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Evaluation of extracts of *Coleus* species for antibacterial activity

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Antibacterial activity of 50%-methanol, water and absolute methanol extracts of *Coleus* species (whole plant) was evaluated using agar, and macro-broth dilution methods. The test microorganisms were strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*. The yields achieved with extracts were 24% for absolute methanol, 22% for the water and 39% for the 50% methanol extracts, respectively. The phytochemical analysis of the extracts revealed the presence of flavonoids, alkaloids, tannins, cardiac glycosides, carbohydrates, steroids, proteins, glycosides and anthracene glycosides. All four (100%) bacterial strains were susceptible to all the extracts with inhibition zone diameter (IZD) ranges of 15-24 mm and minimum inhibition concentrations (MICs) of 1.95-100 mg/ml; and these are consistent with the time kill observed with the extracts. Results authenticate the folklore medicinal usage of *Coleus* species for treatment of diseases such as gastroenteritis and skin infections.

Key words: Antibacterial activity, *Coleus* species, Cameroon.

INTRODUCTION

Plants, once were the only source of medicine in the history of man, that was, before synthetic drugs were introduced and superseded them in orthodox western medicine. However, in recent times herbal remedies have re-emerged as treatments of choice to overcome pathogenic bacterial strains which have developed multiple drug resistance, reduce cytotoxic effects of synthetic drugs on susceptible patients and, of course, meet the need of affordability for those who cannot afford the high cost of efficacious synthetic drugs.

The skepticism surrounding the adoption of herbal remedies in orthodox medicine has been because of the lack of scientific authentication, toxicity evaluation and dose standardization. This work sets out to address some of these problems with one of the common medicinal herbs in sub-Saharan Africa – *Coleus* sp; family, Labiatae.

Species of this genus are edible aromatic perennial herbs which in sub-Saharan Africa, has been used in folklore medicine in treatment of infections and auto-immune diseases, gas and bloating, gastrointestinal and respiratory ailments, athletes foot, skin infections, urinary tract infections, bleeding, fevers, asthma, allergies, sore throat, high blood pressure, arthritis, infertility, red

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blood cell disorders (Cos et al., 2002), and as breast milk stimulant (Muhammad et al., 2013).

Coleus vettiveroides is a bitter cooling diuretic, trichogenous and antipyretic. It is useful in hyperdipsia, vitiated conditions of pitta burning sensation, strangury, leprosy, skin diseases, leucoderma, fever, vomiting, diarrhea and ulcers (Saraswathy and Lavanya, 2013). They are used to treat both fresh cuts and festering wounds, as expectorants and as anti-helmintics ((Kantalak et al., 2003). This report is on the observed antibacterial activity of extracts of the plant.

MATERIALS AND METHODS

Collection of plant materials

Whole plants of *Coleus* species were collected from Kendem in the south west region of Cameroon and authenticated in the Department of Botany, University of Nigeria Nsukka where the voucher specimen has been deposited. The plant was rinsed thoroughly in running tap water, cut into tiny pieces and air dried in the dark. The dried material was then ground to powder in a mortar, weighed and stored in plastic bags in the dark

Extraction of plant materials and phytochemical analysis of extracts

A 100 g weight of powdered plant material was soaked in 400 ml of solvent (absolute methanol, water or 50% methanol in water) in a 1 L conical flask covered with cotton wool plugs. The flask was shaken vigorously at first and then intermittently for 24 h leaving it in a water bath maintained at 40°C between the intervals of shaking. The mixture was filtered, first through three layers of clean muslin cloth, and then through Whatman no 1 filter-paper. The filtrates were evaporated to dryness in a water bath at 56°C. The percentage yields of the crude extract were determined using the principle of Parekh and Chanda (2007).

The phytochemical analysis of the plant extracts was carried out according to the methods described by Trease and Evans (1996).

Test organisms

Strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* used were obtained from the Department of Medical Microbiology, University of Nigeria Teaching Hospital, Enugu, Nigeria. All strains were purified by three successive sub-culturing and re-isolations on Nutrient or MacConkey agar and the identity reaffirmed by standard bacteriological characterization (Cheesbrough, 2006).

Determination of antimicrobial activity of plant extracts

Standardization of the cultures was carried out according to the National Committee of Clinical Laboratory Standards (NCCLS, 1997) to correspond to 0.5 MacFarlaned Standard (approximately 0.5×10^6-1.0×10^8 CFU/ml). The turbidity of the inoculums of each test bacterial strain was adjusted at each time and batch of test.

Susceptibility testing of bacteria was done using the Agar Well Diffusion method (Okeke et al., 2001; Okoli and Iroegbu, 2004). Determination of Minimum Inhibitory Concentrations (MIC) was carried out using the standard methods of European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2000).

