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(RESEARCH ARTICLE)

Phytochemical and antimicrobial activity of ethnomedicinal leaf extract of selected plants in Nigeria

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Abstract

The aim of this research work centered on the antimicrobial and phytochemical analysis of some selected ethnomedicinal leave extract. The phytochemical constituents of methanolic extract of leaf of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* were screened and antimicrobial assay determined using agar well diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against selected food borne pathogen were determined. Analysis of data was carried out using Anova P≥0.05. The results show that 200 g of *Annona muricata* yielded 38.98 g with 19.50% being the highest yield while the least was 16.47 g yield of *Cymbopogon citratus* with 8.24%. *Annona muricata* and *Ocimum gratissimum* yielded highest phytochemical constituents (189996.10±0.03 and 18072.43±0.02 mg/kg of Tannin respectively). All the leaf extract inhibited *Pseudomonas aeruginosa*, *Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia* and *Esherichial coli* to a varying degree. The MIC of the methanolic leaf extract of the ethnomedicinal plant ranges from 6.25 to 25.00 mg/mL and the MBC ranges from 18.75 to 100.00 mg/mL.

Keywords: Ethnomedicinal plants; Pathogenic organisms; MIC; MBC; Methanolic extract.

1. Introduction

Medicinal plants are considered as those plants with chemical properties whose their efficacy has been proved and had been used years long ago as a remedy for curing human ailments most especially those caused by pathogenic organisms [1 and 2]. These plants are known to be associated with substances which could be used to produce drugs [3]. Majority of these plants are used locally to reduce symptoms of illness have been investigated to posses' medicinal relevance, some of which include: *Azadirachta indica, Ocimum gratissimum, Cymbopogon citratus, Moringa oleifera, Allium cepa, Zingiber officinale* and *Vernona amygdalina.* These plants had been investigated and used in the treatment of various ailments such as fever, stomach disorder and cough [4]. The tendency of using most of the medicinal plants for the treatments of different diseases may largely depend on their ability to produce antimicrobial and antioxidant effects from their phytochemicals [5].

The world health organization [6] reported that almost 80% of the world's habitant depends on herbal medicines and treatment for their primary health care. Plants had long formed the root of refined traditional medicine system and supposedly offer outstanding information's for innovative drug development [7 and 8]. Ethno-medicinal plants institute an active source of antimicrobial natural products. The use of medicinal plants all over the world precedes the overview of modern antibiotics and other present drugs into African as a continent [9]. More than twenty years ago, infections as a result of bacterial caused by *Staphylococcus aureus, Escherichia coli*, *Salmonella enteritis* and other pathogens have been resistant progressively to experimental agents of antimicrobial [10].

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From the ancient time in old-style medical system [11], extracts obtained from plants had been successful in treating microbial disease. Owing to the growth of strong resistance to antibiotics by pathogenic micro-organism in modern medical science, resistance to antibiotics is at alarming rates because most them are quite low-cost and are commonly used even without prescription from official health institution [12]. Therefore, attention should be directed towards the use of plant extract as a source of new discoveries in anti-microbial drug. Nair and Chanda [13] reported that the use of medicinal plants as potential sources of antimicrobial compounds can be enhanced if their activity, configuration, composition as well as authentication of the use of the extract obtained from the medicinal plants are appropriately and carefully considered [14]. Majorities of potential chemicals associated with medicinal plants have antimicrobial activities, allowing these plants to be investigated and used for the development and manufacturing of new antimicrobial drugs will be advantageous in pharmaceutical and health sector [15].

There are need to seek for ethnomedicinal plants as an alternative source for commercial drugs due to their high antimicrobial effect. The search for new antimicrobial natural products from plant materials is essential in order to curb the menace of multiple antibiotics resistant pathogens [16].

This has led to the evaluation of other agents that might have antimicrobial activity. The aim of this study is based on the antagonistic activity and quantitative determination of phytochemical compounds of the selected ethnomedicinal plants in Nigeria.

