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Antibacterial Activity and Phytochemical Properties of Selected Medicinal Plants Against *Salmonella Typhi, Salmonella Paratyphi A, B and C,* Clinical Isolates in Lafia, Nigeria

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Abstract

The aim of the research was to investigate the antibacterial activities and the phytochemical properties of aqueous and ethanolic extracts of Mango (*Mangifera indica)* and Neem (*Azadirachta indica*) leaves, on clinical isolates of *Salmonella typhi*, *Salmonella paratyphi A, B and C*, in Lafia, Nigeria. The agar well diffusion technique was used and the analyses were done in triplicates. *Salmonella typhi*, showed inhibition zone of 29.4±0.1mm, at the highest aqueous extract concentration of 80 mg/ml, while *Salmonella paratyphi A, B and C,* exhibited inhibition zones of 14.4±0.2 mm, 21.2±0.4 mm and 13.4±0.1 mm respectively. At the highest ethanolic extract concentration of 80 mg/ml, *Salmonella typhi,* showed inhibition zone of 30.0±0.01mm, while *Salmonella paratyphi A, B and C,* exhibited inhibition zones of 18.0±0.03mm, 20.0±0.04mm and 21.0±0.04mm respectively. At aqueous lower extract concentration of 10mg/ml, *Salmonella typhi*, exhibited inhibition zone of 8.4±0.01mm, while *Salmonella paratyphi A, B and C*, showed inhibition zones of 9.5±0.01mm, 9.1±0.01mm and 6.2±0.01mm respectively as exhibited in the Neem leaf extract. Ethanolic extract at 10 mg/ml, *Salmonella typhi*, showed inhibition zone of 9.0±0.1mm, while *Salmonella paratyphi A, B and C*, exhibited inhibition zones of 9.0±0.01mm, 9.0±0.01mm and 9.0±0.01mm respectively of Neem leaf extract.

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The MIC and MBC, of aqueous and ethanolic extracts against organisms were 2.5 and 5.0 mg/ml, respectively. The qualitative phytochemical results showed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, tannins, terpenoids and saponins. Conclusively, the aqueous and ethanolic extracts of studied medicinal plant exhibited bactericidal activities against all tested organisms, and this supports the claim by traditional medical practitioners and vendors of the use of the plants for the cure of typhoid fever. Further studies are recommended on the toxicity and safe dosage regimen of the plants since the infusion of the plant is taken orally by people for the cure of typhoid fever.

Keywords: *Salmonella* Organisms; Typhoid fever; Phytochemical Constituents; Bacteria; Medicinal plants.

1. Introduction

Infectious diseases which are resistant to antimicrobial agents are one of the major problems confronting developed and developing countries of the world today [1]. Therefore, there is the need to find an efficient and safe alternative to microbial resistance to drugs, and the alternative resides in phytomedicine. Accordingly, various brands of new antibiotics have been manufactured by pharmacological companies every year, but global emergency of multidrug resistant (MDR), bacteria is limiting the potency of these drugs [2]. For instance, multidrug resistant *Salmonella typhi*, showed resistance to all three first line of drugs: Ampicillin, Chloramphenicol and Trimethioprimsulfamethoxazole [3]. This poses challenges to researchers which prompted studies to explore the phytotherapeutic potential of medicinal plants including Mango (*Mangifera Indica*) and Neem (*Azadirachta Indica*), as a source for alternative medicine to microbial resistant drugs which are cheaper and safer than conventional drugs. Medicinal plants such as Mango (*Mangifera Indica*) and Neem (*Azadirachta Indica*), have been used to treat human ailments including malaria, anemia, diabetes and cancer, for many years because they possess organic compounds including alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, cardiac glycosides, that are antibacterial and have definite physiological action on the human body[4,5]. For example, the use of plant extracts for antimicrobial therapy has proven to be a promising remedy in Chinese phytomedicine, Indian Ayurvedic, Arabic and Unani medicines [6]. In Africa, the use of medicinal plants for the treatment of diseases has been since the introduction of modern medicine [7]. In Southern Nigeria, for example, *Acalypha Wilkesiana*, a member of the Euphobiaceae family, is used to treat malaria, dermatological and gastrointestinal disorders and has antimicrobial properties [8,7,9,10]. The interest in plants is because they are available, easily accessible, cheap for both the rich and the poor populace, and safer and more cost-effective sources for alternative medicine [11,12]. At least 25% of drugs in modern pharmacopoeias are derived from compounds isolated from medicinal plants, such as Mango (*Mangifera indica*) and Neem (*Azadirachta indica*),[13]. Accordingly, extracts from medicinal plants have a wide range of bioactive and pharmacological activities which essentially include antibacterial, antifungal and anti-inflammatory properties [14]. Infusion and concoctions prepared from different parts of medicinal plants are used in the treatment of various human diseases, including typhoid fever, a systemic disease which is caused by the *Salmonella* organism, which has been reportedly resistant to both *Trimethioprimsulfamexazole and Chloramphenicol* [15]. Reference [16,17] have reported that plant extracts are traditionally used in most nonindustrialized nations for the treatment of human diseases, including typhoid fever. Reference [18,19,20,21] have demonstrated the bactericidal effect of plant extracts on *Salmonella typhi, Salmonella paratyphi A, B and C,* in the West of province Cameroon; Jos

