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Research Article

Efficacy of Three Tropical Plants for Inhibition of Pathogen Causing Human Diarrhoea

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Abstract

The efficacy of three tropical plants that can be used for the inhibition of pathogens causing human diarrhoea was investigated. Ethanol was used as solvent for extraction. Six human pathogens; *Escherichia coli, Bacillus subtilis, Salmnonella enterica, Pseudomonas aeruginosa, Staphylococcus citrinum* and *Aspergillus niger* were employed in this study. The inhibitory effects of the ethanol extracts of *Psidium guajava L., Mangifera indica L.* and *Vernonia amygdalina L.* on the test organisms were conducted using the agar well diffusion method of antimicrobial assay. Phytochemical screening of the plants was carried out. Antibiotic (Streptomycin) served as the control. Phytochemical screening of the plants revealed the presence of biologically active chemical compounds such as tannins, phenols, saponins, alkaloids, flavonoids and sterols. The quantitative determination of the phytochemicals revealed the different levels of concentrations. The minimum inhibitory concentration(MIC) of the extracts on the organisms ranged between 5.62-15.60mg/mL for *Bacillus subtilis,* 5.30-16.25mg/mL for *Staphylococcus citrinum,* 3.94-12.90mg/mL for *Pseudomonas aeruginosa,* 4.85-13.75 for *Escherichia coli,* 4.50-12.36mg/mL for *Salmonella enteric,* 5.40-14.10mg/mL for *Aspergillus niger.*

The solvent used for extraction varied significantly (P<0.05) among the six test organisms. *Staphylococcus citrinum* and *Bacillus subtilis* were the most susceptible to the extract while *Salmonella enterica* was least inhibited. From the results, it is evident that bark of *Psidium guajava,* bark of *Mangifera indica* and root of *Vernonia amygdalina* possess potential inhibitory activity against human pathogens *in vitro* to varying degrees

Introduction

Diarrhoea means flowing through and it is a condition of having three or more loose or liquid bowel movements per day [1]. The definition of diarrhoea varies but it can generally be taken to mean increase in stool frequency or the passage of soft stools [1].

The passage of greater than three stools per day or a stool volume of greater than 200ml suggests the diagnosis of diarrhoea [1].

Diarrhoea may be defined as acute if the episode is less than two weeks, persistent if it is 2-4 weeks, and chronic if it is greater than four weeks in duration [2]. Acute diarrhoea with a sudden onset is often acquired by faecal-oral transmission via direct contact or through ingestion of food or water contaminated with faecal pathogens.

Thielma and Guerrant (2004), said that diarrhoea is called acute if it lasts for less than two weeks or chronic if it lasts for greater than four weeks [3]. Chronic diarrhoea is seen in conditions such as inflammatory bowel disease, celiac disease, irritable bowel syndrome and diabetic diarrhoea or with certain medications.

Diarrhoea basically develops when there is a defect in absorptive mechanism working in the intestines. This may be due to damage to the mucoso (lining of the bowel) from infections or inflammation or excess secretion of fluid and electrolytes from particular toxins. Sometimes there may be too much sugary material in the lumen of the bowel which draws more fluid across the membranes. In other patients the bowel may just be over active from hormone disorders which causes very frequent bowel motions but the overall volume should remain approximately the same [1].

Diarrhoea can range from a mild discomfort to a severe and life threatening illness due to the risks of dehydration [4].

Thielman and Guerrant (2004), stated that diarrhoea can occur in virtually any person regardless of their age and general health [5]. Children and the elderly are particularly prone to dehydration secondary to diarrhoea. However, the great majority of cases of diarrhoea follow short courses which do not really require specific diarrhoea treatment to resolve.

Wanke (2006), stated that diarrhoeal diseases are one of the top causes of death worldwide and it becomes particularly common in developing countries with poor food practices and hygiene [4].

According to Thapar and Sanderson (2004), diarrhoea has been observed to be one of the leading causes of morbidity and mortality in developing countries [6]. It is most commonly caused by gastrointestinal infections and kills around 4.6million people, including 2.5million children, every year.

By far, the most common cause of diarrhoea is infection [7]. They further stated that most cases are just mild viral infections caused by rotavirus (which some people may refer to as stomach flu). This can be spread easily through daycare centres, schools or families.

According to Presterl *et al.* (2003), the bacterial pathogens usually responsible for diarrhoeal illness include *Escherichia coli, Shigella sp., Salmonella sp., Campylobacter spp., Yersinia sp., Aeromonas sp.* and their cases tend to be more severe than viral diarrhoea and are caused by eating food or drinking water contaminated with these bacteria [7]. Diarrhoea can sometimes be caused by parasites which are transmitted by similar mechanisms.

Giardia lamblia and *Cryptosporidium sp.* are possible parasitic causes of diarrhoea.

Chronic diarrhoea can be caused by a number of medical problems. Irritable bowel syndrome is relatively common and associated with alternating periods of diarrhoea, constipation and bloating [7].

Certain medications, particularly antibiotics and anti-cancer drugs can cause diarrhoea because they disrupt the normal healthy bugs in the intestines and inhibit mucosal cell regenerations respectively. Inflammatory bowel diseases and ulcerative colitis also cause recurrent attacks of diarrhoea often stained with blood and mucus. Colorectal cancer or any other defects in the intestines may also cause diarrhoea [7].

According to Webb and Starr (2005), human diarrhoea could be prevented through food hygiene, hand washing, appropriate exclusion of children with diarrhoea, eg from nurseries, vaccination: *Salmonella typhi* vaccine recommended for travellers to countries with a high incidence [8].