2. Material and methods

2.1. Collection of sample

The plant material of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* was collected at a farm in Boluwaji, Ibadan, Oyo-State, Nigeria where they were growing naturally. The authentication of the plant was done at the Herbarium of Forestry Research Institute of Nigeria (FRIN) Jericho Hill Ibadan. The indicator organisms used in this work were from Food Microbiology unit of the Department of Microbiology, University of Ibadan and were initially isolated and identified from spoilt meat samples.

2.2. Preparation and sterilization of media

The media used in this study are Nutrient Agar (NA), Nutrient broth and Muller- Hinton Agar (MHA). The agars were weighed according to manufacturers' specification. The media were autoclave at 121 °C for 15 mins before commencement of all laboratory work.

2.3. Preparation of leaf extracts of the plants

The air dried leaf samples of selected plants were taken to the feed mill at the Department of Agronomy University of Ibadan for milling. The milled leaf samples were taken to Pharmacognosy at the University of Ibadan for extraction. The milled plant samples (200 g) which were previously oven dried at 40 °C and then blended were extracted using methanol as the solvent [17]. For each leaf sample, 200 g of each blended sample was weighed into different containers and labeled. The crude leaf extract was extracted using 2000 mL of redistilled methanol by cold extraction and decanted at the end of 72 hours. The solvents along with the extracts were drained out with muslin cloth, filtered with no 1 Whatman filter paper and semisolid extracts were obtained with the use rotary evaporator (40 °C) (IKA® RV10, Artisan Technology Group, Champaign, US) with subsequent evaporation to dryness on a water bath. The extracts or yield was stored in a universal bottle under refrigeration (below 10 °C) in order to prevent the deterioration or decomposition and microbial growth.

2.4. Phytochemical screening of the leaves of the plant samples

The leaf extracts of plant (*Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata*) were tested for the presence of different phytochemicals like alkaloids, saponin, tannin, flavonoid, terpenoid and cardiac glycosides etc using standard methods [18].

2.4.1. Determination of alkaloids

Five grams (5 g) of the sample were weighed into a conical flask; 200 mL of 10% acetic acid in ethanol was added, shaken and allowed to stand for 4 hours. The filtrate was allowed to evaporate to about a quarter of its original volume. Few drops of concentration NH_4OH solution were added to precipitate the alkaloid. The precipitate formed was filtered through a weighed filter paper (W1). The filter paper was placed in the oven and allowed to dry to 60 °C till constant (about 30 – 60 mins). The filter paper was weighed again and recoreded as W2 [19].

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{W_1} \times 100 \quad \text{..... equation (1).}$$

2.4.2. Determination of total flavonoids

One gram (1.0 g) of samples was weighed into a conical flask. 50 mL of 80% methanol was added. Extraction was done by placing it on a hot plate at low temperature for 30 min while stirring and allowed to cool. It was then filtered into a 100 mL volumetric flask. It was made up to mark of 100 mL with 80% methanol. 3 mL of the extract was taken with a pipette into a test tube. 0.1 mL of 10% $AlCl_3$, 0.1 mL Na – K tartarate and 3 mL of distilled water were added respectively. The mixture was shaken properly, mixed and the absorbance of the solution was read at 415 nm. The procedure was repeated for Rutin standards of concentrations 5, 10, 15, 20 mg/L. A standard curve for the Rutin Standard was plotted and the concentration of the samples was determined by extrapolating the absorbance down the concentration axis [20].

$$\text{Total Flavonoid (mg/Kg)} = \frac{\text{conc. Obtained (mg/L) x total volume of extract}}{\text{Sample weight}} \quad \text{..... equation (2).}$$