Plateau State; Ebonyi state and Kenya respectively. Reference [21], studied the In-Vitro antimicrobial activity of methanolic leaf extracts from four plants (*Aloe Secundiflora, Bulbine frutescens, Vernonia lasiopus and Tagetes minuta)* against *Salmonella typhi* in Kenya, using a disc diffusion method. They reported *Aloe Secundiflora* bactericidal against tested organisms at low concentration of 5.5 mg/ml; MIC of plant extracts on targeted organism varies from 5 to 9 mg/ml; MBC against organism ranged from 7 to 11mg/ml. Reported qualitative phytochemicals include alkaloids, flavonoids, tannins and saponins. Reference [20] reported herb extracts of *Vernonia amydgalina, Allivm sativum and Allium cepa,* had a strong inhibitory effect against *Salmonella typhi*, in their study on antibacterial activities of some medicinal plants on *Salmonella typhi* isolates in Abakiliki, Ebonyi state, Nigeria, using an agar diffusion method. It records the presence of secondary metabolites, including alkaloids, glucose, tannins, glycosides, flavonoids, steroids and phenolic compounds. Reference [18], recorded MIC values of 6 mg/ml against *Salmonella typhi* and *Salmonella paratyphi* B, and MBC value of 300µg/ml against *Salmonella typhi* and *Salmonella paratyphi B,* in their study of antibacterial agents from methylene chloride and methanol leaf extract of *Crinum purpurascens* herb collected from the West Province of Cameroon, using both agar diffusion and broth dilution methods. The Mango (*Mangifera indica L*) plant belongs to the family of Anacardiaeceae, which consists of about sixty genera and 600 species [22]. Mango, is one of the tropical fruit bearing trees in the world [23]. Mango is a medicinal herb that is used traditionally in the treatment of diseases including mouth infection in children, diarrhea, dysentery, gastrointestinal tract disorders, typhoid fever, sore throat and scurvy [24,25]. Its ground seeds and leaves have been used to treat diabetes, colic and irritation from Scorpions and bee stings [26]. The leaves of *M. indica*, have been reported to contain glycoside and Mangiferin, which is an antimicrobial agent and Mangiferin has been demonstrated to possess antiviral activity against the herpes simplex type 2 virus [27]. The Neem (*Azadirachta indica*) plant, on the other hand, belongs to the family of Melioceae. Neem is a tropical plant, that has adapted to a wide range of climatic, topographic and environmental factors and has immense potentials. In Indian traditional Ayurvedic medicine, different parts of Neem tree have been used for the treatment of various ailments. The Neem oil, bark and leaf extracts have been used to control leprosy, intestinal helminthiasis and respiratory disorders. Mango and Neem plants are medicinal herbs that have been used traditionally in Chinese phytomedicine, Indian Ayurvedic, Arabic, Unani, and African medicines particularly in Nigeria, to treat various human ailments, including typhoid fever, but their antibacterial activity against clinical isolates of *Salmonella* organisms in Lafia, Nigeria, has not been tested. The bacteria, were chosen for the research because of their importance in public health and food poisoning. Therefore, the aim of the research is to study the antibacterial activity and phytochemical properties of Mango and Neem leaf, aqueous and ethanolic extracts against clinical isolates of *Salmonella typhi, Salmonella paratyphi A, B and C,* in Lafia, Nigeria, as an alternative to Medicare for typhoid fever.

2. Materials and Methods

2.1 Collection of Plant Materials

The procedures described by [12]; Good Agricultural collection practice and Field collection practice [28], were the sampling techniques adopted for the collection of plant materials. Fresh leaves of Mango (*Mangifera indica*) and Neem (*Azadirachta indica*), were randomly collected from different locations sites: Gandu and Akunza, in

Lafia local government area, of Nasarawa state, in the morning before sunrise (to avoid degradation of plants biocomponents by ultra violet rays). The leaves were identified by Dr. Enoch, a taxonomist and Botanist in the department of Botany, Federal University of Lafia, Nasarawa State. A voucher specimen was assigned to the samples for record purposes. The leaves were then washed 4 to 5 times with clean water to remove dust and dirt; thereafter dried in shade at room temperature 27^0 C until crisp and brittle to touch; separately grounded to powder using a Laboratory mortar and pestle; separately sieved through a sifter 0.5mm to 10mm to obtain fine particle size [28].The powdered plant samples were stored in a labeled airtight clean, dried opaque polythene container, until ready for analysis.

2.2 Preparation of Plant Extracts (Aqueous and Ethanolic)

Aqueous Extraction

One hundred grams each of dried finely powdered plant samples were weighed separately into different glass beakers using analytical weighing balance (aeAdam, Model PW 124, UK). Then, five hundred milliliters of sterile distilled water were added into each beaker. The mixtures were heated on a hot plate (Model SB 160, UK), at 30 to 40^0C with intermittent stirring for 20 minutes. Thereafter, the aqueous extracts were filtered through Whatman filter paper, number one. The filtrates were evaporated separately in a water bath at 65^0C, and the crudes were labeled and stored in a refrigerator until ready for analysis [29].

Ethanolic Extraction

Using analytical weighing balance (aeAdam, Model PW 124), 100 grams of dried finely powdered Mango and Neem leaves were weighed into separate glass beaker, and 70% ethanol added to each beaker. The mixture was soaked and agitated intermittently for 72 hours. After that the contents were filtered through Whatman filter paper number one. Thereafter, the filtrates were evaporated separately in a water bath at 60^0C. The yield was weighed and recorded. The dried crude was stored in a refrigerator until ready for analysis [29].

