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Resazurin-Based Antibacterial Susceptibility Profile of Standard Antibiotics and Selected Medicinal Plant Extracts from Delta State, Nigeria

Erute Magdalene Adongbede[1*] and Faith Egbule[2]
[1*] University of Lagos, Faculty of Sciences, Department of Botany, University Road, 200213, Akoka, Yaba, Lagos, Nigeria. eadongbede@unilag.edu.ng
[2] Delta State University, Faculty of Sciences, Department of Botany, Abraka, Delta State Nigeria. Faithegbule2000@gmail.com
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Abstract

A rapid and cost-effective approach to screening pathogens and evaluating potential antimicrobials is imperative to solve problem of antimicrobial resistance. The resazurin based broth microdilution and paper disc diffusion assays were used to evaluate the susceptibility pattern of a wild *Escherichia coli* to standard antibiotics, aqueous, ethanol, methanol, chloroform, n-hexane extracts of fifteen medicinal plants from Delta State, Nigeria. The phytochemical composition of medicinal plants' extracts was analyzed with standard chemical methods. Regression analysis was used to establish the Minimum Inhibitory Concentrations (MIC) and Half Maximal inhibitory Concentrations (IC_{50}) of the reference antibiotics and medicinal plant extracts, with the resazurin based broth microdilution method in a six-hour period. The percentage yield of extracts varied from 18.68±1.18% *Aspilia africana* (ethanol extract) to 1.62±0.19% of *Psidium guajava* (n-hexane extract). The predominant phytochemicals in the medicinal plant extracts were alkaloids, flavonoids, phenols, glycosides, and tannins. The IC_{50} of the dose-response curves correlated positively with MIC values of reference antibiotics (r = 0.97). The paper disc diffusion and resazurin-based microbroth dilution assays recorded eight and ten potent antimicrobial plant extracts respectively. *E. coli* was marginally susceptible to gentamycin (MIC~ 4.33±0.54µg/ml) with the resazurin based assay. *Ficus exasperata* and *Ocimum gratissimum* had the most potent antimicrobials (MIC~2.50µg/m; IC_{50}~17.86µg/ml). The medicinal plant extracts of *A. africana*, *F. exasperata*, *O. gratissimum*, *P. guajava*, *Vernonia amygdalina*, *Calapogonium mucunoides* and *Sida acuta* were effective against multi-drug resistant *E. coli*. The resazurin based assay confirmed the susceptibility profile of a wild *E. coli* and identified potential new antimicrobials.

Key words: Antimicrobial activity; Folklore medicine; Multidrug resistance; Plant drugs; Susceptibility test

Introduction

Hundreds of pathogens have acquired resistance to most antibiotics, causing antimicrobial resistance to be an existential treat to man globally (Breijyeh *et al*., 2020). As a result of increasing threat of antimicrobial resistance the world desperately needs new antimicrobial agents (WHO, 2020). There are 50 antibiotics and 10 biologics currently under development worldwide and almost none address the extremely critical gram-negative bacteria such as *Escherichia coli* (WHO, 2021). There is urgent need for rapid testing of existing and potential new antimicrobials with the aim of eliminating drugs that are no longer effective and introducing novel drugs (Breijyeh *et al*., 2020). Most antimicrobial susceptibility tests take 2- 3 days to conclusively show a pattern of susceptibility (Vasala *et al*., 2020). The easy, fast, dependable, and cheap redox action of the electroactive redox dye-resazurin is recommended for screening standard antibiotics and potential new antimicrobial agents (Mishra et al., 2019). In developing Countries like Nigeria, herbal medical practice is accepted and widely popular with extraordinarily little authentication taking place. Medicinal plants have a huge reservoir of potentially effective new drugs that can address antimicrobial resistance globally and particularly in developing countries like Nigeria where access to drugs is difficult and expensive. There is paucity of information or knowledge of how effective the medicinal plants used in Nigeria are and records of the effective ones. Plants, algae, and fungi in their natural environment produce secondary metabolites in response to pathogens and adverse environmental conditions (Kortbeek *et al*., 2019). The secondary metabolites are important sources of natural product medicines (Weli *et al*., 2018). For example, the alkaloid physostigmine extracted from the plant Calabar bean (*Physostigma venenosum*) is used for production of

carbamates compounds for the treatment of various ailments (Batiha *et al*., 2020). Effective drug discovery therefore involves analyzing and taking note of what active compounds are present in the different organisms studied, particularly medicinal plants (Hoffman, 2020). There is an urgent need to investigate the medicinal plants in Nigeria to establish not only a database, but new potential antimicrobials to combat the surge in multidrug resistant strains of deadly gram-negative bacteria such as *Escherichia coli*. The Southern part of Nigeria is rich in plant species used by the locals for food and drugs but has no adequate records of these in literature. The primary health care in rural communities in Nigeria make use of these medicinal plants because they are cheaper and considered safer and more effective. The test plants ~ *Aspilia africana* (Pers.) C.D Adams, *Calapogonium mucunoides* Desv., *Canna indica* Linn., *Chasmanthera dependens* Hochst., *Chromolaena odorata* (L.) R.M. King & H. Rob., *Costus afer* Ker-Gawl, *Culcasia scandens* P. Beauv., *Ficus exasperata* Vahl, *Icacina trichantha* Oliv., *Jatropha curcas* Linn., *Jatropha gossypiifolia* Linn., *Ocimum gratissimum* Linn., *Psidium guajava* L., *Sida acuta* Burm. F., *Vernonia amygdalina* Del., in this study are traditionally used by the Agbor community in Delta State Nigeria for treating various ailments particularly infectious diseases like malaria, diarrhea, and skin infections (Table 1). Reviews of available records from database of Nigerian plants with the ethnomedicinal values of test plants in folklore are documented (Table 1).

Table 1: Ethno-pharmacological Importance of Fifteen Medicinal Plants from Agbor Delta Region of Nigeria