2.4.3. Determination of oxalate

One gram (1.0 g) of samples was weighed into a 250 mL conical flask and 75 mL of 3 N H_2SO_4 was added. The suspension was then filtered through What-man No 1 filter paper after which 25 mL of filtrate was drawn with a pipette into a beaker and two drops of methyl red indicator was added. The mixture was heated to boil and titrated while hot against 0.05 M $KMnO_4$ solution until a faint pink colour persist for at least 30 seconds. Oxalate content was derived by taking 1 mL of 0.05 M $KMnO_4$ as equivalent to 2.2 mg oxalate [21].

$$\text{Oxalate (mg/100g)} = \frac{\text{Titre value x 2.2 x DF}}{W} \quad \text{..... equation (3).}$$

Where 2.2 mg = Mass equivalent oxalate value of 1 mL of 0.05 M $KMnO_4$ solution
DF= Is Dilution Factor. That is total volume of sample divided by volume of portion used for titration.
W = Sample weight in g.

2.4.4. Determination of total phenol

Ten grams (10. 0 g) of powdered sample was weighed into a conical flask. 100 mL of ethanol was added and plugged it with cotton wool and covered with aluminum foil. The content was shaken vigorously and left to stand for 24 h for proper extraction. It was then centrifuged or filtered to obtain clear supernatant. The supernatant was evaporated to get crude extract. The crude extract was stored at 4 °C. 100 mg (0.1 g) of the extract was weighed into a conical flask. 100 mL of distilled water was added to dissolve it. 1 mL of the solution was pipetted into a test tube. 0.5 mL 2N Folin-Ciocalteu reagent and 1.5 mL 20% $NaCO_3$ solution was then added. It was made up to 10 mL with distilled water and shaken vigorously and allowed to stand for 2 h. The absorbance was read at 765 nm. The following concentrations of Garlic acid standard, viz 0.5 mg, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg were prepared. The absorbance's of the above Garlic acid concentrations was read off. A calibration curve for the garlic acid standard was drawn. That is absorbance against concentration. The absorbance of the sample was extrapolated by tracing down the concentration axis to obtain the concentration of the sample [22].

$$\text{Phenol content mg/kg} = \frac{\text{Conc. obtained in mg/L x volume of sample}}{\text{Sample weight}} \quad \text{..... equation (4).}$$

2.4.5. Determination of phytate (phytic acid)

Two grams (2 g) of sample were weighed into a 250 mL conical flask 100 mL of 2% concentrated HCl was added and allowed to soak for 3h, and then filtered. 50 mL of the filtrate was pipetted into a 250 mL beaker. 107 mL of distilled water was added to improve acidity. 10 mL of 0.3% ammonium thiocyanate solution was added as indicator. Titration was then carried out with standard iron (III) chloride ($FeCl_3$) solution which contains 0.00195 g iron/mL until a brownish yellow colour appeared and persisted for 5 mins. The phytic acid content was calculated as shown below [23].

$$\text{Phytic acid g/kg} = \frac{\text{0.00195 x volume of titrant } (FeCl_3) \text{ x vol. of sample}}{\text{Sample weight}} \quad \text{..... equation (5).}$$

2.4.6. Determination of saponin

Twenty grams (20 g) of well blended sample were weighed into a conical flask. 100 mL of 20% aqueous ethanol was added. The content was heated in a hot water bath for 45 mins with continuous stirring at 50 °C filtered and then re-extracted using 200 mL of 20% ethanol. Both extracts were combined. The volume of the extract was reduced to 40 mL by evaporating in a water bath at 90^0 C. The concentrate was transferred into a 250 mL separating funnel. 20 mL of diethyl ether (petroleum ether) was added and shaken vigorously. The clear ether layer was discarded and the aqueous layer was kept. 60 mL of n-butanol was added to the aqueous layer in the separating funnel. The combined butanol layer was washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was collected into a weighed petri dish (W1). The petri dish was dried in an oven at about 90^0 C. The petri dish was re-weighed and recorded as W2 [24].

$$\% \text{ Saponin content} = \frac{W2 - W1}{W0} \times 100 \quad \text{..... equation (6).}$$

2.4.7. Determination of tannin

One gram (1 g) of dried well blended sample was weighed into a flask. 10 mL of distilled water was added and agitated and was left to stand for 30 mins at room temperature, and then centrifuged at 2500 rpm for 15 mins. 2.5 mL of supernatant was measured into a 50 mL volumetric flask. 1 mL of folin Dennis reagent and 2.5 mL of saturated Na_2CO_3 solution were added. The solution was diluted to 50 mL with distilled water and incubated for 90 mins at room temperature [25].