The Minimum Bactericidal Concentrations (MBC) were determined by cutting a 2 mm^2 agar disc from the last three agar plates in each dilution showing growth inhibition and inoculating them into fresh sterile nutrient broth. The broth cultures were incubated for 18 h at 37°C, after which 1 ml of the culture was spread over a fresh nutrient agar medium and then incubated at 37°C for 24 h. The least concentration in which no growth occurred was taken to be the MBC (Olukemi et al., 1997).

For the macro-broth dilution method, the Minimum Bactericidal Concentration (MBC) was determined by taking 100 µl of sample from all the tubes which did not show any growth and sub-culturing it on sterile nutrient agar plates, which were subsequently incubated at 37°C for 24 h. The MBC was taken as the least concentration at which no visible growth occurred.

Time-kill assay of extracts of *Coleus* on test bacterial strains

The effect of the 50% methanol extract of *Coleus* species was further evaluated by the use of a time kill assay performed by the macro-broth dilution technique. The extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and sufficiently diluted such that 1.00 ml of each extract added to 9 ml of nutrient broth, seeded with the appropriate concentration of the test bacteria achieved concentrations which were equivalent to 0.5 MIC, 1 MIC, 2 MIC or 4 MICs values. The bacterial inoculum sizes used depended on the strain of test organism, namely; 5×10^8 cfu/ml for *P. aeruginosa*, 5×10^7 cfu/ml for enterobacteriaceae and 1×10^6 cfu/ml for *S. aureus* (Barry and Lasner, 1979). Two sets of control tubes were included for each experiment; one set was seeded with the organism in broth without extract, and the other set, blank broth with neither test organism nor extract. All the bacterial cultures were incubated under aerobic conditions at 37°C for 24 h. Immediately, after inoculation of the tubes, aliquots of 100 µl of the contents of the negative control tubes contents were taken, serially diluted in saline and seeded on nutrient agar plates to determine the count at zero hour. The same was done for the tubes which contained the test bacteria after 15 min, 1 h, 3 h, 6 h, 9 h and 24 h, respectively. After incubation, the number of emergent colonies on the inoculated agar plate was counted and the mean count (CFU) of each test organism was determined and expressed as $\log_{10}$. The Minimum Lethal Concentration (MLC) of the extract was taken as the lowest concentration that gave approximately100% killing (Barry and Lasner, 1979).

RESULTS

The percentage yields of the crude plant extracts obtained with absolute methanol was 24, 22 with aqueous extract and 39 with 50% methanol. Phytochemical analysis of the whole *Coleus* plant showed presence of alkaloids, flavonoids, tannins, cardiac glycosides, carbohydrates glycosides, proteins and anthracene glycosides and steroids, in all extracts without exception and irrespective of the solvent used in the extraction but no reducing sugars (Table 1).

Inhibition zone diameters (IZDs) achieved with the extracts ranged between 11 and 24 mm. The aqueous extract showed the least zones of inhibition while 50% methanol produced the widest zones of inhibition (Table 2).

The control drug (gentamycin) was active on all the bacteria tested with its highest activity being on *E. coli*

Table 1. Phytochemical Composition of *Coleus* species Extracts.

Metabolites	Absolute methanol	Water	50% methanol
Alkaloids	+	+	++
Flavonoids	+++	++	+++
Tannins	++	++	+++
Saponins	–	–	–
Cardiac glycosides	+	+	+
Glycosides	+	+	+
Proteins	+	+	+
Carbohydrates	+	+	+
Reducing sugars	–	–	–
Steroids	+	+	++
Antracene glycosides	+	+	+

+++ = Strongly positive; ++= moderately positive; + = Weakly positive, - = Negative.

Table 2. Inhibition zone diameters (IZDs) of Coleus species extracts on bacteria.

Coleus species Extract (100 mg)	*S. aureus* IZDs (mm)	*E. coli* IZDs (mm)	*S. typhi* IZDs (mm)	*P. aeruginosa* IZDs (mm)
Absolute Methanol	19±1.16	18±1.40	17±0.00	19±1.40
Aqueous	14±1.60	15 ±1.60	11±1.60	15±0.82
50%-Methanol	24±0.82	22±0.82	21±0.82	21±1.16
Gentamycin (Control) (10 µg/ml)	20±0.00	21 ±0.00	16±0.00	18±1.16

(21 mm) and the lowest on *S. typhi* (16 mm).

S. aureus was the most susceptible of all the bacteria tested with MIC values ranging from 3.9mg/ml to 6.25 mg/ml for the agar dilution and 1.95 - 12.5 mg/ml for the broth dilution methods (Table 3). High MIC values were observed for all the Gram negative bacteria tested in the agar dilution method but the 50% methanol extract showed relatively low MIC values ranging from 4.69 mg/ml for *P. aeruginosa* to 7.80 mg/ml for *E. coli* in the macro broth dilution method (Table 3).