S/N	Plant Species	Family	Local Name	Common Name	Ethno-medical Use
1.	*Aspilia africana* (Pers.) C.D Adams	Asteraceae	Oramejila	Hemorrhage plant, wild sunflower	Stops bleeding, protects, and accelerates wound healing (Okolie et al., 2007)
2.	*Calapogonium mucunoides* Desv.	Fabaceae	Atugbu	Wild groundnut, crab grass	Treatment of diarrhea and skin infections (Enechi and Abugu, 2016)
3.	*Canna indica* Linn.	Cannaceae	Gwangwama	African starch, Indian shot	Anthelmintic, anti-inflammatory, antibacterial, antidiarrheal, hepatoprotective diuretic (Sarje et al., 2019)
4.	*Chasmanthera dependens* Hochst.	Menispermaceae	Ogbo	-	A diuretic (Monier and El-Ghani, 2016); treatment of venereal diseases (Barbosa-Filho et al., 2000)
5.	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Asteraceae	Agbara ohu	Siam weed, Jack in the bush	Treatment of headaches, dysentery, malaria, fever, toothache and skin diseases, antibacterial and antifungal agent (Ngane et al., 2006) (Nwinuka et al., 2009)
6.	*Costus afer* Ker-Gawl.	Costaceae	Okpete	Ginger lily	Treatment of nausea, mild cases of epilepsy, toothache, diarrhea, venereal diseases, malaria (Ezejiofor et al., 2013) An Abortifacient, Aphrodisiac, Arthritis (Finbarrs-Bello et al., 2017).
7.	*Culcasia scandens* P. Beauv.	Arecaceae	Aki okwuru	-	A native plant of Nigeria and used as fish poison, treatment of skin diseases, stomachache, and venereal diseases, used in combination with Costus afer, Sarcocephalus latifolius for the treatment of psychiatric disorders (Finbarrs-Bello et al., 2017).
8.	*Ficus exasperata* Vahl	Moraceae	Ogbu	Sandpaper leaf tree	Treatment of boils, enlarged spleen, jaundice, gonorrhea, scabies; hastens lactation (Sharma et al., 2009)
9.	*Icacina trichantha* Oliv	Icacinaceae	Ututo ogiri	-	Treatment of rheumatism, tooth ache, sterilizing open wounds, purgative, anthelmintic and aphrodisiac (Otun et al., 2015)
10.	*Jatropha curcas* Linn.	Euphorbiaceae	Ugbolu	Physic nut, pig nut, abolition nut	Antimicrobial, anticancer, and anti-HIV activities
11.	*Jatropha gossypiifolia* Linn.	Euphorbiaceae	Akimbogho	Wild cassava, Belly-ache bush	Anti-inflammatory, antimicrobial, a sedative, an analgesic and antidiarrheal agent (Wu et al., 2019)
12.	*Ocimum gratissimum* Linn.	Labiatae	Nchanwu	African basil, Scent leaf	Treatment urinary tract, wounds, skin, and gastrointestinal infections (Nweze and Eze,

					2009)
13.	*Psidium guajava* L.	Myrtaceae	Okwe	Guava	Treatment of fever, diarrhea, stomachache, cough, laxative, dysentery, irregular menstruation, and malaria (Gutierrez et al., 2008; Sanda et al., 2011)
14.	*Sida acuta* Burm. F.	Malvaceae	Udo	Broom weed; Wire weed	An astringent, treatment of urinary diseases, blood disorders and nervous diseases (Benjumea et al., 2016)
15.	*Vernonia amygdalina* Del.	Compositae	Unugbu	Bitter leaf	Treatment of stomachache, gingivitis, toothache, diabetes (Atangwho et al., 2014) pneumonia, malaria, hemostasis, and nervous diseases

The current study used different extracting solvents that targeted different bioactive compounds based on polarity and chemical reactivity of the solvents with aim of eluting different active components (Sasidharan *et al*., 2011; Otun *et al*., 2015). One of the fastest ways of discovering and authenticating potential antimicrobials is by measuring the biological response of a bacterium to a compound with the resazurin-based method (Foerster *et al*., 2017). The resazurin-based method does not require prior growth of the bacterial cells and read outs can be got within hours (Mishra *et al*., 2019).

Escherichia coli the bacterium of interest in this study, occur naturally in the lower intestine of mammals as a gram-negative facultative bacterium (Pitout, 2012). The virulent strains can cause urinary tract infections, gastroenteritis, newborn meningitis, and in rare cases, septicemia, mastitis, hemolytic-uremic syndrome, and gram-negative pneumonia (Pitout, 2012; Kaper *et al*., 2004). Records show that multidrug resistant bacteria have the capability of producing enzymes that make them more infectious and difficult to eradicate within the community, making diseases like urinary tract infections more detrimental (Pitout and Laupland, 2008; Sahm *et al*., 2001). Large scale outbreaks of these infections are common with grave consequences (Buchholz *et al*., 2011). In Nigeria, there is evidence of ESBL-producing *E. coli* in the public with prevalence as high as 45.00% and 60.20% in University of Benin Teaching Hospital, Benin city and Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria respectively and this has grave consequences (Ogefere *et al*., 2015; Afunwa *et al*., 2011; Olowe *et al*., 2013). Steps must be taken to prevent outbreaks of *E. coli* infections and ensure that life-threatening situations caused by MDRS, are prevented. This research is borne out of the need to get replacements for antibiotics, to which pathogenic *E. coli* have become resistant to among local communities in Nigeria. Plants used in folklore medicine in Ika Local Government of Delta state Nigeria for treatment of skin diseases, gastrointestinal, urinary tract diseases, malaria, venereal diseases, as anthelminthic (Table 1) were collected for this study. There are no studies on the validity of the use of these plants in folklore medicine and their antimicrobial properties in this area. The study addresses the emerging problem of multi-drug resistant strains of *E. coli* among the locals and the need for new antimicrobials that are plant based and locally sourced. The study aims to authenticate use of medicinal plant extracts with a quick, objective, and effective way with the resazurin based microdilution assay.

The investigation profiled the resistivity of *Escherichia coli* to extracts from medicinal plants *~Costus afer Culcasia scandens*, *Ficus exasperata*, *Icacina trichantha*, *Jatropha curcas*, *Jatropha gossypiifolia*, *Ocimum gratissimum*, *Psidium guajava*, *Sida acuta*, and *Vernonia amygdalina* , obtaining their minimum inhibitory concentrations (MIC) and effective doses or half maximal inhibitory concentrations (IC_{50} values) within six hours. NOTE

Materials and Methods

Collection of Plant Specimens

Fresh and healthy leaves of 15 medicinal plants (Table 1) were collected from the wild in Agbor in Ika South Local Government Area of Delta State, Nigeria. The local medicinal herb sellers and herbal practitioners were consulted for information. Identification of test plants were confirmed at the Lagos herbarium and herbarium voucher specimens were deposited at the herbarium. Fresh leaves of medicinal plants were washed under running tap water and dried in a food grade dehydrator (Stockli Dorrex Dehydrator, Switzerland) at 40^0C until a constant weight was obtained.

Preparation of Plant Extracts

Ethanol and methanol extracts were got from the plants using a modification of the methods by Vieira, *et al*. (2012). Dried leaves of the plants were ground with a dry mill blender. Ten grams of each ground plant leaves was extracted with 250 ml of 70 % ethanol and methanol by stirring for 1 hour on a magnetic stirrer at room temperature at 150rpm.

The chloroform and n-hexane plant extracts were got by stirring ten grams of plant tissues in 250 ml of chloroform and n-hexane on a magnetic stirrer at 150 rpm while covered with foil paper for one hour. All extraction processes were repeated three times and the extracts were pooled together and concentrated on a rotary evaporator under pressure 10.00 mg/ml for assays. The concentrated chloroform and n-hexane extracts were dried completely over anhydrous sodium sulphate (Pandey *et al*., 2017).

The aqueous extracts were extracted with ten grams of ground plant materials in water bath shaker (Shaking Bath 5B-16; Techne Ltd, UK) at 80ºC for one hour and the extracts were filtered after cooling. The extracts were centrifuged at 4400rpm for ten minutes; the supernatant was collected and was further purified by filtering with Whatman no. 1 filter paper. The residues were reheated, extraction was repeated thrice, and total volume concentrated using a rotary evaporator and the concentrate freeze-dried. The freeze-dried extracts were taken into solution by adding hot water and adjusted to 10mg/ml concentrations separately for assays. All plant extracts taken into solution were sterilized before use for each analysis/assay with a 0.22µm Millipore membrane syringe filter. The percentage yield of the extracts from the plant tissues was found using the formula -

Percentage Yield (%) = W_1 x 100/W_2

Where W_1 = weight of extract residue after removal of solvent

W_2 = Weight of pulverized plant tissue

Phytochemical Evaluation of Plant Extracts

The aqueous, chloroform, ethanol, n-hexane, and methanol extracts of the fifteen medicinal plants were screened for the major phytochemicals using standard methods (Evans, 2009). Alkaloids, carbohydrates, glycosides, phenols, oils, and fats, saponins, tannins and flavonoids were analyzed for using the Dragendroff's test, Molisch's test, modified Borntrager's test, Ferric chloride test, oil and fat stain test, the froth test, the gelatin test, lead acetate test, respectively.

