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Full Length Research Paper

Effect of *Maytenus senegalensis* roots on OVA-induced airway inflammation in a mouse asthma model

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The present work is focused on the evaluation of the *Maytenus senegalensis* roots effect on bronchial hyperleukocytosis and the search of its antioxidant potential both *in vitro* and *in vivo*. The roots of *M. senegalensis* (Celastraceae) are traditionally used for the treatment of cough and asthma. This study evaluates the effect of the hydroalcoholic extract of the roots of *M. senegalensis* on the bronchial hyperleukocytosis that occur during airway inflammation and determine its antioxidant capacity. In an eight days mouse asthma model sensitized to ovalbumin, the effect of the hydroethanolic extract of *M. senegalensis* on infiltration of leukocytes in general and eosinophils particularly in the airways was studied. The antioxidant activity of the extract was evaluated *in vitro* by the tests of DPPH, nitric oxide and AAPH then *in vivo* by the malondialdehyde dosage. The extract significantly inhibited bronchial infiltration of leukocytes in general, and eosinophils particularly (p<0.01; p<0.001). *In vitro* and *in vivo*, antioxidant tests revealed the reducing effect and the inhibitory of membrane lipoperoxidation potential of the extract. Phytochemical tests have shown that the extract contains polyphenols such as flavonoids. These compounds would be partly responsible for the antioxidant and anti-inflammatory activities of the extract. This study suggest that *M. senegalensis* roots would have an anti-inflammatory effect in asthma, which would be partially related to its antioxidant potential.

Key words: *Maytenus senegalensis*, asthma, bronchial inflammation, antioxidant.

INTRODUCTION

Asthma is one of the most common chronic respiratory diseases, which affect people of all ages around the world (Cukic et al., 2012). It is a major public health problem since it is one of the leading causes of hospitalization and death in the world. Approximately 339

million people worldwide develop this disease (The Global asthma report, 2018).

Asthma is defined as a heterogeneous disease characterized by chronic bronchial inflammation, airway hyper responsiveness leading to exaggerated bronchial

smooth muscle contraction and mucus hypersecretion followed by enlarged mucus glands and reversible obstruction of bronchi (Pynn et al., 2012). According to Holgate et al. (2010), airway hyper responsiveness and bronchial obstruction are consequences of bronchial inflammation. In allergic asthma, inflammation is mainly orchestrated by Th2 lymphocytes and is characterized by an infiltration into the airways of leucocytes mainly eosinophils (Lambrecht et al., 2015). Eosinophils are the main effector cells in asthma. By releasing the contents of their granules, they cause epithelial damage, bronchial hyperreactivity, bronchospasm, activation of mast cells and increased vascular permeability (Gleich, 2000; Kay, 2005).

During inflammation in asthma, activated inflammatory cells of the airway excessively produce free radicals in response to the allergen. These free radicals cause in the airways several deleterious effects such as lipid peroxidation (Henricks and Nijkamp, 2001). They also enhance the expression of pro-inflammatory mediators and cytokines and thus the recruitment of leukocytes (Rangasamy et al., 2005); increase of contractile response of bronchial smooth muscle to acetylcholine (Barnes, 1990), bronchial hyperreactivity and mucus hypersecretion (Wood et al., 2003). Damage caused by theses radicals can lead to denaturation of bronchial epithelium and amplification of airway inflammation.

The existing asthma treatments (anti-inflammatories, bronchodilators) control only the symptoms of the disease and this conducts to long-term treatment. The high cost of the treatments weakening the economic situation and their side effects lead to the research of new therapies by people. Nowadays, using medicinal plants has become an attractive option, which populations are turning towards (Park et al., 2011).

Maytenus senegalensis (Lam.) Exell (Celastraceae) is a plant widely used in traditional medicine. Synonym of *Gymnosporia senegalensis* (Lam.) Loes, the plant is commonly called Confetti tree in English. It is known for its antiplasmodial (El Tahir et al., 1999; Malebo et al., 2015); analgesic (Sanogo et al., 2006); anthelmintic (Zangueu et al., 2018) and antibacterial properties (Lindsey et al., 2006, Jain et al., 2008). Its leaves and roots have been shown to possess anti-inflammatory activity *in vitro* and in eodema models induced by carrageenan or croton oil (Sosa et al., 2007; da Silva et al., 2011; Makgatho et al., 2018). Missebukpo et al. (2007) have also demonstrated its antitussive effect. There are no studies performed to evaluate the effect of *M. senegalensis* on airways inflammation that occurs in asthma.

The present study investigated the effect of the hydroetanolic extract of *Maytenus senegalensis* on bronchial inflammation exactly leukocyte infiltration in a murine model of asthma and then analyzed the antioxidant power of the extract.

MATERIALS AND METHODS

Plant material

The roots of *M. senegalensis* were harvested in August 2016 in Tsévié-Boloumondji, an area situated at 35 km at the northern part of Lomé. A specimen is authenticated by the Department of Ecology and Botany of the University of Lome. It is then deposited in the herbarium of the University of Lome (TG 15182). The extraction process is the one used by Metowogo et al. (2011). The roots were washed, cut, dried and crushed. The powder was macerated in a mixture of distilled water / ethanol (1: 1) for 72 h with intermittent manual stirring. The macerate obtained is filtered and then evaporated under vacuum at 45°C using a rotary evaporator (Buchi R120) until a dry extract (8.55% of yield).

Animals

Thirty ICR mice of both sexes weighing between 20 and 25 g from the animal house of the Department of Animal Physiology at the University of Lomé were used for this study. These mice were kept in chamber under a 12 h light / 12 h dark cycle and had free access to food and water. All the tests were carried out following the rules of WHO Guidelines for the care and use of human blood and laboratory animals and were approved by the national bioethics committee of University of Lomé-Togo.

EXPERIMENTAL PROTOCOL

Sensitization, challenge and treatment procedure

The sensitization of the mice was performed according to the method used by Metowogo (2010). Six groups of 5 mice were constituted. On days 0, 1 and 2, all the mice were sensitized by an intraperitoneal injection of 100 µl of a mixture of ovalbumin and aluminum hydroxide dissolved in 0.9% NaCl. On days 5, 6 and 7, the mice received by intranasal route ovalbumin (12,5 µl) for challenge except the control group who received NaCl 0.9%. Two hours earlier, the mice were treated in the following manner.

(i) Groups 1 (control) and 2 (untreated sensitized): the animals received orally distilled water.
(ii) Groups 3, 4 and 5 (sensitized and treated with the extract): the mice of these lots received the extract by single dose gavage respectively at 125, 250 and 500 mg/kg.
(iii) Group 6 (treated with the reference drug): this batch received cetirizine at 10 mg / kg.

Bronchoalveolar lavage and cell count

On day 8, 18 to 24 h after the last ovalbumin administration, all the mice were sacrificed and their trachea intubated. Using a syringe connected to the catheter, 4 ml of 0.9% NaCl was introduced into the lungs and then aspirated back into the syringe. For each mouse, a part of the resulting liquid (bronchoalveolar fluid, BAL) is taken under the microscope for counting total leukocytes using the Malassez cell; the rest is centrifuged at 700g for 10 min and the cells stained with May-Grunwald-Giemsa. The percentage of eosinophils was established after reading the stained slides based on morphological criteria (Missebukpo et al., 2011).

Evaluation of the antioxidant activity of the extract of *M. senegalensis*

DPPH test

The test has been done according to the method used by missebukpo et al., (2013) with some modifications. To DPPH (100 µmol/L), extract's dilutions (62.5 to 500 µg / ml) were added for search its DPPH reduction effect. Ascorbic acid was used as standard control. For each concentration 3 trials were made. The control consists of a mixture of DPPH and methanol. Absorbances were read at 517 nm After 15 min incubation. The percentage of inhibition (PI = (A. control - A. sample / A. control) × 100) of each sample was calculated A = absorbance.

Nitric oxide assay

The nitric oxide scavenging effect of the extract was searched with the method used by Jagetia and Baliga (2004). A solution of sodium nitroprusside 5mM was added to dilutions of the extract and ascorbic acid (50, 100, 200 and 400 µg / ml). After 150 min of incubation, the Greiss reagent was added and the absorbance read at 546 nm. The percentages of inhibition were calculated like described above.

