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Antibacterial and Phytochemical Properties of Crude Leaf Extracts of *Moringa oleifera* Lam., *Pterocarpus santalinoides* L'Herit DC and *Ceiba pentandra* L. on Some Clinical Bacterial Isolates in Nigeria

Reginald C. Njokuocha[1*] and Anthonia E. Ewenike[1]

[1]Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, 410001, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author RCN designed and supervised the study, performed the statistical analysis, wrote the protocol, the first draft of the manuscript, edited the first and final manuscript. Author AEE conducted the laboratory analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was carried out to determine the phytochemical constituents and antibacterial activity of aqueous and ethanolic extracts of fresh leaves of *Moringa oleifera* Lam*., Pterocarpus santalinoides* L'Herit DC and *Ceiba pentandra* L. on bacterial isolates; *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.
Methodology: The plant leaves were dried, pulverized and phytochemical tests were done according to standard laboratory procedure. Aqueous and ethanolic extracts were obtained from 20 g of the of the ground leaves. Antibacterial assay was carried out with Disc diffusion method on seven concentrations of the extracts ;100,50,25,12.5, 6.25,3.125,1.5625 mg/ml and compared with standard antibiotics. Isolated bacterial pathogens; *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (1.0 x 10^5 cfu /ml) were used as test organisms.

**Corresponding author: E-mail: reginald.njokuocha@unn.edu.ng;*

Results: Alkaloids, steroidal aglycones, glycosides, proteins, carbohydrates, reducing sugars, tannins, saponins, vitamins A and E were present in all the plant samples. Flavonoids and cardiac glycosides were not detected in *Pterocarpus santalinoides* and *Ceiba pentandra,* respectively. Anthracene glycoside was absent in all samples. Aqueous and ethanolic extracts of *M. oleifera* showed antibacterial activities against all the bacterial isolates at minimum inhibitory concentration (MIC) of 3.125 mg/ml and 1.5625 mg/ml respectively. *Pterocarpus santalinoides* showed inhibitory activity only on *Salmonella typhi* at 3.125 mg/ml and *Escherichia coli* 1.5625 mg/ml MIC. *Ceiba pentandra* showed spectrum of antibacterial activity against all the bacterial isolates at 1.56 mg/ml MIC with exception of *Salmonella typhi*. *E. coli* was the most susceptible to the leaf extracts. *Salmonella typhi* was not sensitive to the leaf extracts of *Ceiba pentandra,* while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were not sensitive to the leaf extracts of *Pterocarpus santalinoides.*
Conclusion: It can be concluded that both aqueous and ethanolic leaf extracts had antibacterial activity against the test organism, thus justifying their use in folklore medicine.

Keywords: Minimum inhibitory concentration; aqueous and ethanolic extracts; disc diffusion methods; phytochemical constituents; antibacterial activity.

1. INTRODUCTION

The medicinal or dietary administration of herbal plant preparations (extracts, emollients, creams, poultices, decoctions, infusions, oils, powders and concoctions) as a means of treating and controlling various forms of diseases and ailments is an age long tradition [1]. Such indigenous health care practices which were common among ancient people are still popular today among inhabitants of many nations especially in developing countries [2]. The rising demand is not only particularly because they are cheap and accessible, there is strong belief in their holistic and minimal side effects when compared to conventional orthodox medicines. The fact that the practice is enshrined in the tradition and belief of the people makes it readily acceptable.

The therapeutic efficacies of many herbal plant preparations used in the treatment and control of certain diseases and ailments have endeared their use over the years [3,4,5,6]. Despite the numerous successes recorded by orthodox medicine in the treatment of diseases and production of active drugs, the use of herbal plant products and complementary medicine offered as food supplements still persists even in well developed nations of Europe and America [7]. The success achieved in the use of plant extracts in the treatment of infectious diseases and management of ailments [8] is associated with the phytochemical constituents which have been demonstrated to have therapeutic and nutritional qualities [9,10]. These phytochemicals such as alkaloids, tannins, flavonoids, saponin, phenolic compounds and glycosides are potent antimicrobial, antioxidant and bioactive compounds [11,12,13] that have been verified and extracted by many herbal companies for production of drugs used in alternate or complementary medicine [14,15]. This is because they have been adjudged to be nutritionally safe and easily degradable [16]. These phytochemical constituents may function individually and or synergistically in their mode of action to inhibit the growth of pathogenic organisms and positively affect other physiological processes [17]. Isolated chemical compounds from plant extracts such as Tannins, terpenoids, alkaloids, polyphenols, terpinoids and flavonoids have been reported to exhibit antimicrobial activities against microbials [18,19,20,21].

