SCREENING FOR ANTIMICROBIAL, PHYTOCHEMICAL AND ANTIOXIDANT PROFILE OF SOME WILD FRUITS AND SEEDS IN CANAANLAND, OTA, NIGERIA

ABAH, K. A., OWOLABI, A.O. AND ORANUSI, S.*
Department of Biological Sciences, Covenant University, Ota, Nigeria

***Corresponding author**. Email: solomon.oranusi@covenantuniversity.edu.ng

Abstract

The spread of antimicrobial resistance among pathogenic microorganisms has rendered most conventional drugs redundant. New sources of antimicrobial agents are being harnessed to develop novel drugs. The antibacterial activity of aqueous and ethanolic extracts of twenty fruits and seeds were evaluated against some multi-drug resistant clinical isolates Staphylococcus aureus, Salmonella typhii, Pseudomonas aeruginiosa and Escherichia coli. Standard methods of antimicrobial and phytochemical assays, DPPH and nitric oxide radical scavenging, total antioxidant capacity and reducing power assays were employed. Broad spectrum antibacterial activity was shown by extracts of six out of the twenty plants: Caryota mitis, Cassia javanica, Syzygium samarangense, Veitchia merrilli, Bauhinia tomentasa and Cassia fistula with inhibition zones ranging from 20±0.8 to 25±0.1 mm. The minimum inhibitary and bactericidal concentrations of the extracts were between 7.8 to 31.2 mg/ml and 15.6 and 62.5 mg/ml respectively. Phytochemicals present includes saponins, alkalaids, terpenoids, glycosides and high phenol and flavonoid contents in the range of 71.19 - 50.20mg/100g and 47.14 - 34.42mg/100g respectively. The extracts had considerably high antioxidant activity with IC_{50} values of 41.12µg/ml in B. tomentasa and 36.57µg/ml in C. fistula. C. mitis had a total antioxidant capacity of 34.89 mg/100g. High phenol and flavonaid contents of the extracts significantly correlated with high antioxidant capacity. The presence of antimicrobial and antiaxidant praperties in these wild fruits and seeds opens up a new perspective in biotherapy as they could be harnessed as alternative drugs for treatment af micrabial infections and management of diseases.

Keywords: Resistance; Seeds; Fruits; Biotherapy; Antimicrobial; Degenerative diseases

Introduction

Wild plants have been widely employed throughout history as sources of herbal preparations for disease treatment (Cowan, 1999). Ancient man recognized the potential of these plants to protect him from various ailments. Hundreds of these plants have been applied as poultices and infusions for thousands of years and many of them are still in use till date (Corlett, 2016). These plants are a reservoir of antimicrobial agents and have continually served as sources of drugs. Most plant parts including stem, fruit, seed, flower and root have been used for extracts of raw drugs with varied potencies (Mahesh and Satish, 2008). These extracts possess antimicrobial and phytochemical properties of great significance in therapeutic treatments. These properties are conferred on them by the presence of certain compounds most of which are

products of secondary metabolism in the plants. These secondary metabolites have also been identified as the potential sources of antioxidant activity exhibited by some of these medicinal plants (Seifu and Abay, 2012). Research into the activity of these antioxidants is constantly being carried out to determine the role of these compounds as protective dietary constituents. Studies have shown that moderate intake of these plant derived antioxidants over extended periods of time may have positive impact against the occurrence of many chronic ailments (El-Toumy *et al.,* 2011).The presence of these phytochemicals in plant extracts is of great significance in therapeutic treatments. Presently, plants are used in the production of many important drugs. In most industrialized nations, plant derived medicines serve mainly as non-prescription drugs with most laxatives, cold and cough preparations coming from plants (Srivastava *et al.,* 1996). According to the World Health Organization, the primary healthcare needs of about 80% of the population in developing countries are met entirely through folk medicine (WHO, 2002). However, despite this widespread use of plants as sources of drugs, a large number of them are still unexplored.

Antibiotics have been the mainstay of infectious disease therapy since their introduction in the 1940's. However, the emergence of resistance in pathogens such as *Staphylococcus aureus, Streptococcus pyogens* and *Pseudomonas aeruginosa* to many antibiotics since the mid-70's has forced physicians to discard the belief that most bacterial infections can be treated using the available crop of antimicrobial agents (Lowy, 2003). The last few years have seen an increase in an awareness of the different molecular mechanisms by which resistant microorganisms evade drug activity and the identification of new drug targets. This notwithstanding, the race to develop new chemotherapeutic agents that may be effective in the fight against drug resistance hasn't yielded much fruit (Louis and Marie, 2003). In view of this, plants have been identified as sources of antimicrobial compounds that inhibit microbial growth through mechanisms that may differ from those of the antimicrobials in use presently (Oskay *et al.,* 2010). This is predicated by their activity against drug resistant microorganisms. The use of synthetic antioxidants has been continually on the decrease as a result of identified negative health effects, leading to increased interest in antioxidants of natural origin.