Traditional medical (use of plants) practices remain relevant and vital in many areas in Nigeria due to absence or inadequate provision of modern medical services particularly in rural areas [9].

About 80% of people in developing countries use traditional medicines for their health care, including the treatment of diarrhoea [10]. Alawa *et al.* (2002), stated that traditional remedies include the use of plant extracts from different plant parts [11].

Rashid *et al.* (2010), observed that herdsmen in non industrialized nations of the world still use medicinal plants for the treatment of livestock diseases including diarrhoea, either due to lack of access to trained veterinaries and high cost of orthodox medicines, or the held belief that herbal remedies are more efficacious [12]. If plant extracts could be used on animals to treat diseases like diarrhoea and the animals become healed, it could also do well when used on human beings.

Agunu *et al.* (2005), noted that the use of herbal drugs in the treatment of gastrointestinal disorders including diarrhoea is a common practise in many African countries and is usually preferred because it is a cheaper alternative [13].

Furthermore, medicinal plants have proven to be an abundant source of biologically active compounds [10,14].

The herbal drugs which are gotten from different parts of plants contain multiple constituents such as alkaloids, glycoside, flavonoids, terpenes, tannins, etc [14-16]. These constituents have effect-enhancing and/or side effect-neutralizing potential and herbal remedies are considered relatively safe in prolonged use [15].

Medicinal plant according to Sofowora (1993), is a therapeutic resource used by the traditional population of the African continent specifically for health care and which may also serve as precursors for the synthesis of useful drugs [17].

Also the World Health Organisation, defined a medicinal plant as any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological process which may be produced for immediate consumption or as a basis for herbal products [18]. The term 'Herbal drug' determines the part/parts of a plant use for preparing medicines (for example; leaves, flowers, seeds, roots, barks, stem, etc) [19].

Studies on the use of plant extracts for control of diseases have shown the importance of natural chemicals (phytochemicals) as possible sources of non- phytotoxic and easily biodegradable alternative fungicides and antibiotics [20].

Against this background, three tropical plants were selected for this research work to evaluate their efficacy in inhibiting pathogen causing human diarrhoea.

Psidium guajava L. (Guava) is a global plant that belongs to the Myrtle family, Myrtaceae [21]. Guava contains about hundred species of tropical shrubs and small trees. They are native of Mexico, Central America and Northern South America. Guavas are now cultivated and naturalized throughout the tropics and subtropics in Africa, South Asia, Southeast Asia, the Cariibbean, subtropical regions of North America, Hawaii, New Zealand, Australia and Spain [22-23]. Guavas are typical Myrtoidaea with tough dark leaves that are opposite, simple, elliptic to ovate and 5-15cm (2.0-5.9in) long. The flowers are white, with five petals and numerous stamens.

Mangifera indica L (Mango), belongs to genus *Mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae. It is a native of South Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics [24]. The leaves are ever green, alternate, simple (15-35cm), 5.9-14 long and 6-16cm broad. When the leaves are young, they are orange-pink, rapidly changing to a dark, glossy red, then dark green as they mature. The flowers are produced in terminal panicles 10-40cm, each flower is small and white with five petals. The fruits take three to six months to ripen.

Vernonia amygdalina L. (Bitter leaf) is common in West African and Central African countries. Bitter leaf belongs to genus *Venonia* and a member of Asteraceae family. It is a small shrub that grows in the tropical Africa. *V. amygdalina* typically grows to a height of 2-5m. The leaves are elliptical and up to 20cm long. Its bark is rough [25].

Many human pathogens requires serious attention in developing countries like Nigeria [26]. These include *Staphylococcus aureus, Escherichia coli and Candida albicans. Staphylococcus aureus* constitutes a nuisance in post-infection and post-operative wound infection, causing formation and production of wound diseases in both cases [27].

S. aureus and *E. coli* (enteropathogenic) have been frequently incriminated in food poisoning incidence associated with gastroenteritis [27]. Also enterotoxigenic *E. coli* is a common cause of traveller's diarrhoea and also the most common cause of urinary tract infection. *Bacillus aureus* can grow in food and produce an enterotoxin that causes diseases that is more of intoxication than a food borne infection [28]. There are various measures of control employed against the pathogens which include; sterilization, use of chemicals, use of antibiotics and biological measures [16].

However, the prevalence of microbial resistance to existing antimicrobial drugs underscores the need for the continuous search for new antimicrobials [29]. The consequence of drug resistance implies that new drugs, both synthetic and natural, must be sought to treat diseases for which known drugs are no longer useful [27]. One of the avenue for such search is to screen medicinal plants for microbial activities [16].

Therefore, the objectives of this study is to investigate the phytochemical compositions of bark of guava tree, bark of mango tree and root of bitter leaf, find out the antimicrobial properties and its efficacy on the inhibition of some microorganisms causing human diarrhoea compared to standard antibiotic (Streptomycin).

Materials and Methods

Collection of Plant Materials

The plant materials required for this research were; Bark of guava *(Psidium guajava L)* tree, Bark of mango *(Mangifera indica L)* tree, Root of bitter leaf *(Vernonia amygdalina)*. These plant materials were randomly gotten from National Root Crop Research Institute Umudike, Abia State Nigeria and their botanical identities were authenticated by the Institute Plant Taxonomist.

Experimental Site

The research work was carried out in the National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria.

Source of Test Organisms

The test organisms used for this study were *Bacillus subtilis, Salmonella enteric, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus citrinum, Aspergillus niger*. The stock culture of these test organisms were obtained from Pathology Department of National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria. These cultures were identified using gram stain reaction [28], reisolating in nutrient agar three times purified each test bacterial strain and each was maintained on nutrient broth at 4°C [30], until required for study.