Because of the frequent reports on multi-resistant pathogenic bacteria to many commonly administered antibiotics [22,23,24], there is need therefore to explore other possible sources of antimicrobial agents. Urgent action is therefore need in intensification of the search for drug development in view of the threat posed by infectious microorganisms and new emerging diseases ravaging the world such as Severe Acute Respiratory Syndrome (SARS), Coronovirus (COVID-19), Lassa fever and Ebola virus among others [25]. This therefore, entails continuous research and effort at finding plants with bioactive compounds that may have the potential of acting against these multi-resistant pathogenic bacteria and fungi in clinical cases.

Antimicrobial and antiviral properties of the members of Fabaceae, Moringaceae and Bombacaceae families have been documented in

literature [7,18,21,26,27,28]. *Moringa oleifera* Lam. is a pan-tropical tree species of multifunctional purpose. It is a drought resistant tree that grows intensively in semi-arid and subtropical regions [29]. The stem is brittle, with corky whitish-grey and the main trunk grows up to 12m tall and 30cm in diameter and branches profusely. The leaves (30 -60 cm long) are alternate with long petiole, light green, bipinnate and tripinnate bearing opposite ovate leaflets. The flowers are pleasantly fragrant (2.5 cm wide) with white or creamy coloured petals. The fruit is a long pod (15-45 cm), indehiscent, tri-lobed and hanging down from the branches. Each pod contains 12-35 seeds with light brown seed coat [29,30]. It is a multifunctional plant used for a variety of purposes-nutritional, therapeutic and industrial [27,31]. The roots, leaves, flowers, green pods and seeds are used for water purification, biodiesel production among others [32]. The high nutritional qualities have been highlighted [2,33] as well as the therapeutic activities [10,27,29,34] in many scientific journals.

Antimicrobial properties of leaf extracts of *M. oleifera* on different solvents have been widely reported in literature. Methanolic [27] and aqueous [30] extracts of pulverized leaves of *M. oleifera* were reported to show inhibitory activity against isolates of *Enterococcus faecali and Helicobacter pylori* at minimum concentrations of 0.035 µg/ml and µg/ml respectively. Also, Paray et al. [6] reported spectrum of antibacterial activities of aqueous leaf extract of *M. oleifera* against groups of bacterial pathogens (*Escherichia coli*, *Proteus* spp., *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus agalactiae*). Equally, inhibitory activities of water and chloroform leaf extracts of *M. oleifera* against bacterial pathogens were also reported by Abalaka et al. [31] in which chloroform extract had greater activity. In another study, the ethanolic leaf extract was shown to have antibacterial activity against multi-drug-resistant bacterial pathogens [35]. Apart from the leaves, the seeds of *M. oleifera* have been shown to inhibit the growth of eleven bacterial pathogens most of which are enteric organisms known to cause dysentery and other intestinal related infections [33].

Pterocarpus santalinoides L'Herit ex DC is a leguminous tree that grows in most tropical regions of Brazil, Ghana, Nigeria and Senegal [36]. The leaves are alternate, pinnate and newly flushed leaves are normally harvested as vegetable and animal fodder. The flowers are light yellow and zygomorphic and the fruit is winged and dehiscent. The leaves and the dye "santalin" sources are used in the treatment of skin infections, diarrhea and other gastrointestinal disorders as well as an astringent [37,38,39]. In an investigation involving the antibacterial and antifungal properties of the leaf, root, seed and bark of *P. santalinoides,* the results showed considerable levels of inhibitory activities against the tested microorganisms with exception of *Candida albicans* and *Trichophyton rubrum* which were not sensitive to the extracts [40]. Similarly, a study by Odeh and Tor-Anyiin [41] on the leaf extracts of *P. santalinoides* showed antibacterial activities especially the ethanolic extract which exhibited higher zone of inhibition. Apart from bacterial pathogens, the ethanolic leaf extract has been found to show antitrypanosomal activity and exhibited no sign of toxicity on albino rats after acute toxicity test of a single dose of 2000 mg/kg at LC_{50} [37].

Ceiba pentandra L. "kapok" (Malvaceae) is an emergent tall tree of the tropical rainforest. It grows to a height of about 70 m with trunk diameter of about 3 m bearing extensive buttress roots. The trunk and large branches bear large thorns with thick bases. It has palmate leaves which is composed of 5-9 leaflets (20 cm) long. The flowers are creamy white or pale pink in colour and 2.5cm long. The fruit is a pod (15cm) containing many brown seeds surrounded by fluffy whitish wooly fibers [42]. Traditionally, the extracts of the leaves and bark are used in various ways in the treatment of malaria fever, diabetes mellitus, gonorrhea, syphilis, wound healing, gastrointestinal infections and asthma [3,7,42]. The results of phytochemical and antimicrobial screening of the stem bark extracts of *Ceiba pentandra* showed inhibitory activity against fungal and bacterial isolates used in the study. Both the methanolic and ethanolic extracts not only contained the same phytochemicals, they showed higher inhibitory activities compared to that of the petroleum ether [43]. In another study, Lawal et al. [44], demonstrated a considerable level of antibacterial property of the stem and leaf extracts of *C. pentandra* against bacterial strains of *Mycobacterium fortuitum* ATCC 684, M. smegmatis ATCC 19420, *M. abscessus* and *M. phlei* ATCC 19240 under different solvent extracts.

