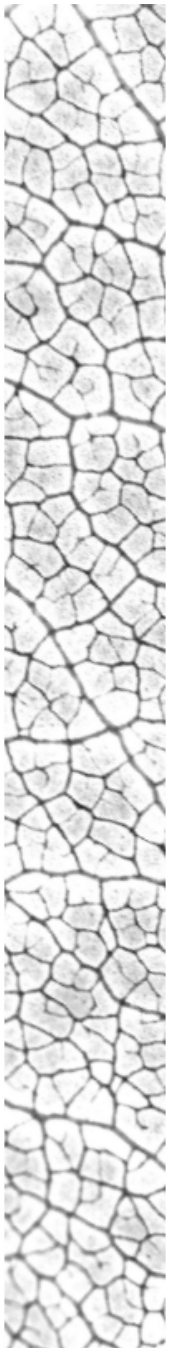

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Inhibitory effect and preliminary phytochemical screening of some ornamental plants against some bacteria pathogens

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Abstract

Background: Bacterial resistance to the drugs known for their treatment is on the increase, therefore suggesting the need to search for dependable natural products as alternative for cure and prevention. Efforts in this regard have focused on plants because of their use historically and the large portions of the world's population relying on plants for the treatment of infectious and non-infectious diseases.
Methods: Some ornamental plants' extracts in compares with commercial antibiotics were tested *in vitro* on *Salmonella typhi, Salmonella typhimurium, Staphylococcus aureus* and *Staphylococcus epidermidis* for medicinal values. Quantitative and qualitative phytochemicals; free radical scavenging capacity, ferric reducing antioxidant property and hydroxyl radical scavenging of the plants' extracts were determined by chemical methods.
Results: Varied degrees of inhibition and in some cases resistance were observed with the extracts on the four bacteria species. However, highest inhibition of 30.33±0.35 mm was recorded on *Salmonella typhimurium*, followed by *S. epidermidis* with zone of 30.00±0.00 mm both with *H. crepitans* extract. Cotrimozazole most inhibited *Salmonella typhimurium* with a zone of 29 mm and followed by ofloxacin with a zone of 27.33 mm on *S. epidermidis*. MIC activity of the extract was between 12.5 – 50 mg/ml and MBC activity from 25 – 100 mg/ml.
Conclusions: Valuable antibacterial effect of the plants' extracts correlating with the phytochemicals and antioxidants potentials, suggest the plants acceptability for folklore and could be of universal recognition for handling diseases that plaque around us mostly in some urban and rural communities where modern medicine are not affordable and accessible by the poor.

Keywords: antibacterial, MIC, ornamental plants, chemicals, disease

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INTRODUCTION

Various human infections are caused by pathogenic organisms, such as *Salmonella typhi, Salmonella typhimurium,* and *Staphylococci* species among others. The continuous growth of bacterial resistance to presently existing antibiotics has necessitated the search for new and more effective antibacterial compounds (Akinnibosun et al. 2008, Mabhiza et al. 2015). Bacterial resistance to almost all available antimicrobials has been recorded, making antibiotics resistance a global concern. Ethnopharmacologists, botanists, microbiologists and natural product chemists are working to discover phytochemicals and leads, which could be developed for treatment of infectious diseases (Kavitha and Padma 2008). Efforts in this regard have focused on plants because of their use historically and the fact that a good portion of the world's population, particularly in developing countries, rely on plants for the treatment of infectious and non-infectious diseases (Aibinu 2006, Bereksi et al. 2018, Girish and Prabhavathi, 2019, Jadeja et al. 2005, Martinez et al. 1996).

Typhoid fever is caused by *Salmonella typhi* and has since 1989 developed simultaneously, resistance to conventional antibiotics of choice in several endemic areas (Greenwood et al. 2009). Chloramphenicol, ampicillin, co-trimoxazole and several other antibiotics are known for their treatment. Several other disease-causing organisms of medical importance have also

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developed resistance to these conventional antibiotics. In undeveloped areas without access to clean drinkable water, good environment and sanitation and hygienic conveniences, the people inhabiting these areas are prone to typhoid fever cases and deaths (Crump and Mintz 2010). *Salmonella typhimurium* on the other hand is the leading cause of Gastroenteritis and food poisoning. The infection is derived mainly from pigs, cattle and chicken as well as environmental contamination from household pets or contaminated birds (Martelli and Davies 2012). This pathogen has been found resistant to oxytetracycline, trimethoprim, sulfamethoxazole and gentamycin (López-Martín et al. 2016). While *S. typhi* infection is strictly limited to humans and higher primates, *S. typhimurium* has a wide range of host such as rodents, cattle and mammals.