AAPH induced hemolysis assay

Blood from rats was centrifuged for separate erythrocytes from plasma. Dilutions of the extract and ascorbic acid with PBS were prepared (100, 150, 200, 250 and 500 µg/ml). To the erythrocyte suspension, was added the extract / ascorbic acid and AAPH (200 mM). For each concentration, 3 trials were made. The control was made up of the erythrocyte suspension, PBS and AAPH. All mixtures were incubated at 37°C for 3 h. After incubation, the degree of hemolysis was determined by measurement of the absorbance of each mixture with spectrophotometer at 540 nm. The percentages of inhibition were calculated (Missebukpo et al., 2013).

In vivo antioxidant activity: malondialdehyde (MDA) assay

At day 8 of the manipulation, two mice were selected respectively in groups 1, 2, 3, 4 and 5 and their lungs were removed. The level of MDA in these lungs was measured out as described previously (Missebukpo et al., 2013). To 175 µl of lung homogenate or MDA dilutions, were added successively 250 µl of 1M HCl; 100µl sodium dodecyl sulfate 9.8%; 1ml of thio-barbituric acid 0.67% and 330µl of distilled water. After incubation at 90°C, 2.5 ml of n-butanol was added and the whole was centrifuged at 1006.2 g for 10 minutes. The absorbance was read at 535nm and the concentration of MDA present in each lung was expressed in ng/mg lung tissue.

Phytochemical tests

Phytochemical screening

Staining tests were carried out on the extract in order to determine the large chemical groups it contains. Alkaloids, flavonoids, tannins, saponins, carbohydrate and reducing compounds were searched in the extract with the methods used by Karumi et al. (2004) and Edeoga et al. (2005).

Quantitative determination of phenols and total flavonoids

Determination of total phenols content: Five ml of folin-ciocalteu diluted to 10% and 5 ml of sodium carbonate were added to 500 µl of extract for determine total phenols it contains. The control and gallic acid dilutions were prepared in the same way. Absorbance was read at 765 nm after 15 min of incubation. The gallic acid curve serves to the extract absorbance calibration. This amount is expressed in mg gallic acid equivalent per gram of extract (Pourmorad et al., 2006).

Total flavonoids determination: 1.5 ml of methanol was added to 500 µl of the extract. Then 100 µl of aluminum chloride, 100µl of potassium acetate and 2.8ml of distilled water were successively added. The standard range was made with quercetin. Absorbance was read at 415nm after 30min of incubation. The absorbance of the extract calibrate with quercetin curve allow the determination of total flavonoids it contains. This quantity is expressed in mg quercetin equivalent per gram of extract (Pourmorad et al., 2006).

Statistical analysis

The data obtained were expressed as mean ± SEM. One way ANOVA followed by the bonferonni test for multiple comparisons serve to the results analysis. A p- value < 0.05 is considered as significant. The statistical software used is the version 6.01 of Graphpad prism (USA).

RESULTS AND DISCUSSION

Evaluation of the effect of the extract on airway inflammation

Figures 1 and 2 show the effect of the extract on the leukocytes (eosinophils) infiltration into the airways. The OVA-sensitization provoked in mice's BALF a significant enhancement of the total leukocytes number and the percentage of eosinophils when compared to the control. Treatment with the extract and cetirizine provoked a significant reduction of these leukocytes number in BALF. The larger reduction's activity is observed at the dose of 500 mg/kg. For both figures, each value represents the mean of the counted cells ± SEM with n = 5. ##p <0.001 (sensitized vs control); ** p <0.01; *** p <0.001 (treated vs sensitized). Control= saline group; sensitized= OVA group; M.S = groups treated with the extract.

Evaluation of the antioxidant activity of the hydro-ethanolic extract of *M. senegalensis*

In vitro tests

The percentages of inhibition of the extract obtained from the DPPH, nitric oxide and AAPH tests were used to determine the IC_{50} value of the extract in each test. This value represent respectively the half maximal DPPH reduction concentration, the 50% nitric oxide scavenging
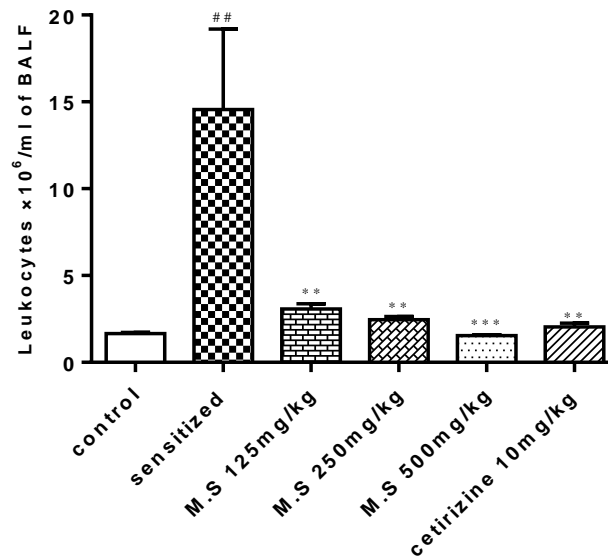
Figure 1. Effect of the *M. senegalensis* extract on leukocytes infiltration enhanced by OVA challenge in the airways of mice.
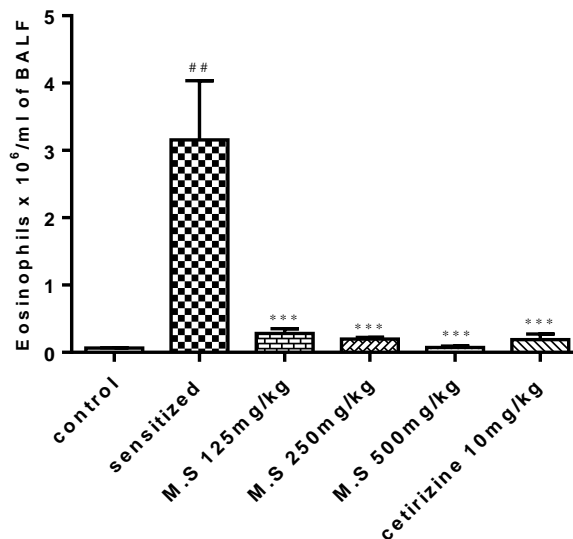
Figure 2. Effect of the *M. senegalensis* extract on the eosinophilic infiltration provoked by OVA challenge into the respiratory tract of mice.

concentration and the half maximal inhibitory concentration of hemolysis induced by AAPH. The extract effect was compared to that of ascorbic acid (Table 1).

Malondialdehyde dosage

This test is carried out in order to evaluate oxidative stress, particularly lipid peroxidation in our asthma model and the effect of the *M. senegalensis* extract on the stress. In sensitized mice, a significant increase ($p < 0.01$) of the MDA concentration is observed when compared to the control group. The extract induced a significant reduction ($p < 0.05$, $p < 0.01$) of this concentration at doses of 250 (38.91%) and 500 mg / kg (55.15%) (Figure

Table 1. IC_{50} values of Ascorbic Acid and Hydro-ethanolic extract of *M. senegalensis* showing their antioxidant effect.

	Tests	Ascorbic acid	*M. senegalensis*
IC50 (μg/ml)	DPPH	170	120
	Nitric oxide	124	184
	AAPH	133	342
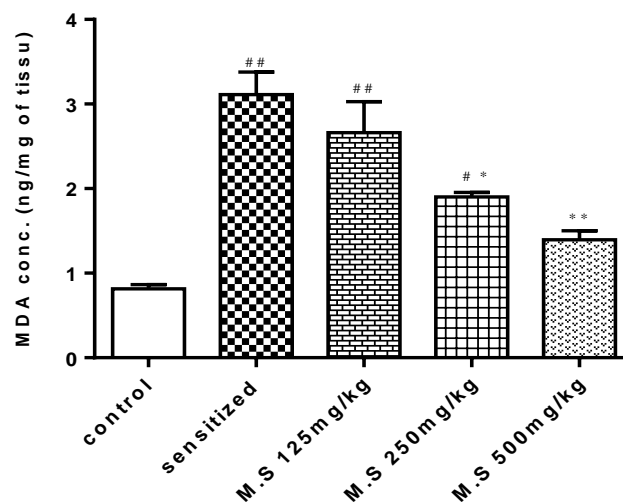
Figure 3. Effect of *M. senegalensis* extract on the membrane lipoperoxidation in lungs. Each value represents the mean ± SEM of 2 mice. ## $p < 0.01$ (sensitized vs. controlled); * $p < 0.05$; ** $p < 0.01$ (treated vs sensitized). Control= saline group ; sensitized= OVA group M.S = groups treated with the extract.

