Original Research Article

Preliminary Phytochemical, Antioxidative and Antibacterial Screening of Selected Medicinal Plants for Possible use in the Treatment of Bacterial Diseases.

ABSTRACT

Development of cheap and reliable diagnostics is pivotal in the control of infectious diseases. Currently, one of the best strategies to tackle infectious disease is the use of natural products from plant origin, due to the perception that long term use of western medicine induces severe complications and also to stop the prevalence of antibacterial infection. This study aims at substantiating the traditional use of selected medicinal plants with antibacterial claim for possible lead for the development of more potent drugs in the treatment of bacterial infections. The preliminary phytochemical content, antioxidant and antibacterial potential of the selected medicinal plants were evaluated using standard procedures. Thirteen plants were obtained through questionnaire administration: *Garcinia kola, Costus afer, Vitellaria paradoxa, Pycnanthus angolensis, Cola acuminata Acanthospermum hipida, Aloe vera, Euadenia trifoliate, Microglossa abzelii, Moringa oleifera, Bryophyllum pinnatum, Baphia nitida* and *Afromonium melegueta.* Thin layer chromatography (TLC) profiling of the various selected plants were carried out using two solvent system consisting of n-Hexane and ethyl acetate in varying ratio (8:2 and 7:3). Eight (8) plants, which showed good chromatographical profiling with the used solvent, were selected for further experiment (*Garcinia kola, Vitellaria paradoxa, Acanthospermum hispidum, Aloe vera, Microglossa abzelii, Baphia nitida, Moringa oleifera* and *Bryophyllum pinnatum*). These plants were subjected to qualitative phytochemistry and five plants (*Moringa oleifera, Vitellaria paradoxa, Baphia nitida, Bryophyllum pinnatum* and *Garcinia kola*) selected for quantitative phyto-screening for alkaloids, flavonoids, saponins and tannins. The antibacterial screening was carried out using Agar well diffusion and minimum inhibitory concentration (MIC) against the following isolate *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Bacillus subtilis.* The antioxidant potential of the selected five plants were evaluated using *in vitro* (DPPH and FRAP) and the best plant was used for *in vivo* (SOD

and MDA) analysis. The phytochemistry of the 13 plants revealed the presence or absence of Alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, carbohydrates and protein in the selected plants, and the best five were selected for quantitative evaluation showed they all have good quantity of alkaloids, flavonoids, saponins and tannins. The plants exhibited mild to strong antibacterial activities, having an MIC value ranging from 2.5 to >20 µg/mL when compared to ciprofloxacin (control) that is 5µg/mL. The plants showed good free radical scavenging activities and efficient ferric reducing power in a concentration dependent manner and a good enzyme activity of the superoxide dismutase (SOD) at a concentration of 100 µ/mL with a reduction in malondialdehyde (MDA) production as a marker of lipid peroxidation. The various plants have potentials for development of drugs for the treatment of infectious diseases; and a potential for further evaluation of their antibacterial claim.

1.0 INTRODUCTION

Medicinal plants have become vital in the fostering of good health. Its potency and low or no cost of accessibility has made greater number of persons especially in the rural areas to venture into using it to treat several diseases. Its potency lies in some of the bioactive compounds embedded in them (Edeoga *et al.*, 2005). In Ohafia, medicinal plant is an integral tool for the treatment of infectious disease. My ethno-botanical survey focused on plants used in the treatment of bacterial disease in Ohafia. Bacterial disease which contribute significantly to the global health challenge responsible for increased morbidity is also a leading infectious disease according to the world health organization. Bacterial disease in its most common form is an airborne disease that invades the alveolar air sac of the lungs and resides in the macrophages and other phagocytic immune cell of the host. Among the multiple factor that contribute to the success of the disease are the recalcitrant nature of the pathogen, and the growing drug resistance acquired by it through decades of treatment with a particular regimen of drugs. Lengthy diagnostic procedure, incorrect diagnosis, followed by inadequate prescription of antibiotics enable resistant strain to spread within and outside community to gain strong hold in the global human health and wellbeing, as it has rendered most of our antibiotics less effective. Continuous usage of a resisted antibiotics poses a health problem, as it can lead to increase in mortality. Therefore, development of a fast and simple method well correlated with medicinal properties will be useful. Thus there is an urgent need to step up research and discovery of novel antibacterial agent from the rich buck list of Nigerian medicinal plants. The global need for

alternative medicine for tuberculosis with minimal side effect is increasing due to prevalence of the disease. Medicinal plants are natural products use for treatment of human disease, which contains organic compounds that produce definite physiological action on the human body and these bioactive compound embedded in them include tannins, alkaloids, carbohydrates, flavonoids, terpenoids and steroids (Edeoga *et al.*, 2005). These compounds plays a significant defensive role against pathogenic bacteria like *Staphylococcus aureus*. Physiological and metabolic processes are disturbed due to oxidative stress produced by reactive oxygen specie (ROS) (Schutezendubel and polle, 2002). Increased generation of ROS decreases the antioxidant defense system (Datta *et al.,* 2000). Plant extracts are bedrock of natural antioxidants that can help sustain the normal functioning of the immune system against infection and diseases like bacterial cellulitis, bacterial food poisoning, tuberculosis and strep throat. In Nigeria, many cases of multi antibiotic – resistant bacteria are reported. To face the multidrug resistance, many studies have proven the effectiveness of medicinal plants in the fight against certain bacteria stains. Faced with the bacterial strain resistance to almost all antibiotics used nowadays, and the incapacity of endogenous antioxidant system to protect without failure biological macromolecules opposite the oxidative stress, research of extract becomes necessary. Meanwhile, as antibacterial resistance from bacteria worsens, our antibiotics becomes less effective, and it is vital that this be put to cognizance as well as factors that can drive the strengthening of infection control practices. And one of the most reliable way is the capitalization of endogenous knowledge through medicinal plant study, and that is why I made a tremendous effort necessary to undertake an ethno-botanical studies to identify plants locally used to treat tuberculosis. Therefore the study aims at substantiating the traditional use of the selected medicinal plants on their antibacterial folkloric usage using TLC profiling, antibacterial effect of some selected bacteria, antioxidant capacity and the phytochemical analysis.

1.1 RELATIONSHIP BETWEEN, OXIDATION AND BACTERIAL DISEASE

Bacterial disease can induce Reactive oxygen species (ROS) production by activating phagocytes which are important part of host defense mechanism against pathogens. ROS production is enhanced by the host cell to clear out bacterial infection, but this becomes

damaging to the host cell itself. Such damage is controlled by introduction of antioxidant. Excessive production of ROS due to bacterial infection may promote tissue injury and inflammation in affected individual. This accentuates immune suppression especially in those with weakened antioxidant capacity. Under normal conditions, during the cellular metabolism due to production of ROS like superoxide anion and hydrogen peroxide (H_2O_2) the lungs are exposed to the basal oxidative stress. These cells possess the capacity to generate huge amount of ROS which are not adequately removed and these ROS induces the lipid peroxidation, rise in calcium ions and DNA damage (Shreewardhan *et al.,* 2017). Also, someone who develops bacterial disease may have subtle immune defects which makes the body system vulnerable to other bacterial infection. (Elizabeth *et al.,* 2019)

2.0 MATERIALS AND METHOD

2.1 Study Area

Ohafia is a local government area of Abia state in the southeastern part of Nigeria situated between 5°37'N and 7°50'E and consist about twenty six villages with a population strength ranging between 800,000 and 916,000 as of 2014. And it is at a distance of 50.1km away from the capital city Umuahia in Abia state. Ohafia is a part of the tropical rainforest with a total annual rainfall of over 1400mm and annual temperature range of 23°C to 32°C. The people of Ohafia practice both subsistence and commercial farming, and they value traditional medicine, either used alone or in combination with other medicine in the treatment of disease. Since some of its inhabitant are rural dwellers, hence they make use of plant based therapies in the management of diseases that include tuberculosis.