Stapylococcus aureus is a commensal and opportunistic pathogen that can cause wide spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive disease (Lowy 1998). *Staphylococcus aureus* is an important pathogen that is part of the normal flora of the human skin and mucous membranes (Chaves et al. 2015). *Staphylococcus* infections are caused by several species of *Staphylococcus* but most importantly, the common species that has acquired multi-drug resistance is *Staphylococcus aureus which* is the cause of several infections namely; urinary tract infection, respiratory tract infection, ureo-genital infection and several others. Another specie; *Staphylococcus epidermidis* infections are associated with intravascular devices, large wounds, catheter infections along with catheter induced UTI's which can lead to serious inflammation and pus secretion. Septicemia and endocarditis are also diseases associated with *S. epidermidis*. *S. epidermidis* have developed resistance to most of the antibiotics known for treatment. For this, man has resulted back to using plants to treat the infections and diseases caused by these organisms. Plants have bioactive constituents present within them which when used against organisms, invokes a negative reaction, thereby inhibiting their growth. The aim of this study was to determine the antimicrobial effect of ornamental plants' extracts against *Salmonella typhi, Salmonella typhimurium, Staphylococcus aureus and Staphylococcus epidermidis,* phytochemicals and *In vitro* antioxidant profile of the plants.

MATERIALS AND METHODS

Collection and Processing of Plant Samples

The leaves of *Acalypha wilkesiana* (Euphobiaceae)*, Acalypha godseffiana* (Euphobiaceae), *Ficus benjamina* (Moraceae), *Ficus thonningii* (Moraceae), *Hura crepitans* (Papaveraceae) and *Terminalia catappa* (Conbretaceae) were collected from Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria, in the month of February, 2017. The leaves were washed and ground with sterile distilled water in a mechanical grinder. The homogenate was filtered through double layers of muslin cloth and centrifuged at 1200 rpm for 5 minutes. The supernatant was thereafter passed through Whatman No 1 filter paper. The filtered aqueous extracts were vaporized using a water bath regulated at 40 ^{o}C to obtain solid concentrate of the extracts and were kept at 4 ^{o}C until required for use. Before use, the extract was reconstituted with sterile distilled water and passed through a membrane filter for sterility.

Collection of Test Bacteria Species

Four clinical bacteria species (*Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi and Salmonella typhimurium)* used for this study were obtained from microbiology laboratory of Afe Babalola University, Ado-Ekiti, Nigeria. They were purified by sub-culturing on Nutrient agar. The sub-cultured plates were incubated at 37 ^{o}C for 24 hours. Thereafter, the cultures were Gram stained for cell uniformity and Gram stain reaction. Several other identification methods to confirm characteristics and identity of each bacteria species were performed with catalase**,** oxidase, Indole test, starch hydrolysis, Vogues-proskauer, triple sugar iron, motility, nitrate reduction and coagulase tests according to Holt et al. (1994).

Preparation of Inoculums

The purified isolates were sub-cultured in peptone water for 18 hours. Cultures were adjusted with sterile saline water to match McFarland's standard of 10^7 Cfu/ml.

Antibacterial Test of Plant Extracts

The antimicrobial activities of the extracts were determined by agar well diffusion method described by Omenka and Osuoha, (2000). Mueller Hinton Agar culture plates were allowed to gel and streaked with test bacteria concentration of 10^7 Cfu/ml. The plates were punched with a sterile cork borer (5.00 mm diameter) to create several open wells which were filled with 0.05 ml of extracts. The plates were kept at room temperature for two hours to permit extract diffusion and bacteria established in the cultures before incubated at 37 ^{o}C for 24 hours. Zones of inhibition were measured and recorded as degree of sensitivity.

Antibiotics Sensitivity Test

Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. Muller Hinton agar was prepared and was autoclaved at 121 ^{o}C for 15 minutes and allowed to cool. 1 ml of 18 hour test bacteria broth culture of MacFarland standard (10^7 Cfu/ml) was pour plated with Molten Mueller Hinton agar and allowed to gel. The seeded plates were left for 2 hour at room temperature before antibiotic disks were overlaid. The culture agar plates were incubated at 37 ^{o}C for 24 hours. Susceptibility of the test bacteria species was by clear

ring, or zone of inhibition around the wafer (James *et al*. 2009).

Qualitative Phytochemical Analysis of the Extracts

Chemical methods by addition to weight of extracts and timing for instant results were used to determine qualitatively the presence of phytochemicals contained in the plants' extracts with the criteria of (Harborne and Williams 2000, Trease and Evans 1989). The such phytochemical screened are Saponins, Flavonoids, Tannins, Steroids, Alkaloids, Phenolic, Cardiac Glycosides, reducing sugar, phlobatanine and terpenoids.