2.3 Phytochemical Evaluation of the Plant Extract

2.3.1 Qualitative Analysis of Phytochemical Constituents in Plant Extracts

2.3.1.1 Test for Alkaloids

The alkaloids content of plant samples was determined by the method described by [30,31,32]. Zero point five (0.5) grams of the crude extract were mixed with 3 ml of 1% HCL, and boiled for 5 minutes. The mixture was cooled and filtered. Thereafter, the filtrate was treated with Mayer's, Wagner's and Dragendroff's reagents. The turbidity of the resulting precipitate was an indication of the presence of alkaloids.

2.3.1.2 Test for Reducing Sugars

Reducing the sugars content of plant extracts was determined by the method described by [30,31,32]. One milliliter of plant extract was treated with a mixture of 2 ml of Fehling's solution (A and B) in a test tube. The setup was gently boiled. The color changes from deep blue to brick red, which indicates the presence of reduced sugars.

2.3.1.3 Test for Glycosides

The method described by [30,31,32], was adopted for glycoside determination in plant crude extracts. One millimeter of plant crude extract was mixed with 2 ml of Chloroform and 2 ml of acetic acid in a test tube. The setup was cooled in an ice bath, followed by the careful addition of concentrated H_2SO_4. **A color change from violet to blue to green indicates the presence of steroidal nucleus, i.e. glycone portion of the glycosides.**

2.3.1.4 Test for Flavonoids

The method described by [30] was adopted for the presence of flavonoids in the plant extract. Two grams of plant extract in a glass test tube was detanned using acetone. After that the setup was placed in a hot water bath for traces of acetone to evaporate. Thereafter, boiling water was added to the detanned sample and the mixture was filtered while hot and allowed to cool. Then 5ml of 20% NaOH solution was added to an equal volume of the filtrate. A yellow solution was evidence of the presence of flavonoids.

2.3.1.5 Test for Tannins/Phenol

The method described by [30] was adopted for the presence of tannins in plant crude extract. Zero point five grams of plant crude extract was dissolved in 1ml of distilled water in a glass test tube, and filtered through Whatman filter paper number one. Three milliliters of the filtrate in a test tube and 2ml of 2% solution of $FeCl_3$, was added. A blue-green or black coloration was an evidence of the presence of tannins.

2.3.1.6 Test for Terpenoids

The method described by [30,31,32], were adopted for analysis of terpenoids in plant crude extract. Zero-point one gram of plant crude extract was dissolved in 2 ml of chloroform in a test tube, then evaporated on a water bath to dryness. Thereafter 2 ml of concentrated H_2SO_4 was carefully added to the residue, then heated for 2 minutes. The grayish coloration was evidence of the presence of terpenoids.

2.3.1.7 Test for Anthraquinones

Procedures described by [30], were the method used to determine anthraquinones in plants crude extract. Zero point five grams of plant crude extract was dissolved in 5 ml of chloroform in a test tube, and the setup was shaken for 5 minutes. The resulting mixture was filtered, and 3 ml of the filtrate was shaken with an equal volume of 100% ammonia solution in a test tube. A pink, violet/red color in the ammoniacal layer indicates the presence of free anthraquinones.

2.3.1.8 Test for Saponins

The method described by [30], was used to determine the presence of saponins in plant crude extracts. Zero point five grams of the extract in a test tube was shaken with distilled water. The presence of saponins in the sample was indicated by persistence of front during warming.

2.4 Quantitative Analysis of Phytochemicals in Plant Crude Extract

2.4.1 Determination of Alkaloids

Reference [31], was the method used for the analysis of alkaloids in the crude extract. The test was done in triplicate. Five grams of the sample in a 250ml beaker and 200 ml of 10% acetic acid in ethanol was added, then covered with a watch glass and allowed to stand for 4 hrs. The mixture was then filtered, and the filtrate was concentrated in a water bath to one quarter of its original volume. Then concentrated ammonium hydroxide was added to the concentrated filtrate drop wise till the precipitate was completed. The precipitate was then collected on a weighed filter paper and washed in diluted ammonium hydroxide(2M). The residue was dried in an oven at 80^0C and the weight was calculated and expressed as a percentage of the weight of the sample.

2.4.2 Assay of Flavonoids

Procedures adopted by [33], were used for the determination. Analysis was done in triplicates. Ten grams of the plant sample was extracted repeatedly with 100 ml of 80% aqueous ethanol at room temperature until the residue become colorless. After that, the whole mixture was filtered through Whatman filter paper number one. Thereafter, the filtrate was transferred into a weighed crucible and was evaporated to dryness in a water bath. The difference in the constant weight of the crucible gave the value of the flavonoids of the assayed sample and the result was expressed as a percentage of the original sample.

2.4.3 Determination of Saponins

The method described by [34], was used for the assay and analysis was carried out in triplicates. Twenty grams of the plant sample was placed in a 250 ml conical flask and 200 ml of 20% ethanol was added. The setup was heated in a water bath at 55^0C for 4 hrs with continuous stirring. After that, the mixture was filtered and the residue was re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml in a water bath at 90^0C. Then, the concentrate was transferred into a glass separatory funnel and 20 ml of diethyl ether was added and the setup was shaken vigorously. After that, the aqueous layer was recovered while the ether layer was discarded. The purification process was carried out once more. Then, 60 ml normal butanol was added and was washed twice with 10 ml 5 % aqueous Sodium chloride. After that, the solution was evaporated in a water bath and the residue was dried in an oven at 80^0C to a constant weight. Saponin content was calculated and the result expressed as percentage of the original sample.