Collection and Identification of Clinical Isolate of *Escherichia Coli*

The test *Escherichia coli* was obtained from the Medical Microbiology Department of the Teaching Hospital, Oghara Delta State Nigeria. The identity of the clinical isolate was confirmed using colony morphology, gram staining technique, catalase indole creation and methyl red positive assays (Mackie *et al*., 1996).

Bacterial Inoculum Preparation (Escherichia coli).

Fresh cultures of the clinical isolate of *E. coli* were grown on tryptic soy broth (overnight). A 0.5 McFarland concentration of all *E. coli* used for the assays and the standardized bacterial suspension was used between 15-60 minutes after preparation (Clinical and Labouratory Standard Institute, 2012).

Antimicrobial Susceptibility Test of Reference Antibiotics and Test Medicinal Plants with the Paper Disc Diffusion (Antibiotic and Test Medicinal Plants Susceptibility Profile of Test *E. coli*)

Commercial antibiotic discs at effective doses of the antibiotics – ampiclox, gentamycin, pefloxacin, erythromycin, co-trimoxazole, streptomycin, ciprofloxacin, ceftriaxone, amoxicillin, and cefuroxime were placed on Mueller-Hinton agar surface already plated with *E. coli*. The 15cm Petri dishes were inoculated with a spiral plater (Microbiology International WASP2, ALT San Diego US) with the automated lawn plating option. The vulnerability of test *E. coli* to fifteen medicinal plant extracts (aqueous, chloroform, ethanol, n-hexane, and methanol extracts) was first evaluated with the paper disc diffusion method. Standardized *E. coli* (0.5 McFarland Standard) cultures in tryptic soy broth was plated uniformly on Petri plates filled with Mueller-Hinton Agar with an automatic spiral plater using the lawn plating option before the different plant extracts (50µl) were aspirated onto sterile filter papers (6mm diameter) and placed on the *E. coli* seeded Mueller-Hinton agar plates and incubated at 37^0C for 18hours. Triplicates of each plant extract was made for all treatments. The zone of inhibition around the plant extract impregnated discs were read to the nearest millimeter. Susceptibility test results were interpreted using the Clinical and Labouratory Standard Institute (2012) guidelines.

Rapid Resazurin-Based Broth Microdilution Antimicrobial Susceptibility Testing/ Dose Response Studies

The rapid resazurin-based broth microdilution assay was performed in 96-well microtiter round bottom sterile plates. The minimum inhibitory concentrations of reference antibiotics (ampiclox, gentamicin, pefloxacin, erythromycin, co-trimoxazole, streptomycin, ciprofloxacin, ceftriaxone, amoxicillin, and cefuroxime) and the ethanol, methanol, chloroform, n-hexane, and water extracts of the fifteen test plants were investigated adopting methods by Elshikh *et al*. (2016). The bacterium suspension only (0.5 McFarland standard) was used as positive control and the antibiotics and plant extracts only were the negative controls. All test antibiotics and plant extracts (50µl) were added to tryptic soy broth (100µl) in the wells of the 96 well microtiter plate. A dose-response assay was designed with the standard antibiotics and plant extracts' concentrations (a four-fold serial dilution- 10.00, 7.50, 5.00 and 2.50µg/ml). The test *E. coli* (50µl) was added to all treatments except the sterile broth control wells. A 0.015% solution of the resazurin dye (5µl) was added to all the wells of the assay and the microplate reader configured to run samples in triplicates. Three blanks with media only and three control wells with media plus resazurin dye were defined in the microtiter plate layout. The plate reader was configured for data reduction, with a transformation that calculated the reduction percentage using the molar extinction coefficients of reduced and oxidized resazurin dye read at 570nm and 600nm. The lowest concentration prior to any noticed change in color is the minimum inhibitory concentration (Elshikh *et al*., 2016). The inoculated microtiter plates were incubated at 37^0C for 75minutes. The absorbance was read in the microplate reader before and after the incubation periods.

Half Maximal Inhibitory Concentration of Antibiotics and Plant Extracts

The half maximal inhibitory concentration (IC_{50}) is defined as the drug concentration that produces 50% of the maximal effect on bacterial growth (Holt, 1975). A dose-response curve was plotted with the signal inhibition induced by the antibiotics and plant extracts as percentage inhibitions at different concentrations against the controls and was expressed using the following formula (Zampini *et al*., 2005) from which the IC_{50} was extrapolated.

Percentage Inhibition (%) = $[(ODc - ODi)/ODc]\ x\ 100$

Where OD_C is the optical density at 570nm and 600nm for the negative control (having no extract or treatment) and OD_I is the optical density for the sample treated with plant extracts and antibiotics.

The relationship between MIC and IC_{50} were analyzed for by log-transforming both values and fitting in a linear regression curve. The slope and intercept of the regression curve was used to predict relationship between MIC and IC_{50}.

Criteria for Determination of Susceptibility

The paper disc diffusion assay data were interpreted according to the Clinical and Labouratory Standard Institute, (2012) critiria for measuring antimicrobial susceptibility. Inhibition zone ≥20mm - Organism is susceptible; Inhibition zone between 15-19mm - susceptibility is termed intermediate; Inhibition zone ≤14mm – Organism is resistant. The data from the resazurin Broth microdilution assays were interpreted as follows- MIC<4µg/ml is break point for susceptibility determination of antimicrobials (Clinical and Labouratory Standard Institute, 2012).

Statistical analysis

Statistical analysis was conducted by One-way analysis of variance with the IBM SPSS Statistics 26 software and the significant difference between means with the Duncan's multiple range test at 5% probability ($p \leq 0.05$) level of significance. The vulnerability of the MDR-*E. coli* was decided following the criteria laid out by the Clinical and Labouratory Standard Institute, (2012).

Results and Discussion

Percentage Yield

The percentage yield of the medicinal plants varied with the plant species and the extracting solvent (Table 2). *Aspilia africana* had the highest percentage yield with the solvent ethanol (18.86%), followed by *Costus afer* (17.85%-methanol extract), *Icacina trichantha* (15.85%-ethanol extract). Low yields were recorded the plants *Ocimum gratissimum* (2.18% ~chloroform extract), followed by *Psidium guajava*~ n-hexane extract and chloroform extracts of same plant (2.19%). The plant with the highest yield with the methanol extract was *Costus afer* (17.85%) and the plant with the least yield was *Jatropha gossypiifolia* (6.24%). Percentage yield of crude aqueous leaves extracts of test medicinal plants varied from 14.75% in *Psidium guajava* to 7.14% in *Jatropha curcas*. Percentage yield of crude chloroform leaves extracts was with the plant *Aspilia africana* ~11.85% and least yield was recorded with the plant *Ocimum gratissimum* (2.18%). The percentage yield of n-hexane was low and comparable to the chloroform extract, as highest yield was recorded with the plant, *Jatropha curcas* (8.64%) and the least yield was with the plant *Psidium guajava* (1.62%) (Table 2). The polar solvents recorded higher yields generally than the non-polar solvents (Table 2). The methanol, ethanol and aqueous extracts recorded higher yields of extracts than the n-hexane and chloroform extracts and this could be attributed to the polarity of the solvents (Do *et al*., 2014). The ethanol and methanol extracts had higher yield than the aqueous extracts because alcohols have the capability to dissolve and elute higher volume from different plants (Galanakis *et al*., 2013). Alcohols are therefore preferred because of their intermediate polarity when compared to water which is more polar (Do *et al*., 2014). However, the methanol extracts performed better than the ethanol extracts and this can be attributed to higher polarity.