Preparation of Medium

Nutrient agar was prepared according to the manufactures instructions. Twenty-eight gram (28g) of the nutrient agar powder was suspended in about 800mL of distilled water and heated in water bath until the agar melted; this was later made up to one litre with distilled water. It was then sterilized in an autoclave at 121°C and 15Psi for 15 minutes. After autoclaving, the medium was allowed to cool at about 45°C. It was then dispensed into sterile glass Petri dishes. The agar plates were allowed to cool and gel in the inoculation room.

Sample Preparation and Extraction

The fresh plant samples were dried using oven at a temperature of 60°C for 2 hours before grinding them using an electric blender (Sonik Blender, Model SB-1212) into a powder form [3].

About 50g of the ground sample was soaked in 400ml of ethanol in other to obtain ethanol extracts at room temperature for 24hrs.

Muslin cloth (40 by 20In) was used to filter the plant residues and the filtrates thus obtained were further purified by filtration through Whatman No 1 filter paper used under aseptic condition. The filtrate collected was then concentrated using rotary evaporator. The extract was then collected in fresh sterile universal bottles and stored in the refrigerator at 4°C until when required for use.

The extract was tested for sterility by introducing 2ml of the supposed sterile extract into 10ml of sterile nutrient broth; incubation was done at 37°C for 24 hrs. A sterile extract was indicated by the presence of turbidity or clearness of the broth after the incubation period. This was done for the plant samples (bark of guava, bark of mango and the root of bitter leaf).

The different extracts of the samples were reconstituted with sterile distilled water and ethanol.

The initial concentration of each plant extracts (50g) was diluted using 50ml ethanol to obtain the stock culture. From this stock culture, different concentrations were obtained such as 100mg/mL, 150mg/mL, 200mg/mL, 250mg/mL and 500mg/mL.

Standardization of Bacterial Cell Suspension

The nutrient broth cultures of the organisms for the study were taken and inoculated on a fresh nutrient agar plate for 24hrs. Sterile distilled water (2ml) was poured on each of them and then mixed with the inoculums. 1ml of each was taken and were transferred into 9mL of sterile distilled water and diluted to 10^4 fold [31].

One hundred micro litre of this was taken and poured on the surface of the agar and then spread evenly with the use of a spreader on the plate to be used for study.

Qualitative Analysis of Test Plants

Analysis for the presence of phytochemicals in each of the test plants of a sample was replicated twice for each of the phytochemical.

Test for Tannins

The presence of tannins in the test sample was determined using the procedure of Trease and Evans (2009) [3]. To 1.0ml of aqueous extract was added one equal volume of bromine water. The formation of greenish to red precipitate is taken as evidence for the presence of tannins.

Test for Alkaloids

This was determined using the test described by Harbone (1998) [32]. Ethanol extract of the samples was used and this was achieved by adding 2g of the ground sample to 10mL of ethanol. The mixture was shaken properly and filtered using filter paper. The resulting filtrate was used for the tests. 3 drops of iodine was added to 2mL of the filtrate and shaken well to mix. The presence of dark brown green colouration in the test sample tube showed the presence of alkaloids.

Test for Flavonoids

This was determined by the acid alkaline test described by Harbone (1998) [32]. An aqueous extract of the test sample was obtained by dispensing 1g of the ground sample in 5mL of distilled water. The mixture was shaken for 5minutes and then filtered using filter paper. The filtrate was used as aqueous extract. 2ml of aqueous extract was dispensed into a test tube and a few drops bench concentrate ammonia (NH_3) was added followed by addition of concentrated Hydrochloric acid (HCl). Yellow solution addition of concentrated NH_3 which turns colourless upon addition of concentrated hydrochloric acid is a positive test for flavonoids.

Test for Saponins

1.0mL of the filtrate (aqueous extract of the sample) was diluted using 4.0mL of distilled water, shaken vigorously and observed on standing for stable froth, which gives a positive test for the presence of saponins [3].

Test for Sterols

One mL of acetic anhydride was added to 2mL of ethanol in a test tube and also 3 drops of concentrated hydrogen tetraoxosulphate (iv) acid, H_2SO_4 was added. The mixture was shaken well and examined for the formation of dark brown-green colouration in the test sample, which is positive for sterols [17].

Test for Phenol

The method described by Pearson (1976) was used. 1.0ml of each of the extract portion was added to 1ml of 10% Ferric Chloride. The formation of a greenish brown or black precipitate or colour was taken as positive for a phenolic nucleus [33].

Quantitative Analysis of Test

Analysis for the quantity of phytochemicals in each of the test plants was replicated thrice for each of the 6

Determination of Tannins

To determine tannin content, the Folin Dennis spectrophotometric method by Pearson (1976) was used [33]. Two g of the powdered sample was mixed with 50ml of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered and the filtrate used for the experiment. Five mL of the filtrate was measured into 50mL volume flask and diluted with 3mL of distilled water. Similarly, 5mL of standard tannic acid solution and 5mL of distilled water was added separately. One mL of Folin-Dennis reagent was added to each of the flask followed by 2.5mL of saturated Sodium Carbonated solution. The content of each flask was made up to mark and incubated for 90minutes at room temperature. The absorbance of the developed colour was measured at 760nm wave length with the reagent blank at zero. The process was repeated two more times to get an average. The tannin content was calculated as shown below:

$$\%\ tannin = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D$$

Where,
W= weight of sample analysed
AY=absorbance of the standard solution
C= concentration of standard in mg/ml
VA=volume of filtrate analysed
AS= absorbance of standard tannin solution
VA= volume of filtrate analysed
VF= total filtrate analysed
D= dilution factor where applicable

Determination of Alkaloids

The quantitative determination of alkaloid was carried out by the alkaline precipitation gravimetric method described by Harborne (1973) [32].