Therefore, the absence of effective antimicrobials for management of resistant infections, as well as diseases arising from the proliferation of free radicals, makes it necessary to carry out investigations to discover new plant components of medicinal importance and evaluate their properties, safety and efficiency against pathogens. This work was designed to screen twenty selected wild plant species from Canaan land, Ota; for antibacterial activity against selected drug resistant pathogens and to study phytochemicals and antioxidant properties of the plants.

Materials and Methods

Collection of plant samples

Fruits of twenty different wild plant species were collected between the months of July to December 2016 from around Canaan land, Ota, Ogun State, Nigeria. The fruits were identified by plant taxonomists in the Botany unit of the Department of Biological Sciences, Covenant University, Ota, to include; *Bauhinia tomentoso, Coryota mitis, Adenanthera povonino, Tecoma capensis, Delonix regia, Caesalpinia pulcherrima, Acacia auriculiformis, Veitchia merillii, Terminalia cattapa, Cassia fistula, Ficus benjamino, Cassia javanica, Syzgium samarangense, Tabebuia rosea, Tabebuia pallida, Murraya exotica, Casuarinae quisetifolia, Bauhinia purpurea, Thuja orientalis* and *Calliandra surinamensis*. The fruits were selected if either the fruit or seed are edible or are growing within the residential areas of Covenant University thus can easily be picked by children for play item. The fruits were thoroughly washed in potable tap water and rinsed twice in normal saline. The seeds were separated from fruits aseptically.

Solvent extraction

The fruits and seeds were oven dried at 40°C for 48-72 hours to gain a constant weight and then grinded to powder using a mechanical grinder (VKP 1024B, Victorio, Utah, USA). Extraction of samples was carried out following the methods as described by Majali *et al.* (2015). A 100g of each powdered sample was mixed with 1000ml of distilled water (aqueous extract) and a 100 ml of 75% ethanol (ethanolic extract) and left standing at 25°C for 72 hours with constant agitation at 100 rpm on a laboratory shaker (MaxQ400, Thermo Scientific, Massachusetts, USA). The solvent extracts were concentrated in a rotary evaporator (RE300B, Stuart, Staffordshire, UK) and left to dry completely. Extracts were weighed and stored at 5°C in air-tight sterile screw capped bottles until further analysis.

Collection and identification of test organisms

Cultures of *Stophylococcus aureus, Salmonella typhi, Pseudomonas oeruginosa and Escherichia coli was obtained from stock cultures of the* Department of Microbiology, Covenant University. The purity and identity of the organisms was confirmed using cultural, microscopic and basic biochemical tests. The antibiotics resistant profile of the test organisms as provided from the culture collection was confirmed using agar well diffusion method, following the description by Cheesbrough, (2006).

Antimicrobial sensitivity assay

The antimicrobial sensitivity assay was performed using agar well diffusion method as described by Cheesbrough, (2006). Gentamicin sensitivity discs (10µg; Rapid Labs Ltd., UK) and DMSO were used as the positive and negative controls respectively.MuellerHinton agar was seeded with bacterial suspensions prepared from fresh cultures of the isolates and standardized to 0.5 scale of McFarland standard ($1.5x10^8$cells/ml). Crude extracts were introduced into uniform wells of 6mm diameter cut on the surface of the agar using

a standard sterile cork borer. The plates were allowed to stand for 1 hour to allow diffusion of the extracts then incubated at 37°C for 18-24 hours Plates were observed for zones of inhibition and zone diameter were measured and recorded in millimeter (mm). Each test was carried out in triplicates.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of the extracts was determined using the tube broth dilution method as described by Mahesh and Satish, (2008).Six concentrations (125, 62.5, 31.2, 15.6, 7.8, 3.9mg/ml) of the extracts were prepared and inoculated with 0.2 ml each of the overnight grown test organisms. The tubes were incubated at 37°C for 24 hours, and the lowest concentration which showed no turbidity was recorded the as the MIC. Tubes that showed no growth during MIC test were selected for MBC determination. Broth from each tube was sub-cultured onto extract-free Müellar-Hinton agar plates and incubated for another 24 hours at 37°C. The lowest concentration that did not produce a single bacterial colony was recorded as the MBC (Ajiboye *et al.,* 2015).

Phytochemical screening

Qualitative phytochemical analysis of the extracts for flavonoids, terpernoids, phenols, tannins, saponins, combine anthraquinone, steroids, alkaloids, phlebotanins and cardiac glycosides was carried out using standard procedures as described by Harborne (1973); Trease and Evans (1989); Sofowora (1993).

Determination of total phenol content

The Folin-ciocalteu method as described by Alothman *et al.,* (2009) with modifications was used. One ml of the extract were mixed with 0.4 ml folin–Ciocalteu reagent and left standing for 5 minutes at room temperature, 4 ml of 7.5% sodium carbonate solution and distilled water was added. The solution was mixed and allowed to stand for 45 minutes and the absorbance was read at 750nm using Spectrophotometer (Model M106, Spectronic Campsec, Leeds, UK). Gallic acid was used as the standard. Results were expressed as mg gallic acid equivalents/100 g of sample.