Quantitative Phytochemical Analysis

Determination of total phenolic compounds

100 mg of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2 N of Folin-ciocalteu reagent and 1.5 ml 20 % of Na_2CO_3 solution were added and ultimately the volume was made up to 100 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using standard calibration curve obtained from various diluted concentrations of garlic acid.

Determination of total flavonoids

The method was based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415 nm.100 µl of 20 % aluminium trichloride in methanol to 5 ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 mL of plant extracts and a drop of acetic acid, and then diluted to 5 ml of methanol. The absorption of standard rutin solution (0.5 mg/mll in methanol was measured under the same conditions. All determinations were carried out in triplicates.

Determination of total alkaloids

Five grams of sample was weighed into 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume .Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloids which was dried and weighed.

Determination of tannin

Tannin from the plant extract was extracted by boiling with distilled water for 1 hour. Colour development was done with Folin-Dennis reagent and sodium carbonate solution. Absorbance was measured at 750 nm spectrophotometically. The tannic acid concentrations were calculated from the tannic acid standard curve.

Determination of saponins

Saponins was extracted for 2 hours in reflux condenser containing pure acetone. Exhaustive re-extraction over heating mantle with methanol in the soxhlet apparatus was done for 2 hours. Extract was weighed after allowing methanol to evaporate. The saponins content was calculated as percentage of the sample.

***In Vitro* Antioxidant Property of Plants' Extracts**

Ferric Reducing Antioxidant Potential (FRAP)

Ferric reducing antioxidant potential (FRAP) of the plants extracts was measured according to the method of Chan et al. (2007). FRAP reagent was prepared by mixing in 2.5 ml phosphate buffer, 2.5 ml potassium ferricyanide (KFeCN) solution. The mixture was incubated for 20 minutes at 50 °C before use. 2.5 ml of TCA solution was added to stop the reaction. The mixtures were then separated into aliquots of 2.5 ml and diluted each with 2.5 ml of distilled water.

0.5 ml of $FeCl_3$ was added to each tube. Gallic acid standard was employed as a standard in this assay; 10 mg Gallic acid into a 100 ml volumetric flask and then dissolve in 50 ml distilled water. The mixture was incubated for 30 minutes in the dark, and its absorbance was measured at 700 nm against a blank reagent.

Free Radical Scavenging Capacity (DPPH)

DPPH radical scavenging activity was determined according to Cavin et al. (1998). 2 ml of the plants' extracts was added to 1 ml of DPPH solution (0.04 mg/ml in methanol). The mixture was vigorously shaken and incubated in the dark for 20 minutes. Thereafter, the reduction of DPPH absorption was measured at 517 nm. A calibration curve was prepared by measuring the reduction in absorbance of the DPPH solution in the presence of different concentrations of Trolox (0-400 µm). Results were expressed as µmol of Trolox equivalents (TE/mg) wet extract. All determinations were performed in duplicate.

Assay for Hydroxyl radical scavenging

The capacity to scavenge hydroxyl radical by the extract was measured according to the modified method proposed by Halliwell et al. (1987) with slight modification. The hydroxyl radicals are generated by iron-ascorbate-EDTA-H_2O_2, which then react with deoxyribose to form thiobarbituric acid reactive substance (TBARS). This substance yields pink chromogen at low pH while heating with trichloroacetic acid (TBA). The reaction mixture contained 4 mM deoxyribose, 0.3 mM ferric chloride, 0.2 mM EDTA, 0.2 mM ascorbic acid, 2 mM H_2O_2 and various concentrations of the plant extract in different tubes. The tubes were capped tightly and incubated for 30 minutes

Table 1. Characterization and identification of test bacteria species

	Staphylococcus aureus	*Staphylococcus epidermidis*	*Salmonella typhi*	*Salmonella typhimurium*
Colour	Yellow	Cream	Cream	Cream
Surface	Smooth	Smooth	Smooth	Smooth
Edge	Circular	Circular	Round	Round
Elevation	Raised	Raised	Raised	Raised
Cell shape	Cocci	Cocci	Rod	Rod
Gram stain	+	+	-	-
Coagulase test	+	-	-	-
Catalase test	+	+	-	-
Starch hydrolysis	-	-	-	-
Motility test	-	-	+	+
Oxidase test	-	-	-	-
Indole test	-	-	-	-
Citrate test	-	-	+	+
Urease test	-	-	-	-
Nitrate reduction test	-	-	+	+
Methyl red test	-	-	+	+
Vogues-proskauer test	-	-	-	-
Triple sugar iron test	-	-	-	-
Glucose	A	A	A	A
Lactose	A	A	-	-
Mannitol	A	-	A	-
Galactose	-	-	A	A
Fructose	-	A	A	-
Maltose	A	A	A	-
Sucrose	A	A	-	-
Sorbitol	-	-	A	A

