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IMMUNOMODULATORY AND ANTIBACTERIAL EFFECTS OF MOMORDICA CHARANTIA AND OCIMUM GRATISSIMUMEXTRACTS ON ISOLATES FROM DIARRHOEIC PATIENTS

[1]Abolarin, Raphael Kayode, [2]Ajibola, Adebayo Thomas, & [3]Omodara, Anthony Mayowa

[1,2]Department of Medical Laboratory Science, College of Health Technology, Ijero Ekiti, Ekiti State
[3]Department of Basic Medical Science, College of Health Technology, Ijero Ekiti, Ekiti State

Abstracts

The assessed immunomodulatory and antibacterial effects of Momordica charantia and Ocimum gratissimum extracts on isolates from diarrheic patients. The ethanol and hot water extract of the leaf of Ocimum gratissimum and Momordica charantia were tested for antibacterial activity against four diarrhea-causing organisms which are Esherichia coli, Salmonella lyphi, Salmonella typhimurium and Vibrio cholerae. The immunomodulatory activities of the two extracts were carried out. The antibacterial activity was carried out using the agar well diffusion method. The result showed that the ethanol extract and hot water extract of Ocimum gratissimum and Momordica charantia had more inhibitory effect on Salmonella typhimurium and Vibrio cholerae at minimum inhibitory concentration of 50mg and 25mg respectively. The two extracts had little or no effect on Escherichia coli and Salmonella typhi. The phytochemical constituent of the extracts was determined qualitatively Flavonoid, alkanoid, saponin, tannin, phiobatarmin, steroid, terpenoid and cardiac glycosides were phytochemical components obtained from both plants. , while those present were analyzed quantitatively for the two extracts. The antioxidant properties such as total phenol, flavonoid, 2,2-dipheny- 1 -picrydraxyl, Iron Chelatoin and 2, 2-azino-bis (3-ethyibenzothiazoline-6-sulphonic acid) of the extracts were also determined. The two extracts were analyzed for acute toxicity of LD two extracts showed very low degree of toxicity at high dose level of 3200mg/kg of the extracts. Furthermore, the histopathological analysis of the liver, spleen and duodenum of the animal showed no damage to the organs. The immunomodulatory activities of the two extracts were carried out in-vivo by studying the health status of the experimental animal using hematological parameters of the experimental animals. The hematological status of the experimental animal on the two extracts caused significant changes in the total RBC, PCV, HB, total differential WBC when evaluated at 400mg, 800mg and 1600mg respectively. There was reduction in WBC count in the group treated with Momordica charantia extract and there were no significant changes in the RBC count. The Ocimum gratissimum caused significant changes ($p<0.0$ in the RBC value and significant increase ($p<0.05$) on the WBC count of the group treated with high dose of the extract, also there was increase in the value of count. While a reduction in the PCV value was observed. The study showed that the extracts have immunomodulatory and antibacterial activities confirming the use of the plants in ethno medicine.

Keyword: *Immunomodulatory, Antibacterial, Momordica Charantia, Ocimum Gratissimumextracts, Diarrhoeic Patients*

Introduction

The world is one full of microbes; the body temperature and nutrient provide an ideal home for these microorganisms to thrive. The human immune system is a remarkably sophisticated defense system which protects them from these invading agents. It is able to generate varieties of cells and molecules capable of recognizing and eliminating limitless varieties of foreign and undesirable agents.

Modulation of the immune system denotes to any change in the immune response that can involve induction, expression, amplification or inhibition of any part of phase of the immune response. Thus immunomodulator is a substance used for its effect in the immune system. There are generally two types immunomodulator based on their effects. They are immune suppressants and Immunostimulators. They have the ability to mount an immune response or defend against pathogens (Mahuddin & Shaikh 2010).

The potential uses of immune modulator in clinical medicine include the reconstitution of immune deficiency (treatment of AIDS) and the suppression of normal or excessive immune function. The treatment of graft rejection or auto immune diseases plants and their active components have been shown to be important sources of immune modulators and antimicrobial agents.(Mahuddin and Shaikh, 2010). Thus the development of drugs for immune modulation, antimicrobial and antitumor activity from natural compound has become an attractive project (Mahuddin and Shaikh 2010).

The use of plant products as immunostimulants has a traditional history and it is currently estimated that almost 50% of the synthetic medicines are derived from or patterned after phytochemicals. Plants synthesize chemicals as part of their defense against pathogens. Natural cumarinolignoids isolated from the seeds of cleome viscose have been recognized for their immune modulatory activity affecting both cell-mediated and humoral immune response (Marjorie, 1999). Some plant extracts for the same purpose has also been reported to show immune-modulatory effect such as barberrin, boswellic acid, flavonoids, alkanoid and they have also been found in vitro to have antimicrobial properties. However the use of plant extracts, as well as other alternative form of medical treatment is enjoying great popularity in the late 90s (Mariorie,1999).

An increasing number of people are adopting alternative system of medicine owing to the irreversible effect of modern drugs and therapies. The use of medicinal plant products for treatment of various acute and chronic diseases is gaining increasing importance around the globe (Benny & Vanitha 2004).