In view of this studies, the aims of this work were to investigate the phytochemical constituents and antibacterial properties of the aqueous and

ethanolic crude extracts of leaves of *Moringa oleifera* L*am, Pterocarpus santalinoides* L'Herit ex DC and *Ceiba pentandra* L. against the clinical isolates of *Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* which are causative agents of common diseases in our environment.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *Pterocarpus santalinoides* L'Herit DC (Herbarium number: INTERCEDD/1573)*, Moringa oleifera* Lam. (Herbarium number: INTERCEDD/501). and *Ceiba pentandra* L. (Herbarium number: INTERCEDD/063) were collected from Nsukka, Enugu State, Nigeria. Voucher specimens of these plants were left in the International Centre for Ethnomedicine and Drug Development, Nsukka branch, Nigeria. The leaves were dried in the air until they became brittle and were pulverized with clean mortar and pestle. The pulverized materials were each divided into two portions for phytochemical and antibacterial tests.

2.2 Phytochemical Tests

Phytochemical tests of the plant materials followed standard qualitative methods of Trease and Evans [45].

Test for alkaloids: This was done by weighing 1.5 g of the pulverized plant materials into a test tube and boiled with 5 ml of 2% hydrochloric acid in a water bath, and the filtrate collected. One ml of the test solution was added to Dragendroff's reagent and observed for formation of precipitate.

Test for flavonoid: About 1.5 g of the powdered sample was added to 10 ml of ethylacetate and boiled in a water bath. One ml of ammonia solution was added to the filtrate and observed for a colour change at ethylacetate layer.

2.2.1 Test for glycosides

One and half grams (1.5 g) of the test sample was mixed with 30 ml of distilled water and heated for 5 minutes. Five ml of the filtrate were added to 0.2 ml of Fehling solution A & B until it turned alkaline. It was heated again for 2 minutes in a water bath and observed for formation of precipitate.

2.2.2 Test for anthracene glycoside

One gram of powered plant sample was added to 5 ml of dilute sulphuric acid and heated in a boiling water for 5 minutes and filtered while hot. The filtrate was shaken with an equal volume of carbon tetrachloride. The lower organic layer formed was carefully separated. The organic layer was gently shaken with 5 ml diluted ammonia.

2.2.3 Test for tannin

Five ml of 45% ethanol were added into 1.5 g of the sample, boiled for 5 minutes and filtered. About 0.5 ml of ferric chloride was added to 1 ml of the filtrate and observed for formation of precipitate.

2.2.4 Test for cardiac glycosides

One ml of the plant sample was warmed with 1 ml of chloroform and filtered and observed for colour formation at the interface.

2.2.5 Test for saponins (Emulsion test)

One gram of the sample was boiled with 5 ml of water for 5 minutes and the filtrate collected while hot. Two drops of olive oil were added to 1 ml of the filtrate and shaken vigorously and observed for formation of emulsion.

2.2.6 Test for protein

One gram of the sample was mixed with 5 ml of distilled water, allowed to stand for 3 hours and filtered. About 0.1 ml of Millions reagent was added to 2 ml of the filtrate and shaken, and observed for formation of precipitate.

2.2.7 Test for vitamin E

One ml of ethanol was added to a pinch of the sample and shaken vigorously and filtered. Ten drops of Nitric acid were added to the filtrate and observed for a colour change.

2.2.8 Test for reducing sugar

Five ml of distilled water was added to 1 g of the sample and the mixture shaken vigorously and filtered. Equal volumes (1 ml each) of Fehling solution A and B were added to 1 ml of the filtrate and shaken vigorously and observed for a colour change.

2.2.9 Test for carbohydrates (Molisch's test)

One gram of the sample was shaken with water and filtered. One drop of Molisch's reagent was added to the filtrate, shaken vigorously and one ml of concentrated sulphuric acid was added and observed for the formation of a layer below the aqueous solution.

2.2.10 Test for vitamin A

Five drops of chloroform and 5 drops of sulphuric acid were added to 5 pinches of the sample and the mixture was shaken vigorously, and observed for a colour change.