Phytochemical Content

The phytochemical screening of plant extracts showed the presence of phenolic compounds, flavonoids, alkaloids, carbohydrates, and glycosides at different degrees of precipitation which varied with the plant species and extracting solvents (Tables 3, 4, 5, 6 and 7). The plants *Canna indica* and *Jatropha curcas* recorded the significant concentrations of alkaloids with the ethanol extracts (Table 3). The current study revealed the presence of secondary metabolites with high degree of precipitation in (+++) in various test plants in the ethanol extract and these include alkaloids (*Canna indica*), flavonoids (*Psidium guajava*, *Costus afer*, *Chromolaena odorata*), tannins (*Psidium guajava*, *Costus afer*, *Ocimum gratissimum*, *Calapogonium mucunoides*), phenols (*Psidium guajava*, *Icacina trichantha*) (Table 3). Plants that had high precipitations of glycosides were *Psidium guajava*, *Costus afer* and *Ficus exasperata* (Table 3)

The plants with the highest degree of precipitation for alkaloids were *Psidium guajava* and *Ocimum gratissimum* with the aqueous extracts and this was not detected in *Canna indica* and *Chasmanthera dependens* (Table 4). Flavonoids were moderately recorded in four plants and not detected in three plants out of the fifteen test plants. High precipitations of saponin and tannin was recorded only in *Chromolaena odorata* (Table 4). Phenols were only recorded in trace and moderate amounts in most test plants but not in *Icacina trichantha* and *Chasmanthera dependens* (Table 4)

The methanol extracts of the fifteen extracts recorded presence of phenolic compounds with the highest degree of precipitation shown in *Ocimum* gratissimum and *Chasmanthera dependens* (Table 5). Saponins and carbohydrates were observed in trace amounts or not present in the methanol extracts. *Ficus exasperata* recorded the highest degree of precipitation with flavonoids but no tannins with the methanol extracts (Table 5).

The n-hexane extracts recorded low or no precipitations of carbohydrates, saponins and tannins. High degree of precipitation of flavonoids was recorded in *Chasmanthera dependens* in the n-hexane extracts (Table 6). The plants *Culcasia scandens* and *Icacina trichantha* had high phenolic contents with the n-hexane extract (Table 6). *Vernonia amygdalina* (chloroform extract) was the only plant among the test medicinal plants that recorded high degree of precipitation with the flavonoids (Table 7). Carbohydrates were only detected in four plants in trace amounts, with moderate levels of saponin recorded in the plant *Vernonia amygdalina* in chloroform extracts (Table 7). The chloroform extracts of the medicinal plants recorded mostly trace to not detected levels of the phytochemicals evaluated.

The preliminary qualitative phytochemical analysis of the leaves of fifteen medicinal plants used in the current research revealed the presence of alkaloids, glycosides, phenols, saponins, tannins, flavonoids which are secondary metabolites, biologically active and with therapeutic properties (Roospahree and Naik, 2019). The test medicinal plants had varying concentrations of polyphenolic compounds, alkaloids, glycosides, tannins, and saponins, indicative of their health promoting activities recorded in this study and previous ones (Seth and Sarin, 2010; Singh *et al*., 2016; Gaur *et al*., 2014; Geidam *et al*., 2007; Odiongenyi *et al*., 2009). The different degree of activity of the plants can be attributed to the presence and concentration of these secondary metabolites (Pang *et al*., 2021). Metabolites like flavonoids and saponins were present in higher concentrations in n-hexane and chloroform extracts and accounts for activities attributed to plants with these compounds (Obasi and Obasi, 2021). It is therefore clear that the degree of polarity of the solvents and species of plants play major roles in successful extraction of secondary metabolites from plants (Altemimi *et al*., 2017). The alcohol extracts were more potent against the test *E. coli* because literature show that, alcohols are better extraction of compounds with antimicrobial activities than water (Madkour, El-Shoubaky, & Ebada, 2019).

Susceptibility Profile of Wild *Escherichia coli* to Standard Antibiotics (Paper Disc Diffusion Assay)

The wild *E. coli* showed multidrug resistance to all antibiotics assessed as shown with the paper disc diffusion assay (Figure 1). The test *E. coli* was not susceptible to all the ten antibiotics evaluated but showed intermediate susceptibility (i.e., response not strong enough to control and could vary with increased concentration; inhibition zone -between 15-19mm) to ampiclox, gentamycin, co-trimoxazole, streptomycin, and amoxicillin (Figure 1). The bacterium was resistant (did not significantly inhibit the growth of the bacterium, inhibition zone ≤14mm) to pefloxacin, erythromycin, ciprofloxacin, ceftriaxone, and cefuroxime (Figure 1; ACCLS, 2012) confirming that the clinical isolate was a multidrug resistant strain.

The resazurin based microdilution method however should that the test *E. coli* can be controlled by the antibiotics ciprofloxacin and gentamycin because they had MIC breakpoints at 4.16±0.76 and 4.33±0.54 µg/ml respectively (Table 8). The effective dose (IC_{50} values) of the two antibiotics were 26.79±1.74 and 32.61±1.72 µg/ml respectively (Table 8).

The positive correlation recorded for the MIC and IC_{50} of the reference antibiotics for the bacterium had been reported by Foerster *et al*., (2017) and was not strictly linear. The dose response curve gives information on the assay specificity and reliability. The Pearson's correlation of r^2 ~0.93 is quite promising (Figure 3). These shows that the method was highly objective and can be particularly useful in conducting experiments involving large library of new compounds, antimicrobials, or a combination of both (Foerster *et al*., 2017).

Eight plant extracts -*Costus afer* (n-hexane), *Vernonia amygdalina* (chloroform), *Canna indica* (n-hexane), *Jatropha curcas* (n-hexane), *Ficus exasperata* (methanol and ethanol), *Calapogonium mucunoides* (methanol) and *Sida acuta* (aqueous extracts) of the 75 plant extracts effectively inhibited the growth of *E. coli* with the paper disc diffusion assay as these recorded inhibition zones >20mm (Figure 2; ACCLS, 2012). The test *E. coli* showed intermediate susceptibility to the plants- *Ocimum gratissimum* (methanol extract), *Chasmanthera dependens* (ethanol extract), *Chromolaena odorata* (methanol extract), *Aspilia africana*, *Costus afer*, *Ocimum gratissimum* and *Vernonia amygdalina* (aqueous extracts) because they all had inhibition zones between15-19mm and cannot effectively control the bacterium's growth (Figure 2; ACCLS, 2012).