2.4.4 Assay of Tannins

For the analysis of tannins in the plants crude extract, reference [35], procedures were used. for analysis of tannins in plant crude extract. The analysis was done in triplicates. Five grams of plant sample was in a 100 ml plastic bottle and 50 ml distilled water was added and the setup was shaken for 1hr on a mechanical shaker. After that, the mixture was filtered into a 50 ml volumetric flask and diluted to the mark with distilled water. Five milliliters of the filtrate were pipetted into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ and 0.1M Hcl and 0.008M $K_4[Fe\ (CN)_6]$. A blank sample was also prepared. The absorbance of the sample was read at 395nm against the blank within 10 minutes of preparation using the T60 UV-Visible spectrophotometer (PG Instrument, UK). A standard curve was prepared using tannic acid to get the 100-ppm measurement limit.

2.4.5 Assay of Total Phenol

The analysis was carried out in triplicates. Ten grams of powdered plant sample was defatted with 100 ml diethyl ether for 2 hrs using a Soxhlet apparatus. The free fat sample was then boiled with 50 ml of ether to extract the Phenolic component before being filtered. Then 5 ml, of the filtrate was pipetted into a 50 ml volumetric flask and 10 ml of distilled water, 2 ml NH_4OH solution and 5 ml amyl alcohol were added and diluted to mark with distilled water. The setup was allowed to react for 30 minutes for color development. Thereafter, the absorbance of the solution was read at 505nm using the T60 UV-VIS Spectrophotometer (PG Instrument, UK). A standard curve was prepared using 0, 25, 50, 75, 100 mg/l solutions of gallic acid in methanol: water (50:50, v/v). The total phenol value was expressed in g gallic acid equivalent per 100g dry weight (g GAE/100g dry mass) [36].

2.4.6 Determination of Cardiac Glycosides

The method described by [37], was used for the analysis of cardiac glycosides of plant extracts. The experiment was done in triplicates with 1gram of fine powdered plant sample used in 250 ml beaker. Ten milliliters of 70% alcohol were added to the setup and allowed to soak for 2 hrs. After that, the mixture was filtered and 8 ml of the filtrate was diluted to 100 ml with distilled water in a 100 ml standard flask. Then 8 ml of the diluted filtrate was transferred into a 100 ml standard flask and 8 ml of 12.5% lead acetate solution (to precipitate resins, tannins and pigments) was added and the setup was mixed by shaking and was diluted to the mark with distilled water and later filtered. After that, fifty milliliters of the filtrate was transferred into a 100 ml standard flask and 8 ml of 4.7% disodium hydrogen phosphate solution was added (precipitate excess lead ions) and the content was diluted to the mark with distilled water and the mixture was filtered twice through Whatman filter paper number one (obtain purified filtrate). Then 10 ml of purified filtrate was transferred into a 50 mL beaker and 10 ml of freshly prepared Baljet's reagent was added into the beaker and the content was mixed and the setup was allowed to stand at room temperature for 1hr for color development. A blank sample was treated similarly. The sample was read at 495 nm against a blank using the T60 UV-Vis Spec (PG Instrument). Differences between the intensity of sample and blank, gave the absorbance which is proportional to the concentration of cardiac glycoside in the analyzed sample and the result was expressed in percentage from the relation: % glycoside = A x 100/17; where A = absorbance of sample at 495nm.

2.5 Preparation of Media

The media that was used include Muller-Hinton agar, Nutrient broth, Nutrient agar, Urea broth, Triple sugar iron agar, Simmon's citrate agar, Motility agar. The media was prepared in accordance with the Manufacturer's instructions. Each batch of prepared media was tested for sterility before being used.

2.6 Bacterial Stains

Clinical isolates of *Salmonella typhi, Salmonella paratyphi A, B and C,* were collected from the Department of Microbiology, Dalhatu Araf Specialist Hospital, Lafia, Nasarawa state, Nigeria. All of the bacterial strains were preserved on Bijou agar slants and stored at 4^0C until they were ready for analysis. The bacteria, were chosen for the research because of their importance in public health and food poisoning.

2.6.1 Bacterial Strain Confirmation

Bacterial Strains were confirmed by biochemical screening including Gram stain, Triple sugar iron (TSI); Urease; Citrate utilization; Motility; Methyl red and Voges-Proskauer tests, as described by [38,39,40], and the Serological test as described by [41,39,38,42,40].

2.6.2 Bacterial Strain Confirmation by Commercial Kit

The procedures adopted by [41,39,42], were the methods used to confirm the bacterial strains. Identification of *Salmonella typhi, Salmonella paratyphi A, B and C,* was performed by slide agglutination tests. Commercial kit was used to confirm serogroup *Salmonella typhi, Salmonella paratyphi A, B and C,* by their somatic (O) and flagella (H) antigens (A, B, C and D). A single pure colony of individual test organism was picked and placed separately on a ceramic tile and was rocked with the corresponding antisera. The reaction was observed for two minutes. The agglutination reaction confirmed the *Salmonella subgroup* and was evidence of a positive organism under test.