Table 2. Chemical groups present in the hydro-ethanolic extract of *M. senegalensis*.

Chemical groups	Results
Flavonoids	+
Tannins	+
Saponins	+
Carbohydrates	+
Reducing compounds	+
Alkaloids	-

- Absent; + Present.

3).

Phytochemical tests

Phytochemical screening: The results obtained from the staining tests realized on the *M. senegalensis* hydro-ethanolic extract are summarized in the Table 2.

Total phenols and flavonoids determination: Gallic acid and quercetin served to determine the quantity of total phenols and total flavonoids present in the hydro-ethanolic extract of *M.senegalensis*. Total phenol amount was 45, 26 mg equivalent of gallic acid/g of extract and

flavonoid was 29,59 mg equivalent of quercetin/g.

DISCUSSION

Bronchial inflammation is the main feature of asthma and its treatment constitute the basis of the disease treatment (Holgate, 2012). This inflammation is characterized by infiltration into the airways of leukocytes mainly mast cells, T lymphocytes and especially eosinophils. In allergic asthma, eosinophils are the foremost effector cells responsible for epithelial damage, chronicity of inflammation, and bronchial hyperreactivity (Gleich, 2000; Kay, 2005). Therefore, substances that limit the accumulation of leukocytes and especially eosinophilia in the airways are therapeutic interest for asthma.

Investigation was done on the effect of the hydro-ethanolic extract of *M. senegalensis* roots on the migration of leucocytes in general and particulary eosinophils in the airways of mice sensitized to ovalbumin. The results obtained showed that the extract significantly reduced the influx of leucocytes and particularly the eosinophilia caused by ovalbumin. The extract of *M. senegalensis* therefore inhibited the recruitment of these leucocytes into the airways. Yang et al.(2008) and Mahajan and Mehta (2011) have shown respectively on guinea-pig and mouse asthma models that the inhibition of leukocyte recruitment in the airways is due to a reduction of the OVA specific IgE number and the inhibition of the expression of Th2-like chemokines and cytokines such as TNF-α, IL-4, 5 and 13 in the airways. The cetirizine is an antiallergic antagonist of the H1 receptor. The extract of *M. senegalensis* would acted by controlling one or more of these parameters. In sum, the *M. senegalensis* hydro-ethanolic extract have an inhibitory effect on airways allergic inflammation in our murine model. However, the mechanisms by which this extract acts remain to be more investigated.

During bronchial inflammation, free radicals produced in the bronchi contribute to the pathogenesis of asthma. These oxidants especially ROS, nitric oxide cause epithelial damage and lead to more   recruitment of leukocytes into the bronchi. Leukocytes in their actions against allergen also produce more radicals and this increase the inflammation (Rangasamy et al., 2005). The use of antioxidants or substances able to restore the oxidant-antioxidant balance would contribute to the treatment of bronchial inflammation (Kirkham and Rahman, 2006). For this, the research analyzed the antioxidant effect of the *M. senegalensis* hydro-ethanolic extract by *in vitro* and *in vivo* tests.

The DPPH test showed that the extract has capacity to reduce DPPH; Bhimrao et al. (2015) also showed this activity of the plant by working on methanolic extracts of the leaves and seeds of *M. senegalensis.* Nitric oxide is involved in the asthmatic reaction. In stress conditions, its reaction with the superoxide anion produces the peroxynitrite responsible for lipid peroxidation on the cells of the bronchial epithelium (Henricks and Nijkamp, 2001). It further tilts the inflammatory reaction on the Th2 side by inhibiting Th1 (Barnes and Liew, 1995). The extract showed an important inhibition of the nitric oxide production. By this effect, the extract would therefore limit the epithelial lesions and the amplification of the Th2 inflammatory response. The AAPH test shows that the extract inhibited AAPH-induced hemolysis. This inhibition of hemolysis would be linked to a protection of the red blood cell membrane due to the trapping of free radicals produced by AAPH. The *M. senegalensis* extract could therefore protect *in vivo* bronchial cells from membrane lipoperoxidation during bronchial inflammation in asthma. To inspect this hypothesis, the malondialdehyde assay was performed.

MDA is a marker of lipid peroxidation whose high concentration in an organ indicates oxidative stress and gives an idea on the ROS effects on this organ (Wood et al., 2003; Michel et al., 2008). The MDA dosage showed that its level increased significantly in the lungs of sensitized mice and that the extract at doses of 250 and 500 mg/g significantly reduced this level of MDA. These results suggest that lipoperoxidation of bronchial cells occurred in our asthma model and that the extract of *M. senegalensis* would have an inhibitory effect on this process *in vivo*. The hydro-ethanolic extract of *M. senegalensis* would therefore have a protective effect of bronchial cells against ROS and their deleterious effects in asthma. Since free radicals are a source of worsening inflammation in asthma, the antioxidant effect of the extract would have contribute to the reduction of bronchial inflammation.

Moreover, a link has been made between the presence of polyphenolic compounds in a plant and its antioxidant and anti-inflammatory activities. Studies have shown that polyphenols, mainly tannins and flavonoids, are potentially important antioxidants and anti-inflammatories (Hagerman et al., 1998; Kim et al., 2004). Phytochemical tests were performed on the extract to see if these compounds are present. The results of the phytochemical screening showed that the hydro-ethanolic extract of *M. senegalensis* contains flavonoids, tannins, saponins, carbohydrates and reducing compounds. As phytochemical screening is a qualitative analysis, one cannot affirm alkaloids absence in the extract. Moreover, this test carried out in Nigeria revealed the presence of alkaloids in the methanolic extracts of the roots and the ethanolic leaves extract of the plant (Tor-Anyiin and Anyam, 2013; Kafu and Adebisi, 2015). Phytochemical screening revealed the presence of polyphenols in the extract; the determination of total phenols and flavonoids has confirmed the presence of these compounds in the plant. Polyphenols present in would probably have contributed to the reduction of oxidative stress and

bronchial inflammation in our asthma model.

The results revealed that the *M. senegalensis* hydro-ethanolic extract prevent bronchial eosinophilia, have antioxidant potential in murine asthma model and possess phenolic compounds. It suggested that the plant would be beneficial in asthma treatment. However, this work remain partial because all the parameters of bronchial inflammation have not been explored yet. Thus, the study must be continued in order to deepen the extract effect on airway inflammation and find the mechanisms by which it acts; to search the effect of the extract on the others asthma parameters like airway hyper-responsiveness, bronchoconstriction and bronchial tissue damage.

Conclusion

The results obtained from this study show that the hydro-ethanolic extract of *M. senegalensis* acts on bronchial inflammation in asthma preventively. The mechanisms by which the extract would have inhibited this inflammation await elucidation. The antioxidant power of the extract both in *vitro* and in *vivo* is also confirmed in this work. This antioxidant activity would be partly involved in the inhibition of the inflammation exerted by the extract. Phytochemical studies of the extract revealed the presence of phenolic compounds known for their anti-inflammatory and antioxidant properties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Antibacterial resistance modulatory properties of selected medicinal plants from Ghana

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Bacterial resistance to antibiotics is a serious challenge to human and animal health and all efforts are being put together to resolve the menace. In this study the antibiotic resistance modifying activity of ten plants was established by determination of the minimum inhibitory concentrations (MICs) of the plant extracts, the MICs of the antibiotics alone (amoxicillin, ciprofloxacin, erythromycin and tetracycline) and the MICs of the antibiotics in the presence of sub-inhibitory concentrations of the methanol extracts of some selected medicinal plants including *Clerodendron splendens, Cyperus esculentus, Duranta plumieri, Kigelia africana, Kyllinga brevifolia, Momordica charantia, Phyllanthus amarus, Pycnanthus angolensis, Secamone afzelii* and *Thuja occidentalis* against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The extracts were found to possess varying degree of antimicrobial activity with MICs between 4 and 50 mg/mL. It was observed that 26.9% of the plant extract-antibiotic combinations/interactions resulted in the reduction of activity of antibiotics. Almost 17% of the extract-antibiotic interactions led to the complete loss of activity of the antibiotics and 30.0% of the extract-antibiotic combinations resulted in resistance modulation. Three per cent of the extract-antibiotic combinations/interactions had antibiotics that were not active when used alone but became active in the presence of the extracts and 23.1% extract-antibiotic combinations/interactions had no modifying effect on the individual *in vitro* activities of the antibiotics. There is need to isolate the bioactive agents from the extracts especially those that potentiated the activity of the antibiotics.