Diarrhea is severe symptoms of failed immune system. It is the passage of loose liquid or watery stools. These liquid stool are usually passed more than three times in a day (Park, 2005). Diarrhea may be acute lasting hours or days, chronic for months. Acute diarrhea is a clinical syndrome of diverse and frequently unidentifiable aetiology present as loose stool and often fever as the most common manifestation. Majorly the aetiological agents include infectious

organism such as Escherichia coli, Vibrio cholerae, Salmonella lyphi, Shigella spp and Entamoeba histolytica. It may also include virus and helminth. The sources of this infection are either infected human beings patient or carrier of the causative agent from where they are transmitted through contaminated food or water to susceptible host (Warren, 2008). The hands of infected person may be highly contaminated with the infectious agent thereby encouraging transmission of agent. Furthermore, lack of proper personal hygiene on the part of the patient may aid transmission of the infections. (Moro et al, 2000).

The treatment of the infection includes oral rehydration therapy, antibiotics and the use of medicinal plant. Hence, the aim of this study is to investigate the immune stimulant and antimicrobial effect of some plant extracts used locally to treat diarrhea. However, two plant extracts will be used for this study. They are *Ocimum gratissimum* and *Momordica charantia. Ocimum gratissimum* (African Basil) belong to the family labiatae, *O.gratissimum* is called efinrin by Yorubas of the southwestern Nigeria, nchanwu by the Igbos and Diadoya by the Hausa. The plant is widely distributed in tropical and warm temperate region. They are commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu & Oladimeji, 2005).

Momordica chanratia is commonly called bitter melon and belongs to the family of (Cucurbitaceae). They are widely distributed and used in Asia and the Amazon in Brasil. Among its many uses is the treatment of skin infection and many others diarrhea inclusive. The fruits and the leaves contain alkaloids and saponin, glycoside like substance (Mwambete, 2009). However, a few previous studies have had evaluated the antimicrobial activity of the plant, hence the aim of this study is to investigate the immuno modulatory and antimicrobial effect of these two plant extracts on isolates from diarrheic patient.

Statement of the Problem

Modern chemotherapy has been responsible for many medical advances, but unfortunately it has also been hampered by the facts that most enteric bacteria that causes diarrhea in man has developed resistance to antimicrobial drugs. Which is attributable to the widespread transmission of resistance plasmids among different genera. Also some drugs such as penicillin are now among the most common causes of hypersensitivity reaction (Mahuddin & Shaikh 2010).

Purpose of the Study

The purpose of the study was to investigate the immunomodulatory effects of *Ocimum gratissmum* and *Momordica charantia* plant extracts and their antimicrobial activities (in vitro and in vivo) on diarrhea isolates. The specific objectives of the research are to:

1. Isolate and identify diarrhea-causing organisms from infected patients
2. evaluate antibacterial activity of Ocimum gratissimum and Momordica charantia plant extracts in vitro
3. determine the phytochemical constituent, free radical scavaging potential of the plants.
4. examine immune stimulating effect of these plants

Research Questions

1. What are the diarrhea-causing organisms from infected patients?
2. What are the antibacterial activity of Ocimum gratissimum and Momordica charantia plant extracts in vitro?
3. What are the phytochemical constituent, free radical scavaging potential of the plants?
4. What are the immune stimulating effect of these plants?

Literature Review

Momordica Chanratia

Momordica chanratia commonly called bitter melon belongs to the family of *Cucurbitaceae*. They are widely distributed and used in Asia and the Amazon in Brasil. Among its many uses is the treatment of skin infection and many others diarrhea inclusive. The fruits and the leaves contain alkaloids and saponin, glycoside-like substance (Mwambete, 2009). Is a tropical plant widely grown in Asia, Africa and the Carribean for its edible *fruits* , which is among the most bitter of all fruits. Its many varieties differ substantially in the shape and bitterness of the fruit. Bitter melon originated on the Indian subcontinent, and was carried to China in the 14th century. (Grover & Yadav, 2004).

This herbaceous, tendril bearing vine grows to height of 5 m. It bears simple, alternate leaves 4-12cm across, with three to seven deeply separated lobes. Each plant bears separate yellow male and female flowers. In the Northern Hemisphere, flowering occurs during June to July and fruiting during September to November. The fruit has a distinct warty exterior and an oblong shape. It is hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large, flat seeds and pith (Grover & Yadav, 2004). The fruit is most often eaten green, or as it is beginning to turn yellow. At this stage, the fruits flesh is crunchy and watery in texture, similar to cucumber, chayote or green bell pepper, but bitter. The skin is tender and edible. Seeds and pith appear white in unripe fruits; they are not intensely bitter and can be removed before cooking. Grover and Yadav (2004)

As the fruit ripens, the flesh (rind) becomes tougher, bitterer, and too distasteful to eat. On the other hand, the pith becomes sweet and intensely red; it can be eaten uncooked in this state, and is a popular ingredient in some Southeast Asian salads. When the fruit is fully ripe, it turns orange and mushy, and splits into segments which curl back dramatically to expose seeds covered in bright red pulp (Beloin et al,2005).