2.7 Standardization of Inoculum

Reference [43], method was used for the standardization of bacterial inoculum. Five colonies of each test organisms were picked aseptically with a wire loop and transferred into separate glass test tubes containing 5 ml of nutrient broth and mixed. The setup was then incubated at 37^0C for 24 hrs. The turbidity that resulted was adjusted to match 0.5 McFarland, Standard which yielded approximately $1 \times 10^7 ml^{-1}$ bacteria.

2.8 Preparation of Various Concentrations of Plant Extracts

Double dilution procedures were used to obtain various concentrations of plant extracts of 80, 40, 20, 10, 5, 2.5 mg/ml, for antibacterial activity, MIC and MBC, using sterile distilled water. Thus, 8 g crude plant extract was reconstituted in 100 ml of sterile distilled water to obtain 80 mg/ml solution (i.e., 8 g crude plant extract = 8000 mg/100 ml distilled water = 80mg/ml). A known volume of 80 mg/ml solution was diluted with equal volume of sterile distilled water to obtain 40mg/ml solution. Double dilution continues until lower concentrations are obtained [43].

2.9 Determination of Antibacterial Activity

The antibacterial screening of aqueous and ethanolic plant crude extracts were carried out using the agar well diffusion method. Several dilutions of crude extracts of Mango and Neem were separately made in separate glass test tubes as described by [43]. The dilutions were 80, 40, 20, 10, 5 and 2.5 mg/ml. A suspension of *Salmonella typhi, Salmonella paratyphi A, B and C,* compared to the 0.5 McFarland, standard was each seeded on separate nutrient agar plates and spread with a glass rod and the excess was drained off. A sterile cork borer of 6 mm diameter was used to bore 8 wells on each plate. Zero point one milliliters of reconstituted extracts were introduced into six labeled well using automatic variable micropipette, and into the remaining 2 wells, one for Ciprofloxacin (250mg/100ml), positive control and the other one distilled water, negative control. The setup was allowed to stay on laboratory bench for one hour for the extracts to diffuse into the agar. Then, the setup was incubated aerobically at 37^0C for 24 hrs. The diameters of inhibition zones were measured with a 120 mm graduated ruler and the results were reported in millimeters [43].

2.10 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibition concentration (MIC) of the plant extract was determined by the method of [43]. Zero point one milliliters of suspension of each standardized *Salmonella typhi, Salmonella paratyphi A, B and C,* was inoculated into different series of sterile labeled test tubes of nutrient broth which contained various concentrations (80, 40, 20, 10, 5, 2.5 mg/ml) of two-fold dilution of plant extract and was incubated at 37^0C for 24 hrs. The minimum inhibition concentration was recorded as the least concentration (highest dilution) that inhibited the growth of tested organisms. The minimum bactericidal concentration (MBC) of the plant extract was determined by the method described by [29]. One milliliters of plant extract was pipetted from tubes which showed no visible growth of MIC, and was sub cultured on freshly prepared nutrient agar plates and incubated at 37^0C for 24 hrs. The MBC was read and recorded as the highest dilution (lowest concentration) of the extract that did not show any colony growth on a new agar plate.

2.11 Statistical Analysis

Quantitative phytochemical parameters and the minimum inhibitory diameter zone of plant crude extracts analyzed were carried out in triplicates. The data was subjected to statistical analysis to evaluate the differences between the phytochemical constituents and inhibitory diameter zone of the studied plants crude extracts. Data were expressed as mean standard error. Comparison of means was analyzed using one-way analysis of variance (ANOVA) on a statistical programme: Statistical Package for Social Science (SPSS) version 16.0 Windows. The difference was significant at $P < 0.05$.

3. Results and Discussion

The qualitative phytochemical constituents' present in the studied medicinal plants are shown in Table 1. Carbohydrates, Tannins, Cardiac glycosides, Terpenoids, Alkaloids, Anthraquinones, Flavonoids and saponins, were the secondary metabolites present in the medicinal plants analyzed. This result agrees with the report by

[44], who documented that plant secondary metabolites exhibit bioactive and physiological activities. These secondary metabolites are pharmacologically important and could account for their antibacterial activity. Compared to aqueous solvent, ethanolic leaf extracts had higher quantity of secondary metabolites with a high degree of precipitation (+++) of alkaloids, tannins and terpenoids in the Mango leaves than in the Neem leaves and in the aqueous solvent (Table 1). Moderate degree of precipitation (++) of carbohydrates, cardiac glycosides, flavonoids, tannins and saponins in both aqueous and ethanolic extracts of Mango and Neem leaves (Table 1). Lesser/slightly degree of precipitation (+) of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids and saponins, in both aqueous and ethanolic extracts of mango and Neem leaves (Table 1). The ethanolic solvent extracts recorded a higher degree of precipitation than the aqueous solvent. This could be ascribed to the ethanolic solvents higher polarity, which contain a greater variety of plant constituents than aqueous solvent [44]. Also, [11], reported that different solvents have different capacities for different phytoconstituents. Thus, the differences in the observed activities of various extracts may be due to varying degrees of solubility of the active constituents in the solvent used. However, aqueous solvent could not extract saponins from the Neem leaves in the study. This does not mean saponins are lacking in the plant. Rather, the analytical technique employed was not sensitive enough to extract the substance. In both aqueous and ethanolic leaf extracts of Mango and Neem leaves, Mango leaf extracts contained higher constituents of phytochemicals than Neem leaf (Table 1). The differences in phytochemical constituents in plants could be due to geographical location, genetic constituents and the extraction method employed. However, the obtained secondary metabolite results agreed with the findings reported by [18,20,21]. Table 2 shows the quantitative phytochemical profile of the studied medicinal herbs. Alkaloids, flavonoids, saponins and tannins contents in Mango leaves are higher than the content found in Neem leaves. Also, the total phenol content in Neem leaves is higher than the content in Mango leaves. The quantitative phytochemical constituents ranged between 0.90±0.01% cardiac glycosides in Mango leaves to 13.11±0.00% tannins in Mango leaves (Table 2).