Legend: A- Acid production; + = Positive; - = Negative

at 37 °C. Then 0.4 ml of 5% (v/v) TBA and 0.4 ml of 1% (v/v) TBA were added to the reaction mixture which was kept in a boiling water bath for 20 minutes. The intensity of pink chromogen developed was measured spectrophotometrically at 532 nm against the blank sample. Ascorbic acid was used as a positive control. Every preparation was carried in triplicates. The hydroxyl radical scavenging activity of the leaf extract was reported as % inhibition of deoxyribose degradation and calculated using the following formula:

$$\%\ Inhibition\ = \frac{A_0\ - A_1}{A_0}\ x\ 100$$

Where A_0 was the absorbance of the control sample and A_1 was the absorbance of the extract or positive control.

Statistical Analysis

Statistical calculations were carried out with the SPSS 10.0 for Windows software package (Statistical). Results are expressed as the mean ± S.E.M. of 5 independent experiments. Student's t-test was used for statistical analyses; P values > 0.05 were considered to be significant.

RESULTS

Inhibition of Bacteria with Plants Extracts

Antibacterial remedies of the six ornamental plants' extracts evaluated *in vitro* on four bacteria pathogens is shown in **Table 1**. Varied degrees of inhibition and in some cases resistance were observed with the extracts on the four bacteria species. However, highest inhibition of 30.01±0.03 mm was recorded on *Salmonella typhimurium* with *H. crepitans* extract and least inhibition of 13.07±0.06 mm on *S. epidermidis* with *F. thonningii* leaf extract. *T. catappa* most inhibited *S. typhimurium*

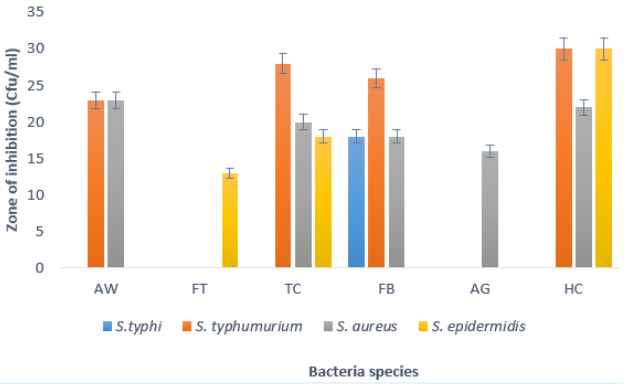

Fig. 1. Zones of inhibition (mm) exhibited by plant extracts on test bacteria

Legend: A.W- *Acalypha wilkesiana*, F.T- *Ficus thoninngii*, T.C- *Terminalia catappa*, F.B- *Ficus benjamina*, A.G- *Acalypha godseffiana* and H.C- *Hura crepitans*.

with zone of 28.33±0.28 mm and least inhibition of 18.33±0.50 mm on *S. epidermidis* while *S. typhi* was observed resistant to this extract. *F. benjamina* most inhibited *S. typhimurium* with a zone of 26.50±0.50 mm, while *S. epidermidis* was resistant to the extract. *A. wilkesiana* only inhibited *S. typhimurium* and *S. aureus* with zone of 23.00±0.00 mm each, while *S. typhi* and *S.epidermidis* were resistant to the extract. *A. godseffiana* only inhibited *S. aureus* with a zone of 16.3±0.18 mm among the tested bacteria species (**Fig. 1**).

Minimum Inhibition and Minimum Bactericidal Activities of Plants Extracts

Valued inhibition was observed with some of the extracts, nonetheless, the extracts in decreased concentrations showed the least concentrations that were inhibitory to the test bacteria species to deduce the minimum inhibitory concentration (MIC) and the

Table 2. Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) of extracts (mg/ml)

	A.W	F.T	T.C	F.B	A.C	H.C
Test bacteria	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC
Gram negative bacteria						
Salmonella typhi	- -	- -	- -	25 50	- -	- -
Salmonella typhimurium	25 50	- -	12.5 25	25 50	- -	25 50
Gram positive bacteria						
Staphylococcus aureus	25 25	- -	12.5 25	25 50	12.5 12.5	25 25
Staphylococcus epidermidis	- -	50 100	12.5 25	- -	- -	25 25

Legend: A.W- *Acalypha wilkesiana*, F.T- *Ficus thoninngii*, T.C- *Terminalia catappa*, F.B- *Ficus benjamina*, A.G- *Acalypha godseffiana* and H.C- *Hura crepitans*