Key words: Bacterial infections, antibiotic resistance, resistance modifying agents; medicinal plants.

INTRODUCTION

Antimicrobial resistance by pathogenic microorganisms (bacteria, viruses, fungi, protozoa and helminths) is one of the biggest clinical problems currently facing humanity (Davies and Davies, 2010). Drug combination, as exemplified by the combination of *β*-lactam antibiotics and *β*-lactamase inhibitors (for example amoxicillin–clavulanic acid) (Reeves et al., 1978) is one way used to overcome resistance by pathogenic

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microorganisms. The mechanisms of action of such combinations vary overtly from that of the same drugs acting individually and isolation of a single phyto-constituent thus may alter its importance in exhibiting this effect (Hemaiswarya et al., 2008).

Nature has provided various sources of substances used as medicines and scientists have found medicines for almost all diseases known to man from natural sources (Newman et al., 2000). Mankind has been using antimicrobial agents for the management of infections for a longer period than initially anticipated (Bassett et al., 1980; Cook et al., 1989; Aminov, 2010).

Phylogenetic reconstruction analysis indicates that antibiotic resistance genes have been around long before the antibiotic era (Aminov and Mackie, 2007; Kobayashi et al., 2007). Structure-based phylogeny of serine and metallo-β-lactamases, for example, show that these ancient enzymes have been around for more than two billion years and that some serine β-lactamases have been present on plasmids for thousands of years (Hall and Barlow, 2004; Garau et al., 2005). The development of the β-lactamase and housekeeping genes in *Klebsiella oxytoca* is highly congruent, meaning that these genes have been evolving for over 100 million years (Fevre, 2005). Thus antibiotic resistance is not a new phenomenon, except that, probably it is now more widespread than before, and it moves round the globe more quickly because of easier and faster movement of mankind.

Antibiotic resistance has become a huge problem in recent times. Patients are dying from infections because the antibiotics used for the treatment of these infections are no more effective because of resistance. Resistance to antibiotics by bacteria and other organisms is now a major public health problem worldwide and antibiotic use has been recognized as the main selective pressure driving the menace.

Plants have been identified as one major source of medicinal agents from nature. Medicinal plants have been used by humans to manage several diseases including infections since time immemorial (Rios et al., 1988). Based on their traditional uses several known medicinal plants have been screened for their antimicrobial activity, phytochemical composition, possible interaction with antibiotics or other medicinal plants, pharmacokinetic, bioavailability and toxicity profiles. However, reports on interactions of antibiotics with plant extracts are scanty and only a few studies have been reported (Rios et al., 1988; Nascimento et al., 2000; Aburjai et al., 2001; Aqil et al., 2005). Natural product scientists have been searching for phytochemicals that could ultimately be developed for use in the treatment of infectious diseases (Cowan, 1999). The antiseptic use of volatile oils has been known to man for centuries and these oils have been obtained from plants using extraction methods such as steam or hydro-distillation. These volatile oils are usually variable mixtures of mostly terpenoids, specifically monoterpenes and sesquiterpenes although

diterpenes may also be present (Dorman and Deans, 2000).

Fractionation of *Glycyrrhiza glabra* L. var. typical, using bioactivity guided methods led to the isolation and characterization of several compounds including 4'-O-methylglabridin, 3'-methoxyglabridin, glabridin and glabrol. Glabrene, hispaglabridin A, hispaglabridin B, 3-hydroxyglabrol formononetin, phaseollinisoflavan, salicylic acid and O-acetyl salicylic acid were also found. Some of these including hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin, glabridin, glabrol and 3-hydroxyglabrol were found to possess significant antimicrobial activity (Mitscher et al., 1980).

Apart from being sources of antimicrobial agents, plants also harbour substances that act to modify the resistance offered by microorganisms to antibiotics (Dapaah et al., 2016; Fankam et al., 2015; Seukep et al., 2016). In a study using the multidrug-resistant *Escherichia coli* that produces extended-spectrum β-lactamases (ESBLs), such as the CTX-M enzymes, the methanol extracts of *Gundelia tournefortii* L. and *Pimpinella anisum* L. enhanced the antibacterial activity of chloramphenicol, neomycin, doxycycline, cephalexin and nalidixic acid (Darwish and Aburjai, 2010). In this study, the antibacterial activity of selected plant extracts as well as their *in vitro* resistance modulatory effects on selected reference antibiotics were determined.

MATERIALS AND METHODS

Collection and identification of plant materials

The plants materials were identified, collected and authenticated by Dr. G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana with voucher specimens kept at the herbarium of the above mentioned institution (Table 1).

Preparation of plant extracts

The various plant parts were washed under running tap water and air dried individually at room temperature (28 to 32°C) for 7 to 14 days (Meyer and Dilika, 1996; Bariş et al., 2006) to a constant weight. The dried plant materials were milled using a laboratory hammer mill into coarse powder. Each powdered plant material (100 g) was cold macerated with 500 mL of 70% v/v methanol for five (5) days with continuous stirring. They were then filtered using Whatman paper No.1 under reduced pressure. They were then evaporated to dryness using rotary evaporator under reduced pressure at 38°C, lyophilized and stored in a fridge at 4°C.

Antibacterial study

Determination of antibacterial activity of extracts

The antibacterial activities of the crude methanol extracts of the various plant parts were determined using the method as described by Das et al. (2011), Bhalodia and Shukla (2011) and Klančnik et al. (2010). One millilitre (1 mL) each of the test organism suspension (*S. aureus* ATCC 25923, *B. subtilis*

Table 1. List of collected medicinal plants.

Plants	Plant part used	Family	Voucher specimen number	Location	Geographical location	
					Latitude ^{o}	Longitude ^{o}
Clerodendron splendens G. Don.	Leaves	Lamiaceae	KNUST/HM/16/L/079	Asokore Mampong Kumasi	6.6990	-1.56914
Cyperus esculentus L.	Aerial parts	Cyperaceae	KNUST/HM/16/S/081	Achiase Nwabiagya, Kumasi	6.78535	-1.67474
Duranta plumieri L.	Leaves	Verbanaceae	KNUST/HM/16/L/082	KNUST, Kumasi	6.67650	-1.56698
Kigelia africana (Lam.) Benth.	Leaves	Bignoniaceae	KNUST/HM/16/L/083	Bobiri Forest, Kubease, Ashanti	6.68290	-1.36449
Kyllinga brevifolia Rottb.	Aerial parts	Cyperaceae	KNUST/HM/16/S/084	Boadi, Knust, Kumasi	6.67972	-1.54737
Momordica charantia L.	Leaves	Cucurbitaceae	KNUST/HM/16/L/085	Asokore Mampong, Kumasi	6.70032	-1.56874
Phyllantus amarus, Schumac & Thonn.	Aerial parts	Euphorbiaceae	KNUST/HM/16/S/088	KNUST, Kumasi	6.67649	-1.56700
Pycnanthus angolensis (Welw.) Warb.	Bark	Myristicaceae	KNUST/HM/16/B/089	KNUST, Kumasi	7.67375	-1.57763
Secamone afzelii, (Schult) K Schum.	Aerial parts	Asclepiadeceae	KNUST/HM/16/S/090	Physique Garden, KNUST, Kumasi	6.67384	-1.56627
Thuja occidentalis L.	Leaves	Cupressaceae	KNUST/HM/16/S/091	FPPS, KNUST, Kumasi	6.67649	-1.56699

NTCC 4853*, E.coli* ATCC 25922*, P. aeruginosa* ATCC 27853) was inoculated into a 10 mL nutrient broth (Oxoid, London UK) and incubated for 24 h and adjusted with normal saline to 0.5 McFarland Standard. Aliquots of 10 µL were applied to the surface of 20 mL of Mueller-Hinton agar (Sigma-Aldrich, Steinheim, Germany) in 10 cm-wide plates. The aliquot was spread over the surface using a sterile cotton wool swab. Three (3) cups were bored in each plate using a cork borer No. 5 (10 mm). Two grams of the extract was added to 1 mL of dimethyl sulfoxide (Sigma-Aldrich, Steinheim, Germany) and diluted to 200 mg/mL with sterile distilled water. It was then serially diluted and the wells filled with 100 µL of 30 and 50 mg/mL of the extract and allowed to diffuse at room temperature (25 to 28°C) for 1 h. A 10 µg/mL solution of tetracycline prepared in water was used as the positive control. The plates were incubated at 37°C for 24 h and zones of growth inhibition recorded. The procedure was performed in triplicates.