5g of the powdered sample was soaked in 20ml of 10% ethanolic acetic acid. The mixture was stood for four hours at room temperature. Therefore, the mixture was filtered through Whatman filter paper (No 42). The filtrate was concentrated by evaporation over a steam bath to (¼) of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper.

After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60°C for 30 minutes, cooled in a desiccators and reweighed. The process was repeated two more times and the average was taken. The weight of the alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown below

$$\%\ Alkaloid = \frac{W_2 - W_1}{Weight\ of\ sample} \times \frac{100}{1}$$

Where
W_1= weight of filter paper
W_2=weight of filter paper+ alkaloid precipitate

Determination of Flavonoid

This was determined by the gravimetric method as was described by Harborne (1998) [32]. Five g of the powdered sample was placed into a conical flask and 50ml of water and t2mL HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through Whatman filter paper (No 42). The ethyl acetate extract with contained flavonoid was recovered, while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filter the second extract. The residue was then placed in an oven to dry at 60°C. It was cooled in a desiccators and weighed. The quantity of flavonoid was determined using the formular

$$\%\ of\ flavonoid = \frac{W_2 - W_1}{Weight\ of\ sample} \times \frac{100}{1}$$

Where:
W_1 = Weight of empty filter paper
W_2 = Weight of paper + flavonoid extract

Determination of Saponin

The saponin content of the sample was determined by double extraction gravimetric method [32].

Five g of the powdered sample was mixed with 50mL of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55°C;

it was then filtered through what man filter paper (No 42). The residue was extracted with 50mL of 20% ethanol and both extract were poured together and the combined extract was reduced to about 40ml at 90°C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted, with 60ml of normal butanol. The combined extracts were washed with 5% aqueous Sodium Chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60°C in the oven and reweighed after cooling in a desiccator. The process was repeated two more times to get an average. Saponin content was determined and calculated as percentage of the original sample as thus,

$$\%\ of\ \ saponin = \frac{W_2 - W_1}{Weight\ of\ \ sample} \times \frac{100}{1}$$

Where:
W_1 = weight of evaporating dish
W_2 = weight of dish + sample

Determination of Sterols

The sterol content of the plant sample was determined using the method described by Harborne (1998) [32].

Five g of the powdered sample was hydrolysed by boiling in 50mL hydrochloric acid solution for about 30 minutes. It was filtered using Whatman filter paper (No 42), the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) was recovered while the aqueous layer was discarded. The extract was later dried at 100°C for 5 minutes in a steam bath. It was again heated with concentrated amyl alcohol in other to extract the sterol. The mixture at this time was reweighed and was filtered properly using Whatman filter paper (No 42). The dry extract was then cooled in a desiccators and reweighed. The process was repeated two more times and an average was obtained. The concentration of sterol was determined and expressed as a percentage thus,

$$\%\ of\ \ sterol = \frac{W_2 - W_1}{Weight\ of\ \ sample} \times \frac{100}{1}$$

Where:
W_1 = weight of evaporating dish
W_2 = weight of dish + sample

Phenol Determination

The concentration of phenol in the plant sample was determined using the Folin-Cio Caltean method as described by Pearson (1976) [33].

0.2g of the powdered sample was added into a test tube and 10ml of methanol was added to it and shaken thoroughly, the mixture was left to stand for 15 minutes before being filtered using Whatman (No 42) filter paper. 1ml of the extract was placed in a test tube and 1ml of folins reagent was added to it with 5ml of distilled water. The colour was allowed to develop for about 1-2 hours at room temperature. The absorbance of the developed colour was measured at 760nm. The process was repeated two more times and an average taken. The content of phenol was calculated as,

$$\%\ Phenol = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{100} \times \frac{D}{VA}$$

Where,

W= weight of sample analyzed
AU= absorbance of test sample
AS= absorbance of standard solution
C= concentration of standard in mg/ml
UF= total filtrate volume
VA= volume of filtrate analyzed
D= dilution factor where applicable

Agar-Well Diffusion Method

The antimicrobial screening of the ethanol's extract was done as recorded by Lino and Deogracious (2006) [34]. Nutrient agar was poured in sterile petri-dishes and allowed to solidify. 1ml of the test culture was spread all over the surface of the agar using a spreader. Wells of approximately 6mm in diameter were made in the surface of the agar medium using a sterile cork borer.

The plates were turned upside down and the wells labelled with a marker. Each well was filled up with the solution of the ethanol extract and care was taken not to allow the solution to spill to the surface of the medium. All the tests were allowed to stand on the laboratory bench for 1-2 hours to allow proper in flow of the solution into the medium before incubating the plates in the incubator at 37°C for 24hours. The plates were later observed for the zones of inhibition.

The effects of the extract on bacterial isolates were compared with those of the standard antibiotics, streptomycin at a concentration of 1mg/mL.

After 24hours, the zones of inhibition were measured using a ruler (AIM(R)) and a pair of divider then results were reported in millimetres (mm).

Minimum Inhibition Concentration (MIC) Evaluation

The MIC was evaluated on plant extracts that showed antibacterial activity in the agar well diffusion assay on any organism. This test was performed at four concentrations for each extract (50mg/mL, 100mg/mL, 150mg/mL, and 200mg/mL) employing doubling dilution of plant extract in Brain heart infusion broth up to the fifth dilution. One mL of the resultant broth was put in test tube and equal amounts of the extracts (1mL) were added to the first test tube and serial dilution done with the last 1ml being discarded.