2.2 Ethno-botanical Survey

The ethno-botanical survey was conducted from October 2019 to January 2020 using an oral interview to the participant ethno-medicinal knowledge of plants use in treatment of various ailments. The oral interview consist of name of plant, plant part used and method of preparation. The people interviewed were twenty five in number both male and female within the age bracket of 40 to 65 years.

2.3 Plant Collection and Identification

The various plant samples: *Bryophyllum pinnatum* (leaves*), Aloe vera* (leaves), *Baphia nitida* (leaves) *Euadenia trifoliate* (root), *Acanthospermum hispidum* (leaves), *Moringa oleifera* (leaves), *Microglossa abzelii* (leaves), *Cola acuminata*(fruit), *Garcinia kola* (leaves), *Garcinia kola* (fruit), *Vitellaria paradoxa* (leaves), *Costus afer* (stem), *Afromomium melegueta* (fruit) and *Pycnanthus angolensis* (bark) were collected after the interview in Ohafia, Abia State. The plants were identified in the department of Plant Science and Biotechnology, Michael Okpara University of agriculture, Umudike. The voucher specimen (JOHNSONVIC 1 – 14) was prepared and kept at the herbarium of the department of pharmacognosy, Nnamdi Azikiwe University, Awka. The best potential five plants were selected for further experiments after simple TLC and qualitative phytochemical screening was carried out on the fourteen plant samples. The five best potential samples are *Garcinia kola, Vitellaria paradoxa, Baphia nitida, Moringa oleifera* and *Bryophyllum pinnatum*.

2.4 Sample Preparation

The collected plant samples were air dried at a room temperature. The plants were pulverized to a fine powder using electric blender and stored in an air-tight container for maceration. One hundred grams (100g) of dried powdered powdered plant material each was extracted with 250 mL of methanol for 48hrs at room temperature. The extract was filtered using Buchner funnel and Whatman number 1 filter paper. The filtrate was allowed to naturally evaporate for some days to ensure the potential active constituents are not lost, distorted or destroyed during application of heat.

2.5 Thin Layer Chromatography (TLC) Profile

TLC was conducted using aluminum pre-coated plates. A drop of methanol was added to the extract to permit easy collection of the extract with the capillary tube. About 10µL of the plant extracts were gently loaded on the base of the plat (4cm above) using capillary tubes. The base of the loaded plates where soaked into a mixture of solvent (n-Hexane : Ethyl acetate) in the ratio of 7: 3 and 8: 2, and the plates were allowed to develop a chromatographic profile. The samples are drawn up via capillary action, because different analyte ascends the TLC plates at different rate,

separation is achieved. The TLC plates are removed and allowed to dry. The spot are detected by treating with iodine vapor and the retention factor calculated using the formula:

$$\frac{\text{Distancetravelledbythecomponent}}{\text{Distance travelled by the solvent}}$$

2.6 Qualitative Phytochemical Screening

The following standard procedure were used to test for the presence of various bioactive compounds in the plant samples

2.6.1 Test for Protein:

To the little portion of the filtrate of the powdered sample in a test tube, two drops of Million reagent was added. A white precipitate indicate the presence of protein.

2.6.2 Test for Carbohydrate:

To the little portion of the filtrate in a test tube, 2 ml of Molisch's reagent was added. Followed by the addition of the concentrated H_2SO_4 on a slanty position. A deep violet or purple color indicates the presence of carbohydrate.

2.6.3 Test for Tannins

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test and then filtered. A few drop of 0.1% Ferric chloride was added and observed for brownish green or a blue black coloration.

2.6.4 Test for Saponins

$20cm^3$ of diethyl ether was added to the filtrate in a $250cm^3$ separating funnel and vigorously agitated from which the aqueous layer was recovered white. The ether layer was discarded. The purification process was repeated twice. 60mls of n-butanol was added and extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes after which the solution was transferred into a crucible and was dried on an oven to a constant weight. The saponin content was calculated as a percentage.

$$\text{Percent saponin} = \frac{\text{Weightofsaponin}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.6.5 Test for Alkaloids

To 2mls of my extract, 2 drops of Wagner reagent was added. A reddish brown color shows the presence of the alkaloids.

2.6.6 Test for Flavonoids

5mls of dilute ammonia solution was added to a portion of aqueous filtrate of each plant extract, followed by addition of H_2SO_4. A yellow coloration observed in each extract indicates the presence of flavonoids. The yellow coloration disappears on standing.

2.6.7 Test for Steroids

2mls of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2mls of H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

2.6.8 Test for Cardiac Glycoside (Keller- Killani test)

5 ml of each extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while the acetic acid layer, a greenish ring form just gradually throughout the layer.

2.6.9 Test for Terpenoids

5 ml of each extract was mixed in 2 ml of chloroform and concentrated H_2SO_4 (3 ml) and carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive result for the presence of terpenoids.

2.7.0 Quantitative Determination of the Phytochemical Constituents

Preparation of fat free sample:

2 g of the sample were defatted with 100ml of diethyl using soxhlet apparatus for 2 hours

2.7.1 Determination of Alkaloid Content

Quantitative determination of alkaloids was done according to the methodology of Harborne J.B. (1973). Exactly 200 ml of 10% acetic acid in ethanol was added to 5 g of the plant sample in a 250 ml beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one quarter of the original volume followed by the addition of 5 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitate was completed immediately

after filtration. After three (3) hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 ml of 0.1m of ammonium hydroxide and then filtered using Gem filter paper. The percentage of alkaloid is expressed mathematically as

$$\% \text{ Alkaloid} = \frac{W2 - W1}{W} \times \frac{100}{1}$$

Where:

W_1 is Weight of filter paper

W_2 is Weight of filter paper + Residue

W is Weight of air dried sample

2.7.2 Determination of Tannin Content

Folin-Denis spectrophotometric method was used. The method is described by Pearson (1976). A measured weight of each sample (1.0g) was dispersed in 10ml distilled water and agitated. This was left to stand for 30mins at room temperature being shaken every 5mins. At the end of the 30mins, it was centrifuged and the extract gotten. 2.5 ml of supernatant (extract) was disposed into a 50 volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate flask. 50 ml flask

A 1.0ml Folin- Denis reagent was measured into each flask, followed by 2.5ml of saturated sodium carbonate solution. The mixture was diluted to mark in the flask (some) and incubated at room temperature. Absorbance was measured at 250nm in a Genway Model 6000 electronic spectrophotometer. Readings were taken with the reagent blank at zero.

The Tannic content was given as follows

$$\% \text{ Tannic} = \frac{An}{As} \times C \times \frac{100}{W} \times \frac{Vf}{Va}$$

Where: An – Absorbance of the test sample

As – Absorbance of standard solution

C – Concentration of standard solution

W – Weight of sample used

Vf – Total volume of extract

Va – Volume of extract analyzed.

2.7.3 Determination of Saponin Content

Saponin quantitative determination was carried out using the method reported by (Ejikeme *et al.*, 2014) and (Obadoni and Ochuko 2002). Exactly 100ml of 20% aqueous ethanol was added to 20 g of the powdered sample in a 250 cm3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a particular temperature of 55^{o}C. The residue of the mixture was re-extracted with another 100ml of 20% aqueous ethanol. After filtration and heater for 4hours at a contact temperature of 55^{o}C with constant stirring. The combine extract was evaporated to 40 cm^3 over water bath at 90^{o}C. The saponin content was calculated as a percentage:

$$\% \text{ Saponin } \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Where:

W_1 is Weight of Empty beaker

W_2 is Weight of beaker + sample

W is Weight of air dried sample

2.7.4 Determination of Flavonoid Content

The method of Harborne was used (Haborne, 1973)

5g of sample was boiled in 100mls of 2M HCl solution for 40 minutes. It was allowed to cool to room temperature before being filtered through Whatmann filter paper. Flavonoid in the extract was precipitated by drop wise addition of concentrated ethyl acetate until in excess following filtration. The flavonoid ppt recovered was oven dried and the weight of flavonoid obtained by different and express as a percentage of the sample analyzed. The percentage of flavonoid is mathematically represented as

$$\% \text{ Flavonoid: } \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Where:

W_1 is Weight of empty filter paper

W_2 is Weight of filter paper + Residue

W is Weight of air dried sample

2.8.0 *In Vitro* Quantification of Antioxidant Activities

2.8.1 DPPH (1, 1, diphenyl-2-picryl hydroxyl) Radical scavenging assay

0.5 mM of DPPH was prepared. Different concentrations of the extract were prepared ranging from 31.25µg to 500 µg. 0.5 ml each of the different concentration of the extract was pipetted into a clean test tube. 0.5 ml of the O.5 mM DPPH was added. 4 ml of methanol was added to the mixture. The absorbance was read at 517 nm using UV-visible spectrophotometer within 30 minutes of the reaction. A blank was used which contains DPPH and methanol only. The percentage DPPH scavenging effect was calculated using the formula:

$$\frac{Abs_{blank} - Abs_{sample}}{Abs\ blank} \times \frac{100}{1}$$

Where Abs blank is the absorbance of blank and Abs sample is the absorbance of sample.