Determination of the minimum inhibitory concentration of extracts

The minimum inhibitory concentration (MIC) of all the plant extracts was determined using the macrobroth dilution method

(Okeke et al*.,* 2001; Wiegand et al*.,* 2008). Solutions of the extracts were prepared by mixing 4 g of extract with 0.5 mL of dimethylsulfoxide (DMSO) and 9.5 mL of distilled water added to produce a 400 mg/mL stock solution. Specified volumes (5 to 250 µL) were then taken from the stock solution and added to 0.5 mL double strength Muller-Hinton broth (Sigma-Aldrich, Steinheim, Germany). Appropriate volume of sterile distilled water was added and 10 µL of test organism containing 1 x 10^5 cells per mL was added to the appropriate well to produce concentrations of 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/mL. They were incubated at 37°C for 24 h, after which 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT 0.1% w/v) was added to each well to detect the presence of growth after 30 min of incubation at 37°C. The MICs were re-determined with a constant difference of 2 mg/mL.

The MICs of reference antibiotics (amoxicillin, erythromycin, tetracycline and ciprofloxacin) were similarly determined against *S. aureus, B. subtilis, E. coli and P. aeruginosa* using the broth microdilution method (Wiegand et al., 2008; Qaiyumi and Wanger, 2007). The reference antibiotics at 1, 2, 4, 8, 16, 32, 64 and 128 µg/mL were prepared in 96-well plates and the volumes adjusted to 190 µL and 10 µL of test organism suspension containing 10^5 cells per mL was added to make it

200 µL per well. They were incubated at 37°C for 24 h. The MIC was recorded as the least concentration that showed no visible bacterial growth which was detected by the absence of purple colour after the addition 10 µL of MTT (0.1% w/v) to each well followed by incubation at 37°C for 30 min.

Screening of extracts for antibacterial resistance modifying activities

The antibiotic resistance modifying activity was determined for all the extracts against the test organisms in the presence of the four reference antibiotics (amoxicillin, erythromycin, ciprofloxacin and tetracycline). The MIC of the reference antibiotics was determined against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* (as described above) in the presence of a sub-inhibitory concentration of 2 mg/mL of extracts by incorporating 10 µL of a 200 mg/mL stock solution of the extract into 100 µL of double strength Muller-Hinton broth. The broth was adjusted to 190 µL with sterile distilled water and 10 µL of test organism was added to produce 200 µL. The plates were then incubated at 37°C for 24 h. The MIC was recorded as the least concentration that showed no visible bacterial growth. This was detected by the absence of purple colour after the

Table 2. Antibacterial activity of plant extracts.

Plant	Part used	Mean zones of growth inhibition (mm)							
		S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
Concentration of extract (mg/mL)		30.0	50.0	30.0	50.0	30.0	50.0	30.0	50.0
C. splendens	Leaves	17.3 ±0.33	19.6±0.33	15.6±0.33	18.6±0.33	14.0±1.00	16.6±0.66	11.3±0.33	14.3±0.33
C. esculentus	Aerial parts	11.6±0.33	14.3±0.33	13.7±0.67	15.7±0.88	14.3±0.33	16.7±0.33	-	12.6±0.33
D. plumieri	Leaves	-	-	12.0±0.58	14.7±0.88	-	12.0±0.58	-	-
K. Africana	Leaves	13.3±0.33	16.6±0.33	13.7±0.33	16.3±0.33	14.7±0.33	16.7±0.33	-	15.0±0.58
K. brevifolia	Aerial parts	-	12.3±0.67	-	13.0±1.15	12.7±0.33	15.7±0.33	-	13.3±0.88
M. charantia	Leaves	-	13.3±0.67	-	13.0±0.58	-	13.3±0.33	-	11.7±0.33
P. amarus	Aerial parts	16.3±0.33	19.3±0.33	14.3±0.33	17.7±0.88	12.3±0.33	15.0±1.00	12.6±0.33	15.6±0.88
P. angolensis	Bark	14.6±0.67	18.0±0.58	14.7±0.33	18.3±0.88	-	-	-	-
S. afzelii	Aerial parts	12.6±0.33	15.3±0.33	15.7±0.33	18.7±0.67	14.7±0.33	17.3±0.67	-	14.3±0.88
T. occidentalis	Leaves	19.6±0.33	23.3±0.33	13.7±0.33	16.3±0.67	14.7±0.67	18.0±0.58	14.0±0.58	16.7±67
Tetracycline (10 mg/mL)		25.8±0.55		32.33±1.4		25.8±0.55		32.33±1.4	

- = no activity

addition 10 µL of MTT (0.1% w/v) to each well followed by incubation at 37°C for 30 min. The experiment was done in three replicates.

RESULTS

Antibacterial activity of extracts

Antibacterial activity showed that the methanol leaf extract of *C. splendens*, methanol aerial parts extract of *P. amarus,* methanol leaf extract of *T. occidentalis* and aerial part methanol extract of *C. esculentus* had activity against all test organisms at test concentrations with zones of growth inhibition in the range of 11.3±0.33 to 19.6±0.33, 12.3±0.33 to 19.3±0.33, 13.7±0.33 to 19.6±0.33 and 11.6±0.33 to 15.7±0.88 mm, respectively (Table 2). Additionally, the methanol leaf extract of *K. africana*, methanol aerial part extract of *K. brevifolia*, methanol leaf extract of *M. charantia* and aerial part extract of *S. afzelii* exhibited activity against all the test organisms at 50 mg/mL. The methanol leaf extract of *D. plumieri* showed no activity against *S. aureus* and *P. aeruginosa* at test concentrations. Methanol bark extract of *P. angolensis* also showed no activity against test Gram-negative organisms (Table 2).

MIC of extracts

The Gram-positive bacteria were more susceptible to the extracts with lower MICs compared to the Gram-negative bacteria (Table 3). *P. amarus* and *C.* *splendens* were also very active against *B. subtilis* with MICs of 4 mg/mL. Methanol extracts of *M. charantia* and *K. brevifolia,* exhibited weak activity against *B. subtilis* with MICs of 36 and 20 mg/mL, respectively (Table 3). Methanol leaf extract of *T. occidentalis* had MIC of 4 mg/mL against *S. aureus*. *S. afzelii, M. charantia* and *K. brevifolia* extracts exhibited weak activity against *S. aureus* with MICs of 26, 30 and 32 mg/mL, respectively. *C. esculentus* showed moderate activity against *S. aureus* and *B. subtilis* with MIC of 16 mg/mL (Table 3).

E. coli was susceptible to the leaf extract of *P. amarus* and *C. splendens* with MICs of 4 mg/mL. The MIC of *C. esculentus* against *E. coli* was 8 mg/mL. Additionally, susceptibility of *E. coli* to extracts of *M arantia* and *D. plumieri* was quite low with MICs of 32

Table 3. MIC of methanol extracts against test organisms.