2.2.11 Test for steroidal aglycone

Five ml of 9% lead acetate was added in one gram and the sample in 10 ml of aqueous ethanol in a conical flask. The was heated in a boiling water for two minutes, cooled and filtered. The filtrate was extracted twice with 15 ml of chloroform. The lower chloroform layer was retained. Five ml of the chloroform layer were evaporated to dryness in a water bath and the resultant residue was dissolved in 2 ml solution of 3,5-dinitrobenzoic acid and 2% ethanol. One ml of 1N sodium hydroxide was added to the solution and observed for a colour change.

2.3 Antibacterial Screening

2.3.1 Extraction of test materials

Fourty grams of the pulverized samples each of *Pterocarpus santalinoides, Moringa oleifera* and *Ceiba pentandra* were weighed. Twenty grams of each sample were mixed with 100 ml of distilled water to obtain aqueous extract and 100 ml of ethanol was used on the remaining 20 g to obtain ethanolic extract. The extracts obtained were evaporated to dryness using rotary evaporator. The concentrates were weighed and stored in a refrigerator at 4^{o}C in sterile bottles. One gram of each extract was dissolved in 5 ml of 1% (v/v) Dimethyl sulfoxide (DMSO; BDH, Milan Italy) and a double serial dilution of each plant extract was made in 1% (v/v) of Dimethyl sulfoxide (DMSO; BDH, Milan, Italy) to obtain different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.5625 mg/ml. Standard antibiotics; Nalidixic acid, Chloramphenicol and Amoxicillin diluted to 50, 30, 25 concentrations were used as control.

2.3.2 Test bacteria

The selected pathogenic test organisms were clinical isolates of different cultures of *Salmonella typhi* (gram-negative), *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram-negative) and *Pseudomonas aeruginosa* (gram-negative) obtained from the Department of Microbiology, University of Nigeria, Nsukka. Each test bacterial strain was maintained in Nutrient Agar medium. The 24-hour bacterial culture was inoculated into Mueller Hinton Broth (MHB) and diluted with sterile physiological saline to a concentration of 1.0×10^{5} cfu /ml and stored at 4 ^{0}C for the antibacterial assay.

2.3.3 Determination of antibacterial activity and minimum inhibitory concentration

The disc diffusion method of Collins and Lyne [46] were used in determining the minimum inhibitory concentrations (MIC) of the plant extracts. Five ml of the pure cultures of *Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa each* were used to inoculate separate Petri dishes containing molten MHB which had been allowed to stand for 1 hour. A sterile cork borer (5 mm) was used to make seven wells aseptically on each plate. Each hole was filled with 2 ml of different concentrations of the leaf extracts. The extracts were allowed to diffuse across the media for 1 hour and the plates incubated aerobically at 35^{o}C for 24 hours. The inhibition zone diameter (IZD) was measured (mm) and the minimum inhibitory concentration (MIC), which is the lowest concentration of the plant extracts that inhibited visible growth of the test organisms was determined based on the readings.

3. RESULTS

The dry yields of the ethanolic and aqueous leaf extracts of the plants in Table 1 showed that *Moringa oleifera* yielded more extracts than *Pterocarpus santalinoides,* and *Ceiba pentandra*. The high yield of ethanol showed that it was probably a better solvent than water in the extraction of the leaf constituents. The phytochemical tests of the pulverized leaf samples of the plants (Table 2) showed that *Moringa oleifera* contained all the phytochemical constituents evaluated. Only flavonoid was not detected in *Pterocarpus santalinoides*, while in *Ceiba pentandra,* cardiac glycoside was not detected.

Table 1. Yield of the plant leaves after extraction

Plant	Dry extract (g)	
	Aqueous	Ethanol
Moringa oleifera Lam.	3.51±0.029	5.51±0.014
Pterocarpus santalinoides L'Herit DC	1.27±0.022	3.60±0.172
Ceiba pentandra L.	1.08±0.016	2.22±0.022

The results of the antibacterial screening showed that both the ethanolic and aqueous extracts exerted considerable antibacterial activities against most of the test organisms (Tables 3, 4, 5). In particular, the ethanolic and aqueous extracts of *Moringa oleifera* showed broad spectrum of inhibitory activity against all the bacterial isolates, followed by that of *Ceiba pentandra* which showed inhibitory action on all the organisms except *Salmonella typhi*. The minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of *Moringa oleifera* were recorded at 1.5625 mg/ml (10 ±0.26 mm -11±0.26 mm) and 3.125 mg/ml (7±0.3 mm - 10±0.14 mm) respectively against all the bacterial isolates. For *Pterocarpus santalinoides*, both ethanolic and aqueous extracts did not have any antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but had inhibitory activity against *Samonella typhi* and *Escherichia coli*. The MIC of the ethanolic extract against *Salmonella typhi* and *Escherichia coli* was observed at 1.5625 mg/ml (IZD -10±0.39 mm - 11±0.37 mm), while that of aqueous extract was 3.125 mg/ml (9±0.29 mm – 10±0.25 mm) against both organisms.