2.8.2 Ferric Reducing Antioxidant Power (FRAP)

Different concentrations of the extract were prepared starting from 10 µg/ml to 50 µg/ml. 2.5 ml of 0.2 M solution phosphate buffer (pH 6.6)

And 2.5 ml of 1% potassium ferricyanide solution was added to each concentration of the extract. The mixture was vortexed (shaken) well and then was incubated for 20 minutes at 50^{o}C. After the incubation, 2.5ml of 10% trichloroacetic acid was added to the mixture and centrifuge for 10 minutes at 300 rpm. 2.5 ml of the supernatant was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The colour formed was read at 700 nm against blank using UV-visible spectrophotometer. The blank that was also used contains all the materials except the extract used. The standard used is ascorbic acid.

2.8.3.0 *In Vivo* Analysis of Antioxidant

2.8.3.1 Animals

About twenty five (25) male Wistar albino rats weighing between 120g were obtained from the Department of Zoology, Nnamdi Azikiwe University, Awka. They were kept in the animal house of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu. These animals were

given with food and water. And was separated into five (5) groups. Group one (1) were dosed with 10ml/kg of distilled water. Group two (2) were dosed with 100mg/kg of ascorbic acid. Group three (3) were dosed with 100mg/kg of the extract. Group four (4) were dosed with 200mg/kg of extract and Group five (5) were dosed with 400mg/kg of extract The animals were allowed to acclimatize for two weeks before their various liver were harvested each, and cut with scissors into a mortar and was crushed. The crushed liver was mixted with 5mls of water, and ready for experiment.

2.8.3.2 Method for SOD (Superoxide Dismutase)

Clean tubes were labelled according to the different groups treated. 0.4 ml of the different samples was added to the respective tubes. 5 ml of 0.05 M carbonate buffer (pH 10.2) was added and mixed. It was allowed to equilibrate for 5 minutes at 37^{o}C. Then 0.6 ml of 0.3 mM of freshly prepared epinephrine was added. The absorbance was reached at 480 nm at 30 seconds and 150 seconds interval. The blank contains everything except the sample that is replaced with distilled water.

The actual OD reading (R) = $\frac{OD_{150} - OD_{30}}{2}$

% inhibition= (R blank – R_{test}/R_{blank}) x 100

Enzyme unit (µ/ml) = $\frac{(\%inhibition)}{50.}$ x Y

Where Y= mg of tissue in the volume of the sample used. Reference Olatosin *et al.*, 2014. Y = 40mg

2.8.3.3 Method of MDA (Malondialdehyde)

Clean test tubes were labelled accordingly to the different groups needed. 0.2 ml of sample was added to the respective tubes. 1ml of 1% thiobarbituric acid (TBA) in 20% sodium hydroxide (NaOH) was added. Then 1ml of glacial acetic acid was added. It was mixed and incubated in a boiling water for 15 minutes. It was cooled and read at 532nm using spectrophotometer. The MDA (nmol/ml) was calculated.

MDA (nmol/ml) = (ODx1,000,000)

156,000

$1.56x10^5$ $M^{-1}cm^{-1}$ is the molar extinction coefficient

2.8.3.4 Acute Toxicity test (LD50)

The acute toxicity test was carried out according to the method of Dietrich Lorke (1983). A total of 13 mice was used and the work was done in two phases.

Phase 1, a total of 9 mice was used, and they were grouped into 3 groups of 3 mice per group. Group 1 received 10mg/kg of the extract. Group 2 received 100mg/kg. While group 3 received 1000mg/kg. The animals were constantly monitored for the next 1hr, intermittently for the next 2hrs, and after 24hrs for behavioral changes and mortality. From the result of phase 1, the second phase was carried out. In this phase, a total of 4 groups of one mice per group. Group 1 received 2000mg/kg of the extract. Group 2 received 3000mg/kg. Group 3 received 4000mg/kg. While group 4 received 5000mg/kg. The animals were monitored as in phase 1, for behavioral changes and mortality.

2.9.0 Antibacterial Activity Screening

2.9.1 Media Preparation

Culture media were prepared according to the instructions of the manufacturers. The manufacturers specifies that the media should be prepared by dissolving 38 grams of MHA, 13 grams of NB in enough distilled water or de-ionized water to make one litre of solution sterilized by autoclaving at 15 psi, 121^{O}C, for 15 minutes. After autoclaving it should be left to cool to 50^{O}C and poured immediately into flat bottom petri dishes on a horizontal surface.

2.9.2 Test Microorganisms

The test organisms used in this work, which include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*, were collected from the Department Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe

University Awka. These organisms were further reconfirmed by sub-culturing and subjecting pure isolates to specific pure culture identifications techniques.

2.9.3 Preparation of Extract for Evaluation

Here, stock concentrations of each of the extract were made by weighing 80 mg each of crude, extract respectively into sterile beakers. Then 4 ml of dimethyl sulfoxide (organic diluent) is added into each of the samples and reconstituted properly. This gives a stock concentration of 20 mg/mL of each extract, thereafter two fold serial-dilution were made from each of the stock concentrations to get graded concentrations (10, 5, 2.5, 1.23 and 0.63 mg/mL) of each of the crude extract.

2.9.4 Determination of Antibacterial Activity

2.9.4.1 Agar Well Diffusion

The antibacterial assay for the crude extracts was carried out using the agar well diffusion assay as described by (Okezie *et al*., 2017) with slight modifications. The antimicrobial activities of the extracts of the plants under study were tested against four standard human pathogenic bacteria species namely *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Bacillus subtilis*. These were standard laboratory cultures whose susceptibility on commonly used antibiotics was already established. *Staphylococcus aureus and Bacillus subtilis* represent Gram positive bacteria while *Escherichial coli and Salmonella typhi* represents Gram negative bacteria.

The bacterial suspensions were adjusted to 0.5 McFarland turbidity standard and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm) while the standardized fungi cultures were inoculated onto Malt Extract Agar plates. A sterile cork borer was used to make five wells (8 mm in diameter) on each of the MHA and MEA plates. Aliquots of 80µl of each extract dilutions, reconstituted in DMSO at concentrations of 20, 10, 5, 2.5, 1.23 and 0.63 mg/mL, and were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin (50 µg/mL) served as the positive control against the test organisms. The cultures were incubated at 37ºC for 24 h. The antimicrobial potential for each extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the

well). For each of the crude extract, three replicates were conducted against each organism. Each of the sample was tested against all the test isolates.

2.9.4.2 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate Petri dishes. Here, agar dilution method was adopted.

Stock solutions of 200 mg/ml of the various extracts were prepared. Then, two-fold serial dilutions were made to get 100, 50, 25, 12.5 and 6.25 mg/mL thereafter 10-fold dilutions of each of the concentration was made using 9 mL sterile molten agar this was allowed to solidify. The microbial inoculums which has been standardized to 0.5 McFarland turbidity is streaked on the agar appropriately. The plates are incubated at 37 ^{O}C for 24hrs for the bacteria plates.