Determination of total flavonoids

The method of Zhishen *et al.,* (1999) was used following the descriptions of Oranusi *et al.,* (2013). 1ml of the extract was added to 4ml of distilled water, followed by 0.3ml of 5% sodium nitrite and 0.3ml of 10% aluminum chloride. The solution was incubated at room temperature for 5min, 2ml of 1M sodium hydroxide was added followed immediately by the addition of 2.4 ml of distilled water to make up the volume to 10 ml and vortex thoroughly. Absorbance was read at 570nm. The results were expressed as mg catechin equivalents/100 g of sample.

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The method of Alothman *et al.,* (2009) was used with modifications. One ml of extract was added to 2ml of 1M methanolic solution of DPPH. The mixture was vortex-mixed and incubated for 30 minutes at 37°C in the dark. The absorbance was

measured at 571 nm, against a blank of methanol without DPPH. The percentage of the DPPH radical scavenging of the extracts and the standard was calculated.

Nitric oxide scavenging activity

The Nitric oxide scavenging activity of the extracts was determined using the method of Chanda and Dave (2009) with modification. Aliquot 2ml of 10 mM sodium nitroprusside and 0.5ml of phosphate buffer was added to 2.0 ml of extract. The solution was then incubated at 25°C for 150 minutes. 0.5ml of Grease reagent (1% sulphanilamide, 0.1% naphthyethylenediamine dihydrochloride in 2% H3PO3) was added to the solution and incubated again for 30minutes and absorbance was read at 540nm. A similar procedure is repeated with methanol as blank, which serves as control. Percentage of inhibition of the Nitric oxide was calculated.

Reducing power activity

The reducing power of the extracts was determined by the method of Athukorala *et al.* (2006) with modifications. Aliquot 1.0 ml extract was mixed with 1ml of distilled water, 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide and incubated for 20 min at 50°C. After incubation, 2.5 ml of trichloroacetic acid was added. The reaction mixture was centrifuged for 10 min at 3000 rpm, 2.5 ml of the supernatant was added to 2.5 ml of distilled water and 0.5 ml of FeCl3. Absorbance was read at 700 nm against blank. Ascorbic acid was used as standard.

Statistics

Statistical Package for Social Sciences (SP5S), version 13, was used for the data analyses. Results were expressed as the Mean ± SD and tests of statistical significance were carried out using one-way analysis of variance (ANOVA). Statistical significance was defined as $P<0.05$.

Results

The results of the antimicrobial sensitivity assay is shown in Tables 1 and 2.Table 1 reveals that the ethanol extracts of six plants (two fruit and four seed extracts) exhibited effective inhibition against the test isolates. The broadest inhibition diameter of 25±0.1mm was shown by the seed extract of *S. samarangense* and fruit extract of *C. fistula* against *S. aureus* respectively. Table 2 shows that the aqueous plant extracts had no significant inhibitory effect against the test isolates. The gentamicin antibiotic disc showed 28±0.8mm, 25±0.6mm, 21±0.1mm and 24±0.5mm diameter inhibition zones against *S. aureus, E. coli, S. typhii* and *P. aeruginosa* respectively which are not significantly different from the results obtained against *S. aureus* with the ethanol extracts. Tables 3 show the results of the MIC and MBC determinations. *S. samarangese, C. fistula and V. merrilli* extracts had an MIC of 7.8mg/ml for *S. aureus, P.aeruginosa* and *E. coli.* Table 4 shows the results of qualitative phytochemical screening of the extracts. Results revealed the presence of phenols, terpenoids, tannin and flavonoids in all the extracts. The table also shows the total phenol content in milligram gallic acid equivalent/100 g extract. *S. samarangense* and *C. fistula* extracts had higher phenol

contents compared to the other extracts. Table 4 reveals the total flavonoid content in milligram catechin equivalents/100 g of sample *C. fistula* (fruit) and *C. mitis* (fruit) had higher flavonoid contents. The DPPH radical scavenging activity of the extracts expressed as % inhibition is shown in Figure 1. All the extracts has good DPPH radical scavenging activity, however, *V. merrilli, B. tomentosa* and *C. fistula* had better activity. Figure 2 shows the scavenging of nitrogen oxide values as % inhibition by the extracts. It reveals varying degrees of scavenging capacities. *V. merrilli, B. tomentosa and C. fistula* extracts had the highest radical scavenging activity. The lowest activity was shown by the *C. javanica* extract. The Reducing power activity of the extracts is shown in Figure 3. *C. javanica, S. samarangense* and *C. mitis* had the highest reducing power activity compared to the other extracts.