Table 1: Qualitative Analysis of Phytochemical Screening of Aqueous and Ethanolic Crude Extracts of Medicinal Plants Investigated

Plant parts	Solvents	Alkaloids	Anthraquinones	Carbohydrates	Cardiac glycosides	Flavonoids	Tannins	Terpenoids	Saponins
Mango leaves	Aqueous	+	+	+++	+++	+++	++	++	+
	80 % Ethanol	+++	+	++	++	++	+++	+++	++
Neem leaves	Aqueous	+	+	+	+	+	++	++	ND
	80 % Ethanol	++	+	++	++	++	++	++	++

Key: + = Slightly present; ++ = Moderately present; +++ = Heavy present; ND = Not detectable

This demonstrate that the studied plants contain compounds which exhibited biological and physiological activities. As well as pharmacological significance that is directly related to secondary metabolites and accounts for their antibacterial activities [18,20,21]. Also, [45], documented that plants with a higher amount of phenolic content have the ability to be used to treat inflammatory diseases and can be implicated in wound healing. For instance, the plants under study. The presence of phytochemical compounds probably justifies the use of the selected plants for the treatment of typhoid fever.

The differences in the phytochemical profile of the studied samples could be due to geographical location, genetic constituents, soil condition, variation in the season cycle, the age of plants and extraction method employed. However, results of the study, agree with the findings reported by [18,45,20,21]. Notably, there are significant differences between the phytochemical constituents of the medicinal herbs studied ($P < 0.05$) (Table 2). Antibacterial activities of aqueous and ethanolic extracts of Mango and Neem leaves against *Salmonella typhi and Salmonella paratyphi A, B and C*, at different extract concentrations are shown in Table 3 and Table 4 respectively.

The extracts inhibited the growth of tested organisms at varying degrees of extract concentrations as shown by their diameter (mm) inhibition zones on Tables 3 and 4 respectively. *Salmonella typhi* showed inhibition zones of 29.4 ± 0.1^{b}mm at the highest aqueous extract concentration of 80 mg/ml, while *Salmonella paratyphi A, B and C,* exhibited inhibition zones of 14.4±0.02mm, 21.2±0.04mm and 13.4±0.01mm respectively (Table 3). Also, *Salmonella typhi* exhibited inhibition zones of 30.0±0.1mm at the highest ethanolic extract concentration of 80 mg/ml, while *Salmonella paratyphi A, B and C*, showed inhibition growth of 18.0±0.03 mm, 20.0±0.04 mm and 21.0±0.04 mm respectively (Table 4). At aqueous lower extract concentration of 10mg/ml, *Salmonella typhi*, showed inhibition zone of 8.4±0.01mm, while *Salmonella Paratyphi A, B and C,* exhibited inhibition growth of 9.5±0.01mm, 9.1±0.01mm and 6.2±0.01mm respectively as exhibited by Neem leaf extracts (Table 3). Similarly, ethanolic extract at a lower concentration of 10 mg/ml, *Salmonella typhi*, showed an inhibition zone of 9.0±0.01mm, while *Salmonella Paratyphi A, B and C,* exhibited inhibition zones of 9.0±0.01 mm, 9.0±0.01 mm and 9.0±0.01 mm respectively in Neem leaf extracts (Table 4). Essentially, the Neem leaves exhibited the highest diameter (mm) inhibition zones compared to Mango leaves at all extract concentrations. This could be attributed to the genetic heterogeneity of plant species. In general, this study suggests that the plant extracts possess antibacterial potential for the tested organisms and demonstrates that inhibition zones increase with increasing extract concentrations, indicating that concentration influences the activities against the test organisms. The extracts of these plants could be alternative Medicare for typhoid fever. The positive control, Ciprofloxacin (2.5mg/ml), showed an inhibition zone of 31.0±0.3mm, while the negative control, sterile distilled water, recorded no inhibition zone. However, the zone of inhibition exhibited by the standard drug Ciprofloxacin, is higher than the plants extracts. This could be due to the crude nature of plant extracts which contained other constituents that do not possess antibacterial properties. Also, the ability of plant extracts to diffuse through the gel agar may be hindered because of the large molecules. Even at a higher extract concentration, the inhibition zones are not comparable with the zones of standard drugs. However, the results of the study are in agreement with the findings documented by [18,46,47,20,21]. At different extract concentrations, there are significant differences between the mean diameter (mm) inhibition zones of aqueous and ethanolic plant extracts against tested organisms ($p <0.05$).