The Bitter melon plant contains several biologically active compounds, chiefly momordicini and momordicin 11 and cucurbitacin B. It also contains several bioactive (including momordin, charantin. Charatosides, goyaglycosides, momordicosides) and other terpenoid compound (including momordicin-28, momordicinin, momordicilin, momordenol and momordol). There is also the presence of cytotoxic (ribosome-inactivating) proteins such as momorcharrin and momordin. (Beloin et al, 2005). Two compounds extracted from bitter melon a-eleostearic acid (from seeds) and dehydrxy-a-eleostearic acid (from the fruit) have been found to induce apoptosis of leukemia cells in vitro. Diets containing 0.01% bitter melon oil (0.006% as a-

eleostearic acid) were found to prevent azoxymethane induced colon carcinogenesis in rats (Beloin et al, 2005).

Laboratory tests suggest compounds in bitter melon might be effective for treating HIV infection as most compounds isolated from bitter melon that impact HIV have either been proteins or lectins, neither of which are well-absorbed, it is unlikely that oral intake of bitter melon will slow HIV in infected people. Oral ingestion of bitter melon possibly could offset negative effects of anti-WV drugs, if an in vitro study can be shown to be applicable to people (Annaya & Sarmistha, 2010).

Other compounds in bitter melon have been found to activate the AMPK, the protein that regulates glucose uptake (a process which is impaired in diabetics). Bitter melon also contains a lectin that has insulin-like activity due to its nonprotein-specific linking together to insulin receptors. This lectin lowers blood glucose concentrations by acting on peripheral tissues and, similar to insulin's effects in the brain, suppressing appetite. The lectin is likely a major contributor to the hypoglyceamic effect that develops after eating bitter melon. As bitter melon is extremely bitter if eaten raw, it must be cooked to make it palatable (Annaya & Sarmistha, 2010).

Ocimum gratissimum

Ocimum gratissimum - (African Basil) belong to the family Labiatae, *O.gratissimum* is called efinrin by Yoruba's of the southwestern of Nigeria, nchanwu by the Igbos and Diadoya by the Hausa. The plant is widely distributed in tropical and warm temperate region. They are commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu & Oladimeji,2005).

Moreover, a lot of work has been done to show the antimicrobial properties of the plant to some selected pathogen. For example 0. gratissimum has been reported to be active against several bacteria and fungi. (Nwosu and Okafor, 1995, Nakurama et al, 1999). It has also been reportedly done for some selected diarrhea causing bacteria by (Adebolu & Oladimeji (2005), although, much has been documented on the antimicrobial properties of this plant.

The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity Adebolu and Oladimeji (2005). Leaf extract of 0. gratissimurn showed antidiabetic properties in streptozocin-induced in diabetic rats. A test on guinea pigs found evidence that the essential oil relaxes the muscles of the small intestine, consistent with the traditional use of the plant to treat gastrointestinal disorders Adebolu and Oladimeji (2005). Antitumor and anti-cancer effects have been reported in in o experiments. A study on rats also found evidence that a leaf extract of the plant prevented diarrhea. Ocimum gratissimum has anti-fertility effects in male mice. Ocimum gratissimum ethanolie extracts showed a hepatoprotective effect in rats (George & Charturvedi 2008).

A polyherbal preparation of a water extract obtained from the leaves of *Gongronema latifolia, Vernon/a amygdalina and Ocimum gratissimum* showed analgesic activity. 0. gratissimum has mosquito-repellent and mosquitocidal potential. A study on goats also found that the essential oil has antihelmintic activity (George & Charturvedi, 2008). The ethanolic extract of Ocimum gratissimum produced a significant and sustained increase in the sexual activity of normal male mice, without any adverse effects. "Thus, the resultant aphrodisiac effectivity of the extract lends support to the claims for its traditional usage in sexual disorders" The essential oil has potential for use as a food preservative, and is toxic to leishmania. (Blinking 2000).

Phytochemical

Phyto-chemicals are chemical compounds that occur naturally in plants hyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke, or metabolic syndrome (Liu, 2004).

Without specific knowledge of their cellular actions or mechanisms, phytochemicals have been considered as drugs for millennia. For example, the bark of the white willow tree was originally extracted and leaf were prescribed to abate fever and pain-relieving properties and later synthetically produced became the staple over-the-counter drug. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of, possibly due to, and effects (Brown, et al 2001).

Specific phytochemicals, such as, are allowed limited health claims by the US Food and Drug Administration (FDA). An important cancer drug Taxol (Paclitaxel) is a phytochemical initially extracted and purified from the Pacific yew tree.

Some phytochemicals with properties may be elements rather than complex organic molecules. For example selenium which is abundant in many fruits and vegetables, is involved with major metabolic pathways, including metabolism thyroid hormone and immune function. Particularly, it is an essential nutrient and cofactor for the enzymatic synthesis of glutathione an endogenous antioxidant. (Brown, et al 2001)

Immune System Overview

The immune system is a complex network of cells and glands that interact through a multitude of cytokines and cell receptors. In general, the immune system is divided into two sub systems: the innate immune system and the adaptive immune system. Both are vital for protecting our bodies from invading organism.(kaiko et al 2000).