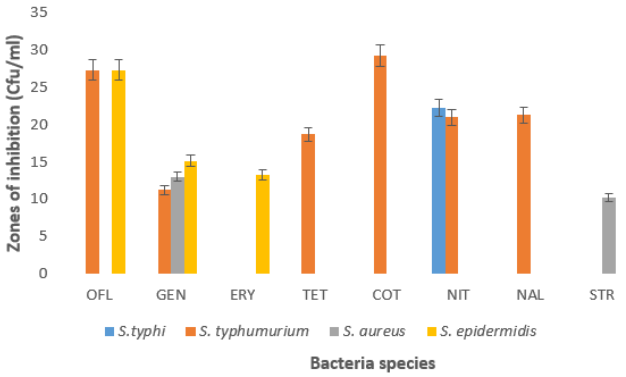

Fig. 2. Zones of inhibition (mm) exhibited by test bacteria on commercial antibiotics

Legend: OFL-Ofloxacin, GEN-Gentamycin, ERY- Erythromycin, TET-Tetracycline, COT-Contrimoxazole, NIT-Nitrofuratoin, NAL-Nalidixic acid, STR-Streptomycin

minimum bactericidal concentration (MBC) of the extracts. The MIC of *A. wilkesiana* on the test bacteria species was 25 mg/ml and MBC of between 25 to 50 mg/ml. *F. thoninngii* MIC was 50 mg/ml and MBC activity with 100 mg/ml. *T. catappa* MIC and MBC was 12.5 mg/ml and 25 mg/ml respectively. *F. benjamina* MIC was 25mg/ml and MBC of 50 mg/ml. *A. godseffiana* MIC and MBC was 12.5 mg/ml each while; *H. crepitans* on the inhibited bacteria has MIC activity of 25 mg/ml and MBC activity of between 25 to 50 mg/ml (**Table 2**).

Inhibition of Bacteria with Commercial Antibiotics

As observed with the plant extracts where the tested bacteria species were susceptible and resistant in some cases, the employed referenced antibiotics also did not perform total inhibition on all tested bacteria species. Meanwhile, cotrimoxazole showed highest inhibited of 29 mm on *S. typhimurium* while other tested bacteria species were observed resistant. Ofloxacin inhibited *S. typhimurium* and *S. epidermidis* with zone of 27 mm each, while other tested bacteria were resistant. *S. typhi* and *S. typhimurium* were susceptible to nitrofurantoin with zones of 22 mm and 21 mm respectively. *S. typhimurium, S. aureus and S. epidermidis* were inhibited with gentamycin with zones of 11 mm, 13 mm and 15 mm respectively. Tetracycline only inhibited *S. epidermidis* with a zone of 13 mm and nalidixic acid inhibited only *S. typhimurium* with a zone of 21 mm while other tested bacteria species were resistant. However, least inhibition of 10 mm was recorded on *S. aureus* with streptomycin (**Fig. 2**).

Qualitative Phytochemicals of Plants Extracts

In quantitative phytochemical analysis, alkaloids was more in *H. crepitans* with value of 9.07±0.19 mg/g, followed by *A. godseffiana* with 7.23±0.09 mg/g and least in *F. benjamina* with 0.63±0.04 mg/g. Flavonoids was quantitatively more in *A. godseffiana* and *H. crepitans* with values of 4.29±0.15 mg/g and 4.28±0.14 mg/g respectively. This was followed by *A. wilkesiana* with value of 3.05±1.15 mg/g and least flavonoid value of 0.15±0.05 mg/g from *F. benjamina*. Tannin was more in *A. godseffiana* with value of 0.19±0.00 mg/g, followed by *H. crepitans* with 0.14±0.01 mg/g, while *A. wilkesiana* and *F. benjamina* shared equal values of 0.12±0.01 mg/g each. However, least value of 0.09±0.00 mg/g was detected from *T. catappa*. Phenol was quantitatively more in *A. godseffiana* with value of 0.65±0.05 mg/g, followed by *F. benjamina* and *H. crepitans* with values of 0.63±0.03 mg/g and 0.63±0.04 mg/g respectively. Least value of 0.48±0.02 mg/g was present in *T. catappa*. Saponins was more in *A. godseffiana* with value of 24±0.00 mg/g, followed by *A. wilkesiana* with quantity of 24.11±0.00 mg/g. Least value of 18.21±0.00 mg/g was recorded from *T. catappa* (**Table 3**).