Table 2: Quantitative Assays of Phytochemical Crude Extracts of Medicinal Herbs Studied

Plant Parts	% Alkaloids	% Cardiac glycosides	% Flavonoids	% Total Phenol	% Saponins	% Tannins
Mango leaves	1.62±0.13^{d}	0.90±0.01^{a}	11.37±0.20^{b}	9.68±0.03^{c}	13.27±0.04^{c}	13.11±0.00^{c}
Neem leaves	1.42±0.11^{d}	0.91±0.01^{a}	10.79±0.20^{b}	10.52±0.13^{b}	12.62±0.02^{d}	11.32±0.01^{e}
Overall mean	0.30	0.18	2.21	2.02	2.59	2.44
±SEM	0.05	0.01	0.16	0.16	0.06	0.10

Key: a-e Mean in the same column with different superscript differ significantly (P<0.05)

•±SEM = Standard Error Mean. • Values are means of three determinations.

Table 3: Antibacterial Activity of Aqueous Extracts of Medicinal Plant Studied Showing Diameters (mm) Inhibition Zones at Different Extract Concentrations Against Tested Organisms

Diameters (mm) Inhibition Zones

	Salmonella typhi						*Salmonella Paratyphi A*						*Salmonella Paratyphi B*						*Salmonella Paratyphi C*					
	Concentrations (mg/ml)						Concentrations (mg/ml)						Concentrations (mg/ml)						Concentrations (mg/ml)					
Samples	80	40	20	10	+C	-C	80	40	20	10	+C	-C	80	40	20	10	+C	-C	80	40	20	10	+C	-C
Mango leaves	29.4±0.1^{b}	25.5±0.05^{b}	7.6±0.01^{b}	7.5±0.01^{b}	31.0±0.3^{b}	0.0±0.0^{b}	14.4±0.02^{b}	13.3±0.02^{b}	13.2±0.02^{b}	8.4±0.02^{b}	31.0±0.3^{b}	0.0±0.0^{b}	21.2±0.04^{c}	19.2±0.03^{c}	12.2±0.02^{b}	8.3±0.01^{c}	31.0±0.3^{b}	0.0±0.0^{b}	13.4±0.01^{g}	10.5±0.01^{g}	10.6±0.01^{g}	3.4±0.01^{g}	31.0±0.3^{a}	0.0±0.0
Neem leaves	29.1±0.1^{a}	27.7±0.05^{a}	9.7±0.01^{a}	8.4±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}	15.2±0.03^{a}	13.5±0.02^{a}	3.5±0.01^{a}	9.5±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}	20.5±0.03^{a}	20.1±0.03^{a}	19.1±0.03^{a}	9.1±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}	14.1±0.02^{b}	15.2±0.02^{b}	11.2±0.01^{b}	6.2±0.01^{b}	31.0±0.3^{a}	0.0±0.0
Overall mean	5.9	5.3	1.7	1.8	6.2	0.0	3.0	2.7	1.9	2.0	6.2	0.0	4.2	3.9	3.1	1.9	6.2	0.0	2.6	2.6	2.2	1.0	6.2	0.0

Key: •a-g Mean in the same column with different superscript differ significantly (p < 0.05). • Values are means of three determinations. • ±= Standard Error. + = Positive control (2.5mg/ml, Ciprofloxacin). - = Negative control (Sterile distilled water).

The Minimum inhibition concentration (MIC, mg/ml) and Minimum bactericidal concentration (MBC, mg/ml) of aqueous and ethanolic extracts of studied medicinal plants against tested organisms are shown in Tables 5 and 6 respectively. At different plant extract concentrations, tested organisms were inhibited by both aqueous and ethanolic extracts. Aqueous extracts showed MIC against *Salmonella typhi, Salmonella Paratyphi A*, *B and C,* at 2.5mg/ml extract concentration, while at 5mg/ml concentration, the aqueous extract showed MBC against the tested organisms (Table 5). Similarly, at 2.5 mg/ml ethanolic extract concentration, the extracts exhibited MIC against tested organisms, while at 5 mg/ml ethanolic extract concentration the extracts showed MBC, against tested organisms (Table 6). The lowest MBC of 2.5 mg/ml, was exhibited by all tested organisms, and shows that the organisms are more sensitive to the extracts (Tables 5 and 6). This supports the claim by traditional medical practitioners and vendors that the crude extracts of the investigated medicinal plants are a remedy for the cure of typhoid fever. All tested organisms showed a higher MBC than the MIC (Tables 5 and 6). This demonstrate that higher concentrations of extracts were needed to kill the bacteria than to inhibit their growth. However, the results of the study differ with the findings by [18,19,21].

Table 4: Antibacterial Activity of Ethanolic Extracts of Medicinal Plant Studied Showing Diameters (mm) Inhibition Zones at Different Extract Concentrations Against Tested Organisms