The rapid resazurin-based broth microdilution assay indicated that the *E. coli* was susceptible to ten (*Psidium guajava* (n-hexane), *Aspilia africana* (water), *Costus afer* (n-hexane), *Ocimum gratissimum* (water), *Vernonia amygdalina* (chloroform & water), *Canna indica* (water), *Jatropha curcas* (n-hexane), *Ficus exasperata* (ethanol & methanol), *Calapogonium mucunoides* (methanol) out as these recorded susceptibility breakpoints >4µg/ml using the MIC values (Table 9). The most potent plants extracts were methanol and ethanol extracts of *F. exasperata*, Aqueous extracts of *V. amygdalina* and *A. Africana* as these recorded MIC values between 2.5-3.33 µg/ml (Table 9).

Conversely, the IC_{50} of methanol extracts with the most active plants varied from 20.27 µg/ml in Ficus exasperata to 28.30 µg/ml in *Chromolaena odorata* (Table 10). The plants with less activity recorded higher IC_{50} concentrations such as 115.53 µg/ml in chloroform extracts of Chromolaena odorata and 375.38 µg/ml in aqueous extracts of *Calapogonium mucunoides* (Table 10).

The resazurin-based broth microdilution used in this study showed within hours, that the wild *E. coli* was a multi-drug resistant strain. It discriminated within a broad range of compounds while showing which could be potential replacements for standard antibiotics to which the bacterium had become resistant. The method can therefore be used for surveillance of pathogenic organisms spreading in the public to ascertain drug resistance emergence and screen plants for new potentially more effective drugs (Foerster *et al*., 2017). A total of 10 plant extracts of the 75 extracts investigated were selected for further purification and study as potential new sources of antimicrobials particularly for multidrug resistant strains of *E. coli*. The resazurin-based method had been used in susceptibility profile of *Neisseria gonorrhoeae* to antibiotics successfully in a 2-4hours period in previous research (Foerster *et al*., 2017). The redox action of the resazurin dye had also been used by Lescat *et al*. (2019) to distinguish between colistin susceptible and resistant strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from over a hundred and eighty samples successfully within a 4 hour period. In the current study, the method was used to screen 75 plant extracts for potential new drugs in a fast and efficient way successfully. The study demonstrates that the huge reservoir of natural resources in the wild in Nigeria could be quickly investigated, and potential beneficial compounds identified in a timely and effective manner (Sreejith *et al*., 2017).

Ficus exasperata was one of the most active plants particularly with the ethanol and methanol extracts, commonly known as sandpaper plant. *F. exasperata* is a medicinal plant used to treat malaria and typhoid in Nigeria when mixed with other herbs ((Enogieru, Charles, Omoruyi, Momodu, & Ezeuko, 2015). The plant is extraordinarily rich in bioactive compounds particularly phenolic compounds as shown here in this study and has been recommended for use as functional food or nutraceuticals (Mouho, et al., 2018).

The aqueous extracts of *Canna indica* activity was potent against the test *E. coli* comparable to results of Singh *et al*. (2016) where the ethanol extract elicited significant activity with the bacterium. *C. indica* commonly referred to as African arrowroot has rhizomes that are edible and have been used to produce vermicelli, white wine, and ethanol (Yadav and Sisdia, 2019). The roots are also used as feed for livestock and has scores of pharmacological activities (Yadav and Sisdia, 2019). All parts of the plant have been exploited with success for different activities and the antibacterial activity of the aqueous extract is justified in the current study.

The report of Vieira *et al*. (2001) reported high degrees of inhibition against *E. coli* with the plant *Psidium guajava* as seen in this study. *P. guajava* is a popular tree crop valued for its fruits and medicinal leaves and stem. The plant has history for its use for treating diarrhea, dysentery and even for relieving pain (Naseer, Hussain, Naeem, Pervaiz, & Rahman, 2018). All extracts of the plant evaluated were active against the MDR-*E. coli* with the n-hexane extract being the most active. The activity can be attributed to secondary metabolites present in the plant (Naseer, Hussain, Naeem, Pervaiz, & Rahman, 2018). According to Naseer et al., (2018), the leaf of the plant has fungistatic and bacteriostatic compounds. The chloroform and n-hexane extracts of the plant *Icacina trichantha* were more active than extracts of same solvent from other test plants as previously shown by Otun *et al*., (2015*). I. trichantha* is indigenous to West Africa and used as food and medicine. The plant has been used and proven to be effective as emetic and antimicrobial agent (Che, *et al*., 2016). The medicinal plants' extracts evaluated performed better than the reference antibiotics and showed promise as deposits of novel antimicrobial compounds.

Conclusion

The current study showed the rapid resazurin-based broth microdilution assay to be a high-thorough put method and was successfully used to identify potential antimicrobials among the medicinal plants evaluated. The growth of the wild Escherichia coli a multidrug resistant strain was effectively inhibited by extracts of the plants *Ficus exasperata*, *Calapogonium mucunoides*, *Costus afer*, *Canna indica Jatropha curcas*, *Vernonia amygdalina* and *Sida acuta.* These plants have bioactive compounds that can replace antibiotics used for the treatment multi-drug resistant bacteria. The resazurin based microdilution assay outperformed the paper disc diffusion assay and could be useful for surveillance of antimicrobial resistance among clinical isolates and evaluation of novel antimicrobials in a fast, effective, and dependable manner. The plants used in folklore medicine in Nigeria are potential sources of new antimicrobials.

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Declarations

Ethics approval and consent to participate.
Not applicable

Consent for publication
Not applicable

Availability of data and materials
The data generated or analyzed during the current study are included in this article.

Competing Interest
The authors have no competing interests.

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Authors Contributions
EMA: Conceptualized and designed the experiment, performed the microbroth dilution assays, analyzed data from experiments. FE performed the paper disc diffusion assays and contributed to writing and editing the manuscript.
All authors read and approved the final manuscript.

ABBREVIATIONS

MIC-Minimum Inhibitory Concentration
IC_{50}- Half Maximal Inhibitory Concentration
MDRS- Multiple Drug Resistant Strain
GC-MS-Gas Chromatography-Mass Spectrophotometer
CLSI- Clinical Laboratory Standard Institute
ESBL-producing E. coli- Extended-Spectrum Beta Lactamase producing Escherichia coli

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Table 2: Yield of Extracts of Fifteen Medicinal Plants from Niger Delta State, Nigeria Using Different Extractants (%)