Quantitative Phytochemicals of Plants Extracts

The analysis on qualitative phytochemicals of the plant extracts denotes that alkaloids were present in *A. wilkesiana, T. catappa, A. godseffiana* and *H. crepitans*. Saponins was present in all plant extracts except *H.*

Table 3. Phytochemical qualitative analysis on plant extracts

	A.W	F.T	T.C	F.B	A.G	H.C
Alkaloids	+	-	+	-	+	+
Saponins	+	+	+	+	+	+
Tannins	+	-	+	-	+	+
Phenols	+	-	+	-	+	+
Cardiac glycoside	-	-	-	-	-	+
Terpenoids	-	+	-	+	+	+
Steroids	-	-	+	-	+	+
Flavonoids	+	+	+	-	+	+
Phlobatanins	-	-	-	-	-	-
Reducing sugar	+	+	+	-	+	+

Key: A.W- *Acalypha wilkesiana*, F.T- *Ficus thoninngii*, T.C- *Terminalia catappa*, F.B- *Ficus benjamina*, A.G- *Acalypha godseffiana* and H.C- *Hura crepitans*

Table 4. Phytochemical quantitative analysis on plant extracts

	A.W	F.T	T.C	F.B	A.C	H.C
Alkaloids (mg/g)	4.29±0.30	1.43±0.12	1.99±0.03	0.63±0.03	7.23±0.09	9.07±0.19
Flavonoids (mg/g)	3.05±0.15	2.46±0.11	2.41±0.18	0.15±0.03	4.29±0.15	4.28±0.14
Tannins (mg/g)	0.12±0.01	0.10±0.00	0.09±0.00	0.12±0.02	0.19±0.05	0.14±0.01
Phenol (mg/g)	0.60±0.03	0.53±0.04	0.48±0.02	0.63±0.03	0.65±0.04	0.63±0.04
Saponin (mg/g)	24.11	23.83	18.21	20.23	24.38	-

Key: A.W- *Acalypha wilkesiana,* F.T- *Ficus thoninngii,* T.C- *Terminalia catappa,* F.B- *Ficus benjamina,* A.G- *Acalypha godseffiana* and H.C- *Hura crepitans*

Table 5. *In vitro* antioxidants of the plants' extracts

Plant species	FRAP (MgGAE/100g)	FRAS (DPPH) %	HRS (%)
Acalypha wilkesiana	76.73±4.06	3.62±0.36	0.65±0.00
Ficus thonningii	65.57±3.04	2.18±0.00	0.44±0.00
Terminalia catappa	69.83±3.21	4.78±0.43	0.86±0.18
Ficus benjamina	66.57±4.19	4.53±0.14	0.53±0.45
Acalypha godseffiana	69.91±3.15	4.26±0.64	0.63±0.17
Hura crepitans	160.48±4.20	5.13±0.43	0.61±0.15

crepitans. Terpenoids was present all plant extracts except *A. wilkesiana*. Steroids was present in *T. catappa, A. godseffiana* and *H. crepitans*. Flavonoids and reducing sugar were present in all plant extracts except *F. benjamina*. Meanwhile, cardiac glycoside was only present in *H. crepitans* while phlobatanins was not detected from any of the plant extracts (**Table 4)**.

Antioxidant Profile of the Plants Extracts

Table 5 shows the antioxidant properties of the plant extracts. *H. crepitans* had the highest ferric reducing antioxidant property of 160.48±4.20%, followed by *A. wilkesiana* with value of 76.73±4.06% and least value of 66.57±4.19% from *F. benjamina*. Free radical scavenging (DPPH) activity of the plant extracts was also more in *H. crepitans* with value of 5.13±0.43%, followed by *T. catappa* with value of 4.78±0.43% and least value of 2.18±0.00% from *F. thonningii*. Hydroxyl radical scavenging activity was more in *T. catappa* leaf extract with value of 0.86±0.18%, followed by *A.wilkesiana* having a value of 0.65±0.00% and least value of 0.44±0.00% from *F. thonningi* leaf extract.

DISCUSSION

Despite plants as valuable medicines were not discovered through scientific studies in the ancient times, people of different cultures relied on plants for food, curing and prevention of diseases. Many diseases of microbial and non-microbial origins such as cancer, diabetes, malaria, anaemia and host of others were successfully treated with plant derivatives. Scientific studies have proved plants to have medicinal value in health care system for healing of diseases. This is based on their components such as vitamins and minerals that are essential for human health; phytochemicals and antioxidants which are capable of neutralizing free radicals and maintenance of health status.

Ornamental medicinal plants are as important as wild medicinal plants. Ornamental plants will be of easy access to majorities of people in developed countries where deforestation has mitigated the extinction or scarcity of valuable wild medicinal plants. In future this will as well affect the underdeveloped countries of the world to resolve on the use of available ornamental plants with medicinal values. Medicinal plants application for pharmaceutical, industry and economic purposes has advanced beyond the employment of wild medicinal plants only.