The innate immune system is considered the first-line of defense and is general non specific. This system involves mechanical barriers to pathogens (skin, mucous etc). Chemical barriers (stomach acid), secretory barriers (enzymes, immunoglobulin (sLga) and the inflammatory

processes. Within the innate immune system are cells such as neutrophils, macrophages and natural killer (NK) cells which are non-antigen specific and have no “memory”, these barriers and cells often prevent pathogens from getting a foothold within sensitive tissues, limiting the need for the adaptive immune system.(kaiko et a! 2000)

In contrast to the innate immune system, the adaptive immune system “adapts” toinvading organism over time. The primary cells involved in this system are the T and B lymphocytes, which organize invading organism with high degree of specificity using T-cell receptors and immunoglobulin (antibody) proteins. The adaptive immune cells also have “memory” allowing a second invasion of the same (or cross-reactive) antigen to stimulate a quicker and more potent response (kaiko et al 2000)

The maturation and specificity of the adaptive immune response is partially centered on the differentiation of a specific set of CD4+ T-helper cells (TH). The two most well understood are the Th1 and Th2 subsets, but newer research has defined a Th17 subset (named for its expression of 1L- 17) as well. The interaction of a native T-helper cell (Th0) with an antigen (in the context of an antigen presenting cell) causes the permanent differentiation to one of this T helper cell subset, since T-helper cell coordinate how the rest of the adaptive immune system will respond to antigen, their differentiation determines which portions of the immune system will mount a response. In general, Th1 cells secrete interferon-gamma and TNF-B, stimulating a cellular response against viruses, bacterially-infected macrophages and cancer (Mahhudin & Shaikh, 2010)

Th2 cells, on the other hand, secrete cytokines that up-regulate antibody production (e.g the IgE allergic response) and protection against parasites, Th17 cells may be involved in stimulating a portion of the inflammatory response while activating neutrophils. (Kaiko, *et al* 2000)

Many factors can influence a shift in immune system Thl/Th2 ratio, including maternal diet and immune challenges during fetal development, early childhood exposure to antigens and allergens, diet gut microflora and immunization. The so-called “hygiene hypothesis” suggest that children with more exposure to pathogens earlier in life will preferentially develop a Th1 profile which result in less allergic susceptibility. The inverse relationship between atopic disease and exposure to child hood pathogens seems to confirm the hygiene hypothesis, although the relationship is far from scientific agreement other factors that have been shown to be associated with increase allergic potential are urban living, exposure to diesel exhaust, use of antibiotics fewer siblings and vaccination programs. (Mahhudin and Shaikh, 2010).

Both the adaptive and innate inmiune system need to be working properly in order to maintain protection against infectious agents and malignant cells. Both systems are vulnerable to nutritional deficiencies and stress: however, they are both candidates for improvement with proper diet, exercise and nutritional supplementation. When reviewing the agents one could use to improve immune function, it is important to understand that there is no one single end-point or surrogate marker for measuring immune’ function: certainly not an end point that can be used in every patient and in all circumstances. For instance, in HIV positive patients, measuring CD4+

T- cells is a fairly good measure of immune health: however, in most other individual, this measurement is less predictive of overall immune strength (Rush and Niculescu, 2005). Likewise, measuring the number of various immune cells, cells activity or the concentration of various cytokines or antibiotics needs to be tailored to each condition. There are laboratories that have developed various immunology panels, which include test such as lymphocytes subpopulation analysis (Including TH1/Th2ratios), immunoglobulin levels (including secretary IgA), lymphocyte immune function, natural killer cytotoxicity activity, interferon production and many others. These tests can be valuable for diagnostic and follow-up analysis in patients with recurrent infections or immune system challenges (chronic fatigue, fibromyalgia, cancer etc.) but is not yet widely used in the clinical setting (Rush & Niculescu, 2005).

While immunology test can add to our understanding of immune function in the individual, the prevention of recurrent infections (cold, flus, UTLs etc) is a clinical end point which most considered to be a good functional marker for immune health. Every generation and every culture has remedies for preventing or limiting these sorts of recurrent illness (chicken soup, salt water gargles, herbal teas e.t.c). In the past several decades, many of these traditional remedies have been scrutinized at the clinical level (do they really prevent disease in a controlled trial?) and the basic science level (can we explain how these remedies might affect immune cell function?) (Rush and Niculescu, 2005). What is emerging is very promising, but often times contradictory, due to complexity of the Research process, the individuals being studied and the immune system itself (Rush and Niculescu, 2005).

Methodology

Collection and Identification of Plant Materials

The fresh leaves of *Ocimum gratissimum and Momordica charantia* used for this study were collected from Ado-Ekiti and Akure Farm Settlements. The taxonomic identification of the plants materials were confirmed at Forestry and Wood Technology Department of the Federal University of Technology, Akure.