2.4.8. Standard tannic acid

The procedure was repeated for tannic acid standards 0.5 mg, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg excluding centrifugation. The absorbance's of the above Tannic acid concentrations was read off at a wavelength of 750 nm. A calibration curve for the tannic acid standards was drawn, that is absorbance against concentration. The absorbance of the sample was extrapolated by tracing down the concentration axis to obtain the tannic acid concentration of the sample [25].

$$\text{Tannic Acid content (mg/kg)} = \frac{\text{Conc. obtained in mg/l x volume of sample}}{\text{Sample weight}} \quad \text{..... equation (7).}$$

2.4.9. Determination of terpenoid

The powdered of the sample (0.1 g) was weighed into a conical flask; 25 mL of petroleum ether was added. It is allowed to extract by constant shaking for 15 mins and then filtered. The volume of the filtrate is noted and the absorbance of the filtrate is read at 538 nm. A standard curve is plotted. The absorbances of the samples on the standard graph were extrapolated to obtain the terpenoid concentration of the samples [26].

$$\text{Terpenoid (mg/kg)} = \frac{\text{Conc. Obtained (mg/l) x total volume of extract}}{\text{Sample weight}} \quad \text{..... equation (8).}$$

2.5. Antagonistic activity of leaf extracts of the plants against indicators organisms

The antibacterial activity of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* were determined by the agar well diffusion method [27 and 28]. The bacteria were sub-cultured from the preserved slants for 24 hours before use. Mueller-Hinton Agar was prepared, sterilized, allowed to cool to room temperature and then poured into plates to about 4 mm depth under an aseptic condition. 24 hours old culture of each test organism was standardized to 0.5 McFarland standards (10^6 CFU/mL). About 100 µL of the standardized cell suspensions was spread on Mueller-Hinton agar plates in triplicates. Well was dug on each plate with a sterile 6 mm diameter cork borer; 100 µL of the crude extracts were introduced into the wells, allowed to stand at room temperature for about 30 minutes. The standard drugs Streptomycin and Gentamycin were used as positive control and DMSO solution only as negative control. The plates were incubated at 37 °C for 24 h and then observed for inhibition zone diameter (mm).

2.5.1. Determination of minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration test was carried out using the tube dilution method with Mueller Hinton broth. The tube dilution susceptibility test was used to determine the MIC values of the plant extracts. A series of Mueller-Hinton broth tubes containing varying two-fold concentrations of the various plants extract samples, in the range of 100 mg/mL to 6.25 mg/mL were prepared and incubated with a previously standardized density of the test organisms (0.5

mL). The lowest concentration of the plant extract sample resulting in no growth following visual inspection after 18-24 h of incubation for bacteria and 24-72 h using a spectrophotometer was recorded as the MIC [29].

2.5.2. Determination of minimum bactericidal concentration (MBC)

Minimum bactericidal concentration was determined from all wells showing no growth as well as from the lowest concentration showing growth in the MIC assay for all the samples. Bacterial cells from the MIC test tube were sub-cultured on freshly prepared solid Mueller-Hinton agar by making streaks on the surface of the agar. The plates were incubated at 37 °C for 24 h overnight. Plates that did not show growth were considered to be the MBC for the extract or drug used [29]. The experiment was carried out in triplicate.

2.6. Statistical analysis

One way analysis of variance (ANOVA) was worked out to find out the significance of the treatments. The treatments were separated by least significance different (LSD) at P≥0.05 level.