After incubation the plates were examined for microbial growth by checking for growths using a plus sign (+) indicating growth while a negative sign (-) indicates no growth. * indicates no MIC carried out because there was no antibacterial activity

2.10 STATISTICAL ANALYSIS

Statistical analysis was performed using Graph pad instat software version 3.10. The results were expressed as mean ± Standard Error Mean (SEM). Data were analyzed using one-way Analysis of variance (ANOVA) followed by Dunnett multiple comparison test $p<0.01$ were considered.

3.0 RESULTS

3.1 Description of the Medicinal Plants Obtained from the Ethno-Botanical Survey

Table 1 Medicinal plants that are used traditionally in the treatment of tuberculosis.

The various medicinal plants were gotten from my ethno-botanical survey conducted in Ohafia, Abia state, Nigeria.

S/N	Plant Sample	Plant Part	Plant Weight(g)	Extract Weight (g)	Common Name	Family	Method of preparation
1	*Acanthospermum hispidum*	Leaves, whole plant	141.67	4.50	Starburr	*Compositae*	Infusion
2	*Euadenia trifoliate*	Root	153.77	6.17	Euadenia	*Capparaceae*	Decoction
3	*Microglossa abzelli*	Leave,	109.27	3.77		*Compostitae*	Maceration
4	*Bryophyllum pinnatum*	Leave	190.22	5.0	Life plant	*Crassulaceae*	Infusion
5	*Aloe vera*	Leave	124.03	4.31	Aloe	*Asphodelaceae*	Infusion
6	*Baphia nitida*	Leave	144.62	6.96	Camwood	*Fabaceae*	Infusion
7	*Moringa oleifera*	Leave, flower	134.20	9.09	Drumstick	*Moringaceae*	Maceration
8	*Cola acuminate*	Fruit	152.78	8.53	Kola nut	*Malvaceae*	Infusion
9	*Garcinia kola*	leaves	119.02	4.20	Bitter kola	*Clusiaceae*	Decoction
10	*Vitellaria paradoxa*	Leave,	210.50	8.41	Shea tree	*Sapotaceae*	Decoction
11	*Costus afer*	Stem	122.31	4.45	Ginger lily	*zingiberaceae*	Decoction or infusion

12	|*Pycnanthus angolensis*	Stem, bark	262.95	8.05	African nutmeg	*Myristicaceae*	Maceration
13	*Afromomum melegueta*	Fruit	112.36	3.32	Alligator pepper	*Zingiberaceae*	Maceration

3.2. Thin Layer Chromatography profiling Analysis

The table below shows the Retention factor values of the used plant extract, alongside with their migration picture on the glass plate coated with silica gel. The TLC was carried out using two different ratio on two solvent system.

First Ratio 7 : 3

N-Hexane: Ethyl acetate

Solvent front = 6.3

Table 2: Thin layer chromatographic values using the ratio of 7:3 of N-Hexane and Ethyl acetate

S/N	**Plant Extracts**	**Number of detected compounds**	**Distance travelled by compounds**	**Retardation Factor**
1	*Garcinia kola*	6	1.4, 2.9, 3.5, 4.0, 4.7, 5.5	0.22, 0.46, 0.56, 0.63, 0.75, 0.87
2	*Moringa oleifera*	6	0.5, 2.8, 3.9, 4.6, 5.0, 5.8	0.079, 0.44, 0.62, 0.73, 0.79, 0.92
3	*Vitellaria paradoxa*	9	1.6, 2.0, 2.4, 2.8, 3.2, 3.9, 4.6, 5.0, 5.5	0.25, 0.32, 0.38, 0.44, 0.51, 0.62, 0.73, 0.79, 0.92
4	*Baphia nitida*	6	2.7, 3.8, 4.3, 4.7, 5.0, 5.8	0.43, 0.60, 0.68, 0.75, 0.79, 0.92

5	*Bryophyllum pinnatum*	7	1.9, 2.8, 3.1, 3.7, 4.4, 4.9, 5.7	0.30, 0.44, 0.49, 0.59, 0.70, 0.78, 0.90

Second Ratio 8 : 2

N- Hexane: Ethyl acetate

Solvent front = 6.6

Table 3: Thin layer chromatographic values using the ratio of 8:2 of N-Hexane and Ethyl acetate

S/N	Plant Extracts	Number of detected compounds	Distance travelled by compounds	Retardation Factor
1	*Garcinia kola*	8	0.9, 1.5, 2.0, 3.0, 3.5, 3.8, 4.0, 5.3	0.14, 0.23, 0.30, 0.45, 0.53, 0.58, 0.61, 0.80
2	*Moringa oleifera*	5	1.5, 2.1, 3.1, 4.0, 5.9	0.23, 0.32, 0.47, 0.61, 0.89
3	*Vitellaria paradoxa*	5	0.6, 1.1, 1.6, 2.8, 3.6	0.09. 0.17, 0.24, 0.42, 0.55
4	*Baphia nitida*	8	1.0, 1.5, 2.0, 2.4, 3.0, 3.8, 4.1, 5.9	0.15, 0.23, 0.30, 0.36, 0.45, 0.58, 0.62, 0.89
5	*Bryophyllum pinnatum*	6	1.0, 1.1, 2.1, 2.9, 3.8, 5.9	0.15, 0.17, 0.32, 0.44, 0.58, 0.89

3.3.0 Phytochemistry

3.3.1 Qualitative Phyto-screening

After performing the phytochemical analysis of the studied medicinal plant extracts, the results are as shown on the table below. Bioactive compounds tested for are alkaloids, saponins, tannins, flavonoids, steroids, Terpenoids, cardiac glyucosides, carbohydrates and protein.

Table 4: Qualitative analysis of the phytochemicals of the medicinal plants

S/N	SAMPLE	ALK	SAP	TAN	FLA	STE	TERP	CARD GLY	CARB	PROT
1	*Garcinia kola*	++	+	++	+	-	+	+	+	++
2	*Vitellaria paradoxa*	+	++	+	+	-	-	+	+	++
3	*Acanthospermum hispidum*	+	-	-	++	-	-	++	+	++
4	*Aloe vera*	+	-	-	++	-	+	+	-	-
5	*Microglossa abzelii*	++	-	+	+	-	-	+	+	-
6	*Baphia nitida*	+	++	+	++	-	-	+	+	++
7	*Moringa Oleifera*	+	++	+	+	-	+	++	++	++
8	*Bryophyllum pinnatum*	++	+	+	++	-	+	++	+	++

Key to the table below: ALK is Alkaloids, SAP is saponins, TAN is Tannins, FLA is flavoinoids, STE is Steroids, TERP is Terpenoids, CARD GLY is Cardiac glycosides, CARB is Carbohydrate and PROT is Protein. Also minus (-) sign signifies absence of the compound, Plus (+) sign signifies presence of compound, while double plus (++) sign signifies the compound presence in high concentration.

3.3.2 Quantitative Phytochemical Analysis some selected Nigerian Medicinal Plants

Table 5: Quantitative analysis of the phytochemicals of the medicinal plants

PLANT EXTRACT	ALKALOID (%)	FLAVONOID (%)	SAPONIN (%)	TANNIN (%)
Bryophyllum pinnatum	8.8	4.4	5.5	3.4
Vitellaria paradoxa	3.8	3.0	8.95	3.2
Baphia nitida	4.0	3.2	8.6	4.3
Moringa oleifera	2.4	5.0	7.8	7.2
Garcinia kola	10.6	5.0	4.6	7.2

3.4.0 Result of the *In Vitro* Antioxidant Activities of the Plants Using DPPH and FRAP

3.4.1 DPPH radical scavenging effect on plant extract

Blank = 0.636

Control (Ascorbic acid)

The DPPH radical scavenging effect of the plant extracts were determined using 1,1-diphenyl picrylhydrazyl radical. The plant extracts showed good radical scavenging property in a concentration dependent manner as shown in the table below. Ascorbic acid serves as its control.