Discussion

The results of this study show that the fruit and seed extract of most of the plants exhibited antimicrobial activity against the test isolates. Six of the 20 plants extracts namely *V. merrilli* (seed), *B. tomentosa* (seed), *C. fistula* (fruit), *C. mitis* (seed), *C. javanica* (fruit) and *S. samarangense* (seed) exhibited broad spectrum antibacterial activity and significant inhibition compared to the positive control antibiotic used. On the other hand *F. benjamina, M. exotica, C. equisetifolia, T. orientalis* and *B. purpureo* were the least active plants showing little or no antibacterial activity. The sensitivity of the test isolates to the extracts was in the order *S. aureus* > *E. coli* > *P. aeruginosa* > *S. typhii*. *S. samarangese* seed extract had the highest inhibitory activity in relation to the other extracts. Venkata *et al.* (2008) reported that ethanol, methanol and water extracts of *S. samarangense* fruit exhibited high inhibitory activity against both the gram positive and gram negative bacteria. Oyinlade (2014) reported the presence of bioactive compounds in extracts of *S. samarangese* that confer antimicrobial properties on these plants. Pawar *et al.* (2015) also reported that bark extracts of *S. samarangense* had activity against some pathogenic bacteria. The antibacterial activity of *S. samarangense* extract reported in this work could be due to the high number of phytochemicals present. *B. tomentosa* extract in this report, showed reasonable inhibitory activity. Various extracts of this plant have reportedly shown antimicrobial activity against the test isolates. It has been reported that *B. tomentosa* aqueous extracts showed potent inhibitory effect against the test isolates compared to the ethanol extracts .This is contrary to findings in this study where *B. tomentosa* aqueous extract showed little inhibitory effect. This variation may be attributed to environmental factors (Govindan and Muthukrishnan, 2013).

The result for the phytochemical screening showed that the plants extracts contain some phytochemical compounds possessing good antimicrobial properties. Analysis of the plant extracts indicated flavonoids, phenols, saponin, tannin, alkaloids and terpenoids as being present. Although mechanism by which phytochemicals inhibit bacteria have not been fully understood, it is inferred that they probably bring about their

antibacterial activity through one of many mechanisms which include protein synthesis inhibition, disruption of nucleic acid synthesis, blockage of cell wall synthesis or lysis of microbial cells (Khoo *et al.,* 2016).

Alkaloids possess a number of biological activities and exhibit strong antibacterial potentials. They have been known to act as drug precursors and are employed as an anti-hypertensive. Tannins have the ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic enzymes (Zohra *et al.,* 2012).Saponins are used in the management of conditions like hypercholesterolemia and hyperglycemia, they also exhibit antioxidant and anti-inflammatory activity (Oyinlade, 2014). The existence of these phytochemicals in the extracts provides a basis for further development of these plants and their products as antimicrobial agents to be used in diseases therapy.

The total phenol and flavonoid contents of the selected plant extracts was between 71.19±0.2 and 50.02±0.6mg/100g. *S.samarangense* seed extract had the highest total phenol content. This corroborates findings by Khandaker *et al.,* (2015) who reported that the bark, fruit and seed extracts of *S. samarangense* possess very high phenolic content. The *C. fistula* and *C. javanica* extracts also showed high phenolic contents compared to the other extracts, which corroborate the findings of Bhalerao and Kelkar (2012). The flavonoid contents of the extracts in terms of the cathecin equivalent were between 34.42 and 47.14 mg/100g. *C. fistula* had the highest flavonoid content. This corresponds to the findings by Kumar *et al.,* (2008). *V. merrilli* extracts had the lowest flavonoid content. Studies have shown a correlation between total flavonoid and total phenol contents of plant extracts and free radicals scavenging activity whereby scavenging activity tends to increase as total phenol and total flavonoids increases (Khandaker *et al.,* 2015). Zohra *et al.,* (2012) reported that phenolics and flavonoids retain significant antioxidant and antimicrobial activities.

Results of this study show that the antioxidant activity by DPPH assay were in the order of *B. tomentosa* > *V.merrilli* > *C. fistula* > *S. samarangense* > *C. mitis* > *C. javanica*. DPPH scavenging assay method is an easy, rapid and sensitive method for antioxidants in plant extracts (Aliyu *et al.,* 2013).

The reduction of the radical to give a stable un-reactive diamagnetic molecule forms the premise on which this assay is based, a process which may be mediated by phenolic acids (Oranusi *et al.,* 2013). Govindan and Muthukrishnan (2013) reported that high levels of phenol compounds in plant extracts are related to increased radical scavenging activity for DPPH. Our results are on par with those findings as *B. tomentosa, V. merrilli and S. samarangense* extracts were shown to possess high a content of phenolics.

The results of this report showed that nitric oxide inhibition of the plant extracts was between 21.67 and 84.60µg/ml. *V. merrilli S, B. tomentosa* and *C. fistula* had the highest inhibition of all the extracts. *S. samarangense* extract also had high NO scavenging activity when compared with the other extracts. Pawar *et al.,* (2015) also reported that *S. samarangense* possess good NO scavenging activity. At high tissue levels, nitric oxide has been implicated as a causal agent of carcinoma and ulcerative colitis (Govindan and Muthukrishnan, 2013). These plants and their products have the potential to negate the deleterious effects of nitrite formation. This is very promising as regards management of *in vivo* NO generation.