Equally both ethanolic and aqueous extracts of *Ceiba pentandra* did not show any antibacterial activity against *Salmonella typhi*, but showed inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at a minimum inhibitory concentration of 1.5625 mg/ml with inhibitory zone diameter varying from 10±0.36 mm - 11±0.16 mm for ethanolic extract and 7±0.12 mm - 10±0.29 mm) for the aqueous extract. For the control, the bacterial isolates were found to be sensitive to all antibiotics (Chloramphenicol, Nalidixic acid and Amoxicillin) at concentration of 30 µg/ml in which the range of IZD for Chloramphenicol was 11±0.22 mm - 13±0.26 mm; Nalidixic acid was10±29 mm - 14±0.25 mm and Amoxicillin was14±0.29 mm - 16±0.32 mm. The Comparative results of the antibacterial activity of the crude plant extracts showed comparable bioactive activity with the synthetic antibiotics drugs.

4. DISCUSSION

The presence of notable bioactive compounds such as tannins, alkaloids, flavonoids, saponins, proteins and vitamins A and E detected in the aqueous and ethanolic leaf extracts of *M. oleifera, P. santalinoides* and *C. pentandra* have also been reported to be present in part or full in the leaf, bark, root and seed extracts of these plants in related studies elsewhere [29,33,41,42]. This study showed that the leaf extracts of *Moringa oleifera, Pterocarpus santalinoides* and *Ceiba pentandra* exerted inhibitory activities against the clinical isolates of *Salmonela typhi, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. This indicates that the plant extracts contain active complex organic substances some of which have effect on the developmental functions of the organisms. The presence of such compounds like alkaloids, flavonoids, tannins, cardiac glycosides, saponins among others have been reported to have bioactive activities against bacterial and fungal pathogens [15,47]. The extent to which plant extracts demonstrate their antimicrobial activity have been reported to be influenced by the nature and type of active constituents present in the plants [8,10]. This may possibly explain the spectrum of activity exhibited by the leaf extracts especially that of *Moringa oleifera* and *Ceiba pentandra.*

The antibacterial activity of the leaf extracts of *Moringa oleifera* at 1.5625 mg/ml and 3.125 mg/ml against all the gastrointestinal bacterial isolates, demonstrated the strong medicinal properties of this plant. *M. oleifera* has been reported to have multifunctional properties including nutritional and medicinal values [29]. The presence of potent compounds such as alkaloids, flavonoids, glycosides, saponins and tannins among others in the leaf extract of *M. oleifera* may be responsible for its strong activity since these compounds have been found to exert a spectrum of activity against gram + and gram – bacteria [10,48]. Comparatively in another study, aqueous and ethanolic leaf extracts of *M. oleifera* were found to show antibacterial effect against *S. aureus, Vibrio parahaemolyticus, Enterococcus*

faecalis and *Aeromonas caviae* [49]. but, unlike the present study, *E. Coli and P. aeruginosa* were resistant to the extracts. Paray et al. [6], working on six medicinal plants found that aqueous leaf extract of *M. oleifera* exhibited antibacterial activity against six bacterial pathogens subjected to test. Othman and Ahmed [35], demonstrated susceptibility of five multi drug resistant isolates of *E. coli* and *S. aureus* on the ethanolic leaf extract of *M. oliefera*. Also, antibacterial studies on the aqueous and chloroform [31], aqueous and methanolic [30] and methanolic [27] leaf extracts showed remarkable inhibitory activity against isolates of *E. coli* and *S. typhi; Enterococcus faecalis* and *Helicobacter pylori* respectively. However, in contrast to the present study *P. aeruginosa* according to Abalaka et al [31] was not susceptible to their aqueous leaf extract. Equally, isolated substances such as pterygosperma, 4-(α-L-rhamnopy-ranosyloxy) benzyl glucosinolate and its cognate isothiocyanate from *M. oleifera* have been shown to act against a wide range of bacterial and fungal pathogens [34,50,51].