The antibacterial activity of *A. godseffiana* has been reported against some pathogens such as *Escherichia coli*, *Proteus mirabilis, Enterococcus feacailis, Staphylococcus aureus* and *Klebsiella pneumonia* (Sahoo et al. 2014). The aqueous extract of *A. wilkesiana* has also been reported as effective against *E. coli, Pseudomonas aureuginosa, Proteus vulgaris* and *S. aureus* (Iyekowa et al. 2016). From this study, only *S. aureus* falls among their tested bacteria species and was inhibited with higher value as reported in literature. But Salami and Afolabi (2014) reported higher inhibition of *S. aureus* with hot water extract of *A. wilkesiana* than the value reported in this study. However, *A. wilkesiana* is medically useful in the treatment of skin diseases (Akinyemi et al. 2006, Oyelami et al. 2003). From earlier study by Chanda et al. (2012), lower inhibition with *Terminalia catappa* on *S. epidermidis* ATCC 12228 was reported. However, Mohammed and Mudir, (2011) could not achieve potency with *T. catappa* on *S. aureus*, but reported inhibition of *S. typhi* with *T. catappa* leaf extract. From this study, *S. aureus* and *S. typhi* were inhibited with the extract of *H. crepitans* which was not valid in the study by Abdulkadir et al. (2013). *T. catappa* leaves have been found helpful in treatment of liver diseases, Hepatitis; the bark extract is used in healing wounds and fungal infections of the mouth, throat and intestine.

Meanwhile, David et al. (2014) has reported on the seed extract of *H. crepitans* to be of antibacterial agent against *S. aureus*. This plant leaf extract in our study, inhibited *S. typhimurium* and *S. epidermidis* and it is in correlation with the report of Oyeleke *et al*. (2014). Oloyede and Olatinwo (2014) have reported on the antifungal activity of *H. crepitans* and concluded that it may be a source of medicinally important plant drug. The leaves and stem extracts of *H. crepitans* have been found to significantly reduce the level of biochemical abnormalities in rats, indicating prevention against hepatocellular injury (Gajanan and Sonwane 2016).

Hura crepitans is medically useful where the leaves are used as body-rub for leprosy treatment and the bark serves as purgative and emetic.

No literature has reported on the antimicrobial activity of *F. benjamina* and *F. thonningii* except its chemical and biological studies. Meanwhile, in this study, *F. benjamina* leaf extract exhibited valuable inhibition on *S. typhi, S. typhimurium* and *S. aureus* while *F. thonningii* leaf extract was effective on *S. epidermidis*.

The differences in inhibition observed during this study in compare with related literatures could be the methods in extraction, preparation, age of plants, origin of the plant and environmental conditions of each region. However, the leaves and bark of *F. benjamina* pound together are applied as poultice for headache and rheumatic treatment, bruises and wounds. In some part of the world, the latex is used for curing liver diseases. *Ficus thonningii* is used in Africa medicine for the treatment of mental illness, diarrhoea, diabetes mellitus, gonorrhoea, urinary tract and respiratory infections.

Not many authors have used antibiotics along line with the employed plant extracts as studied. However, Haruna and co-workers (2013), have reported on *S. aureus* ATCC 25923 to be inhibited with chloramphenicol, tetracycline, ofloxacin, augumentin, nitrofurantoin, amoxicillin and cotrimoxazole. Similar result was reported in this study with ofloxacin, gentamycin, tetracycline, nitrofurantoin and nalidixic acid.

The differences in inhibition between antibiotics and plants' extracts may be due to the various plant chemical compounds present in the plants and the purified bioactive compounds present in the commercial antibiotics. Though *S. aureus* was the only bacteria extensively reported upon and few words on *S. typhi*, it is because there is no literature that has reported on the employed plant extracts as did in this study on *S. typhimurium* and *S. epidermidis*.

Madziga and co-workers (2010) have reported the presence of tannin, flavonoids, saponin, cardiac glycosides, alkaloids and terpenoids but without anthraquinone. Iyekowa et al. (2016), have also screened tannins, flavonoids, saponins, cardiac glycosides, alkaloids and terpenoids from the leaves of *A. wilkesiana*, a similar result in this study. Kanika et al. (2014), Amina et al. (2017) have reported the presence of alkaloids, flavonoids, tannins, saponins, steroids and cardiac glycosides from the leaf of *T. catappa*. These reports are similar to the result obtained in this study.

Phytochemicals such as tannin, phenol, saponins, cardiac glycosides, alkaloids, terpenoids and anthraquinone have been reported to be present in the leaves of *A. wilkesiana*, but the results obtained in this study determined higher values in phenol, tannins, saponins and alkaloids than in the report of Omage and Azeke (2014). Salami and Afolabi (2014) reported quantities of tannin, flavonoids, glycoside, alkaloids and saponins from *A. wilkesiana* leaf extract. They reported higher values in tannin, flavonoids and alkaloids while we recorded a lower value in saponins in this study.