The order of the reducing power activity of the extracts was*: S. samarangense* > *C.javanica*> *C. mitis* > *V. merrilli* > *B. tomentosa* > *C. fistula*. The high reducing power observed in *S. samarangense* extract may be due to the presence of strong reducing agents such as reductones in its structure which have strong antioxidant activity. Reduction cleaves the free radical chain and donates a hydrogen atom to bring about their antioxidant effect (Kumaran and Karunakaran, 2006). The reducing power of *S. samarangense*, *C. javanica* and *C. mitis* where not significantly differed from that of ascorbic acid, the reference compound.

Conclusion

In conclusion, most of the wild plants screened had varying degrees of antibacterial activity against the test isolates. However six extracts showed significant activity compared to the standard control. Qualitative phytochemicals analysis of these extracts indicated a large number of active compounds distributed among the extracts which may be mediators of the positive antibacterial activity observed in the extracts. The significant number of phenolic compounds and flavonoids extracted from the plants extracts is an indicator of antioxidant activity. This was confirmed in the series of antioxidant assays carried out on the extracts which indicated very high activity. This is of great importance because of the associated health benefits of natural antioxidants as regards scavenging for free radicals. Results from this study have established plant phenol and flavonoid contents bear a positive correlation with any potential antioxidant activity that that plant species may possess. In this regard *S. samarangense, B. tomentosa, C. fistula, C.mitis, V. merrilli* and *C. javanica* have emerged as promising sources of antimicrobial and antioxidant agents that could be investigated further in the development of new drugs.

Table1: *In vitro* antibacterial activity of ethanol plant extracts against clinical isolates

Samples	Zone of inhibition (mm)* for ethanol plant extracts (1000mg/ml)							
	S. aureus		*S. typhi*		*P. aeruginosa*		*E. coli*	
	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds
Bauhinia tamentalis	14±0.4^{c}	24±0.1^{a}	12±1.2^{c}	20±0.8^{d}	11±1.2^{bc}	19±0.4^{d}	15±0.5^{d}	23±1.4^{de}
Caryatamitis	13±1.3^{c}	23±0.3^{e}	11±1.0^{c}	17±0.4^{c}	12±0.3^{c}	18±0.7^{d}	16±0.2^{d}	23±0.9^{de}
Adenantherapavonina	13±0.9^{c}	23±0.6^{e}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	8±0.3^{b}	7±0^{b}
Tecomacapensis	8±0.2^{b}	7±0^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Delanixregia	13±0.1^{c}	16±0.5^{d}	9±0.4^{bc}	15±1.3^{c}	10±0.5^{bc}	12±1.3^{c}	0±0^{a}	9±1.1^{b}
Caesalpiniapulcherrima	9±0.5^{b}	11±1.2^{bc}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	8±0.6^{b}	8±0.4^{b}
Acacia auriculiformis	10±1.3^{bc}	9±0.2^{b}	0±0^{a}	0±0^{a}	8±0.2^{b}	7±1.3^{b}	0±0^{a}	0±0^{a}
Veitchiamerillii	16±0.6^{cd}	23±0.6^{e}	9±0.7^{bc}	17±1.1^{c}	0±0^{a}	16±0.6^{d}	11±0.3^{bc}	21±0.3^{d}
Terminaliacattapa	0±0^{a}	0±0^{a}	0±0^{a}	6±0.9^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Cassia fistula	25±0.1^{e}	17±0.6^{d}	18±0.2^{d}	10±0.3^{b}	20±1.4^{d}	11±0.3^{bc}	24±0.2^{e}	14±0.9^{c}
Ficusbenjamina	0±0^{a}	6±0^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Cassia javanica	20±0.9^{d}	11±0.5^{bc}	9±1.4^{bc}	16±0.5^{c}	17±0.9^{d}	8±0.2^{b}	23±0.9^{e}	12±1.1^{c}
Syzgiumsamarangense	16±0.2^{cd}	25±0.1^{e}	10±0.3^{bc}	18±1.2^{c}	11±0.3^{b}	23±1.2^{de}	9±0.3^{b}	21±1.2^{d}
Tabebuirosea	9±0.3^{b}	9±0.8^{b}	0±0^{a}	0±0^{a}	7±0.8^{b}	8±0.5^{b}	7±0.5^{b}	7±0.5^{b}
Tabebuipallida	14±1.4^{c}	12±0.6^{bc}	0±0^{a}	9±1.2^{b}	10±1.3^{b}	8±0.1^{b}	12±0.1^{c}	11±0.4^{bc}
Murraya exotica	7±0.1^{b}	9±0.2^{b}	0±0^{a}	0±0^{a}	0±0^{a}	7±0.4^{b}	0±0^{a}	8±0.5^{b}
Casuarinaequisetifolia	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Bauhinia purpurea	7±0.3^{b}	7±1.2^{b}	7±1.1^{b}	7±1.2^{b}	9±0.2^{b}	8±1.5^{b}	7±0.2^{b}	7±0.3^{b}
Thurjaorientolis	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	8±0.3^{b}	0±0^{a}	0±0^{a}	0±0^{a}
Gentamicin10µg	28±0.8^{e}		25±0.6^{e}		21±0.1^{d}		26±0.5^{e}	