The leaves of *Pterocarpus santalinoides* selectively inhibited the growth of *Salmonella typhi* and *Escherichia coli.* The antibacterial activity demonstrated against these two bacteria pathogens affirms to its use in folklore medicine in southern Nigeria. Comparatively, similar findings have been reported by Okpo *et al* [39], Osuagwu and Akomas [36], Odeh et al. [38] and Odeh et al. [52] in studies conducted at various locations in Nigeria. In a study conducted by Odeh and Tor-Anyiin [41] involving multiple solvents, the ethanolic and aqueous leaf extracts exhibited strong antibacterial and antifungal activities. Like the present work, both studies did not show any inhibitory effect on *P. aeruginosa*. Of particular interest is the antibacterial activity demonstrated by the ethanolic and aqueous leaf extracts of *Ceiba pentandra* at 1.5625 mg/ml against *S. aureus, E. coli* and *P. aeruginosa.* This inhibitory activity may be related to the type and concentration of the particular bioactive properties present in the extracts. Similar antibacterial activities of the leaf and stem bark extract of *Ceiba pentandra* have been reported [1, 9,42,43]. It has also been shown that the oil extract from *Ceiba pentandra* seed demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungal pathogens [53]. Unlike the other plants, both solvent extracts showed activity at the lowest minimum concentration (1.5625 mg/ml) against the same bacterial isolates. This shows that the active constituents responsible for these inhibitory activities may have considerably solubility potential in both ethanolic and aqueous media. The solubility and diffusion rates of the active ingredients may have resulted to increased concentration of the active agents and level of contact with the bacterial isolates, leading to the biological activity recorded at lower concentration of the extracts [54].

The phytochemical analysis showed that the leaf extract of the test plants contains the commonly reported biochemical compounds which have been reported to be valuable therapeutic properties against bacterial pathogens. These findings therefore, justify their use in folklore medicine for the treatment of dysentery, diarrhea, typhoid fever and stomach disorder, among others. The differences observed in the antibacterial activity of the leaf extracts of the plants may be attributed to multiple factors such as the chemical constituents, diffusing ability of the extracts, concentration of bioactive compounds and the ability of the extracts to lower the internal pH and affect the membrane of the bacteria.

The susceptibility of the bacterial isolates to the leaf extracts of *Moringa oliefera, Pterocarpus santalinoides and Ceiba pantandra* showed that the plant extracts contain potentially active principles which can possibly take care of the multiple antibiotic resistant strains of *S. aereus* and *P. auriginosa* that occur commonly in clinical situations worldwide [51]. The mechanism of action of the leaf extract of the plants can be explained by the ability of some of the constituent phytochemical compounds to inhibit the bacterial enzymes or bind to their enzyme substrate. It can also damage the bacterial cell membrane thereby altering their physiological processes such as lowering of their internal pH and membrane hyperpolarization [17,55]. Specifically, isolated plant tannins have demonstrated strong antimicrobial effects against multiple genera of microorganisms such as yeasts, fungi and bacteria [56]. The mode of action of tannins involves the precipitation of proteins, bringing about inactivation of microbial adhesin and cell transport proteins, binds to metals and other macromolecules making them unavailable to the microorganisms [57,58]. Other plants isolate such as steroids, alkaloids and flavonoids have been reported to show significant antimicrobial effects against infectious organisms in addition to their other roles as anti- oxidants, anti-diarhoetic, anti-inflammation and enzyme inhibitors among others [11,12,19,20,22,59].

Table 2. Results of the phytochemical screening of the leaf extracts of *M. oleifera, P. santalinoides* and *C. pentandra*

Phytochemical constituents	***Moringa oleifera***	***Pterocarpus santalinoides***	***Ceiba pentandra***	***Procedure***	***Reference***	***Screening Results***
Alkaloids	+	+	+	Test sample+ 5ml HCl + heat + Dragendroffs reagent	Trease and Evans [45]	Red precipitate formed
Flavonoids	+	-	+	Test sample in 10 ml ethylacetate + heat, filtered + 1ml of ammonia solution	‘’	Yellow colouration at ethylacetate layer
Glycosides	+	+	+	Filtrate of heated test sample + 0.2ml Fehling solution A & B + heat	“	Brick red precipitate formed
Cardiac glycosides	+	+	-	Test sample + 45% ethanol + heat + Ferric chloride test	“	Pale brown precipitate formed
Proteins	+	+	+	Solution of test sample, filtered + Millions reagent + agitation	“	Yellow precipitate formed
Anthracene glycosides	-	-	-	Test sample + 5 ml dilute HCl + heat, filtered + carbon tetrachloride + lower organic layer + equal volume of ammonia	“	Ammonia layer turns pink
Carbohydrates	+	+	+	Molisch’s test	“	brown ring precipitate formed
Reducing sugar	+	+	+	Test sample + Fehling solution A & B + agitated	“	Blue precipitate turns brick red
Steroidal aglycones	+	+	+	Test sample + lead acetate solution + ethanol +heat + extract filtrate with chloroform + dry + residue with 3,5-dinitrobenzoic acid & ethanol + 1N Sodium hydroxide solution	“	Red colour is formed
Tannins	+	+	+	Test sample + ethanol + boil + Ferric chloride test	“	greenish colour formed
Saponins	+	+	+	Emulsion test	“	Emulsion formed
Vitamin A	+	+	+	Test sample + drops of chloroform + H_2SO_4 + agitation	“	Blue colour formed
Vitamin E	+	+	+	Test sample + ethanol + agitation, filtered + nitric acid	“	Red colour formed