Medicinal Plants	Ethanol	Methanol	Aqueous	Chloroform	n-Hexane
Psidium guajava	12.04±0.8^{a}	10.27±0.63^{b}	14.75±0.8^{a}	2.19±0.52	1.62±0.19
Aspilia africana	18.68±1.18^{a}	13.45±0.6^{a}	9.72±0.38^{b}	11.85±0.71^{b}	10.91±0.55^{b}
Costus afer	13.91±1.21^{a}	17.85±1.3^{a}	12.48±0.81^{a}	6.01±0.27^{c}	4.05±0.15^{cd}
Ocimum gratissimum	8.10±0.71^{c}	9.02±0.33^{b}	11.35±1.51^{b}	2.18±0.25	3.84±0.41^{cd}
Vernonia amygdalina	9.81±1.02^{b}	14.61±1.4^{a}	12.92±2.04^{a}	5.85±0.79	4.17±0.32^{cd}
Culcasia scandens	8.29±0.55^{c}	11.81±0.37^{b}	10.28±0.56^{b}	7.01±0.25^{c}	4.27±0.50^{cd}
Icacina trichantha	15.85±1.0^{a}	8.17±0.62^{b}	10.72±0.90^{b}	3.25±0.11^{d}	6.04±0.22^{c}
Canna indica	7.51±0.82^{c}	5.92±0.55^{c}	9.21±0.73^{b}	4.11±0.19	5.68±0.82^{c}
Chasmanthera dependens	6.79±0.88^{c}	5.21±0.31$^{c\,d}$	9.04±0.69^{b}	3.14±0.25^{d}	2.72±0.18^{d}
Jatropha gossypiifolia	6.24±0.92^{c}	4.61±0.77^{c}	8.05±1.03^{b}	3.38±0.21^{d}	5.74±0.94^{c}
Jatropha curcas	11.08±1.86^{b}	12.95±0.89^{a}	7.14±0.95$^{b\,c}$	4.28±0.72^{cd}	8.64±1.02^{b}
Ficus exasperata	10.15±0.91^{b}	13.08±1.06^{a}	7.35±0.86$^{b\,c}$	4.22±0.71^{cd}	2.58±0.18^{d}
Chromolaena odorata	11.27±1.21^{b}	13.61±0.97^{a}	9.08±0.82^{b}	2.95±0.54^{d}	3.75±0.82^{d}
Calapogonium mucunoides	14.18±2.0^{a}	11.98±0.85^{b}	10.64±1.03^{b}	8.11±0.45^{b}	5.62±0.33$^{c\,d}$
Sida acuta	11.71±0.9^{b}	13.09±0.76^{a}	10.55±0.59^{b}	6.29±0.17^{c}	4.18±0.41$^{c\,d}$

*Mean (n=3) ±SD followed by the same letter superscripts are not significantly different (Duncan's multiple range test α = 0.05); -

Table 3: Phytochemical Composition of Ethanol Extracts of Fifteen Plants Used in Folklore Medicine in Delta State, Nigeria

Medicinal Plants	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Carbohydrate	Glycosides
Psidium guajava	++	+++	-	+++	+++	++	+++
Aspilia africana	+	++	+	+	+	+	-
Costus afer	+	+++	+	+++	++	+	+++
Ocimum gratissimum	++	+	+	+++	+	+	+
Vernonia amygdalina	++	-	+	++	+	+	+
Culcasia scandens	+	+	-	-	+	+	+
Icacina trichantha	++	+	-	++	+++	+	++
Canna indica	+++	++	-	+	++	++	++
Chasmanthera dependens	+	-	-	+	-	+	+
Jatropha gossypiifolia	+++	-	+	+	+	+	+
Jatropha curcas	++	-	+	+	+	+	+
Ficus exasperata	+	++	+	+	+	+	+++
Chromolaena odorata	++	+++	-	-	+	-	-
Calapogonium	++	++	++	+++	+	+	+

mucunoides							
Sida acuta	+	++	-	-	+	+	+

* -: Not detected, +: Trace amount, ++: moderately present, +++: strongly present (+-shows degree of precipitation)

Table 4: Phytochemical Composition of Aqueous Extracts Fifteen Plants Used in Folklore Medicine in Delta State, Nigeria

Medicinal Plants	Alkaloid	Flavonoid	Saponin	Tannin	Phenol	Carbohydrate	Glycoside
Psidium guajava	+++	++	-	++	++	++	++
Aspilia africana	+	+	+	+	+	+	+
Costus afer	+++	++	-	+	++	+	++
Ocimum gratissimum	+	-	+	-	+	+	-
Vernonia amygdalina	+	++	+	+	+	+	-
Culcasia scandens	+	+	+	+	++	+	+
Icacina trichantha	++	-	-	-	-	+	-
Canna indica	-	+	++	-	+	+	+
Chasmanthera dependens	-	-	-	+	-	-	-
Jatropha gossypiifolia	++	+	+	-	+	+	+
Jatropha curcas	++	+	+	+	++	+	+
Ficus exasperata	+	++	-	-	++	+	++
Chromolaena odorata	++	++	+++	+++	+	+	+
Calapogonium mucunoides	+	+	+	+	+	+	+
Sida acuta	+	+	+	+	+	+	+

*-: Not detected+: Trace amount, ++: moderately present; +++: strongly present (+-shows degree of precipitation)

Table 5: Phytochemical Composition of Methanol Extracts Fifteen Plants Used in Folklore Medicine in Delta State, Nigeria

Medicinal Plants	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Carbohydrates	Glycosides
Psidium guajava Linn.	+	++	+	++	++	+	+
Aspilia africana	+	++	+	+	++	-	+
Costus afer	+	+	-	-	++	+	+
Ocimum gratissimum	+	+	+	++	+++	+	+
Vernonia amygdalina	++	+	+	++	++	+	+
Culcasia scandens	+	++	+	+	++	+	++
Icacina trichantha	+	+	+	-	+	+	+
Canna indica	-	+	-	+	++	+	+
Chasmanthera dependens	-	++	+	+	+++	-	-
Jatropha gossypiifolia	+	+	+	++	++	+	++
Jatropha curcas	+	+	+	+	++	-	+
Ficus exasperata	++	+++	-	-	++	+	++
Chromolaena odorata	+	++	+	-	++	-	+
Calapogonium mucunoides	+	+	+	+	++	-	+
Sida acuta	++	+	+	++	++	+	+

*-: Not detected+: Trace amount, ++: moderately present; +++: strongly present.

Table 6: Phytochemical Composition of n-Hexane Extracts Fifteen Plants Used in Folklore Medicine in Delta State, Nigeria

Medicinal Plants	Alkaloid	Flavonoids	Saponins	Tannins	Phenols	Carbohydrate	Glycoside
Psidium guajava	-	-	-	-	-	-	-
Aspilia africana	-	-	+	-	+	-	-
Costus afer	+	+	+	+	+	+	++
Ocimum gratissimum	+	-	-	-	-	-	+
Vernonia amygdalina	++	-	-	-	+	-	++
Culcasia scandens	-	+++	-	+	+++	-	++
Icacina trichantha	+	++	-	+	+++	-	+ +
Canna indica	-	+	-	-	++	-	-
Chasmanthera dependens	-	-	+	+	-	+	+
Jatropha gossypiifolia	-	+	+	-	+	-	+
Jatropha curcas	+	+	-	-	+	+	+
Ficus exasperata	-	++	+	+	++	-	+
Chromolaena odorata	-	-	+	-	+	-	-
Calapogonium mucunoides	-	+	-	-	+	+	+
Sida acuta	-	+	+	-	+	-	-

*-: Not detected+: Trace amount, ++: moderately present; +++: strongly present.

Table 7: Phytochemical Composition of Chloroform Extracts Fifteen Plants Used in Folklore Medicine in Delta State, Nigeria

Medicinal Plants	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Carbohydrates	Glycosides
Psidium guajava	-	-	-	+	+	-	-
Aspilia africana	+	+	+	+	+	+	+
Costus afer	-	++	-	+	++	-	+
Ocimum gratissimum	-	+	-	+	+	+	-
Vernonia amygdalina	++	+++	++	++	+	-	++
Culcasia scandens	-	-	-	+	+	-	-
Icacina trichantha	-	+	-	-	+	-	-
Canna indica	-	-	+	-	-	-	+
Chasmanthera dependens	+	-	+	+	+	-	-
Jatropha gossypiifolia	-	-	+	-	-	+	+
Jatropha curcas	-	+	+	-	-	-	+
Ficus exasperata	+	++	+	-	+	-	-
Chromolaena odorata	-	-	+	-	-	-	-
Calapogonium mucunoides	+	-	+	+	++	-	-
Sida acuta	+	+	+	-	++	+	+

* -: Not detected+: Trace amount, ++: moderately present; +++: strongly present.

