of 2.9×10^5 .

SAMPLE CODE	DILUTION FACTOR	VOLUME OF INOCULUM	COLONIES COUNT	CFU/ML
A	1.0	10-6	240	2.4×10^5
B	1.0	10-6	270	2.7×10^5
C	1.0	10-6	250	2.5×10^5
D	1.0	10-6	250	2.5×10^5
E	1.0	10-6	280	2.8×10^5

Table 5 below shows the total coliform count obtained from herbal mixtures. The total coliform count obtained ranged from 1.5×10^5 - 2.9×10^5 . Sample A has the highest count of 2.9×10^5 while Sample D has the least count of 2.9×10^5 . **Total 5: Coliform count obtained from herbal mixtures.**

SAMPLE CODE	DILUTION FACTOR	VOLUME OF INOCULUM	COLONIES COUNT	CFU/ML
A	1.0	10-6	290	2.9×10^5
B	1.0	10-6	250	2.5×10^5
C	1.0	10-6	190	1.9×10^5
D	1.0	10-6	150	1.5×10^5
E	1.0	10-6	280	2.8×10^5



The table below shows the Total Salmonella Shigella Count obtained from samples of herbal mixtures. The Total Coliform Count obtained ranges from 1.5×10^5 - 2.9×10^5 . Sample A has the highest count of 2.9×10^5 while Sample D has the least count of 1.5×10^5 .

TABLE 6: Salmonella Shigella Count Obtained from Samples

SAMPLE CODE	DILUTION FACTOR	VOLUME OF INOCULUM	COLONIES COUNT	CFU/ML
A	1.0	10-6	250	2.5×10^5
B	1.0	10-6	240	2.4×10^5
C	1.0	10-6	190	1.9×10^5
D	1.0	10-6	150	1.5×10^5
E	1.0	10-6	220	2.2×10^5

TABLE 7: Biochemical Properties of Isolated Bacteria from Samples Herbal Mixtures

Isolates	Morphological appearances	Bacteriological Tests			Biochemical test										Probable organism
		Gram reaction	Cellular mobility	Motility test	Catalase test	Citrate Test	Indole Test	Oxidase test	Coagulase test	Voges Proskue	Methyl red Test	Glucose test	Lactose test	Sucrose Test	
1	Creamy, pin size, circular, entire, transparent and flat.	+	Cocci	-	+	+	-	-	+	+	+	+	+	+	<i>Staphylococcus spp.</i>
2	Pinkish red, entire, raised, opaque and small.	-	Rod	+	+	-	+	-	-	-	+	+	+	+	<i>Escherichia coli</i>
3	Creamy, circular, entire, raised, opaque and small.	+	Cocci	-	-	-	-	-	-	-	-	+	+	+	<i>Streptococcus spp.</i>



4	Creamy, pin size, slightly raised, circular, entire and translucent.	+	Rod	+	+	+	-	-	-	+	-	+	-	+	<i>Bacillus spp.</i>
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DISCUSSION

The aim of this study is to isolate and identify microorganisms from herbal mixtures sold in Owerri, South east of Nigeria. Five samples of herbal mixture were selected at random from the herbal shop in Imo State metropolis and were analyzed in the microbiology laboratory, Imo State University. And all the herbal medicine was sparingly contaminated. For the Goko cleanser, the mixture is a good combination of some tropical quality herbs formulated to enhance body energy, vitality and youth fitness. It also removes harmful toxins from the body systems, thereby reducing accumulated fat and cholesterol level, regulates monthly menstrual cycle, and prevents oedema and numbness. It regulates blood sugar level, pile, waist pain and also boosts the immune system. It also serves as a very good prevention for typhoid fever by removing the accumulated toxin from the body system. It then enhances appetite and restores the balance of the cell structure by correcting the metabolic disturbance. It eliminates worms.

Gogorigo Herbal Mixtures cures many ailments like:

1. For Belly Trouble: You take 1 spoon or 1/2 spoon and mix with warm water and drink or you may lick it.
2. For Chest Pain: Rub it on the Chest and lick some.
3. For Fresh Cut: Put into a cotton wool and band it.
4. For Smallpox: Mix with palm oil and rub on the body.
5. For Snake or Scorpion Bite: Give blade cut and rub.
6. For Cough: Mix with palm oil and sugar and then lick.
7. For Pile: Take 1 spoonful with hot water and drink.
8. For Fever: Take 1 spoonful with hot water and drink.
9. Dysentery: Take 1 spoonful with raw pap and drink.
10. Dog Bite or Nail Wound: Rub on the place and so on.
11. For Convulsion: Mix the same with kernel oil, rub it on the child and give him or her to lick.

However, the presence of microbial contaminants in non-sterile pharmaceutical products in some herbal mixtures can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients taking the medicine. This fact that is supported by the article review on “rapid monitoring of microbial contamination” by Nakayima et al. (2005),



Okunlola et al. (2007), The possible contaminated organisms were isolated from these products, are *E coli*, *Staphylococcus aureus*, *Bacillus* sp., *Aspergillus* sp., *Proteus* sp., *Rhizopus*, *penicillin*. And the organisms isolated are the same with other finding results from other researchers that work on isolating microorganisms from herbal mixtures. And these microorganisms may occur as a result of inadequate heat processing, improper handling of products and contamination of processing equipment, supported by the work of Frazier and Westhoff (2003). Biochemical analysis of the products showed that some herbal products had some pathogenic microorganisms which are very harmful and can bring toxins to the body, like *Aspergillus* for example; it is very harmful. Other findings have shown that apart from possible microbial degradation of the active constituents contained in the herbal preparations, the presence of these contaminating microorganisms could constitute a source of infection and serious health risk to the consumers of herbal preparations who were initially indicated (Mangram et al., 1999; Bowler et al., 2001).

CONCLUSION

Some herbal medicines bought from Owerri, Imo State, South East Nigeria showed significant microbial growth; some were contaminated with microorganisms, while some were not. Good manufacturing processes have not been applied to the manufacturing of the herbal mixtures, and also, the raw materials used for this production may have not been properly sterilized, which might lead to an increase in the microbial content, as well as poor handling in preparing and packaging this herbal product.

RECOMMENDATIONS

Herbal medicine practitioners should be educated on the need to produce good and sterile herbal products, thereby reducing the source of infection and serious health risk to the consumers taking it. Moreover, the regulatory agency, NAFDAC, should carry out more detailed and regular analyses on these herbal preparations to prevent the uninformed consumers from buying what may worsen their ailment. Validation of equipment cleaning procedures should be practiced in herbal products industries to prevent cross contamination of drug products. At every stage of processing, raw materials, intermediate or printing materials should be free from microbial and other contaminants.

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