Key:+ = present - = not detected

Table 3. Antibacterial activity of the of *Moringa oleifera* showing the inhibitory zone diameter (mm) and the various concentrations (mg/ml and ug/ml)

Sources (leaf extracts)	Solvent	Bacterial isolates	Leaf extract concentration (mg/ml) / IZD |(mm)							Control concentration (µg/ml)/IZD (mm)		
			100	50	25	12.5	6.25	3.125	1.5625	50	30	25
Moringa oleifera	Aqueous	*Salmonella typhi*	13 ± 0.16	12 ± 0.36	12 ± 0.52	11 ± 0.37	10 ± 0.27	10 ± 0.14	0	-	-	-
		Staphylococcus aureus	12 ± 0.22	12 ± 0.22	11 ± 0.36	10 ± 0.37	19 ± 0.14	8 ± 0.16	0	-	-	-
		Escherichia coli	10 ± 0.22	9 ± 0.29	9 ± 0.18	8 ± 0.29	8 ± 0.18	7 ± 0.29	0	-	-	-
		Pseudomonas aeruginosa	12 ± 0.26	11 ± 0.26	11 ± 0.08	10 ± 0.29	10 ± 0.18	9 ± ± 0.37	0	-	-	-
	Ethanol	*Salmonella typhi*	14 ± 0.26	14 ± 0.18	13 ± 0.29	13 ± 0.18	11 ± 0.18	10 ± 0.18	10 ± 0.26	-	-	-
		Staphylococcus aureus	14 ± 0.29	14 ± 0.18	13 ± 0.29	12 ± 0.18	12 ± 0.26	11 ± 0.18	11 ± 0.26	-	-	-
		Escherichia coli	13 ± 0.18	13 ± 0.26	13 ± 0.12	12 ± 0.29	11 ± 0.18	10 ± 0.18	10 ± 0.27	-	-	-
		Pseudomonas aeruginosa	13 ± 0.18	13 ± 0.27	12 ± 0.37	12 ± 0.18	11 ± 0.37	11 ± 0.18	10 ± 0.32	-	-	-
Control	Chloramphenicol	*Salmonella typhi*	-	-	-	-	-	-	-	0	13 ± 0.26	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	11 ± 0.22	0
		Escherichia coli	-	-	-	-	-	-	-	0	12 ± 0.37	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	13 ± 0.16	0
	Nalidixic acid	*Salmonella typhi*	-	-	-	-	-	-	-	0	14 ± 0. 25	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	13 ± 0.37	0
		Escherichia coli	-	-	-	-	-	-	-	0	11 ± 0.18	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	10 ± 0.29	0
	Amoxicillin	*Salmonella typhi*	-	-	-	-	-	-	-	0	14 ± 0.29	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	15 ± 0.25	0
		Escherichia coli	-	-	-	-	-	-	-	0	14 ± 0.50	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	16 ± 0.32	0

Table 4. Antibacterial activity of the of *Pterocarpus santalinoides* showing the inhibitory zone diameter (mm) and the various concentrations (mg/ml and ug/ml)

Sources (leaf extracts)	Solvent	Bacterial isolates	Leaf extract concentration (mg/ml) / IZD |(mm)							Control concentration (µg/ml)		
			100	50	25	12.5	6.25	3.125	1.5625	50	30	25
Pterocarpus santalinoides	Aqueous	*Salmonella typhi*	11 ± 0.34	11 ± 0.26	10 ± 0.36	10 ± 0.26	10 ± 0.18	9 ± 0.29	0	-	-	-
		Staphylococcus aureus	0	0	0	0	0	0	0	-	-	-
		Escherichia coli	13 ± 0.25	12 ± 0.25	12 ± 0.25	11 ± 0.18	10 ± 0.50	10 ± 0.25	0	-	-	-
		Pseudomonas aeruginosa	0	0	0	0	0	0	0	-	-	-
	Ethanol	*Salmonella typhi*	14 ± 0.26	13 ± 0.18	12 ± 0.22	12 ± 0.22	11 ± 0.37	11 ± 0.22	10 ± 0.39	-	-	-
		Staphylococcus aureus	0	0	0	0	0	0	0	-	-	-
		Escherichia coli	11 ± 0.34	11 ± 0.26	10 ± 0.36	10 ± 0.26	10 ± 0.18	9 ± 0.29	0	-	-	-
		Pseudomonas aeruginosa	0	0	0	0	0	0	0	-	-	-
Control	Chloramphenicol	*Salmonella typhi*	-	-	-	-	-	-	-	0	13 ± 0.26	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	11 ± 0.22	0
		Escherichia coli	-	-	-	-	-	-	-	0	12 ± 0.37	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	13 ± 0.16	0
	Nalidixic acid	*Salmonella typhi*	-	-	-	-	-	-	-	0	14 ± 0. 25	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	13 ± 0.37	0
		Escherichia coli	-	-	-	-	-	-	-	0	11 ± 0.18	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	10 ± 0.29	0
	Amoxicillin	*Salmonella typhi*	-	-	-	-	-	-	-	0	14 ± 0.29	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	15 ± 0.25	0
		Escherichia coli	-	-	-	-	-	-	-	0	14 ± 0.50	0