Table 6: The DPPH percentage inhibition for Ascorbic acid.

SAMPLE	100µg	50µg	25µg	12.5µg	6.25µg	IC50 µg/ml
Ascorbic acid	0.152±0.006**	0.184±0.002**	0.218±0.005**	0.446±0.004**	0.478±0.002**	31.62

% inhibition	76.18%	71.54%	65.72%	29.95%	24.84%	

Values apart from the percentage inhibition are mean ± SEM. Signficant with the blank *P<0.05 and **P<0.01

Table 7: The DPPH percentage inhibition for the plant extracts

SAMPLE	**500µg**	**250µg**	**125µg**	**62.5µg**	**31.25µg**	**IC50 µg/ml**
Garcinia kola	0.186±0.004**	0.355±0.006**	0.521±0.005**	0.530±0.006**	0.587±0.004**	331.53
%inhibition	70.83%	44.26%	18.16%	16.74%	7.74%	
Vitellaria paradoxa	0.125±0.003**	0.132±0.006**	0.194±0.006**	0.441±0.012**	0.468±0.002*	127.63
%inhibition	80.34%	79.32%	69.49%	30.73%	26.41%	
Baphia nitida	0.398±0.008**	0.456±0.004**	0.545±0.012**	0.554±0.013**	0.585±0.004**	670.72
%inhibition	37.42%	28.38%	14.38%	12.97%	8.01%	
Moringa oleifera	0.454±0.005**	0.538±0.012**	0.549±0.014**	0.574±0.011**	0.597±0.006**	993.0
%inhibition	28.69%	15.40%	13.67%	9.83%	6.21%	
Bryophyllum pinnatum	0.131±0.004**	0.144±0.003**	0.288±0.008**	0.447±0.004**	0.559±0.008**	188.30
%inhibition	79.48%	77.43%	54.79%	29.79%	12.10%	

Values apart from the percentage inhibition are mean ± SEM. Signficant with the blank *P<0.05 and **P<0.01. Values with different superscript on the same row differs significantly

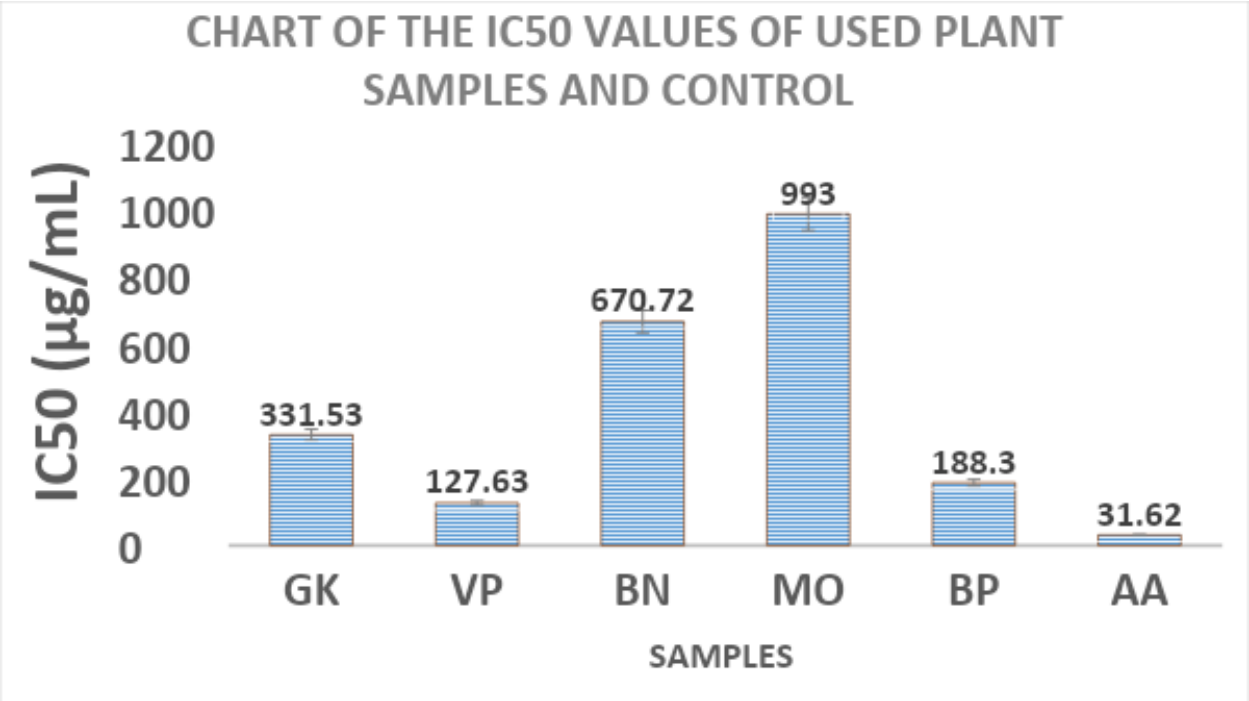


Figure 1: Showing the IC50 values of the various plant extracts on a bar chart, when tested against DPPH radicals.

Where: GK is *Garcinia kola*, VP is *Vitellaria paradoxa*, BN is *Baphia nitida*, MO is *Moringa oleifera*, BP is *Bryophyllum pinnatum* while AA is the ascorbic acid which is the control.

3.4.2 FRAP (Ferric reducing antioxidant power)

Blank = 0.305±0.00

The ability of the plant extract to reduce Fe3+ to Fe2+ shows the antioxidant capability. This is formed by the action of electron donating antioxidants at low pH. An intense blue color complex was formed when ferric tripyridyl triazine (Fe3+) complex was reduced to ferrous (Fe2+) form. And from the result, there is a good ferric reduction.

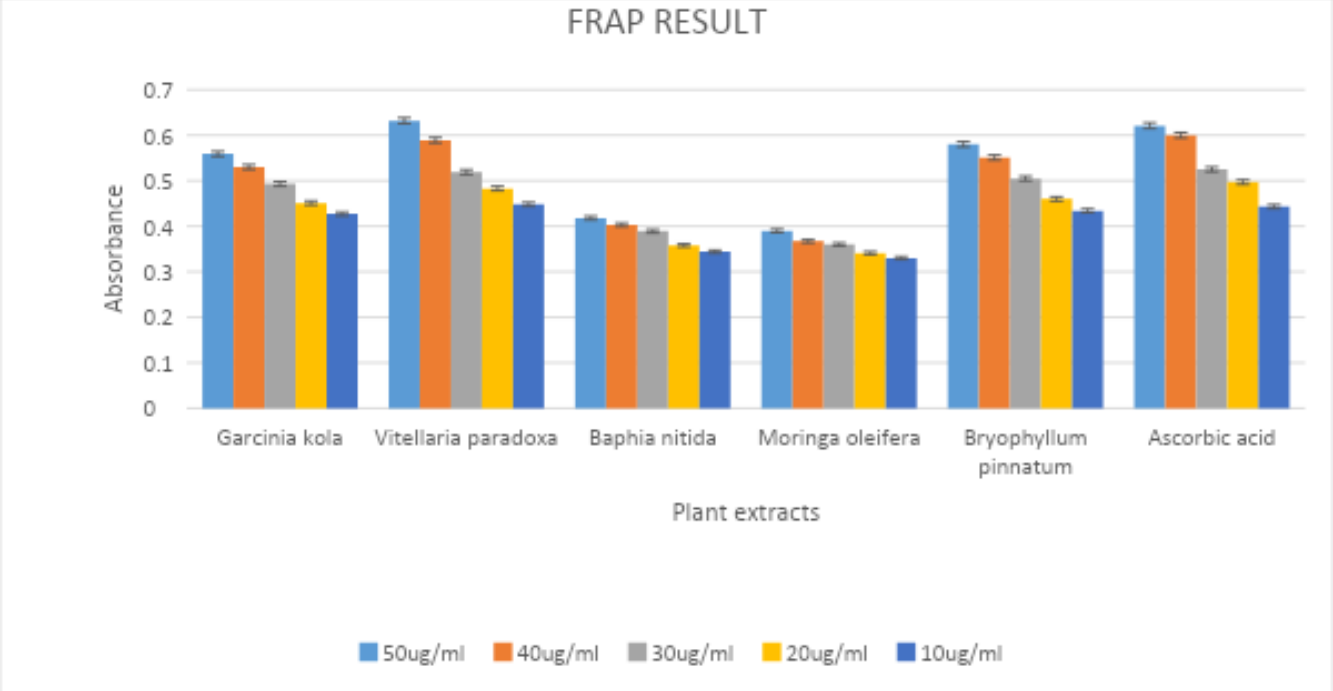
Table 8: The spectrophotometric absorbance of ferric reducing antioxidant power (FRAP)