To complete the test, each organism was separately suspended in 5ml of Brain heart infusion broth and incubated overnight, after which 0.1ml was added to all the test tubes and preparation incubated at 37°C for 18 hours. After incubation, a loop full from each tube was sub cultured on nutrient agar to see if bacteria growth was inhibited. Growth of bacteria on solid media indicated that particular concentration of the extract was unable to inhibit the bacteria. The MIC was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation [35].

Statistical Analysis

The statistical analysis was based on the method of statistical analysis system(SAS). Data generated were subjected to two-way analysis of variance (ANOVA) using multiple least test and Fisher's least significant difference (FLSD) at 5% probability to separate the treatment.

Results

Qualitative Analysis of Test Plant Samples

Qualitative phytochemical screening indicated that the ethanol plant extracts of bark of *Psidium guajava L.,* bark of *Mangifera indica L.* and root of *Vernonia amygdalina L.*, contained classes of compounds (Table 1). These compounds include Tannins, Alkaloids, Flavonoids, Saponins, Steroids and Phenols. These compounds were all found to be present in ethanolic plant extracts but Alkaloids were deeply present in the bark of *P. guajava,* bark of *M. indica* and root of *V. amygdalina* (Table1).

Table 1: *Phytochemical Qualitative Result*

Plant	Tannin	Alkaloid	Flavonoid	Saponin	Sterol	Phenol
P. guajava	+	++	+	+	+	+
M. indica	+	++	+	+	+	+
V. amygdalina	+	++	+	+	+	+

Where + represents present
And ++ represents deeply present

Quantitative Analysis of Test Plant Samples

The phytochemicals in each test plant samples varied in quantity (Table 2). The percentage quantity of each of the phytochemical vary significantly in each of the test plant samples. Mango recorded the highest percentage quantity of each phytochemical followed by guava and then bitter leaf. Mango recorded the highest percentage of alkaloid. Followed by saponin, flavonoid, tannin, sterol and phenol; which had the least percentage. Alkaloid was the highest in guava followed by saponin, tannin, flavonoid, sterol and phenol which had the least percentage. The content of alkaloid in *V. amygdalina* was the highest, followed by flavonoid, saponin, tannin, phenol and sterol; which had the lowest percentage.

Table 2: *Mean Percentage of Phytochemical Composition of three Plant Extract (Guava, Mango and Bitter Leaf)*

Phytochemicals	Guava	Mango	Bitter Leaf
Tannin (%)	1.33±0.042	1.18±0.021	1.24±0.023
Alkaloid (%)	3.16±0.025	3.43±0.049	2.74±0.055
Flavonoid (%)	1.27±0.010	1.43±0.021	1.78±0.038
Saponin (%)	1.84±0.015	1.66±0.067	1.44±0.047
Sterol (%)	0.25±0.021	0.26±0.030	0.160±0.020
Phenol (%)	0.17±0.012	0.19±0.006	0.170±0.00
Total (Mean ± St.D)	1.33±1.052[a*]	1.36±1.006 [a]	1.26±0.931 [a]

*LSD test (columns with the same superscript are not significantly different at 5% level of significance)

The Result of Growth Inhibition of Human Pathogens by Each of the Three Plant Extracts at 100mg/Ml

The three plant extracts demonstrated antibacterial activity against *E. coli, Staphylococcus citrinum, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella enteric* and *Aspergillus niger* (Table3). Amongst the three plant extracts, the mean total zone of inhibition of the human pathogens by guava extract was higher (7.19) than those of mango (5.64) and bitter leaf (6.23). The mean total zone of inhibition of the human pathogens was higher in the control than in each of the plant extracts (Table3) while amongst the pathogens, the mean total zone of inhibition of *Bacillus* (9.87) and *Staphylococcus* (9.57) were higher than in other pathogens.

There is significant difference in the inhibition effect of the plant extracts and the susceptibility of the human pathogens($P<0.05$).

Table 3: *Growth Inhibition of Human Pathogens by Three Plant Extract at 100mg/mL*

Zone of Inhibition by Plant Extract (mm)					
Human Pathogens	**Guava**	**Mango**	**Bitter Leaf**	**Streptomycin (Control)**	**Total (Mean ± SD)**
E. Coli	6.67±0.021	4.30±0.015	5.71±0.015	13.64±0.099	7.59±3.760^{b*}
Staphylococcus	8.44±0.036	6.83±0.031	7.20±0.000	15.80±0.000	9.57±3.810^{f}
Bacillus	9.12±0.000	6.92±0.015	7.40±0.020	15.84±0.093	9.82±3.732^{e}
Pseudomonas	7.20±0.00	5.74±0.000	5.89±0.036	13.81±0.165	8.16±3.459^{c}
Salmonella	5.80±0.020	4.65±0.000	4.74±0.023	12.94±0.200	7.03±3.593^{a}
Aspergillus	5.90±0.020	5.42±0.020	6.95±0.017	16.52±0.00	8.70±4.752^{d}
Total (Mean ± SD)	7.19±1.271^{c*}	5.64±1.017^{a}	6.32±0.974^{b}	14.76±1.383^{d}	
P-value **Plant Extract		0.000			
P-value **Human Pathogen		0.000			

*LSD test (columns with the same superscript are not significantly different at 5% level of significance)

The Results of Growth Inhibition of Human Pathogens by Three Plant Extracts at 150mg/ml

The mean total zone of inhibition of the human pathogens by guava extract was higher (7.98) than those of mango (6.92) and bitter leaf (7.38). When compared with the control, the mean total zone of inhibition was higher than that of each of the plant extracts. Amongst the pathogens, the mean total zone of inhibition of *Bacillus* (11.49) and *Staphylococcus* (11.43) were higher than in other pathogens (Table 4). There are significant differences in the inhibition effects of the plant extracts and the susceptibility of the human pathogens ($P<0.05$).