Table 8: The Minimum Inhibitory and Half Maximal Inhibitory Concentrations of Antibiotics on a Clinical Isolate of Escherichia coli

S/N	Antibiotics	Minimum Inhibitory Concentration (MIC) (µg/ml)	Half Maximal Inhibitory Concentration (IC_{50}) (µg/ml
1.	Ampiclox	7.82 ± 0.78^{c}	78.51 ± 2.02^{b}
2.	Gentamycin	4.33 ± 0.54^{a}	26.79 ± 1.74^{a}
3.	Pefloxacin	5.45 ± 0.91^{a}	30.05 ± 1.45^{a}
4.	Erythromycin	6.03 ± 1.44^{ab}	31.15 ± 1.88^{a}
5.	Co-trimoxazole	6.71 ± 1.25^{b}	75.29 ± 2.08^{b}
6.	Streptomycin	9.04 ± 1.05^{c}	86.51 ± 2.15^{bc}
7.	Ciprofloxacin	4.16 ± 0.76^{a}	32.61 ± 1.72^{a}
8.	Ceftriaxone	8.33 ± 1.04^{bc}	79.79 ± 2.35^{b}
9.	Amoxicillin	9.16 ± 0.81^{c}	82.78 ± 2.18^{b}
10.	Cefuroxime	9.38 ± 0.55^{c}	87.35 ± 2.32^{bc}

*Mean (n=3) ±SD followed by the same letter superscripts are not significantly different within the same column (Duncan's multiple range test $\alpha = 0.05$); - No activity, susceptibility breakpoint <4.00µg/ml

Table 9: Minimum Inhibitory Concentration of the Different Medicinal Plant Extracts on *Escherichia coli* (µg /ml)

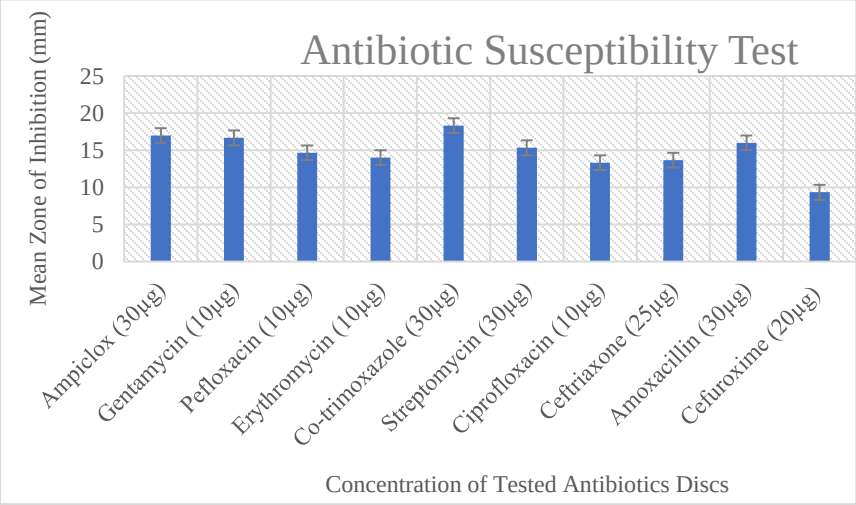
S/N	Medicinal Plants	Ethanol	Methanol	n-Hexane	Chloroform	Water Aqueous
1.	*Psidium guajava*	8.33±0.24^{bc}	7.50±0.00^{bc}	4.16±0.74^{a}	6.67±0.54^{ab}	6.67±0.44^{ab}
2.	*Aspilia africana*	9.17±0.92^{cb}	6.67±0.64^{ab}	10±0.00^{d}	9.16±0.74	3.33±0.44^{a}
3.	*Costus afer*	9.17±0.88^{cb}	8.33±0.95	4.16±0.61^{a}	6.67±0.42^{ab}	5.0±0.00^{a}
4.	*Ocimum gratissimum*	8.33±0.72^{bc}	6.67±0.84^{ab}	7.50±0.00^{ab}	7.50±0.00^{ab}	2.50±0.00^{a}
5.	*Vernonia amygdalina*	9.16±0.81^{cb}	9.16±1.04^{cd}	6.67±0.84^{ab}	3.33±0.14^{a}	4.16±0.31^{a}
6.	*Culcasia scandens*	8.33±0.84^{bc}	10.00±0.00^{d}	7.50±0.00^{ab}	7.50±0.00^{ab}	-
7.	*Icacina trichantha*	9.38±0.95^{d}	9.16±0.94	7.50±0.00^{ab}	6.67±0.34^{ab}	7.50±0.00^{ab}
8.	*Canna indica*	-	10.00±0.00^{d}	5.00±0.00^{a}	6.67±0.51^{ab}	4.17±0.42^{a}
9.	*Chasmanthera dependens*	8.33±0.55^{bc}	9.17±0.75^{cd}	6.67±0.64^{ab}	-	-
10.	*Jatropha gossypiifolia*	7.50±0.00^{b}	-	7.50±0.00^{ab}	-	9.17±0.48^{cd}
11.	*Jatropha curcas*	9.16±0.44^{cb}	6.67±0.54^{ab}	4.16±0.24^{a}	-	10±0.61^{d}
12.	*Ficus exasperata*	2.50±0.00^{a}	3.33±0.42^{a}	9.16±0.96^{cd}	-	-
13.	*Chromolaena odorata*	7.50±0.00^{ab}	6.67±0.41^{ab}	-	10±0.00^{cd}	8.33±0.74^{bc}
14.	*Calapogonium mucunoides*	6.67±0.81^{ab}	4.16±0.62^{a}	10±0.00^{d}	9.16±0.86^{cd}	-
15.	*Sida acuta*	6.67±0.94^{ab}	7.5±0.18^{ab}	-	6.67±0.45^{ab}	5.00±0.25^{a}

*Mean (n=3) ±SD followed by the same letter superscripts are not significantly different within the same column (Duncan's multiple range test α = 0.05); - No activity, susceptibility breakpoint <4.00µg/ml

Table 10: Half Maximal Inhibitory Concentration (IC_{50}) of Different Medicinal Plant Extracts on Escherichia coli (µg /ml)