3. Results

The weight of the total yield of the methanolic extract of the leaf samples of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* from 200 g of each sample is shown in Table 1. The methanolic extract of *Ocimum gratissimum* leaf had the highest weight of extract yield of 28.59 g with a percentage yield of 14.3%, while that of *Cymbopogon citratus* had the lowest weight of extract yield of 16.47 g with a percentage yield of 8.24%.

Table 1 Total yield of selected ethnomedicinal leaves extract using methanolic extraction.

Plant samples	Weight of plant soaked {g}	Weight of extract yield {g}	Percentage of extract yield {%}
Cymbopogon citratus	200	16.47	8.24
Annona muricata	200	38.98	19.50
Azadirachta indica	200	22.54	11.27
Ocimum gratissimum	200	28.59	14.30

The phytochemicals present in the selected ethnomedicinal plants was alkaloids, Terpenoids, Saponins, Tannins, Flavonoids, Cyano glycosides, Phenols and Phytate which were all existed in considerable amounts in the methanolic extract of the plant leaves as shown in Table 2.

Table 2 Quantitative phytochemical analysis of ethnomedicinal leaf extracts using methanolic extraction

Phytochemicals	*Cymbopogon citratus*	*Annona muricata*	*Azadirachta indica*	*Ocimum gratissimum*
Tannin (mg/kg)	7619.05±0.00	189996.10±0.03	12916.66±0.02	18072.43±0.02
Phytate (mg/kg)	14.55±0.02	38.35±0.02	38.58±0.02	63.71±0.01
Cyano. Glycosides (mg/kg)	2.74±0.00	14.35±0.01	10.62±0.01	18.57±0.01
Alkaloids (%)	17.24±0.01	36.42±0.01	18.47±0.02	22.11±0.01
Antioxidant (% DPPH scavenged)	249.20±0.03	211.76±0.01	203.34±0.02	154.47±0.01
Phenol(mg/kg)	1560.00±0.02	3814.65±0.02	3646.55±0.02	3930.00±0.02
Flavonoids (mg/kg)	4848.18±0.00	4744.00±0.02	4446.05±0.03	4891.59±0.04
Terpenoids (mg/kg)	390.48±0.00	952.27±0.01	625.00±0.00	865.22±0.00
Saponin (%)	1.19±0.02	1.17±0.03	1.22±0.01	6.18±0.02

The antagonistic activity of the methanolic extract of the leaf of Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus and Annona muricata against P. aeruginosa, E. coli, Proteus mirabilis, Klebsiella pneumonia and S. aureus are shown in Table 3. The methanolic leaf extract of Cymbopogon citratus, Annona muricata and Ocimum gratissimum are not significantly different having the highest zone of inhibition (20 – 21 mm) against P. aeruginosa. Azadirachta indica had the highest zone of inhibition (15.33±0.58 mm) against E. coli and the lowest zone of inhibition (6.33±0.58 mm) against P. mirabilis. However, Annona muricata recorded the highest zone of inhibition against P. aeruginosa (20.67±1.15) and the least zone of inhibition (10.33 mm) against S. aureus. More so, Azadirachta indica had the highest zone of inhibition (15.33 mm) against E. coli and the least zone of inhibition (6.33mm) against P. mirabilis. Ocimum gratissimum recorded the highest zone of inhibition (21.00±0.00 mm) against Pseu. aeruginosa and the least zone of inhibition (12.00±0.00 mm) against S. aureus while Ocimum gratissimum had the highest zone of inhibition (21.00±0.00 mm) against Pseu. aeruginosa and the least (12.00±0.00 mm) against S. aureus.