Diameters (mm) Inhibition Zones																								
	Salmonella typhi						*Salmonella Paratyphi A*						*Salmonella Paratyphi B*						*Salmonella Paratyphi C*					
	Concentrations (mg/mL)						Concentrations (mg/mL)						Concentrations (mg/mL)						Concentrations (mg/mL)					
Samples	80	40	20	10	+C	-C	80	40	20	10	+C	-C	80	40	20	10	+C	-C	80	40	20	10	+C	-C
Mango leaves	30.0±0.1^{b}	28.0±0.05^{b}	16.0±0.02^{b}	8.0±0.01^{b}	31.0±0.3^{b}	0.0±0.0^{b}	17.0±0.03^{b}	19.0±0.03^{b}	16.0±0.03^{c}	6.0±0.02^{b}	31.0±0.3^{b}	0.0±0.0^{b}	16.0±0.04^{c}	19.0±0.03^{c}	18.0±0.03^{b}	8.0±0.01^{c}	31.0±0.3^{b}	0.0±0.0^{b}	19.0±0.04^{g}	20.0±0.04^{g}	18.0±0.01^{g}	8.0±0.01^{g}	31.0±0.3^{b}	0.0±0.0^{b}
Neem leaves	30.0±0.1^{a}	26.0±0.05^{a}	18.0±0.02^{a}	9.0±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}	18.0±0.03^{a}	20.0±0.03^{a}	18.0±0.03^{a}	9.0±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}	20.0±0.04^{a}	20.0±0.03^{a}	19.0±0.03^{a}	9.0±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}	21.0±0.04^{a}	21.0±0.04^{a}	16.0±0.03^{a}	9.0±0.01^{a}	31.0±0.3^{a}	0.0±0.0^{a}
Overall mean	6.0	5.4	3.4	1.7	6.2	0.0	3.5	3.9	3.4	1.5	6.2	0.0	3.6	3.9	3.7	1.7	6.2	0.0	3.0	4.1	3.4	1.7	6.2	0.0

Key: •a-g Mean in the same column with different superscript differ significantly (p < 0.05). • Values are means of three determinations. •±= Standard Error. + = Positive control (2.5mg/ml, Ciprofloxacin). - = Negative control (Sterile distilled water).

This could be due to genetic heterogeneity of plant species, soil factor, variation in season cycle, the age of plants, climatic influences and different geographical locations where plants were collected. Notably reference [48], reported that there is a relationship between chemical composition of plants and their geographical location.

Table 5: Minimum Inhibition Concentration (MIC, mg/ml) and Minimum Bactericidal Concentration (MBC, mg/ml) of Aqueous Extract of Studied Medicinal Plants Against Test Organisms

Sam	*Salmonella typhi*						*Salmonella Paratyphi A*						*Salmonella Paratyphi B*						*Salmonella Paratyphi C*					
	MIC			MBC			MIC			MBC			MIC			MBC			MIC			MBC		
	Extract Conc (mg/ml)			Extract Conc (mg/ml)			Extract Conc (mg/ml)			Extract Conc (mg/ml)			Extract Conc (mg/ml)			Extract Conc (mg/ml)			Extract Conc (mg/ml)			Extract Conc (mg/ml)		
	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5
ML	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+
NL	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+

Key: Sam = Samples. ML = Mango Leaf. NL = Neem Leaf. MIC = Minimum Inhibition Concentration. MBC = Minimum Bactericidal Concentration. Conc = Concentration(mg/ml). + = Growth/No Inhibition. - = No growth/ Inhibition of growth.

Table 6: Minimum Inhibition Concentration (MIC, mg/mL) and Minimum Bactericidal Concentration (MBC, mg/mL) of Ethanolic Extracts of Studied Plants Against Tested Organisms

Sam	*Salmonella typhi*						*Salmonella Paratyphi A*						*Salmonella Paratyphi B*						*Salmonella Paratyphi C*					
	MIC			MBC			MIC			MBC			MIC			MBC			MIC			MBC		
	Extract Conc (mg/mL)			Extract Conc (mg/mL)			Extract Conc (mg/mL)			Extract Conc (mg/mL)			Extract Conc (mg/mL)			Extract Conc (mg/mL)			Extract Conc (mg/mL)			Extract Conc (mg/mL)		
	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5
ML	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+
NL	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+	+	+	_	+	_	+

Key: Sam = Samples. ML = Mango Leaf. NL = Neem Leaf. MIC = Minimum Inhibition Concentration. MBC = Minimum Bactericidal Concentration. Conc = Concentration(mg/ml). + = Growth/ No Inhibition. - = No Growth/ Growth Inhibition.

4. Conclusion

The aqueous and ethanolic plant extracts of investigated medicinal plants showed activities against all tested organisms at different extract concentrations. The aqueous and ethanolic plant extracts exhibited MIC and MBC against all tested organisms at 2.5mg/ml and 5mg/ml concentrations, respectively. The qualitative and quantitative profile of studied medicinal plants is rich in phytochemical compounds which exhibit biological and physiological activities. This demonstrate that the plants have pharmacological significance that is directly related to the secondary metabolites that account for their antibacterial properties. The diameter (mm) inhibition zones of the medicinal plants studied indicate that they are good candidate for typhoid Medicare alternative. However, there are significant differences between the quantitative phytochemical content and diameter (mm) inhibition zones of aqueous and ethanolic extract concentrations against tested organisms ($p < 0.05$).

5. Recommendations

Further studies are recommended on the toxicity and safe dosage regimen of the plants since the infusion of the plants are taken orally by local people for the treatment of typhoid fever. Traditional medical practitioners and vendors should be educated about modern and traditional medicine through the use of plants compounds. This will eliminate the challenges to phytomedicine, such as the lack of reproducibility of biological activity of individual herbal extracts after the success of the initial screening process, toxicity, contamination and adulteration, standardization and drug interaction issues. The loss of medical plant species due to risk of extinction as a result of high harvest and destruction of habitats, decrease in wildlife reservoir due to growing human population and excessive conservation of plants should be avoided and should be backed up by legislation.

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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