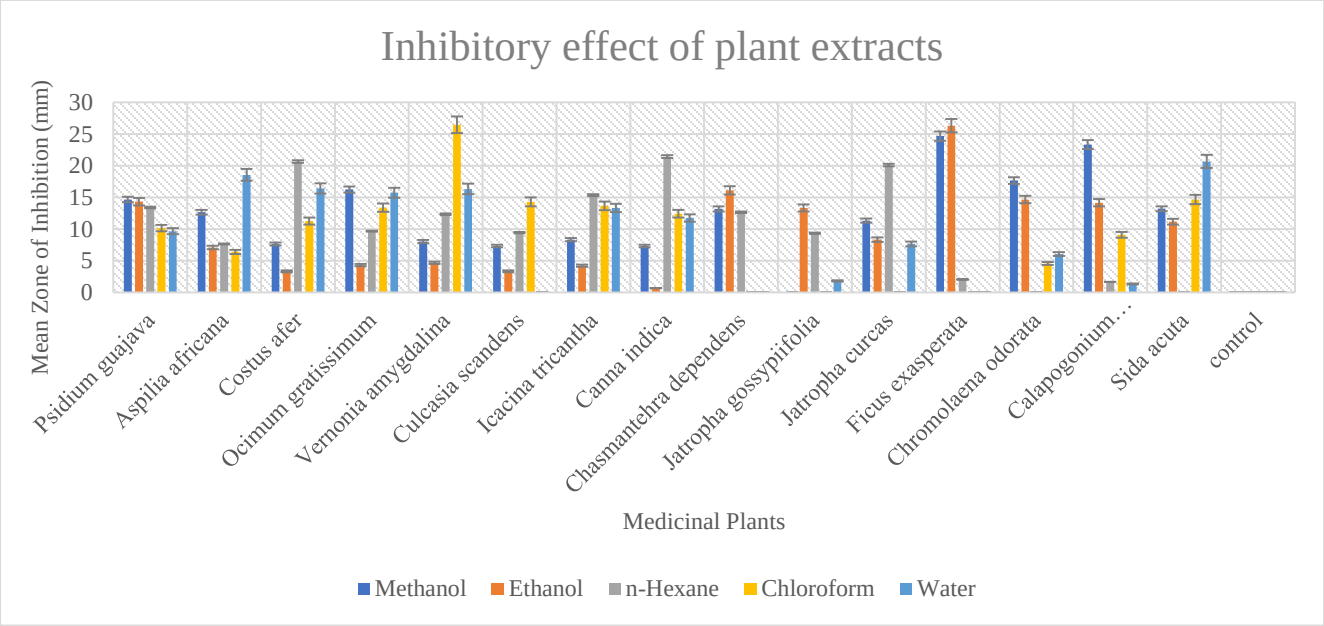
S/N	Medicinal Plants	Ethanol	Methanol	n-Hexane	Chloroform	Water Aqueous
1.	*Psidium guajava*	30.61±1.72^{b}	34.09±1.11^{b}	37.50±0.98^{b}	48.39±0.51^{d}	51.73±1.35^{d}
2.	*Aspilia africana*	55.56±2.05^{d}	39.48±0.82^{b}	65.23±1.51^{e}	78.96±2.08^{e}	27.78±0.68^{ab}
3.	*Costus afer*	93.77±1.58^{f}	65.23±1.22^{e}	24.19±0.66^{a}	44.12±1.22^{d}	31.25±1.11^{b}
4.	*Ocimum gratissimum*	78.82±0.94^{e}	31.25±0.74^{b}	51.73±2.19^{d}	37.50±2.08^{b}	33.33±0.97^{b}
5.	*Vernonia amygdalina*	75.03±2.16^{e}	62.50±2.01^{e}	40.54±1.07^{c}	18.99±0.73^{a}	30.61±1.21^{b}
6.	*Culcasia scandens*	93.55±1.85^{f}	38.46±0.58^{b}	54.28±2.01^{d}	34.89±1.02^{b}	-
7.	*Icacina trichantha*	83.33±1.09^{f}	44.12±0.97^{c}	32.61±0.45^{b}	36.59±1.61^{b}	37.50±1.38^{b}
8.	*Canna indica*	-	20.27±0.63^{a}	23.44±0.88^{a}	40.54±0.91^{c}	42.86±2.01^{b}
9.	*Chasmanthera dependens*	27.78±0.92^{a}	38.46±1.47^{b}	39.48±0.94^{b}	-	-
10.	*Jatropha gossypiifolia*	32.61±1.21^{c}	-	53.58±1.09^{d}	-	300.45±2.85^{h}
11.	*Jatropha curcas*	48.39±1.72^{c}	44.12±1.28^{c}	25.00±0.84^{a}	-	65.23±1.97^{e}
12.	*Ficus exasperata*	17.86±1.01^{a}	20.27±1.19^{a}	25.00±1.05^{a}	-	-
13.	*Chromolaena odorata*	31.25±0.88^{b}	28.30±2.01^{a}	-	115.53±2.92^{f}	83.33±0.95^{f}
14.	*Calapogonium mucunoides*	30.00±1.52^{b}	21.43±1.91^{a}	300.45±2.35^{h}	55.56±2.14^{d}	375.38±2.28^{i}
15.	*Sida acuta*	38.46±2.01^{b}	45.45±2.22^{c}	-	34.09±1.05^{b}	24.19±0.62^{a}

*Mean (n=3) ±SD followed by the same letter superscripts are not significantly different within the same column (Duncan's multiple range test α = 0.05); - No activity, susceptibility breakpoint <4.00µg/ml



*Susceptibility breakpoint: Inhibition zone- ≥20mm- Susceptible; 15-19mm -Intermediate susceptibility; ≤14mm - Resistant

Figure 1: Antibiotic Susceptibility Profile of Wild *Escherichia coli*



*Criteria for susceptibility: Inhibition zone- ≥20mm- Susceptible; 15-19mm -Intermediate susceptibility; ≤14mm - Resistant

Figure 2: Inhibitory Effect of Medicinal Plant Extracts on Growth of MDR- Escherichia coli

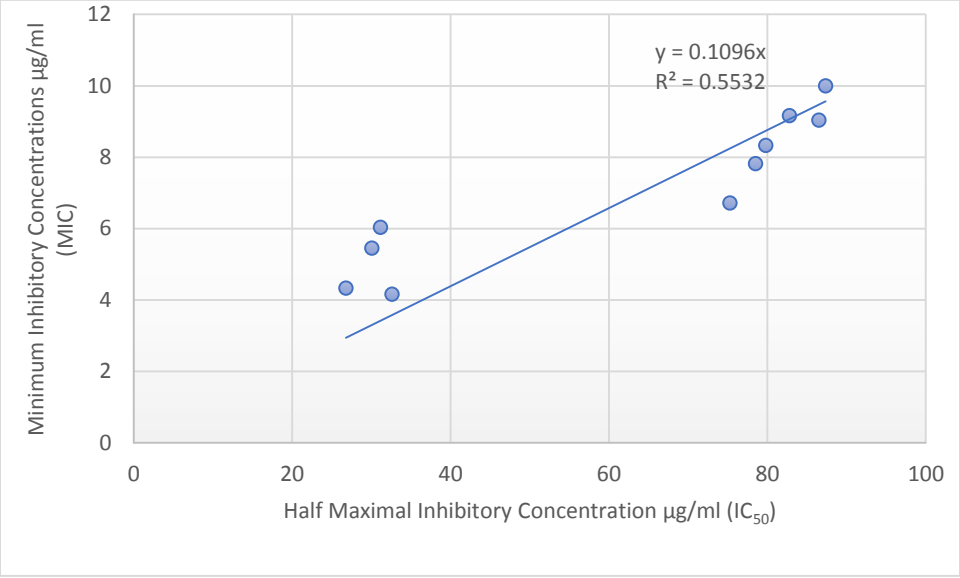


Figure 3: Correlation between Minimum Inhibitory Concentrations (MIC) and Half Maximal Inhibitory Concentrations (IC_{50}) of Antibiotics Against Wild *E. coli*