Plant	Part used	MIC (mg/mL)			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
C. splendens	Leaves	8.0	4.0	4.0	26
C. esculentus	Aerial parts	16.0	16.0	8.0	32.0
D. plumieri	Leaves	>100.0	12.0	48.0	>100
K. africana	Leaves	16.0	8.0	10.0	22.0
K. brevifolia	Aerial parts	32.0	20.0	14.0	8.0
M. charantia	Leaves	30.0	36.0	32.0	38.0
P. amarus	Aerial parts	6.0	4.0	4.0	6.0
P. angolensis	Bark	8.0	6.0	>100	>100
S. afzelii	Leaves	26.0	8.0	6.0	22.0
T. occidentalis	Leaves	4.0	8.0	6.0	10.0

and 48 mg/mL, respectively. *P. aeruginosa* was comparably, the most resistant organism to all the extracts but was most sensitive to *P. amarus* with MIC of 6 mg/mL. It showed very little susceptibility to *C. esculentus*, *S. afzelii*, *M. charantia*, *K. africana* and *C. splendens* with MICs of 32, 22, 38, 22, 32 and 26 mg/mL, respectively (Table 3).

Some of the extracts did not show activity at all against the test organisms within the range of concentrations used. The methanol extract of *D. plumieri* was not active against *S. aureus* and *P. aeruginosa* while *P. angolensis* showed no activity against the Gram-negative bacteria (Table 3).

Antibiotic resistance modifying/modulation activity of extracts

Influence of C. splendens extract on activity of selected antibiotics

Sub-inhibitory concentrations of methanol leaf extract of *C. splendens* had minimal effects on the activities of the antibiotics (Table 4). The extract enhanced the activity of tetracycline against *P. aeruginosa* by reducing the MIC by 8 folds (Table 14). Most of the interactions between the organisms and the antibiotics in the presence of the sub-inhibitory concentration of methanol extracts of *C. splendens* resulted in marginal changes in their MICs (Tables 4 and 14).

Influence of C. esculentus extract on activity of some selected antibiotics

The MICs of the reference antibiotics were determined with and without the sub-inhibitory concentration (2.0 mg/mL) of methanol aerial parts extract of *C. esculentus.* It was established that the antibacterial activities of all the reference antibiotics against all the

organisms were reduced (Tables 5 and 14).

Influence of D. plumieri extract on activity of selected antibiotics

The presence of sub-inhibitory concentration of methanol leaf extract *D. plumieri* (2 mg/mL) reduced the activity of the antibiotics against most of the test organisms (Table 6). With the exception of *E. coli, S. aureus* and *P. aeruginosa* that saw increased sensitivity to ciprofloxacin, erythromycin and tetracycline producing 2.5, 2 and 8-folds reduction in resistance respectively almost all the interactions produced resistance enhancement (Table 14).

Influence of K. africana extract on activity of selected antibiotics

Methanol leaf extracts of *K. africana* modulated the susceptibility of the test organisms to the antibiotics. The resistance of *B. subtilis* reduced against all the antibiotics with amoxicillin showing the greatest increase in activity producing a 15-fold decrease in MIC. In the presence of the extract, *E. coli* lost sensitivity to all the antibiotics. The sensitivity of *P. aeruginosa* to tetracycline increased by 8-fold (Tables 7 and 14).

Influence of K. brevifolia extract on activity of selected antibiotics

The methanol aerial parts of extract of *K. brevifolia* nullified the activity of amoxicillin against *S. aureus* and *B. subtilis* and also tetracycline against *B. subtilis*. The extract made *P. aeruginosa* sensitive to amoxicillin with an MIC of 32 μg/mL, while producing 2 and 4 fold enhancement in the activities of erythromycin and

Table 4. Influence of *C. splendens* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
SA	8.0	2.0	2.0	1.0	1.0	2.0	2.0	2.0	2.0
BS	4.0	2.0	4.0	0.5	1.0	1.0	2.0	2.0	4.0
EC	4.0	4.0	2.0	0.5	0.5	4.0	4.0	4.0	4.0
PA	26.0	>128.0	>128.0	2.0	1.0	>128.0	>128.0	32.0	4.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus*, BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*

Table 5. Influence of *C. esculentus* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
SA	16.0	2.0	4.0	1.0	4.0	2.0	16.0	2.0	32.0
BS	16.0	2.0	>128.0	0.5	>128	1.0	>128.0	2.0	>128.0
EC	8.0	4.0	32.0	0.5	2.0	4.0	8.0	4.0	32.0
PA	32.0	>128.0	>128.0	2.0	>128	>128.0	>128.0	32.0	>128.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 6. Influence of *D. Plumieri* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
SA	>100.0	2.0	2.0	1.0	16.0	2.0	1.0	2.0	8.0
BS	12.0	2.0	4.0	0.5	16.0	1.0	>128.0	2.0	4.0
EC	48.0	4.0	4.0	0.5	2.0	4.0	8.0	4.0	4.0
PA	>100.0	>128.0	>128.0	2.0	8.0	>128.0	>128.0	32.0	4.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*

tetracycline against E. coli, respectively Tables 8 and 14).

Influence of *M. charantia* extract on activity of selected antibiotics

In the presence of sub-inhibitory concentration (2 mg/mL) of methanol leaf of M. charantia, S. aureus and B. subtilis lost their sensitivity to tetracycline completely (Table 9). The resistance of S. aureus was reduced 66 folds to amoxicillin while that of P. aeruginosa and E. coli were reduced 15 and 13 folds with MICs of 0.13 and 0.3 µg/mL, respectively (Table 14).

Influence of *P. amarus* extract on activity of selected antibiotics

In the presence of sub-inhibitory concentration (2 mg/mL) of *P. amarus,* the organisms were more resistant to amoxicillin with higher MICs than theamoxicillin alone. With the exception of *S. aureus,* all the organisms were more sensitive to ciprofloxacin in the presence of the extract. Erythromycin lost activity completely against *S. aureus* rather had lower MIC of 0.13 µg/mL against *P. aeruginosa* (Table 10). With the exception of *S. aureus*, the rest of the organisms including *B. subtilis, E. coli* and *P. aeruginosa* became more sensitive to tetracycline with 4, 8 and 16 folds

Table 7. Influence of *K. africana* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
SA	16.0	2.0	1.0	1.0	1.0	2.0	0.5	2.0	2.0
BS	8.0	2.0	0.13	0.5	0.13	1.0	0.5	2.0	0.3
EC	10.0	4.0	>128.0	0.5	>32.0	4.0	>128.0	4.0	>128.0
PA	22.0	>128.0	>128.0	2.0	1.0	>128.0	>128.0	32.0	4.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 8. Influence of *K. brevifolia* extract on the antibacterial activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
SA	32.0	2.0	>128.0	1.0	1.0	2.0	64.0	2.0	2.0
BS	20.0	2.0	>128.0	0.5	0.3	1.0	32.0	2.0	>128.0
EC	14.0	4.0	128.0	0.5	1.0	4.0	2.0	4.0	1.0
PA	8.0	>128.0	32.0	2.0	2.0	>128.0	>128.0	32.0	64.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 9. Influence of *M. charantia* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
SA	30.0	2.0	0.03	1.0	1.0	2.0	1.0	2.0	>128.0
BS	36.0	2.0	2.0	0.5	0.13	1.0	0.13	2.0	>128.0
EC	32.0	4.0	2.0	0.5	0.13	4.0	0.5	4.0	0.3
PA	38.0	>128.0	>128.0	2.0	0.13	>128.0	>128.0	32.0	128.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

reduction in MICs respectively (Table 14).

Influence of *P. angolensis* extract on activity of selected antibiotics

Methanol bark extract of *P. angolensis* had marginal effects on the activities of the antibiotics (Table 11). Amoxicillin, ciprofloxacin and erythromycin were more active against *S. aureus* with 2-fold reduction of their MICs. In the presence of the extract, amoxicillin and ciprofloxacin were less active against *B. subtilis* (Table 14).