Collection of Organisms Used

The four organisms used for this work were those implicated with diarrhea disease and they were isolated from diarrhea patient namely, *Salmonella typhi, Salmonella typhimurium and Escherichia coli* which were obtained from Ekiti State University Teaching Hospital Ado-Ekiti, While Vibrio cholerae was obtained from Obafemi Awolowo University Teaching Hospital Ile Ife, Osun State. The typed culture isolates of *Salmonella lyphi and Escherichia coil* were obtained from Pharmacy Department of Obafemi Awolowo University Ile-Ife.

Media Used and Sterilization Method

The media used for this practical work were Mueller Hinton Agar, Desoxycholate Citrate Agar, Eosin Methylene Blue Agar and Thiosulfate-citrate-bile-sucrose agar. The selective media was used to isolate the organisms while Mueller Hinton agar was used to prepare agar slant and

antimicrobial activity .All the media used were prepared according to the instruction of the manufacturers, they were all autoclaved at 121'Oc for 15 minutes.

All the used organisms were maintained by routine culture on Mueller Hinton agar slant and kept at 4^0 C. Peptone water was used to revive the isolate before biochemical test. Sterilization of glass wares. The glass wares used in this study include Conical Flask, Mackertney bottles, Glass slides and Test tubes. They were sterilized in the autoclave at 121°C for 1 5minutes and in the oven at 60°C for 1 hour.

Biochemical test

Catalase test - A small amount of the bacteria to be tested was placed on a clean slide with a sterile ioop and then covered with drops 3% hydrogen peroxide, Intense formation of gas bubbles indicates the presence of catalyst enzyme (Fawole & Oso, 1988).

Action on Simple Carbohydrates (Production of sugar with &without gas formation. Sterile broth medium was prepared in test tube. To each of the test tube different sugar were added with few drops of methyl red indicator. The indicator sugar broth was inoculated with the bacteria and a control test tube was left uninoculated. The test tube were incubated for five days and observed daily. Acid production was shown by a change in the color of the indicator from red to yellow and gas production was shown by the displacement of Durham tubes in each test tube. The reaction that occurred within the first and second day are known as early fermentation, those that later occurred were considered late.

Preparation of Extract.

The plant materials were air- dried at room temperature for 5days and blended by using blender.

Exactly 300g of Occirnum gratissimum powder was weighed and 300g of Momordica charantia. Ethanol and hot water were used as extracting solvent using ratio 1:3 volume. 98% of 900ml of ethanol was added to each and 900m1 hot water respectively. They were allowed to soak for 72hours, after which it was sieved using muslin cloth and concentrated using Rotatory Evaporator for ethanol while hot water extract was concentrated by exposing it to air at room temperature.

The oily form of ethanol extract was stored inside a sterile bottle and the powdered form of hot water extract was stored in a dark nylon and kept inside refrigerator for further used.

Reconstitution and sterilization of extract

The reconstitution of the extract depend on concentration to be used for the anti microbes activities. In this project work various concentration were used for the anti microbial activities of the extract.

Procedures

A known gram of the powder according to the concentration to be used were weighed and dissolved in known volume of DMSO as dissolving solvent e.g 100mg/mi 0.lg of the extract was

dissolved in 1m1 of the solvent. The dissolved extract was sterilized and later filtered using filter membrane paper into sterile bottles. The filter extract was also filtered to another sterile bottles using Millipore filter membrane and then keep in refrigerator for further used

Test for anti microbial activities of the extract

The anti microbial activities was measured using the agar well diffusion method of Schinning and Lucke (1989) well were made with 8mm cork borer in plates containing solidified Muellertin agar. After making the well .a sterilize swab stick was dipped in to 24 hours old both culture of test organism and the swab was use to inoculate the organism in to agar surface by spreading. It was left for 1-2 hours to ensure proper adhension of the organism on the surface of the medium after which 0.2ml of the extract was pippeted by using 2m1 needle and syringe in to the well while centre well served as control and left for 30minutes before incubating it at 37°C for 24hours. The antibacterial activities of the extracts was evaluated by measuring the zone of inhibition.

linmunomodulatory Activities

The immunomodulatory activities and effect of the two extracts on the experimental animals was done by looking into health status of the animals using hernatological, histopathological and immunological parameter.

Experimental animals - Albino rat weighing between 80 to 85g were used for this work. Rats were gotten from Ibadan and Ife. The animals were maintained under standard condition in an animal house of Animal production and health department of Federal University of Technology Akure. The animals were given pellet food and water and allow to acclimatized for some days.

A total number of sixty rats were used for this work. The rats were grouped into twelve groups each group containing five rats for two extracts i.e. *Momordica charantia and Ocimum gratissimum.* The tested organism for invivo activities was *Salmonella typhimurium.*

Statistical Analysis

All the data was subjected to analysis of variance test means of different group were compared using Duncan's multiple range test described by Steel and Forrie (1981). SPSS Package was used in the Analysis.