Table 4: *Growth Inhibition of Human Pathogens by Three Plant Extract at 150mg/mL*

Human Pathogens	Guava	Mango	Bitter Leaf	Streptomycin (Control)	Total (Mean ± SD)
E. coli	6.36±0.053	6.45±0.025	6.69±0.010	17.59±0.042	9.27±5.016[b*]
Staphylococcus	9.64±0.000	7.90±0.000	8.29±0.042	19.91±0.036	11.43±5.155[e]
Bacillus	10.30±0.020	8.16±0.000	8.67±0.070	18.84±0.032	11.49±4.506[f]
Pseudomonas	8.45±0.000	6.80±0.020	6.913±0.023	16.79±0.036	9.74±4.307[c]
Salmonella	6.20±0.000	5.65±0.000	5.82±0.020	16.33±0.031	8.50±4.728[a]
Aspergillus	6.95±0.010	6.51±0.031	7.90±0.040	17.45±0.025	9.70±4.702[d]
Total (Mean ± SD)	7.98±1.639[c*]	6.92±0.893[a]	7.38±1.019[b]	17.82±1.251[d]	
P-value **Plant Extract		0.000			
P-value **Human Pathogen		0.000			

*LSD test (columns with the same superscript are not significantly different at 5% level of significance)

The Results of Growth Inhibition of Human Pathogens by Three Plant Extracts at 200mg/ml

The mean total zone of inhibition of human pathogens by guava extracts was higher (9.49) than those of mango (8.84) and bitter leaf (9.20). The mean total zone of inhibition of the human pathogens was higher in control (18.44) than in any of the plant extracts. Amongst the pathogens, the mean total zone of inhibition of *Bacillus* (13.66) and *Staphylococcus* (13.22) were higher than in other pathogens (Table5).

There are significant differences in the inhibition effects of the plant extracts and the susceptibility of the human pathogens ($P<0.05$).

Table 5: *Growth Inhibition of Human Pathogens by Three Plant Extract at 200mg/mL*

Zone of Inhibition by Plant Extract (mm)					
Human Pathogens	**Guava**	**Mango**	**Bitter Leaf**	**Streptomycin (Control)**	**Total (Mean ± SD)**
E. coli	7.48±0.010	8.42±0.020	8.80±0.020	13.72±0.0252	9.61±2.533^{a*}
Staphylococcus	11.42±0.025	9.79±0.041	10.33±0.031	21.35±0.110	13.22±4.939^{c}
Bacillus	12.32± 0.025	10.28±0.126	10.43±0.031	21.60±0.000	13.66±4.863^{c}
Pseudomonas	9.44±0.047	8.88±0.0529	8.47±0.419	17.94±0.306	11.18±4.096^{b}
Salmonella	7.90±0.020	7.80±0.000	7.71±0.168	17.39±0.301	10.20±4.339^{a}
Aspergillus	8.40±0.000	7.87±0.0819	9.45±0.020	18.61±0.036	11.08±4.578^{b}
Total (Mean ± SD)	9.49±1.856^{b*}	8.84±0.958^{a}	9.20±1.021^{ab}	18.44±2.725^{c}	
P-value **Plant Extract		0.000			
P-value **Human Pathogen		0.000			

*LSD test (columns or rows with or sharing similar superscript are not significantly different at 5% level of significance)

The Results of Growth Inhibition of Human Pathogens by Three Plant Extracts at 250mg/ml

The mean total zone of inhibition of the human pathogens by guava extracts was higher (11.16) than those of mango (10.25) and bitter leaf (10.72). The mean zone of inhibition of the human pathogen was higher in control (21.56) than in any of the plant extracts. Amongst the pathogens, the mean zone of inhibition of *Aspergillus* (15.23) and *Staphylococcus* (15.13) were higher than in other pathogens (Table 6).

There are significant differences in the inhibition effects of the plant extracts and the susceptibility of the human pathogens ($P<0.05$).

Table 6: *Growth Inhibition of Human Pathogens by Three Plant Extract at 250mg/mL*

Zone of Inhibition by Plant Extract (mm)					
Human Pathogens	**Guava**	**Mango**	**Bitter Leaf**	**Streptomycin (Control)**	**Total (Mean ± SD)**
E. Coli	10.62±0.020	9.68±0.053	10.23±0.025	19.53±0.031	12.52±4.247^{b}
Staphylococcus	12.30±0.000	10.34±0.000	11.56±0.100	26.26±0.053	15.13±6.760^{d}
Bacillus	12.54±0.053	11.30±0.132	10.86±0.060	21.57±0.093	14.06±4.565^{c}
Pseudomonas	10.35±0.000	9.84±0.020	10.18±0.020	18.79±0.114	12.29±3.926^{b}
Salmonella	7.86±0.060	8.80±0.000	8.91±0.031	19.68±0.000	11.31±5.065^{a}
Aspergillus	13.27±0.012	11.54±0.053	12.56±0.000	23.55±0.042	15.23±5.059^{d}
Total (Mean ± SD)	11.16±1.857^{b}	10.25±0.975^{a}	10.72±1.184^{ab}	21.56±2.702^{c}	
P-value **Plant Extract		0.000			
P-value **Human Pathogen		0.000			

*LSD test (columns or rows with or sharing similar superscript are not significantly different at 5% level of significance)

The Results of Growth Inhibition of Human Pathogens by Three Plant Extracts at 500mg/ml

The mean total zone of inhibition of the human pathogens by bitter leaf extract was higher (14.55) than those of guava (14.47) and bitter leaf (12.43). When compared with the control, the mean total zone of inhibition of the human pathogens was higher in control (24.94) than in any of the plant extracts. Amongst the pathogens, the mean total zone of inhibition of *Aspergillus* (18.25) and *Staphylococcus* (18.10) were higher than in other pathogens (Table 7).