SAMPLE	**50 µg/ml**	**40 µg/ml**	**30 µg/ml**	**20 µg/ml**	**10 µg/ml**
Garcinia kola	0.559±0.002**	0.530±0.001**	0.493±0.005**	0.451±0.001**	0.427±0.003**
Vitellaria paradoxa	0.632±0.006**	0.589±0.005**	0.519±0.005**	0.483±0.004**	0.448±0.005**

Baphia nitida	0.418±0.005**	0.403±0.005**	0.389±0.004**	0.358±0.002**	0.344±0.003**
Moringa oleifera	0.390±0.003**	0.367±0.003**	0.360±0.001**	0.341±0.001**	0.330±0.001**
Bryophyllum pinnatum	0.580±0.002**	0.551±0.002**	0.505±0.004**	0.460±0.001**	0.434±0.003**
Ascorbic acid	0.621±0.006**	0.600±0.004**	0.525±0.002**	0.497±0.003**	0.443±0.004**

Values are mean ± SEM. Significant with the blank *P<0.05 and **P<0.01. Values with different superscript on the same row differs significantly.

Fig 2: The spectrophotometric absorbance of ferric reducing antioxidant power (FRAP)



3.5.0 The Results of the *In vivo* Antioxidant Activities of *Vitellaria paradoxa*

3.5.1 Superoxide Dismutase (SOD) Assay

Actual absorbance of blank = 0.042±0.00

Table 9: Spectrophotometric absorbance of Superoxide Dismutase (SOD) on test samples

TEST GROUPS	ACTUAL READING (nm)	PERCENTAGE INHIBITION (%)	ENZYME ACTIVITY(µ/mL)
10ml/kg distilled water	0.0460±0.007**	-9.52	-7.62
100mg/kg ascorbic acid (control)	0.0260±0.001	38.10	30.48
100 mg/kg Extract	0.0085±0.003*	79.76	63.81
200 mg/kg Extract	0.0127±0.002^{ns}	70.24	56.19
400 mg/kg Extract	0.0385±0.003^{ns}	8.33	6.66

Values are mean ± SEM. Significant with the control *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same column differs significantly

3.5.2 Malondialdehyde (MDA) Assay

Table 10: Spectrophotometric absorbance Malondialdehyde (MDA)

GROUP	ABSORBANCE (nm)	MDA(nmol/ml)
10 ml/kg distilled water	0.366±0.021**	2.346
100 mg/kg Ascorbic acid(control)	0.057±0.003	0.365
100 mg/kg Extract	0.007±0.003*	0.045
200 mg/kg Extract	0.010±0.002*	0.064
400 mg/kg Extract	0.143±0.007**	0.917

Values are mean ± SEM. Signficant with the control *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same column differs significantly

3.53 Acute Toxicity Test (LD50)

Table 11: Result of acute toxicity test (LD50)

PHASE	DOSES	NO OF BEHAVIORAL CHANGES AND DEATH

1	10mg/kg 100mg/kg 1000mg/kg	0/3 0/3 0/3
2	2000mg/kg 3000mg/kg 4000mg/kg 5000mg/kg	0/1 0/1 0/1 0/1

Thus LD50 > 5000mg/kg

3.6.0 Antibacterial Activities of Plant Extracts

3.6.1 Agar well diffusion assay (Susceptibility test)

The results of the Agar well diffusion are shown below, which has *Byophyllum pinnatum* as the most active, followed by *Garcinia kola, vitellaria paradoxa, Moringa oleifera* and *Baphia nitida*. The plant extract were acted on *Staphylococcus aureus, Bcillus subtilis, Escherichial coli* and *Salmonella typhi*. Ciprofloxacin was used as a standard antibiotics

Table 12: Result of the Antimicrobial assay of *Bryophyllum pinnatum*

Test organisms	**Concentration (mg/mL) / Inhibition zone diameter (mm)**						
	20	10	5	2.5	1.25	0.63	Control
S. a	**4.33±0.03****	**3.07±0.03****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
B. s	**4.70±0.06****	**3.73±0.03****	**2.03±0.03****	0±0.00**	0±0.00**	0±0.00**	**11.3**
E. c	**5.0±0.23****	**4.07±0.12****	**2.03±0.03****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
Sal	**4.3±0.06****	**4.07±0.03****	**2.07±0.03****	0±0.00**	0±0.00**	0±0.00**	**9.3**

Key: *S. a: Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; Sal: Salmonella typhi,* Ctrl: Positive controls: ciprofloxacin 5 µg/mL. Values are mean ± SEM. Significant with

the standard *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same row differs significantly

Table 13: Result of the Antimicrobial assay of *Garcinia kola*

Test organisms	**Concentration (mg/mL) / Inhibition zone diameter (mm)**						
	20	10	5	2.5	1.25	0.63	Control
S. a	**4.70±0.06****	**3.70±0.03****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
B. s	**12.07±0.03****	**9.8±0.06****	0±0.00**	0±0.00**	0±0.00**	0±0.00**	**11.3**
E. c	**9.0±0.03****	**7.0±0.06****	**5.1±0.21****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
Sal	**12.13±0.01****	**12.03±0.03****	**7.8±0.06****	0±0.00**	0±0.00**	0±0.00**	**9.3**

Key: *S. a: Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; Sal: Salmonella typhi*, Ctrl: Positive controls: ciprofloxacin 5 µg/mL. Values are mean ± SEM. Significant with the standard *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same row differs significantly

Table 14: Result of the Antimicrobial assay of *Vitellaria paradoxa*

Test organisms	**Concentration (mg/mL) / Inhibition zone diameter (mm)**						
	20	10	5	2.5	1.25	0.63	Control
S. a	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**0**
B. s	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**11.3**
E. c	**8.71±0.07****	**4.3±0.01****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
Sal	**4.0±0.03****	0±0.00**	0±0.00**	0±0.00**	0±0.00**	0±0.00**	**9.3**

Key: *S. a: Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; Sal: Salmonella typhi*, Ctrl: Positive controls: ciprofloxacin 5 µg/mL. Values are mean ± SEM. Significant with

the standard *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same row differs significantly

Table 15: Result of the Antimicrobial assay of *Baphia nitida*

Test organisms	**Concentration (mg/mL) / Inhibition zone diameter (mm)**						
	20	10	5	2.5	1.25	0.63	Control
S. a	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**0**
B. s	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**11.3**
E. c	**5.07±0.07****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
Sal	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**9.3**

Key: *S. a: Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; Sal: Salmonella typhi*, Ctrl: Positive controls: ciprofloxacin 5 µg/mL. Values are mean ± SEM. Significant with the standard *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same row differs significantly

Table 16: Result of the Antimicrobial assay of *Moringa oleifera*

Test organisms	**Concentration (mg/mL) / Inhibition zone diameter (mm)**						
	20	10	5	2.5	1.25	0.63	Control
S. a	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**0**
B. s	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	**11.3**
E. c	**3.3±0.06****	**2.73±0.03****	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	0±0.00^{ns}	**0**
Sal	**2.1±0.06****	0±0.00**	0±0.00**	0±0.00**	0±0.00**	0±0.00**	**9.3**

Key: *S. a: Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; Sal: Salmonella typhi*, Ctrl: Positive controls: ciprofloxacin 5 µg/mL. Values are mean ± SEM. Significant with

3.6.2 Minimum Inhibitory Concentration (MIC)

Below is the MIC result of methanol extract of the plant samples against *Staphylococcus aureus, Bacillus subtilis, Escherichial coli* and *Salmonella typhi*.