Results

Colonicall Morphological and Biochemical Characteristics of the isolates

The table 1 showed morphological and biochemical reaction of the tested organisms. The colony morphology of the organisms were examined on selective media. EMB agar was used to isolate and identified *Esherichia coli*. The colony morphology of the organism showed metallic sheen, circular convex, distinct edges blue black on the agar. Also the Gram staining reaction showed negative reaction and rod shape. Biochemical test was carried out on acid production and catalase reaction. The organism showed no acid production but positive for catalase test.

Salmonella typhi and Salmonella typhimurium were cultured on DCA agar. The colony morphology of *Salmonella typhi* on the DCA showed Convex, glossy,transparent yellow and black centre on the agar. The organism showed negative gram reaction with rod shape. There was production of acid on fermentation of simple carbohydrate with catalase reaction. The colony morphology *Salmonella typhimurium* on DCA showed convex, glossy entire cleaning around the colony. The organism showed acid production on simple sugar and catalase positive.

Vibrio cholerae-The organism was cultured on TCBS agar. The colony morphology showed convex,Smooth round yellow colony opaque on the agar. The Gram reaction is negative with coma shape. **Note:-** AP-Acid production, -veR Negative Rod, + Positive, -ye coma=Negative comas.

Anti bacterial Activity of the Two Plants Extract on the Isolates

The antibacterial activity of *Ocimum gratissisum* and Momordica charantia extracts against the tested organisms was assessed by the presence or absence of inhibition zones. The presence, and minimum inhibition concentration of the extract to those organisms that the extract were sensitive to were shown in table 2.

Momordica charantia at different level of concentration of the extract show no level of significant on Escherichia coli both water and ethanol extract.

Momordica charantia at different level of concentration of the extract show a level of significant different on *Salmonella lyphimurium* at level of 100 and 200g. There is significant different for both water and ethanol extract.

Momordica charantia at different level of concentration of the extract show no level of significant different on salmonella typhi of both water and ethanol extract.

Momordica charantia at different level of concentration show a level of significant different on vibrio cholera especially at concentration level of 75 — 200g of both water and ethanol extract.

Ocimum gratssimum at different level of concentration of the extract it show no of significant different on Esherichia coli of both water and ethanol extract. Ocimum gratissimum at different level of concentration of the extract show a level of significant different of Salmonella typhimum.

Ocimum gratissimum at different level of concentration of the extract it show no level of significant on Salmonella typhi of both water and ethanol extract.

Ocimum gratissimum at different level of concentration of the extract it show level of significant on Vibrio cholera of both water and ethanol extract.

Table 1: Colonicall Morphological and Biochemical Characteristics of the Isolates

Morphology on Selective Media	Gram Reaction	AP	Catalase	Identity
Metallic sheen, circular, Convex, Distinct edges, Blue black on EMB agar	-ye R	-	+	*E.coli*
Convex, glossy, transp yellow,black centre on DCA	-ve R	+	+	*s.tphy*
Convex, glossy,Entire. Cleaning around colony on DCA	-veR	+	+	*S.typhimurium*
Convex, Smooth, round,raid,yellow colonies opaque on TCBS	-ve coma	-	-	*V.cholera*

Key. AP-Acid production,

-veR= Negative Rod,

+= Positive,

-ve coma=Negative coma.

Table 2: Result of Minimum Inhibitory Concentration (MIC) of the extracts against the four test organisms

Treatment	Organism	Level	HWE	EE	Control
M.charantua	***E.coil***	25	0.00±0.00	0.00±0.00	0.00±0.00
M.charantua	***E.coil***	50	0.00±0.00	0.50±0.50	0.00±0.00
M.charantua	***E.coil***	75	0.50±0.50	1.50±0.50	0.00±0.00
M.charantua	***E.coil***	100	1.50±0.50	5.00±1.00	0.00±0.00
M.charantua	***E.coil***	200	3.00±0.58	6.00±1.5	0.00±0.00
M.charantua	***S.tyhpimurium***	25	0.00±0.00	0.50±0.50	0.00±0.00
M.charantua	***S.tyhpimurium***	50	0.05±0.50	1.50±0.50	0.00±0.00
M.charantua	***S.tyhpimurium***	75	2.50±0.50	5.00±1.00	0.00±0.00
M.charantua	***S.tyhpimurium***	100	6.00±1.15	12.00±1.65	0.00±0.00
M.charantua	***S.tyhpimurium***	200	7.00±1.00	13.00±1.91	0.00±.00
M.charantua	***S.typhi***	25	0.00±0.00	0.00±0.00	0.00±0.00
M.charantua	***S.typhi***	50	0.00±0.00	0.00±0.00	0.00±0.00
M.charantua	***S.typhi***	75	0.00±0.00	0.00±0.00	0.00±0.00
M.charantua	***S.typhi***	100	0.00±0.00	0.00±0.00	0.00±0.00
M.charantua	***S.typhi***	200	0.00±0.00	0.00±0.00	0.00±0.00
M.charantua	***V.cholera***	25	0.00±0.00	0.50±0.50	0.00±0.00
M.charantua	***V.cholera***	50	0.50±0.50	2.00±0.82	0.00±0.00
M.charantua	***V.cholera***	75	2.00±0.82	6.00±1.15	0.00±0.00
M.charantua	***V.cholera***	100	5.00±1.00	17.00±1.91	0.00±0.00
M.charantua	***V.cholera***	200	7.00±1.00	20.00±1.63	0.00±0.00