Influence of *S. afzelii* extract on activity of selected antibiotics

P. aeruginosa was very sensitive to amoxicillin when combined with methanol leaf extract of *S. afzelii* with an MIC of 16 µg/mL while amoxicillin alone at a concentration of 128 µg/mL had no effect on *P. aeruginosa* (Table 12). *S. aureus*, *B. subtilis* and *P. aeruginosa* also became less resistant to ciprofloxacin in the presence of the extract. The extract made all the organisms more resistant to erythromycin except *P. aeruginosa* that became very sensitive to the antibiotic in the presence of the extract with MIC of 8 µg/mL while

Table 10. Influence of *P. amarus* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	MIC							
		Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	6.0	2.0	8.0	1.0	2.0	2.0	>128.0	2.0	>128.0
BS	4.0	2.0	4.0	0.5	0.13	1.0	0.13	2.0	0.5
EC	4.0	4.0	32.0	0.5	0.13	4.0	8.0	4.0	0.5
PA	6.0	>128.0	>128.0	2.0	0.3	>128.0	0.13	32.0	2.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus* ; BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 11. Influence of *P. angolensis* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	MIC							
		Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	8.0	2.0	1.0	1.0	0.5	2.0	1.0	2.0	4.0
BS	6.0	2.0	4.0	0.5	1.0	1.0	0.5	2.0	2.0
EC	-	4.0	8.0	0.5	1.0	4.0	4.0	4.0	4.0
PA	-	>128.0	>128.0	2.0	0.5	>128.0	>128.0	32.0	32.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa;* - = no activity.

Table 12. Influence of *S. afzelii* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	MIC							
		Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	26.0	2.0	1.0	1.0	0.13	2.0	4.0	2.0	>128.0
BS	8.0	2.0	1.0	0.5	0.13	1.0	>128.0	2.0	1.0
EC	6.0	4.0	0.5	0.5	0.5	4.0	>128.0	4.0	>128.0
PA	22.0	>128.0	16.0	2.0	0.3	>128.0	8.0	32.0	8.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

the antibiotic alone has no activity within the concentrations used. Methanol leaf extract of *S. afzelii* demonstrated resistance modulation activity on amoxicillin against all the test organisms with 2-fold potentiation of amoxicillin against *S. aureus* and *B. subtilis* and an 8-fold potentiation against *E. coli*. In the case of tetracycline, *S. aureus* and *E. coli* lost their sensitivity completely while *B. subtilis* and *P. eruginosa* became more susceptible in the presence of the extract (Table 14).

Influence of *T. occidentalis* extract on activity of selected antibiotics

The methanol leaf extract of *T. occidentalis* suppressed the activity of all the antibiotics against all the test organisms except in some few cases. *S. aureus* and *B. subtilis* lost sensitivity completely to amoxicillin while *S. aureus, E. coli* and *P. aeruginosa* lost their sensitivity completely to tetracycline in the presence of the extract. *B. subtilis* showed a 33 folds reduction in resistance to

Table 13. Influence of *T. occidentalis* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	4.0	2.0	>128.0	1.0	32.0	2.0	16.0	2.0	>128.0
BS	8.0	2.0	>128.0	0.5	32.0	1.0	0.03	2.0	0.06
EC	6.0	4.0	8.0	0.5	32.0	4.0	0.5	4.0	>128.0
PA	10.0	>128.0	>128.0	2.0	32.0	>128.0	1.0	32.0	>128.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*

Table 14. Ratio of MIC of antibiotic alone to MIC of antibiotic in the presence of sub-inhibitory concentration of extract.

Plant extract	Amoxicillin				Ciprofloxacin				Erythromycin				Tetracycline			
	SA	BS	EC	PA	SA	BS	EC	PA	SA	BS	EC	PA	SA	BS	EC	PA
C. splendens	-	0.5	2	-	-	0.5	-	2	-	0.5	-	-	-	0.5	-	8
C. esculentus	0.5	↓	0.125	-	0.25	↓	0.25	↓	0.13	↓	0.5	-	0.06	↓	0.13	↓
D. plumieri	-	0.5	-	-	0.06	0.03	2.5	0.25	2	↓	0.5	-	0.25	0.5	-	8
K. africana	2	15.38	↓	-	-	3.85	↓	2	4	2	↓	-	-	6.67	↓	8
K. brevifolia	↓	↓	0.03	↑	-	1.67	0.5	-	0.03	0.03	2	-	-	↓	4	0.5
M. charantia	66.7	-	2	-	-	3.85	3.85	15.39	2	7.69	0.8	-	↓	↓	13.33	0.25
P. amarus	0.25	0.5	0.13	-	0.5	3.85	3.85	6.67	↓	7.69	0.5	↑	↓	4	8	16
P. angolensis	2	0.5	0.5	-	2	0.5	0.5	4	2	2	-	-	0.5	-	-	-
S. afzelii	2	2	8	↑	7.69	3.85	-	6.67	0.5	↓	↓	↑	↓	2	↓	4
T. occidentalis	↓	↓	0.5	-	0.03	0.02	0.02	0.06	0.13	33.33	8	↑	↓	33.33	↓	↓

SA, *S. aureus*; BS, *B. subtilis*; EC, *E. coli*; PS, *P. aeruginosa*; ↓= antagonism; ↑= potentiation; - = no activity.

erythromycin and tetracycline while *E. coli* also showed an 8 folds reduction in resistance to erythromycin. *P. aeruginosa* became more sensitivity to erythromycin in the presence of the extract with an MIC of 1 µg/mL (Tables 13 and 14).

Ratio of activity of antibiotic to activity of antibiotic in the presence of extract

The antibacterial activity of the extracts and reference antibiotics were expressed as MIC, the smaller the MIC the more active the substance. Thus the effect of the sub-inhibitory concentration of the extracts on the activity of the test antibiotics was determined by comparing the ration of MIC of the antibiotic to the MIC of the antibiotic in the presence of sub-inhibitory concentrations of the extract (Table 14).

DISCUSSION

Some plant extracts have been shown over the years to exhibit antimicrobial activity and hence the agar diffusion method was employed to determine the activity of the selected plant extracts against test Gram-positive and negative bacteria. The study revealed that the methanol leaf extract of *C. splendens* (11.3±0.33 - 19.6±0.33 mm), methanol aerial part extract of *P. amarus* (12.3±0.33 - 19.3±0.33 mm)*,* methanol leaf extract of *T. occidentalis* (13.7±0.33 to 19.6±0.33 mm) and aerial part methanol extract of *C. esculentus* (11.6±0.33 to 15.7±0.88 mm) had activity against all test organisms at test concentrations (Table 2). Methanol leaf extract of *C. splendens* have been reported to have antibacterial and antiprotozoan activity, the former is in agreement with our studies (Gbedema et al.*,* 2010; Abouzid et al.*,* 2013). MIC of *C. splendens* methanol extract was determined to be 4.0 to 26 mg/mL against test Gram-positive and negative organisms (Table 3). However, Gbedema et al. (2010) reported a MIC of 128 to 516 µg/mL for extract of *C. splendens,* indicating a better antimicrobial activity than our findings. The difference may be due to method of extraction, the part of the plant used, site of collection and variation in storage conditions may contribute to the variations in activity (Inbathamizh and Padmini, 2013; Khattak, 2015). The extract of *C. splendens*

enhanced the activity of tetracycline against *P. aeruginosa* by reducing the MIC by 8 folds (Table 14). This reduction may due to blockade of tetracycline efflux pump activity or modification of the binding site which have been identified as the main mechanism of bacteria resistance to antibiotics including the tetracyclines (John, 2005; Lin et al., 2015).

Aerial parts extract of *C. esculentus* showed antibacterial activity with zones of inhibition and MIC determined to be between 11.6±0.33 to 16.7±0.33 mmand 8 to 32 mg/mL, respectively for test organisms. This is agreement with the findings of Adeniyi et al. (2014) in which the zone of inhibition was reported to be within the range of 7.5±0.41 to 25.0±0.16 mm for aqueous and ethanol whole plant extracts of *C. esculentus*. It was also established that the antibacterial activities of all the antibiotics against all the organisms were reduced (Tables 5 and 14). This may probably be due to the extracts ability to reduce efflux pump activity, increase drug uptake and accumulation (Lin et al., 2015; Munita and Arias, 2016).