Hence, there is significant difference in the inhibition effect of the plant extracts and the susceptibility of the human pathogens ($P<0.05$).

Table 7: *Growth Inhibition of Human Pathogens by Three Plant Extract at 500mg/mL*

Zone of Inhibition by Plant Extract (mm)					
Human Pathogens	**Guava**	**Mango**	**Bitter Leaf**	**Streptomycin (Control)**	**Total (Mean ± SD)**
E. coli	13.45±0.000	11.60±0.000	14.80±0.020	23.25±0.000	15.78±4.661^{b*}
Staphylococcus	15.23±0.031	12.74±0.036	14.82±0.025	29.62±0.020	18.10±7.014^{c}
Bacillus	14.80±0.020	13.59±0.042	16.85±0.050	26.42±0.020	17.91±5.272^{c}
Pseudomonas	14.92±0.020	11.65±0.000	13.72±0.000	22.92±0.020	15.80±4.463^{b}
Salmonella	11.62±0.020	10.72±0.000	11.16±0.000	21.60±0.020	13.78±4.730^{a}
Aspergillus	16.80±0.020	14.34±0.010	15.92±0.020	25.85±0.050	18.23±4.688^{c}
Total (Mean ± SD)	14.47±1.65^{b*}	12.43±1.281^{a}	14.55±1.85^{b}	24.94±2.755^{c}	
P-value **Plant Extract		0.000			
P-value **Human Pathogen		0.000			

*LSD test (columns or rows with sharing similar superscript are not significantly different at 5% level of significance)

Analysis of Variance of the Effects of Plant Extracts at Various Concentrations on the Inhibition of Human Pathogens

There existed significant difference among the inhibition effects of the plants extracts (Table 8).

Table 8: *Analysis of Variance of the Effect of Plant Extract at Various Concentrations on the Inhibition of Human Pathogens*

Sources of Variation	F-ratio	P-value*
Plant Extract	656962.750	.000
Human Pathogen	32179.122	.000
Concentrations	206671.080	.000
Plant Extract * Human Pathogens	2111.195	.000
Plant * Concentration	2287.630	.000
Human Pathogen * Concentration	1565.582	.000
Plant * Human Pathogen * Concentration	596.411	.000

*Significant difference exist at P<0.05

The susceptibility of the various human pathogens to each of the plant extracts also significantly differed (P<0.05). At various concentration there was also a significant difference in the inhibition effects of the plant extract (P<0.05).

The Inhibition Effect of Various Plant Extracts at Various Concentration

At higher concentration, the inhibition strength of the various plant extracts increased. At 100mg/ml, 150mg/ml and 200mg/ml, the inhibition of guava extract was higher than those of bitter leaf and mango but at 250mg/ml and 500mg/ml, the inhibition effect of guava extract was the same with that of bitter leaf.

The Susceptibility of Human Pathogen to Various Concentrations of the Plant Extracts

At higher concentrations, the susceptibility of the human pathogens increased. The susceptibility of *Staphylococcus* and *Bacillus* were higher than others in most of the concentrations of the plant extracts, though at 250mg/ml and 500mg/mL, the susceptibility of *Aspergillus* slightly matched those of the former.

Result of the Minimum Inhibitory Concentration of Bark of Guava

The mean total inhibition of bark of guava extract was highest at 200mg/mL (36.90), followed by 150mg/mL (28.06) and then minimum at 50mg/mL (11.42). There was significant difference in the inhibitory concentration of bark of guava plant (P<0.05) (Table 9).

Table 9: *Minimum Inhibitory Concentration of Bark of* ***Psidium guajava*** *Plant*

Minimum Inhibition at various Concentration (in 50mg/ml)					
Organisms	**0**	**50**	**100**	**150**	**200**
Bacillus	Nil	5.62	9.05	10.30	12.35
Staphylococcus	Nil	5.30	6.84	7.92	9.75
Pseudomonas	Nil	3.94	5.80	6.94	8.93
E. coli	Nil	4.85	6.60	8.30	10.40
Salmonella	Nil	4.50	4.62	5.68	7.92
Penicillin	Nil	5.74	6.25	7.28	8.95
Mean Total inhibition	Nil	11.42^{a*}	19.88^{b}	28.06^{c}	36.90^{d}
F-ratio	54.05				
P-value	0.00				

*LSD test (columns or rows with the same superscript are not significantly different at 5% level of significance)

Result of the Minimum Inhibitory Concentration of Bark of Mango Plant

The mean inhibition of bark of mango plant was highest at 200mg/mL (9.38) followed by 150mg/mL (7.21) and then minimum at 100mg/ml (5.53). There was significance difference in the minimum inhibitory concentration of bark of mango plant (P<0.05) (Table 10).