O.Gratissinium	***E.coil***	25	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***E.coil***	50	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***E.coil***	75	0.00±0.00	1.00±0.50	0.00±0.00
O.Gratissinium	***E.coil***	100	0.0±0.00	2.50±0.50	0.00±0.00
O.Gratissinium	***E.coil***	200	0.25±0.25	7.00±1.00	0.00±0.00
O.Gratissinium	***S.tyhpimurium***	25	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***S.tyhpimurium***	50	0.00±0.00	1.50±0.50	0.00±0.00
O.Gratissinium	***S.tyhpimurium***	75	0.50±0.50	2.50±0.50	0.00±0.00
O.Gratissinium	***S.tyhpimurium***	100	1.50±0.96	5.00±1.00	0.00±0.00
O.Gratissinium	***S.tyhpimurium***	200	5.00±1.00	10.00±1.15	0.00±0.00
O.Gratissinium	***S.typhi***	25	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***S.typhi***	50	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***S.typhi***	75	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***S.typhi***	100	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***S.typhi***	200	0.00±0.00	0.00±0.00	0.00±0.00
O.Gratissinium	***V.cholera***	25	0.00±0.00	0.50±0.00	0.00±0.00
O.Gratissinium	***V.cholera***	50	0.00±0.00	2.50±0.50	0.00±0.00
O.Gratissinium	***V.cholera***	75	2.50±0.50	6.00±1.15	0.00±0.00
O.Gratissinium	***V.cholera***	100	5.00± 1.00	15.00±1.91	0.00±0.00
O.Gratissinium	***V.cholera***	200	9.00±1.00	18.00±1.15	0.00±0.00
Level of significant			**	***	**NS**
Treatment			***	***	**NS**
Organism			***	***	**NS**
Treatment*Organism			***	*	**NS**
Organism*Levels			***	***	**NS**
Treatment*levels			NS	*	**NS**
Treatment*Organism*level			**	***	**NS**

Key. HWE-Hot Water Extract

EE-Ethanol Extract NS-Not Significant

Table 3: Zones of Inhibition of Standard Antibiotics Sensitivity to The Tested Organisms (mm).

Organism	**Standard Antibiotics**							
	1	2	3	4	5	6	7	8
Escherichia Coil	0	18	8	16	8	0	0	0
	0	22	8	14	6	2	0	0
	0	18	7	15	8	0	0	0
	0	22	8	14	7	2	0	0
Salmonella typhi	0	8	4	10	0	0	0	0
	0	10	6	12	0	0	0	0
	0	8	6	8	0	0	0	0

	0	8	8	10	0	0	0	0
Salmonella typhimurium	0	52	12	32	26	40	0	4
	0	48	10	32	30	36	0	4
	0	46	12	30	30	42	0	4
	0	48	10	32	26	40	0	6
Vibrio Cholerae	0	16	10	20	4	8	0	8
	0	14	10	16	6	10	0	4
	0	12	8	18	8	12	0	8
	0	12	10	16	6	10	0	8

KEY. 1=Augmentin. 2=Ofloxacin, 3=Gentamycin, 4=Nalidixic Acid, 5=Nitofurantoin, 6=Cotrjmoxazole, 7=Amoxycillin, 8=Tetracycl1n.

Phytochemical screening on the quantitative analysis of the plant extracts was determined the cardiac glycosides was determined by Keller Killani test, salkowski test, legal test. The test revealed that both extract had cardiac glycosides. Also there were present of saponin, tannin and flavonoid compound on the two extract both water and ethanol. The phytochemical screening on quantitative analysis of the plant extracts was determiuned. The quality of traiming on ocimum gratissimum ethanol extract was 0.1221mg on the average. The quality of training on Momordica charantia hot water extract was 0.2094mg on the average. Ocimum gratissimum hot water extract the quality of taning was 0.2538mg on the average.

The quantity of flavonoid on Ocimum gratissimum ethanol extract was 2.727mg/g on the average. The quantity of flavonoid on Momordica charantia hot water extract was 2.005mg/g on the average. The quantity of flavonoid on Momordica charantia ethanol extract was 0.9755mg/g on the average. The quantity of flavonoid on Ocimum gratissimum hot water extract was 3.7905mg/g on the average. The quantity of saponin on Ocimum gratissimum ethanol extract was 2.0798mg/g on the average. The quantity of saponin on Momordica charantia hot water extract 2.0938mg/g on the average. The quantity of saponin on Momordica charantia ethanol extract was 3.8182mg/g on the average the quantity of saponin on Ocimum gratissimum hot water extract was 2.53 125mg/g on the average. All these were subjected to statistical analysis using ANOVA Duncan multiple value to find stand error means. S The mean value along the column with difference subscript indicates significant difference at P(0.05). There is more yield in flavonord in Ocimum gratissimum hot water extract and high yield of saponin in Momordica charantia ethanol extract.