Table 17: Minimum inhibitory result of the plant extracts against the used bacteria

Test Organisms	**MIC (mg / mL)**						
	BP	GK	VP	BN	MO	Neg ctrl	Pos ctrl
S. a	10	5	>20	>20	>20	Na	>5 µg/mL
B. s	2.5	5	>20	>20	>20	Na	5 µg/mL
E. c	5	5	10	10	10	Na	>5 µg/mL
Sal	2.5	2.5	10	>20	20	Na	5 µg/mL

Key: *S. a: Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; Sal: Salmonella typhi*, Ctrl: Positive controls: ciprofloxacin 5 µg/mL. Values are mean ± SEM. Significant with the standard *P<0.05, **P<0.01 and ns is not significantly different. Values with different superscript on the same row differs significantly.

4.0 DISCUSSION

4.1 Ethno-botanical survey

During my ethno-botanical survey, about twenty five (25) persons were interviewed in the study area. Most of the people interviewed (18) were within the age bracket of 45 to 70 years old. While the remaining people (7) were within the age bracket of 30 to 40 years. Dominated by

females (20) while the males were five (5) in number. A total of about 14 medicinal plants belonging to 12 families were identified to be used traditionally in treatment of tuberculosis as shown in Table 1. The leaves recorded (75%) the most commonly used part followed by the fruit (10%), and then the bark, stem and roots, where are (5%) each. The respondents used did not indicate any possible side effects caused by any of these plants. The most common method of preparation used include infusion, decoction and maceration. These tradition healers often collect the plants from the field, dry and grind them into powder before storing them in bottles to prevent people not in their field from identifying the plants used for the treatment. Most of the traditional healers request for payment before they can provide any information on the plant use, otherwise it becomes a desecration of their belief.

4.2 Thin layer Chromatography (TLC)

Thin layer chromatography (TLC) is one of the most common vital techniques use by researchers to get a quick result of the different constituents of a crude extract which will be followed by further screening to purify the bioactive compounds available. The TLC was done using aluminum pre-coated plates (Stationary phase), which various samples was spotted on. A solvent system (mobile phase) is drawn up via capillary action on the TLC plate at different mobility rate depending on the phytochemical compositions. The separated spots with different colors were detected by the use of iodine vapour. The different colored spots detected in the crude extracts indicates the presence of different phytochemical that are responsible for the pharmacological potentiality of the plants used in the treatment of tuberculosis as acclaimed by the traditional healers. I quantified the result by measuring the distance travelled by the plant compound divided by the total distance traveled by the mobile phase. It is obvious that the mobile phase consisting of N-Hexane and ethyl acetate in the ratio of 7 : 3 had a good separation as well as the Table 3 which consist of N-Hexane and ethyl acetate in the ratio of 8 : 2. The methanolic extract of *Vitellaria paradoxa* has the highest number of bands of about nine (9) using the ratio of 7 : 3 of N-Hexane and ethyl acetate. The retardation factor values (R_f) of *Vitellaria paradoxa* are 0.25, 0.32, 0.38, 0.44, 0.51, 0.62, 0.73, 0.79, and 0.92. While *Garcinia kola* and *Baphia nitida* had the highest band using the ratio of 8 : 2 of N-Hexane and ethyl acetate, and there are about

eight(8) bands in each of them. For *Garcinia kola* the various retardationtion factors (R_f) are 0.14, 0.23, 0.30, 0.45, 0.53, 0.58, 0.61, 0.80. Whereas, the retention factor for *Baphia nitida* are 0.15, 0.23, 0.30, 0.36, 0.45, 0.58, 0.62, 0.89. The plant extracts with lesser bands may suggest improved solvent system.

4.3 The phytochemical Analysis

The findings of this study shows that the methanolic extracts of the various plant samples used contains different bioactive compounds, which are known for their therapeutic capability. *Garcinia kola* was seen to contain Alkaloid, Saponin, Tannin, flavonoid, Terpenoid, Cardiac glycoside, Carbohydrate and protein. The amount of alkaloid, tannin, and protein present was in a high concentration. *Vitellaria paradoxa* was seen to contain alkaloid, saponin, tannin, flavonoid, cardiac glycoside, carbohydrate and protein. This phytochemical findings in *Vitellaria paradoxa* disagrees with (Oyedum *et al* 2018) whose result showed the presence of steroid as one of the phytochemical present. Meanwhile, the level of saponin, and protein was in a high concentration. *Baphia nitida* was also seen to contain alkaloids, saponin, tannin, flavonoids, cardiac glycoside, carbohydrate and protein. Where the level of saponin, flavonoid, and protein was in a high concentration. *Moringa oleifera* was also seen to have contain alkaloid, saponin, tannin, flavonoid, terpenoids, cardiac glycoside, carbohydrate, protein. Where saponin, cardiac glycoside, and protein are in a high level of concentration. *Bryophyllum pinnatum* was seen to contain alkaloid, saponin, tannin, flavonoid, terpenoid, cardiac glycoside, carbohydrate and protein. Where the level of alkaloid, flavonoid, cardiac glycoside and protein is on a high concentration. The five plant samples used all contains protein in a high concentration. Tannin is one of the major active ingredient found in medicinal plants. Tannin has been reported to selectively inhibit HIV replication (Kashiwada *et al.,* 1992). Appreciable quantity of tannin was found in the five plants used which are 3.4%, 3.2%, 7.2%, 8.9% and 4.3% in *Bryophyllum pinnatum, Vitellaria paradoxa, Moringa oleifera, Garcinia kola,* and *Baphia nitida* respectively. And *Garcinia kola* has the highest percentage of tannin. Flavonoid have been known to have antioxidant effect and have been shown to inhibit the initiation, promotion and progression of tumours (Kim *et al.,* 1994). Reduction of coronary heart disease has been reported to be associated with intake of flavonoids (Hertog *et al.,* 1993). Flavonoids possess protection against

platelet aggregation, hepatotoxin, and inflammation (Barakat *et al.,* 1993). A good quantity of flavonoid was found in the various plants. They are 4.4%, 3.0%, 5.0%, 9.8% and 3.8% in *Bryophyllum pinnatum, Vitellaria paradoxa, Moringa oleifera, Garcinia kola,* and *Baphia nitida* respectively. *Garcinia kola* has the highest amount of flavonoid, followed by *Moringa oleifera.* Alkaloid have a widely pharmacological activities including antimalarial, anticancer, antibacterial, antihyperglycermia (Qiu *et al.,* 2014) The various plant extract showed a good quantity of alkaloid which are 8.3%, 3.8%, 2.4%, 10.6% and 4.0% for *Bryophyllum pinnatum, Vitellaria paradoxa, Moringa oleifera, Garcinia kola,* and *Baphia nitida* respectively. Having *Garcinia kola* with the highest quantity of alkaloid. Saponin been one of the useful bioactive compound is use to protect against microbial attack. An appreciable quantities of saponin was seen in the various plants, which are 5.5%, 8.95%, 7.8%, 4.6% and 8.6% for *Bryophyllum pinnatum, Vitellaria paradoxa, Moringa oleifera, Garcinia kola,* and *Baphia nitida* respectively. *Vitellaria paradoxa* has the highest quantity of saponin, followed by *Baphia nitida*, and then *Moringa oleifera*.