Values are mean±SD of three replicates; Super script abcde= values with different superscript f or the same sample (within the same column) are significantly different

Table2: *In vitro* antibacterial activity of aqueous plant extracts against clinical isolates

Extracts	Zone of inhibition (mm)* for aqueous plant extracts (1000mg/ml)							
	S. aureus		*S. typhi*		*P. aeruginosa*		*E. coli*	
	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds
Bauhinia tomentalis	9±1.2^{bc}	13±0.4^{c}	9±0.3^{bc}	11±0.2^{c}	7±0.5^{a}	10±1.1^{c}	0±0^{a}	13±1.2^{c}
Coryotamitis	7±0.6^{b}	11±1.3^{c}	0±0^{a}	7±0.6^{b}	7±0.2^{b}	9±0.4^{bc}	0±0^{a}	10±0.2^{c}
Adenontherapavonina	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Tecomacapensis	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	7±0.4^{b}	0±0^{a}
Delonixregia	0±0^{a}	0±0^{a}	7±0.4^{b}	7±0.6^{b}	9±0.8^{bc}	0±0^{a}	0±0^{a}	7±1.2^{b}
Caesalpiniapulcherrima	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Acacia auriculiformis	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	8±1.2^{b}	7±0.3^{b}
Veitchiamerillii	9±1.2^{bc}	12±0.3^{c}	0±0^{a}	9±0.1^{bc}	9±1.1^{bc}	11±1.3^{c}	0±0^{a}	13±1.4^{c}
Terminaliacatta pa	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	11±1.3^{c}	0±0^{a}
Cassia fistula	10±1.2^{c}	7±0.4^{b}	11±1.3^{c}	0±0^{a}	12±1.3^{c}	9±0.2^{bc}	6±0^{b}	8±0.5^{b}
Ficusbenjamina	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	9±0.2^{bc}	7±0.1^{b}
Cassia javanica	12±1.2^{c}	8±0.5^{b}	9±0.1^{bc}	0±0^{a}	10±0.2^{c}	7±0.4^{a}	0±0^{a}	0±0^{a}
Syzgiumsamarangense	7±0.3^{b}	11±0.5^{c}	0±0^{a}	9±0.5^{bc}	0±0^{a}	10±0.2^{c}	0±0^{a}	9±1.4^{bc}
Tabebuirosea	7±0.4^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Tabebuipallida	7±0.4^{b}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Murraya exotica	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Casuarinaequisetifolia	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Bauhinia purpurea	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Thurjaorientalis	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
Gentamicin10µg	28±0.8^{d}		25±0.6^{d}		21±1.0^{d}		26±0.5^{d}	

Values are mean±SD of three replicates; Super script abcde= values with different superscript for the same sample (within the same column) are significantly different

Table 3: Minimum inhibitory and bactericidal concentration of plant extrac ts

Extracts	MIC (mg/ml)				MBC (mg/ml)			
	S. aureus	*S. typhii*	*P. aeruginosa*	*E. coli*	*S. aureus*	*S. typhii*	*P. aeruginosa*	*E. coli*
V. merrilli (seed)	7.8^{a}	15.6^{a}	31.2^{c}	15.6^{b}	15.6^{a}	31.2^{a}	62.5^{c}	62.5^{c}
B. tomentosa (seed)	7.8^{a}	15.6^{a}	15.6^{b}	7.8^{a}	15.6^{a}	31.2^{a}	62.5^{c}	15.6^{a}
C. fistula (fruit)	7.8^{a}	15.6^{a}	15.6^{b}	7.8^{a}	15.6^{a}	62.5^{b}	31.2^{b}	15.6^{a}
C. mitis (seed)	7.8^{a}	31.2^{b}	31.2^{c}	7.8^{a}	15.6^{a}	125^{c}	62.5^{c}	15.6^{a}
C. javanica (fruit)	15.6^{b}	31.2^{b}	31.2^{c}	7.8^{a}	31.2^{b}	125^{c}	62.5^{c}	31.2^{b}
S. samarangense (seed)	7.8^{a}	15.6^{a}	7.8^{a}	7.8^{a}	15.6^{a}	31.2^{a}	15.6^{a}	15.6^{a}

Super script abc= values with different superscript and within the same column are significantly different