Table 5. Antibacterial activity of the of *Ceiba pentandra* showing the inhibitory zone diameter (mm) and the various concentrations (mg/ml and ug/ml)

Sources (leaf extracts)	Solvent	Bacterial isolates	Leaf extract concentration (mg/ml) / IZD |(mm)							Control concentration (µg/ml)		
			100	50	25	12.5	6.25	3.125	1.5625	50	30	25
Ceiba pentandra	Aqueous	*Salmonella typhi*	0	0	0	0	0	0	0	-	-	-
		Staphylococcus aureus	12 ± 0.18	11 ± 0.26	11 ± 0.26	10 ± 0.29	10 ± 0.22	10 ± 0.29	10 ± 0.29	-	-	-
		Escherichia coli	11 ± 0.34	11 ± 0.25	10 ± 0.32	10 ± 0.55	10 ± 0.40	9 ± 0.18	8 ± 0.18	-	-	-
		Pseudomonas aeruginosa	12 ± 0.22	12 ± 0.29	12 ± 0.26	11 ± 0.18	11 ± 0.14	8 ± 0.25	7 ± 0.12	-	-	-
	Ethanol	*Salmonella typhi*	0	0	0	0	0	0	0	-	-	-
		Staphylococcus aureus	15 ± 0.26	15 ± 0.22	14 ± 0.18	13 ± 0.18	13 ± 0.22	12 ± 0.18	11 ± 0.16	-	-	-
		Escherichia coli	14 ± 0.18	13 ± 0.08	13 ± 0.26	12 ± 0.37	11 ± 0.18	11 ± 0.26	10 ± 0.36	-	-	-
		Pseudomonas aeruginosa	14 ± 0.14	14 ± 0.18	13 ± 0.26	13 ± 0.16	12 ± 0.12	11 ± 0.29	11 ± 0.08	-	-	-
Control	Chloramphenicol	*Salmonella typhi*	-	-	-	-	-	-	-	0	13 ± 0.26	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	11 ± 0.22	0
		Escherichia coli	-	-	-	-	-	-	-	0	12 ± 0.37	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	13 ± 0.16	0
	Nalidixic acid	*Salmonella typhi*	-	-	-	-	-	-	-	0	14 ± 0. 25	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	13 ± 0.37	0
		Escherichia coli	-	-	-	-	-	-	-	0	11 ± 0.18	0
		Pseudomonas aeruginosa	-	-	-	-	-	-	-	0	10 ± 0.29	0
	Amoxicillin	*Salmonella typhi*	-	-	-	-	-	-	-	0	14 ± 0.29	0
		Staphylococcus aureus	-	-	-	-	-	-	-	0	15 ± 0.25	0
		Escherichia coli	-	-	-	-	-	-	-	0	14 ± 0.50	0

5. CONCLUSION

The phytochemical results showed that the leaf extracts of *M. oleifera, P. santalinoides* and *C. pentandra* possessed all the phytoconstituents analyzed with exception of anthracene glycosides that was not detected in all the plants, while flavonoids and cardiac glycosides were not detected in *P. santalinoides* and *C. pentandra* respectively. The antibacterial assay of the aqueous and ethanolic leaf extracts of the plants showed that all the bacterial isolates were susceptible to the extracts of *M. oleifera*, while *S. aureus* and *P aeruginosa* were not susceptible to that of *P. santalinoides* and *S. typhi* was not susceptible to that of *C. pentandra*. Therefore, the presence of the bioactive constituents and the extent of antibacterial activity shown by these plants justify their use in traditional medicine. However, further studies need to be carried out at lower concentrations and to isolate and characterize the active agents which may serve as bases for formulation and development of new drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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