Discussion

The ethanol and hot water extract of the two medicinal plants used in this study possess varying degrees of phytoconstituents and antibacterial action. The ethanol and hot water extract of Ocimum gratissimum and Momordica charantia were tested for antibacterial activity on four organisms namely Esherichia coli, Salmonella typhi, Salmonella lyphymurium and Vibrio

cholerea. The ethanol and hot water solvent of leaf extract of Ocimum gratissimum had moderate inhibitory effect on Salmonella lyphymurium and Vibrio cholerea at 200mg/ml concentration. The inhibitory effect of *Ocimum gratissimum* ethanol extract is likely to be due to eugenol property present in the *Ocimum gratissimum* . This component has been demonstrated to have both antibacterial activities reported by (Nakaruma et al 1999) and antihelmintic activities by Pessoa et al (2002)

Also the saponin, tannin, flavonoid compounds present in the extract both water and ethanol extract were know to show medicinal properties as reported by (Victinck and Peters 2005), and (Mwambete 2009)

The weak inhibitory effect of Ocimum grattissimum of ethanol and hot water extract on Esherichia coli at 200mg/mi concentration may be due to insufficient release of the oil during extraction reported by Adebolu and Oladimeji (2005). Also (Ndounga and Quamba 1997) in their study reported that the antibacterial activity of ethanolic leaf extract exhibited mild degree of activity against Esherichia coli and this may be due to low affinity of the active agent on the target molecules (Mwambete, 2009).

The non-inhibitory effect of the Ocimum gratissimum ethanol and hot water extract even at high concentration on Salmonella typhi may be due to genetic component of the organisms and probably excessive intake of antibiotics by the patients which may cause the isolates to build resistance. It may also be due to insufficient release of antimicrobial property during extraction which may not be enough for antibacterial activity against the isolate (Adebolu et al 2005). In addition the resistance of this organisms may also be attributable to the presence of more active enzyme in this microbe which deactivate the active antibacterial agents or low affinity of the active agent on the target molecules (Mwambete 2009)

The ethanol and hot water solvent of leaf extract of Momordica charantia had strong inhibitory effect on Salmonella lyphimurium and Vibrio cholerea at 200mg/ml and moderate at 100mg/mi. the inhibitory effect of the ethanol and hot water extract on the two organisms may be due to presence of some compound such as saponin, tarmin, flavonoid, terpenoid in Momordica charantia which has come medicinal properties (Mwambete, 2009) previous studies had also demonstrated that M charantia is very rich in triterpenes, proteins and steroids, those of major interest include momordin, alpha — and beta momor charm cucurbitacin B 1 and oleanolic acid. It is speculated that the antimicrobial activities of triterpenes depend on interaction between their lipid component with the surface of microbial membrane (Mwambete 2009). The presence of all these compounds may be responsible for the antibacterial activity of the extract. The weak and non — inhibitory effect of antibacterial activities of the extract on Esherichia coli and Salmonella lyphi may be due to low affinity of the active agent on the target molecules (Mwambete 2009). It may also due to genetic component of the organisms and probably excessive intake of antibiotics by the patients which may cause the isolates to build resistance. It may also be due to insufficient release of antimicrobial property during extraction which may not

be enough for antibacterial activity against the isolate Adebolu and Oladimeji (2005). In addition the resistance of this organisms may also be attributable to the presence of more active enzyme in this microbe which deactivate the active antibacterial agents.

However when comparing the inhibitory effect of the two extracts with inhibitory effect of standard antibiotics it was observed that the standard antibiotics hard high degree of inhibitory effect on the four isolates used in this study especially Ofloxacin and Nalidixic, reason for this may be due to actual dose been presence in the drug while the plant extracts do not have actual dose used for the antibacterial activity except varying concentration. Also the high effectiveness of standard antibiotics may also be as a result of actual active component for antibacterial activities while the plant extract APIs were neither quantified nor isolated. The presence of more than one APIs with either antagonistic pharmacological property could ascribe for this observation (Mwambete 2009).

Conclusion and Recommendations

The effects of the extracts on Salmonella typhimurium and Vibrio cholerae may suggest that the two extracts possess compounds with antibacterial properties. Also the changes in the hematological parameters of the experimental animals treated with the extracts suggest that the extracts especially Ocimum gratissimum which causes significant increase in the white blood cell may modulate immune function. While Momordica charantia may modify the immune response via reduced lymphocyte. It is therefore concluded that the extracts can be used in the treatment of infection caused by these organisms and also be used as immune stimulants. Based on the results of this study, it was recommended that the antibacterial effects of the two extracts Momordica charantia and Ocimum gratissimum on diarrheic isolates could be increased upon by partial purification and subsequent concentration of the biological active properties of the plants. More work should be done on comparison between cold water and hot water as extractive solvent on the two extracts.

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