The phytochemicals isolated from the leaves aqueous extracts of the employed plants are relatively essential in human health. Tannins are effective in protecting the kidneys; hence the leaves may therefore have protective effects on the kidney, a major organ in the regulation of homeostatsis in human and animal bodies. It also has been used for the immediate relief of sore throat, diarrhoea, dysentery, haemorrhage, skin ulcer and as a cicatrizant. Saponins have been reported as an antioxidant and anticancer. Flavonoids are the combinations of flavonols and flavanols and are known for their antimicrobial, anticancer, anti-inflammatory and anti- allergic activities. Steroids have activities in medicine for their cardiotonic, antimicrobial, insecticidal, nutrition and cosmetics. Terpenoids are valued for their medical importance in traditional herbal remedies, anti-neoplastic, antibacterial and pharmaceuticals. Cardiac glycosides are known drugs for the treatment of congestive heart failure and cardiac arrhythmia. Anthraquinones are good laxative compounds. Alkaloids are chemical derivatives that function for protection and psychoactive while phenol are known precursors for many drugs, anticancer, anti-inflammatory, analgesic, antioxidant also contribute in inducing apoptosis by arresting cell cycle regulation, carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell division, migration, proliferation or differentiation and block signaling pathways (Huang et al. 2010). These phytochemical properties possessed by the plants extracts in this study stand them as potential and useful medicinal plants to be employed for production of effective new drugs in treatment of human disease and as herbal remedies for traditional medicine.

The *in vitro* antioxidant activity of the aqueous plants' extracts investigated with DPPH free radical scavenging assay showed valuable results. This further strengthened the statement that the model systems of scavenging DPPH free radicals are acceptable methods to evaluate the antioxidant activity. Evidence has it that DPPH free radical scavenging by antioxidants is due to their hydrogen density ability (Hareesh et al. 2010).

The reducing power of the plants extracts yielded results of interest for the plants to be supported for traditional use. However, variations in reducing power ability were noticed among the different plants. This reducing power ability of the aqueous plants extracts according to Omorayi et al. (2012) could be attributed to the presence of hydrophobic polyphenolic compounds. The results obtained are in line with the previous study of Park and Jhon (2010). The High FRAP activity of the aqueous leaf extracts may be due to the tannin content since the antioxidant activity of tannins is mediated through reducing power and scavenging activity as reported by Minussi et al. (2012). The reducing power

ability of this extract may suggest that ROS such as H_2O_2, O_2 and OH could be neutralized via hydrogen atom transfer (Boakye et al. 2016).

The presence of phenolic compounds like flavonoids and tannins may present the aqueous leaf extracts the potential free radical scavenging effects observed; hence they act as primary antioxidants (Belguidoum et al. 2015). However, plants extracts have been reported as potential human antioxidant defense system and are antioxidant of choice because of their lower toxicity than synthetic antioxidants (Imran et al. 2014) and also the readily availability and of low cost.

Staphylococcus aureus has been known as one of the leading bacteria that causes serious infections such as blood stream (bacteremia), pneumonia (bone and joint infection) and also pneumonia of the lungs infection. Hence these are serious infections that typically requires hospitalization, treatment with available antibiotics is necessary for those that can afford them but the leave extracts of *A. wilkesiana* or *A. godseffiana* may be used to manage these diseases as alternative where an individual cannot afford the modern treatment. Also, in situations with severe infections with Methicillin-resistant *Staphylococcus aureus* (MRSA), *A. wilkesiana* and *A. godseffiana* extract could serve as leading source of drug to combat the menace caused by MRSA in humans.

S. epidermidis has been reported upon to be equipped with genes assumed to provide protection (Chu et al. 2009, Rogers et al. 2007) and has been suspected to be involved in prosthetic joint, vascular graft, surgical site, central nervous system shunt and cardiac device infections (Rogers et al. 2009). *S. epidermidis* therefore, must be taken into consideration as a pathogen to be mindful of. It therefore required to be guided, control and treat with potential antibacterial agents that may be obtained from plants derivatives as proved by some of the plants extracts in this study.

S. typhi and *S. typhimurium* are also human threatening pathogens that have caused severe infections resulting to hospitalization and even death. Alternative medicine for their cure and prevention is needed hence some of the available commercial antibiotics known for their treatment are now becoming ineffective and even with side effects in some cases.

CONCLUSION

The present study has provided scientific rationale for use of ornamental plants as medicine in folklore. Hence potential bioactive molecules such as phenols, steroids, flavonoids, tannins and saponins were isolated from the plants, composition for development of new drugs for the therapy of infectious diseases could be possible with the plants. Toxic effect on human or animal of the employed plants has not been reported since extracts from these plants are been used externally and internally on man and animals successfully. Meanwhile, the *Ficus*, *Terminalia catappa* and *Acalypha* species are good forage for ruminants especially goats and sheep.

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