In the case of *D. plumieri* leaf extract, the zones of inhibition were determined to be 12.0±0.58 and 14.7±0.88 mm for *B. subtilis*. No activity was observed for *S. aureus* and *P. aeruginosa* but for *E. coli* (12.0±0.58 mm) at a concentration of 50 mg/mL. This indicates that the extract has weak antibacterial activity which is in agreement with the findings of Adu et al. (2011). The presence of sub-inhibitory concentration of *D. plumieri* leaf extract reduced the activity of the antibiotics against most of the test organisms (Tables 6 and 14). The reduced or nullified activity of the antibiotics may be as a result of interactions between the phytochemicals in the extract and the antibiotics or the microorganisms. The phyto-constituents may react chemically with the antibiotics leading to loss of activity (Dapaah et al., 2016; Adu et al., 2009).

The methanol leaf extract of *K. africana* showed activity against test organisms which is consistent with report by Agyare et al. (2013). The ethanolic extracts of *K. africana* stem bark and fruit extracts have also been reported to possess activity against *B. subtilis, E. coli, K. pneumoniae* and *S. aureus* with MICs of 0.63 to 2.5 mg/mL (Grace et al., 2002). The resistance of *B. subtilis* was reduced against all the antibiotics with amoxicillin showing the greatest increase in activity producing a 15-fold reduction in the MIC. Sensitivity of *P. aeruginosa* to tetracycline increased by 8-folds. This observed potentiation in activities of the antibiotics may be due to increased drug uptake and its accumulation in bacteria (Lin et al., 2015; Munita and Arias, 2016).

Extracts of *K. brevifolia* nullified the antibacterial activity of amoxicillin against *S. aureus* and *B. subtilis* and also tetracycline against *B. subtilis* which may be due to the phyto-constituents reacting chemically with the antibiotics leading to loss of activity (Dapaah et al., 2016; Adu et al., 2009). The extract enhanced the sensitivity of *P. aeruginosa* to amoxicillin with an MIC of 32 μg/mL, while producing 2 and 4 fold potentiation of

the activities of erythromycin and tetracycline respectively, against *E. coli* (Tables 8 and 14). This indicates that the extract possesses phyto-constituents that make the organisms more susceptible to the antibiotics which may be due to increased drug uptake and efflux pump activity inhibition as well as β lactamase inhibition in the case of penicillins (Munita and Arias, 2016; Adu et al., 2009).

For *M. charantia*, the MIC and zones of growth inhibition was respectively determined to be in the range of 30 to 38 mg/mL and 11.7±0.33 to 13.3±0.33 mm against test organisms. These findings are in agreement with the reported antimicrobial activity of leaf, seed and fruit extracts of *M. charantia* (Costa et al., 2010; Ozusaglam et al., 2013; de Lucena et al., 2015). In the presence of sub-inhibitory concentration of *M. charantia* extract, *S. aureus* and *B. subtilis* lost their sensitivity to tetracycline completely (Table 9). The resistance of *S. aureus* was reduced 66 folds to amoxicillin while that of *P. aeruginosa* and *E. coli* were reduced 15 and 13 folds with MICs of 0.13 and 0.3 μg/mL, respectively (Table 14). This indicates that the extract contains phytochemicals that have either synergistic or inhibitory effect when combined with the test antibiotics (Adu et al., 2009; Costa et al., 2010).

The aerial part methanol extract of *P. amarus* was determined to possess antibacterial activity with MIC in the range of 4 to 6 mg/mL which is in agreement with the findings of Babatunde et al. (2014) and Oluwafemi and Debiri (2008). With the exception of *S. aureus,* the test organisms were more sensitive to ciprofloxacin in the presence of the extract indicating a lowering of efflux pump activity and reduced drug uptake which are the major players in bacterial resistance to the flouroquinolones (Munita and Arias, 2016; Fabrega et al., 2009). With the exception of *S. aureus* that lost activity completely in the presence of the extract *B. subtilis, E. coli* and *P. aeruginosa* became more sensitive to tetracycline with 4, 8 and 16-folds reduction in MICs, respectively (Tables 10 and 14). The observed effect may due to blockade of tetracycline efflux pump activity or modification of the binding site which have been identified as the main mechanism of bacteria resistance to tetracycline (John, 2005; Munita and Arias, 2016).

The methanol bark extract of *P. angolensis* was active against only *S. aureus* and *B subtilis* but not *E. coli* and *P. aeruginosa*. The findings of Olademeji et al. (2006) which give credence to our studies indicate that the stem, leaves and root bark extracts of *P. angolensis* have activity against Gram-positive bacteria but not Gram-negative bacteria. However, Kuete et al. (2011) reported that the root bark extract has antimicrobial activity. In the presence of the extract *B. subtilis* became more resistant to amoxicillin and ciprofloxacin with the resistance increasing 2 folds (Tables 11 and 14). However, Lambert (2002) reported that some phyto-constituents may act as protein activators or co-enzymes which bind to and activate enzymes or genes

responsible for resistance in an organism.

Methanol aerial part extract of *S. afzelii* was observed have antibacterial activity which is in agreement with previous studies (Lagnika et al*.,* 2011). The observance of both resistance and synergistic effects from the methanol aerial part extract of *S. afzelii* (Tables 12 and 14) may be due the presence of myriad compounds. These compounds may either potentiate or inhibit the activity of the test antibiotics by activating or inhibiting certain enzymes or genes responsible for their resistance (Dapaah et al*.,* 2016; Adu et al*.,* 2009).

Methanol leaf extract of *T. occidentalis* had antibacterial activity with MIC of 4 to 10 mg/mL which is in agreement with the findings of Eltayeb and Hamid (2017). However, Sah et al. (2017) reported better antibacterial activity for *T. occidentalis* extract which may be due to the solvent system (a mixture of ethylacetate chloroform and ethanol in a ratio of 30:30:40) used in their extraction protocol. Additionally, Khubeiz et al*.* (2016) reported a poorer antibacterial activity of *T. occidentalis* compared to our findings which may be due to method of extraction (aqueous extraction) and also the geographical as well as the season of collection of plant material which have been reported to influence the levels of the various secondary metabolites in the plants (Inbathamizh and Padmini, 2013; Khattak, 2015). Additionally, *B. subtilis* showed a 33-fold reduction in resistance to erythromycin and tetracycline while *E. coli* also showed an 8 folds reduction in resistance to erythromycin. *P. aeruginosa* gained sensitivity to erythromycin in the presence of the extract with an MIC of 1 µg/mL (Tables 13 and 14). The increased sensitivity of the test organisms to erythromycin and tetracycline may be due to decreased efflux pump activity, target site modification and reduce drug uptake (John, 2005; Munita and Arias, 2016; Leclercq and Courvalin, 2002).

In general, there was almost 30% reduction in *in vitro* activities of the antibiotics when they were combined with the plant extracts. This means that the sub-inhibitory concentrations of the extracts inhibited some mechanisms of action of the antibiotics. In some situations, the antibiotic alone had activity against the organism but in the presence of the extract, the activity of the antibiotic was lost completely thus making the organism very resistant to the antibiotic. This may mean that, the extract might have prevented the antibiotic from entering the organism, prevented the antibiotic from reaching its target site after entering the organism, reacted with the antibiotic to produce a non-active compound(s) or, inhibited one or more enzymes that were involved in the mechanisms of antimicrobial action (Dapaah et al*.,* 2016; Adu et al*.,* 2009; Adu et al*.,* 2014).

The study clearly shows that when medicinal plants are combined with antibiotics, microbial resistance which is a global problem can be reduced. Synergistic combinations of medicinal plants and antibiotics may help to reduce emergence of multidrug resistant mutants, toxicity, exhibit more antimicrobial activity and more effective against mixed infections (Bhardwaji et al*.,* 2016; Abascal and Yarnell, 2004). The above findings show the need to exercise caution when it comes to the indiscriminate combination of herbal medicines with antibiotics.

Conclusion

Potentiation and antagonistic activities were observed among the plant extract-antibiotic combinations. Synergistic activity observed from combinations of plant extract and antibiotics could help reduce the emergence and effect of multidrug resistant strains responsible for some bacterial infections and these extracts possess phytochemicals which could be exploited for their synergistic or antagonistic effect when combined with antibiotics. It is recommended that further studies be carried out to identify the phytocompounds responsible for the observed potentiation or antagonism when these plant extracts are combined with the selected reference antibiotics.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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