4.4 Antioxidant Analysis

Antioxidants (free radical scavengers) are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system. The body makes some of the antioxidants internally which is use to neutralize free radicals, and they are called endogenous antioxidants. The body also depend on external sources to get antioxidant to assist the internally generated once. These external sources are fruits, vegetables, grains that are rich in antioxidants. The *in vitro* antioxidant activities of the five plants I used was assayed using DPPH and FRAP assay, while the *in vivo* antioxidant activities was evaluated using serum superoxide dismutase (SOD) and malondialdehyde (MDA) level assay. The in vitro antioxidant assay of these plants reveal that it has a potent antioxidant activities when compared with ascorbic acid which was used as a reference standard. For the DPPH, the method of action is based on transfer of electron. The concentrations of the plant were prepared from 500µg/ml to 31.25µg/ml. At 500µg/ml, the percentage DPPH scavenging effects of *Bryophyllum pinnatum, Vitellaria paradoxa, Moringa oleifera, Garcinia kola,* and *Baphia nitida* were 79.48%, 80.34%, 28.69%, 70.83%, and 37.42% respectively. Showing that *Vitellaria paradoxa* is the most potent,

followed by *Bryophyllum pinnatum* and *Garcinia kola.* In the investigation, the concentration (IC_{50}) at which 50% of the free radicals scavenged were calculated from the plot of percentage inhibition against plant extract concentration. The plants was confirmed to have a good, moderate to low scavenging activities through a good IC_{50} value which are 127.63µg/ml, 188.30µg/ml, 331.53µg/ml, 670.72µg/ml and 993.0µg/ml for *Vitellaria paradoxa, Bryophyllum pinnatum, Garcinia kola, Baphia nitida* and *Moringa oleifera*. *Vitellaria paradoxa* and *Bryophyllum pinnatum* showed a good IC50, *Garcinia kola* showed a moderate IC50 while *Baphia nitida* and Moringa oleifera showed a low IC50. The ferric reducing power is use to check the capacity of antioxidants to donate either an electron or hydrogen atom to unpaired electron (Kuo *et al.*, 2009). In this assay, plant extracts exerts antioxidant activities through the reduction of Fe^{3+} (ferric cyanide) to Fe^{2+} (ferrous form). From my result, the reducing power of the methanolic extraxts and the standard ascorbic acid increased with increase in concentration. At 50µg/ml, the extracts from *Garcinia kola, Vitellaria paradoxa, Baphia nitida, Moringa oleifera*, and *Bryophyllum pinnatum* had absorbances value of 0.559, 0.632, 0.418, 0.390, and 0.580 respectively. While that of *Vitellaria paradoxa* (0.632) is higher than the standard ascorbic acid (0.621). *Vitellaria paradoxa* exhibited a remarkable antioxidant capacity which can be as a result of richness of phytochemicals. This result has shown that this plants especially *Vitellaria paradoxa* are good materials to minimize the rate of oxidative stress. The sequence of antioxidant potentiality in FRAP assay is the same with that of DPPH assay, which is seen as *Vitellaria paradoxa > Bryophyllum pinnatum > Garcinia kola > Baphia nitida > Moringa oleifera.*

The acute toxicity test showed that the LD50 of the extract is greater than 5000mg/kg. The *in vivo* assay was carried out with the extract that has the best *in vitro* antioxidant result, and *Vitellaria paradoxa* happens to give the best *in vitro* antioxidant result. The *in vivo* antioxidant assay of *Vitellaria paradoxa* showed that the extract increased the activity of superoxide dismutase (SOD) and decreased the level of Malondialdehyde (MDA) produced. The best result was seen at 100mg/kg, while the higher concentration of the extract (200 and 400mg/kg) reduced the the activity of the superoxide dismutase (SOD) which invariably increased the Malondialdehyde production. This result is in contrast with (Ali monahi 2019) that shows that at

higher concentration (200mg/kg) it increased the SOD activity than the 100mg/kg. This shows that *Vitellaria paradoxa* is potent to scavenge free radicals and can stop lipid peroxidation. SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitite. The increased serum activities of SOD as seen in this study suggest that the extract has an *in vivo* antioxidant activity and is capable of stopping their reactive oxygen specie (ROS).

4.5 Antibacterial Activities

The result of the agar well diffusion assay showed the inhibition zone diameter (IZD) as shown from table 12 to 16. The inhibition zone diameter varied from specie to specie of organism and also according to the type of extract. The plant extracts were tested against the organisms at concentration of 20, 10, 5, 2.5 and 1.25mg/mL. The *Bryophyllum pinnatum* and *Garcinia kola* were seen to have broad spectrum activity against bacteria. The IZD of *Bryophyllum pinnatum* against the bacteria at 10mg/mL were 3.03±0.03, 3.73±0.03, 4.07±0.12, and 4.07±0.03mm for *Staphylococcus aureus, Bacillus subtilis, Escheriche coli* and *Salmonella typhi* respectively. The IZD of *Garcinia kola* against the bacteria at 10mg/mL were 3.70±0.03, 9.8±0.06, 7.0±0.06, and 12.03±0.03 for *Staphylococcus aureus, Bacillus subtilis, Escheriche coli* and *Salmonella typhi* respectively. Meanwhile, *Vitellaria paradoxa* only had activity on gram negative bacteria, with an IZD of 8.71±0.07 and 4.0±0.03mm at 20mg/mL of extract against *E.coli* and *Samonella typi*. While *Baphia nitida* had IZD of 5.07±0.07 at 20mg/mL of extract only on *E.coli*. *Moringa oleifera* had activity only on gram negative bacteria, with an IZD of 3.3±0.06 and 2.1±0.06mm at 20mg/mL of extract against *E.coli* and *Salmonella typhi*. The susceptibility test result showed that *Garcinia kola* have the best inhibition zone against all the four bacteria used. The MICs of the extracts are, *Bryophyllum pinnatum* against *S. aureus, B. subtilis. E.coli, and S. typhi* are 10, 2.5, 5, and 2.5mg/mL respectively. *Garcinia kola* against *S. aureus, B. subtilis. E.coli,* and *S. typhi* are 5, 5, 5 and 2.5mg/mL respectively. *Vitellaria paradoxa* against *S. aureus, B. subtilis. E.coli,* and *S. typhi* are >20, >20, 10 and >20mg/mL respectively. *Baphia nitida* against *S. aureus, B. subtilis. E.coli,* and *S. typhi* are >20, >20, 10, >20mg/mL respectively. *Moringa oleifera* against *S. aureus, B. subtilis. E.coli,* and *S. typhi* are >20, >20, 10, 20mg/mL respectively. Among the five plants that were tested, *Garcinia kola* was found to show the

overall best antimicrobial activity using Agar well diffusion and MIC in all the organisms tested. The antimicrobial result is similar to those of other studies that reported antimicrobial activities of these plants. However, contrary to my result, Elijah parker *et al.,* 2020 reported antimicrobial activity of *Baphia nitida* against *Staphylococcus aureus* and *Bacillus subtilis*. Also Sandabi and Abu zaid 2011 reported *Moringa oleifera* had antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*. The difference in the result could be due to the use of plant extract in less concentration (20mg/mL) compared to that used by them (100mg/mL).

5.0 SUMMARY

This study shows that the crude extracts of methanol of these selected five plants contains many phytochemical: alkaloid, flavonoid, saponin, tannin, carbohydrates, cardiac glycosides, protein and terpenoid (for some). The antioxidant result is encouraging as the tested extracts revealed potential antioxidant activity, especially in Vitellaria paradoxa. The antibacterial activity of the plant extracts is worth harnessing, most especially in *Garcinia kola* and *Bryophyllum pinnatum* which have the best MIC values compared to others.

6.0 CONCLUSION

This study have provided scientific evidence of traditional medicinal use. The result reveal the presence of medicinally active constituents and it indicates the potentials of this plants for treatment of diseases. The moderate to high free radical scavenging, good antimicrobial activities demonstrated by these plants is worth harnessing for proper evaluation and can be seen as a potential source of useful drug.

7.0 RECOMMENDATIONS

The following recommendations and future research areas are forwarded below:

Further work is highly recommended to isolate, purify and possibly characterize the active compounds responsible for their activities.

1. A comprehensive anti-tubercular screening showed be done on these plants to fully ascertain their ethno-medicinal claims.

2. Also addition work is recommended upon a view to elucidate the possible mechanism of action of these plant extracts.

NOTE:

The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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