Table 10: *Minimum Inhibitory Concentration of Bark of* ***Mangifera indica*** *Plant*

Minimum Inhibition at various Concentration (in 50mg/mL)					
Organisms	**0**	**50**	**100**	**150**	**200**
Bacillus	Nil	Nil	6.95	8.02	12.35
Staphylococcus	Nil	Nil	6.86	7.90	9.74
Pseudomonas	Nil	Nil	5.74	6.78	8.80
E. coli	Nil	Nil	4.30	6.42	8.45
Salmonella	Nil	Nil	4.65	5.68	7.80
Penicillium	Nil	Nil	4.80	9.16	10.65
Aspergillus	Nil	Nil	5.40	6.48	7.90
Mean Total inhibition	Nil	Nil	5.53^{a}	7.21^{b}	9.38^{c}
F-ratio	17.45				
P-value	0.001				

*LSD test (columns with the same superscript are not significantly different at 5% level of significance)

Result of the Minimum Inhibitory Concentration of Root of Bitter Leaf

The mean inhibition of root of bitter leaf was highest at 200mg/ml (14.15) followed by 150mg/mL (12.82) and then minimum at 100mg/mL (9.94). There was a significant difference in the minimum inhibitory concentration of root of bitter leaf (P<0.05) (Table11)

Table 11: *Minimum Inhibitory Concentration of Root of* ***Vernonia amygdalina*** *Leaf*

Minimum Inhibition at various Concentration (in 100mg/mL)					
Organisms	**0**	**50**	**100**	**150**	**200**
Bacillus	Nil	Nil	10.95	13.80	15.60
Staphylococcus	Nil	Nil	10.76	14.94	16.25
Pseudomonas	Nil	Nil	8.60	11.75	12.90
E. coli	Nil	Nil	8.74	12.56	13.75
Salmonella	Nil	Nil	7.90	11.32	12.36
Penicillin	Nil	Nil	11.46	12.68	14.10
Aspergillus	Nil	Nil	11.20	12.68	14.10
Mean Total inhibition	Nil	Nil	9.94^{a}	12.82^{b}	14.15^{b}
F-ratio	14.84				
P-value	0.001				

*LSD test (columns with the same superscript are not significantly different at 5% level of significance)

Discussion

The efficacy of the ethanolic extracts of three tropical plants namely: *Psidium guajava L.*, *Mangifera indica L.* and *Vernonia amygdalina L.* was evaluated on the inhibition of the selected human pathogens- *Escherichia coli, Bacillus subtilis, Salmonella enteric, Pseudomonas aeruginosa, Staphylococcus citrinum* and *Aspergillus niger* which cause human diarrhoea.

Ethanol was used because it is an organic solvent and would dissolve organic compounds better, hence liberate the active compounds required for antimicrobial activity [16,30,36].

The phytochemical analysis on the extracts of the test plants revealed the presence of saponins, tannins, sterols, flavonoids, phenol and alkaloids [17]. Most of these compounds have been shown to display physiological activity against most microorganisms [37-38].

This agrees with the finding made by Zhu *et al.* (1997) [39] when they stated that tannins were very useful in medicine because of their astringent properties and observed that herbs that contain tannins were recommended for a wide range of treatment such as liver injury, kidney problems and also as diuretics. Alkaloids were reported to be toxic and often elucidate strong physiological changes in the body, hence they were widely used in medicine [32,17] for their analgesic, antispasmodics and antibactericidal effects [40,41]. Tannins possess astringent properties while saponins were used as expectorant and emulsifying agents in medicine [41,42]. Phenols are antimicrobial agents which are inhibitory to the growth of pathogens [41].

Each of the plant extracts was more inhibitory to *Staphylococcus citrinum* followed by *Bacillus subtilis* and *Aspergillus niger*. This is in accordance with reports of Desta (1993); Okigbo and Mmeka (2008) [43,20]. They reported that plant extracts show stronger retardation effects on the gram positive test strain than on the gram negative ones. It can also be observed in the antibiotic (control) used in which streptomycin had more effect on *Staphylococcus citrinum* than on *E. Coli.*

The antimicrobial property of these plants justifies their use by traditional medical practitioners for the treatment of various ailments [20]. In humans, the leaves and barks of the *P. guajava* tree is use to treat diarrhoea [44]. Various parts of *M. indica* plant are used as a dentrifice, antiseptic, astringent, vermifuge and diuretic, and to treat diarrhoea, dysentery, anaemia and cough [45]. The bark, leaves and roots of *V. amygdalina* have ethno medicinal value use for treating various diseases like fever, diarrhoea, stomach discomfort [46].

The inhibitory effects of plant extracts were highly dependent on concentration. At higher concentration, wider zone of inhibition was produced which reduced as the concentration reduced. The minimum inhibitory concentration (MIC) was done using the agar well diffusion method. With bark of guava plant, all the test organisms were inhibited at all the concentrations; bark of mango plant and root of bitter leaf inhibited also at all the concentrations except at 50mg/ml. The interaction of extraction medium and concentration of extracts was significant ($P<0.05$) on the inhibition of test organisms [30,26].

In all, ethanol extracts at 200mg/ml concentration gave the highest inhibitory effect on the test organisms.

Conclusion and Recommendation

From the results obtained in this research work, it is evident that bark of *Psidium guajava,* bark of *Mangifera indica* and root of *Vernonia amygdalina* possess potential inhibitory activity against human pathogens *in vitro* to varying degrees. From this work, the ability of each of the extracts to inhibit each of the test organisms indicates that *P. guajava, M. indica* and *V. amygdalina* can be used to treat infections arising from the activities of these pathogens. The possible chemotherapeutic usefulness of these three plants has therefore been demonstrated in this work.

Hopefully, the use of plants with chemotherapeutic properties will contribute to the development of antimicrobial agents in Nigeria.

Further investigation can combine the plants extracts for possible synergetic effects. Also the plant extracts can be tested on other human pathogens to elucidate and ascertain their uses.

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