Table 3 Antagonistic activity (mm) of Ethno-medicinal plants against selected food borne pathogens

Test organisms	Cymbopogon citratus	Annona muricata	Azadirachta indica	Ocimum gratissimum	Dimethyl sulphoxide
P. aeruginosa	20.00±1.00b	20.67±1.15b	8.00±0.00a	21.00±0.00b	00.00±0.00.00a
S. aureus	12.33±0.58b	10.33±0.58a	14.67±0.58c	12.00±0.00b	00.00±0.00.00a
P. mirabilis	16.00±1.00c	19.33±0.58d	6.33±0.58a	12.33±0.58b	00.00±0.00.00a
K. pneumonia	12.67±0.58a	16.00±1.00b	12.67±0.58a	17.33±0.58c	00.00±0.00.00a
E. coli	14.00±0.00b	15.00±1.00bc	15.33±0.58c	12.33±0.58a	00.00±0.00.00a

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.0).

The minimum inhibitory concentration (MIC) exhibited by the methanolic leaf extract of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* are shown in Table 4. *Ocimum gratissimum* had MIC of 6.25 mg/mL against *P. aeruginosa* and MIC of 12.5 mg/mL against *K. pneumonia* while MIC of 25.00 mg/mL was recorded against *Proteus mirabilis*, *E. coli* and *S. aureus* each. *Annona muricata* had MIC of 6.25 mg/mL against *P. aeruginosa* and *Proteus mirabilis* while MIC of 12.5 mg/mL was documented against *K. pneumonia* and *E. coli,* while *S. aureus* had MIC of 25 mg/mL. However, *Azadirachta indica* had MIC of 25.00 mg/mL against *S. aureus*, *Proteus mirabilis* and *E. coli* while MIC of 6.25 mg/mL and MIC of 12.50 mg/mL was recorded against *P. aeruginosa* and *K. pneumonia* respectively. *Ocimum gratissimum* had MIC of 6.25 mg/mL against *P. aeruginosa* and MIC of 25 mg/mL against *S. aureus*, *Proteus mirabilis* and *E. coli.* The Minimum Bactericidal Concentration (MBC) of the methanolic leaf extract of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* are equally shown in Table 4. The MBC of the methanolic leaf extract of the selected ethnomedicinal plants ranges from 18.75 to 100.00 mg/mL.

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (mg/ml) performance of different extracts of ethnomedicinal leaf extract using methanolic extraction.

Test organism		LG	SS	N	CB
P. aeruginosa	MIC	6.25	6.25	6.25	6.25
	MBC	31.25	31.25	18.75	31.25
S. aureus	MIC	25.00	25.00	25.00	25.00
	MBC	75.00	75.00	100.00	75.00
Proteus mirabilis	MIC	12.50	6.25	25.00	25.00
	MBC	50.00	25.00	50.00	75.00
Klebsiella pneumoniae	MIC	12.50	12.50	12.50	12.50
	MBC	37.50	50.00	37.50	50.00
E. coli	MIC	12.50	12.50	25.00	25.00
	MBC	50.00	50.00	100.00	75.00

KEY: LG = Cymbopogon citratus; SS = Annona muricata; N = Azadirachta indica; CB = Ocimum gratissimum; Minimum Inhibitory Concentration = MIC; Minimum Bactericidal Concentration = MBC

4. Discussion

Phytochemical and antimicrobial activity of methanolic extracts of selected ethnomedicinal plants against food borne pathogens was investigated. The phytochemicals such as alkaloids, Terpenoids, Saponins, Tannins, Flavonoids, Cyano glycosides, Phenols and Phytate were all present in considerable amounts in the methanolic extract of the plant leaf. Ghosh *et al*. [30] demonstrated that strong polar solvents such as absolute alcohol or water (polarity index of 10.2) extract phytochemicals and biochemical with higher molecular weight compound [30] because during bioassay, solvents extract polar molecules.