Table 4: Qualitative and quantitative phytochemical screening of plant extracts

Phytochemical tests	Extracts					
	V. merrilli (seed)	*B. tomentosa* (seed)	*C. fistula* (fruit)	*C. mitis* (fruit)	*C. javanica* (fruit)	*S. samarangense* (seed)
Terpenoid	+	+	+	+	+	+
Phenol	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Steroid	+	+	-	+	+	+
Cardiac Glycosides	-	+	-	+	+	+
Combine Anthraquinone	-	+	+	+	+	+
Saponin	-	+	-	+	+	+
Phlebotanin	-	+	+	+	+	-
Alkaloid	+	-	-	+	+	-
Total phenol content (mg/100g)	51.60±0.3	50.20±0.6	61.83±0.9	52.46±1.3	69.56±0.8	71.19±0.2
Total Flavonoid content (mg/100g)	34.42±0.5	42.23±0.6	47.14±0.9	47.14±0.8	41.78±1.2	44.60±0.6

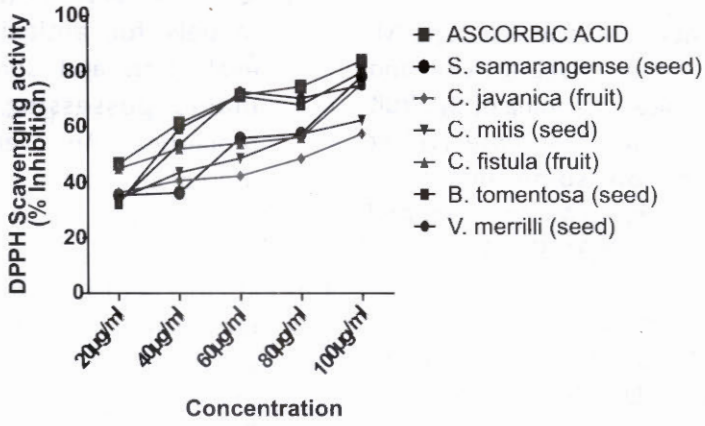

Figure 1: DPPH % inhibition in different concentrations of plant extracts

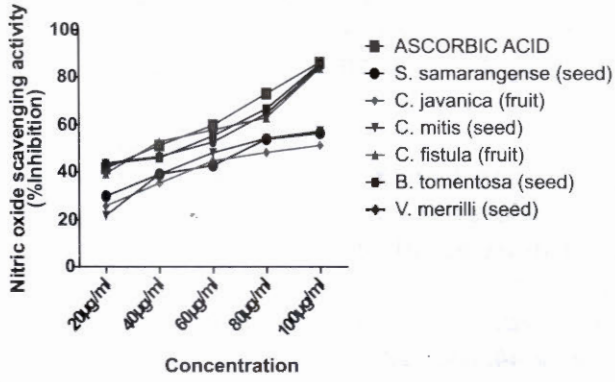

Figure 2: Nitric oxide % inhibition in different concentrations of plant extracts

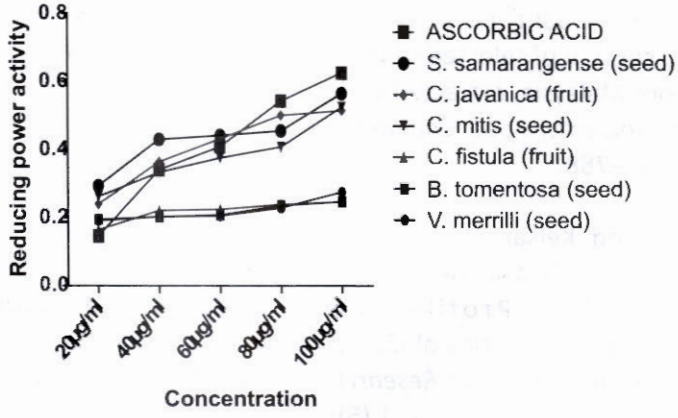

Figure 3: Reducing power activity of plant extracts.

References

Ajiboye, A., Ameen, M. and Adedayo, M. (2015). Antimicrobial activity and phytochemical screening of the fruit pulp of *Dialium guineense* (Velvet Tamarind) on some microbial isolates. *Journal of Microbiology and Antimicrobiols*. **7** (7): 33–41.

Aliyu, A. B., Ibrahim, M. A., Musa, A. M., Musa, A. O., Kiplimo, J. J.and Oyewale, A. O. (2013). Free radical scavenging and total antioxidant capacity of root extracts of *Anchomanes difformis* Engl. (*Araceae*). *Acta Poloniae Pharmaceutica - Drug Research*. **70** (1): 115–121.

Athukorala Y., Kim, K. N. and Jeon, Y. J. (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga *Ecklonia cava*. *Food and Chemical Toxicology*. **44**: 1065-1074.

Alothman, M., Bhat, R. and Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry,* **115** (3): 785–788.

Bhalerao S. A, and Kelkar T. S. (2012). Traditional Medicinal Uses, Phytochemical Profile and Pharmacological Activities of *Cassia fistula* Linn. *International Reseorch Journal of Biological Sciences*. **1** (5): 2278–3202.