Flavonoid which has been classified as part of the phytochemical constituents of the methanolic extract of the leaf samples of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* display an extensive range of biological activities, one of which is their ability to hunt for superoxide anion radicals, hydroxyl radicals and is of health stimulating in deed. Flavonoid also exhibit anti-angionic, anti-allergic effects, anti-inflammatory, antioxidant and analgesic properties [31]. All phytochemical compounds present in these plants such as tannins, saponins, glycosides, terpenoids, flavonoids and alkaloids are known to play important roles in biological activity of medicinal plants in the human system. The activities of the compound thus explain the usefulness of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* in folklore as remedies for the treatment of various infections. Cardiac glycosides are an important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure [32]. This compound has been reported to be a novel cancer therapeutic agent [33].

Another phytochemical compound present in the plant is tannins. Tannins exert antimicrobial activities through iron deprivation, binding or specific interactions with vital proteins, such as enzymes in microbial cells [34]. Herbs that have tannins are astringent in nature and are often used for the treatment of intestinal disorders, such as diarrhea and dysentery [35]. This may further indicate why *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* is among the medicinal plants used for the treatment of microbial infections. Tannins have been demonstrated to have remarkable activity in cancer prevention [36]. Besides, tannins have been useful in the treatment of inflamed or ulcerative tissues. Thus the presence of tannins in *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* leaves strengthened the traditional medicinal use of these plants in the treatment of ailments caused by microorganisms.

Just *et al.* [37] reported the inhibitory effect of saponins on inflamed cells. Saponin is also present in *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* leaf extract. Saponins which are glycosides with soapy characteristic are often reported to possess bioactive agents [38].

Alkaloids were also present in the methanolic extract of the leaf of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata*. Trease and Evans [39] reported alkaloids as naturally occurring compounds containing basic nitrogen atoms which are beneficial to plants having high inhibitory effects on bacterial and also act as repulsive agent to predators and pests. The noteworthy activity observed in this study could thus be credited to the interaction of one or more of the known metabolites against the test organisms. Just *et al.* [37] described that plant extract can be useful as anti-cancer, antioxidant, weight loss agent and anti-inflammatory.

The antimicrobial activity of the methanolic extract of the leaf of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* successfully inhibited the growth of both Gram-positive and Gram-negative organism used in this study. It was observed that the antibacterial effect of *Cymbopogon citratus*, *Annona muricata* and *Ocimum gratissimum* are not significantly different having the highest antagonistic activity against *P. aeruginosa* while *Azadirachta indica* had the least. *Azadirachta indica* had the highest antagonistic activity against *S. aureus* while *Annona muricata* had the least. *Annona muricata* recorded the highest antagonistic activity against *Proteus mirabilis* while *Azadirachta indica* had the least. *Ocimum gratissimum* documented the highest antagonistic activity against *Klebsiella pneumonia* while *Azadirachta indica* had the least. *Azadirachta indica* had the highest antagonistic activity against *E. coli* while *Ocimum gratissimum* had the least. This finding confirmed the report that medicinal plants such as Ocimum gratissimum, Azadirachta indica, *Cymbopogon citratus* and *Annona muricata* have high antimicrobial activities against Gram positive and Gram negative bacteria [40 and 41].

The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) exhibited by the methanolic leaf extract of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* were determined. The minimum inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC) had close similarity with disc diffusion results. This result aligns with Bartelt *et al*. [42] who reported that MIC and MBC interrelated with agar diffusion method. MIC could help a physician in choosing from among a group of similar drugs for the treatment of an ailment of diseases [12]. The phytochemical components solubility of methanolic extract of leave

of plants used in this study contributed to the developed antagonistic activity experienced in this work and could be of better low-cost benefit in pharmaceutical industry [28].

5. Conclusion

The result of the present study revealed that the methanol extract of *Ocimum gratissimum, Azadirachta indica, Cymbopogon citratus* and *Annona muricata* contains essential phytochemicals such as flavonids, saponins, tannins, alkaloids, phenols, cardiac glycosides, and terpenoids etc which have been reported to be of abundant benefit to human health with a great antimicrobial activity against food borne pathogens as reveald in this reseach.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest in this research article.

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