Chanda, S. and Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties : An overview. *African Journal of Microbiology Research*. ***3*** (13): 981–996.

Cheesbrough, M. (2006). District laboratory practice in tropical countries. District laboratory practice in tropical countries Part 2 (Vol. Part 1).

Corlett, R. (2016). Plant diversity in a changing world. *Plant Diversity*, **1**: 11–18.

Cowan, M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. ***12*** (4): 564–82.

El-Toumy, S. A., Mohamed, S. M., Hassan, M. S. and Mossa, A, M. (2011). Phenolic metabolites from *Acacia nilotica* flowers and evaluation of its free radical scavenging activity. Journal of American Science. **7**(3): 287-295.

Govindan, P. and Muthukrishnan, S. (2013). Evaluation of total phenolic content and free radical scavenging activity of *Boerhavia erecta*. *Journal of Acute Medicine*. **3** (3): 103–109.

Harborne, J. B. (1973). Phytochemical methods. Chapman and Hall Ltd, London. pp 49-188

Kumar, A. and Schweizer, H. P. (2005). Bacterial resistance to antibiotics: Active efflux and reduced uptake. *Advanced Drug Delivery Reviews,* **57**: 1486–1513.

Kumaran, A., and Joel Karunakaran, R. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus. Food Chemistry,* **97**(1): 109–114

Louis, S. and Marie, A. (2003). European antimicrobial resistance surveillance as part of a community strategy. University of Groningen/UMCG research database.

Lowy, F. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus. Journal of Clinical Investigation,* **111**(9): 1265–1273.

Khandaker, M. M., Sarwar M. J. and Mat, N. (2015). Bioactive constituents , antioxidant and antimicrobial activities of three cultivars of wax apple (*Syzygium samarangense* L.) fruits. *Research Journal of Biotechnology,* **10**(1): 435-441.

Khoo, H. E., Azlan, A., Kong, K. W. and Ismail, A. (2016). Phytochemicals and medicinal properties of indigenous tropical fruits with potential for commercial development. *Evidence-Based Complementary and Alternative Medicine.*

Kumar, A. and Schweizer, H. P. (2005). Bacterial resistance to antibiotics: Active efflux and reduced uptake. *Advanced Drug Delivery Reviews,* **57**: 1486–1513.

Kumaran, A. and Joel Karunakaran, R. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus. Food Chemistry,* **97**(1): 109–114.

Mahesh, B. and Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. *World Journal of Agricultural Sciences,* **4**: 839–843.

Oranusi, S., Nwachukwu, C., Temitope, A. B., Samuel, D. O. and Oladipupo, A. A. (2013). Microbial profile, antibacterial and antioxidant activities of some imported spices in Nigeria, **3**(6): 193–202.

Oskay, M., Oskay, D., and Kalyoncu, F. (2010). Activity of some plant extracts against multi-drug resistant human pathogens. *Iranian Journal of Pharmaceutical Research,* **8**(5): 293–300.

Oyinlade, O. C. (2014). Phytochemical and physicochemical analysis of three different types of apples. *International Journal of Scientific Research and Reviews,* **3**(3): 67–78.

Pawar, M. M., Patil, S. D., Jadhav, A. P., Kadam, V. J., and Jadhav, A. P. (2015). *In vitro* antimicrobial activity and phytochemial screening of selected indian medicinal plants. *Indo American Journal of Pharmaceutical Research.* Retrievedfrom http://www.ejmanager.com/mnstemps/36/36-1424512720.pdf

Seifu, D., Assefa, F. and Abay, S. M. (2010). Understanding their mechanism of action and therapeutic efficacy. Retrieved from http://trnres.com/ebook/uploads/capassocontent/T_13599719966Capasso.pdf

Sofowora, A. (1993). Plants and Traditional Medicine in Africa (2nd edn.). Spectrum books Ltd, Ibadan, Nigeria. pp 9-25.

Srivastava, J., Lambert, J., Vietmeyer, N. and Mei, I. (1996). Medicinal Plants: An expanding role in development. Perspective. Retrieved from http://documents.worldbank.org/curated/en/293821468

Venkata, R. R. R., Ratnam, K. V. and Venkata P. R. (2008). In vitro Antimicrobial Screening of the Fruit Extracts of Two Syzygium Species (Myrtaceae). *Advances in Biological Research,* **2** (12): 17–20.

Trease, G. E., and Evans, W. E. (1989). Pharmacognosy (11th edn.). Braille Tirida Canada Macmillan publisbers, Canada. pp 257.

World Health Organization (WHO). (2002). WHO Traditional Medicine Strategy 2002-2005. *World Health Organisation Geneva,* 1–74. https://doi.org/WHO/EDM/TRM/2002.1

Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry,* **64**: 555–559.

Zohra, S. F., Meriem, B., Samira, S.and Alsayadi Muneer, M. S. (2012). Phytochemical Screening and Identification of Some Compounds from Mallow. *Journal of Natural Plant Products Resources,* **2**(4): 512–516.