The bactericidal activity of the 50%-methanol extract was better when determined with the macro broth dilution method (MBC, 12.5 mg/ml for the Gram negative bacteria and 3.13 mg/ml for *S. aureus,* respectively) than when determined with the agar dilution method (MBC, 50 mg/ml, for the Gram negative bacteria and 6.25 mg/ml for *S. aureus*) (Table 3).

The MIC-MBC indices recorded obtained ranged from 0.25 to 0.75 (Table 3). The absolute methanol extract showed intermediate activity on all Gram-negative bacteria tested in both methods while the aqueous extract showed the highest MIC values in both methods used, (100 mg/ml for *P. aeruginosa*, 100 mg/ml for *S. typhi* in the agar dilution, and 50 mg/ml for *S. typhi,* and 100 mg/ml for *P. aeruginosa* in the broth dilution method (Table 3).

The exposure of *S. aureus* to 4 and 2 MIC (7.8 and 3.9 mg/ml) of 50%-methanol extract of *Coleus* species reduced the viable cell count to undetectable levels in 15 min and 1 h, respectively (Figure 1). The MIC (1.95 mg/ml) reduced the cell count to $0.9Log_{10}$ after 24 h while sub-inhibitory (0.5 MIC) concentrations had no effects on *S. aureus.* The control drug, Gentamycin, (at 1 µg/ml), also, killed the test organism within 1hour of exposure (Figure 1). However, the Minimum Lethal Concentration (MLC) was taken to be 3.9 mg/ml, the lowest concentration which achieved 100% killing.

E. coli was inhibited within 15 min by the 4 and 2 MIC concentrations (31.3 and 15.7 mg/ml) of 50%-methanol extract of *Coleus* species. The MIC (7.8 mg/ml) inhibited it in 24 h while 0.5 MIC reduced the viable cell count to$1.2Log_{10}$ in 24 h. At 1 µg/ml, the control drug (gentamycin) also inhibited it in 3 h (Figure 2). The Minimum Lethal Concentration (MLC) was considered to be 7.8 mg/ml for *E. coli.*

S. typhi was inhibited within 15 min by the 4 and 2 MIC concentrations (25 and 12 mg/ml) of 50% methanol extract of *Coleus* species. The 1 MIC (6.25 mg/ml) inhibited it in 6 h while 0.5 MIC (3.125 mg/ml) showed no bactericidal activity on it. The control drug (gentamycin 2 µg/ml) inhibited it in 1 h (Figure 3) The Minimum Lethal Concentration (MLC) was taken to be (6.25 mg/ml) for *S. typhi.*

The exposure of *P. aeruginosa* to 4 MIC (18.76 mg/ml)

Table 3. The MIC, MBC and MIC-MBC indices of *Coleus* species extracts on bacteria.

Coleus extracts	Agar dilution method			Macro broth dilution method		
	MIC±SD (mg/ml)	MBC (mg/ml)	MIC-MBC Index	MIC±SD (mg/ml)	MBC (mg/ml)	MIC-MBC Index
Absolute methanol						
S. aureus	4.6 9±1.80	6.25	0.75	4.69±1.80	6.25	0.75
E. coli	25±.0.00	50	0.50	12.50±0.0	25	0.50
S. typhi	50 ± 0.00	100	0.50	12.50±0.00	50	0.25
P. aeruginosa	25± 0.00	50	0.50	12.5±0.00	25	0.50
Aqueous						
S. aureus	6.25±0.00	12.5	0.50	12.5±0.00	12.5	1.00
E. coli	50±.0.00	100	0.50	50±0.00	100	0.50
S. typhi	100±0.00	100	1.00	50±0.00	100	0.50
P. aeruginosa	100± 0.00	100	1.00	100±0.00	100	1.00
50% Methanol						
S. aureus	3.9±1.60	6.25	0.63	1.95±0.78	3.13	0.63
E. coli	15.63±0.63	50	0.31	7.8±0.00	12.50	0.62
S. typhi	25±0.00	50	0.50	6.25±0.00	12.50	0.50
P. aeruginosa	25.0± 0.00	50	0.50	4.69±1.80	12.50	0.50
Gentamycin (10 µg/ml) (Control)						
S. aureus	1±0.00	1	1.00	0.50±0.00	1	0.50
E. coli	1±0.00	2	0.50	1.00±0.00	1	1.00
S. typhi	1±0.00	2	0.50	1.00±0.00	2	0.50
P. aeruginosa	2.5±1.00	4	0.62	2.00±0.00	4	0.50

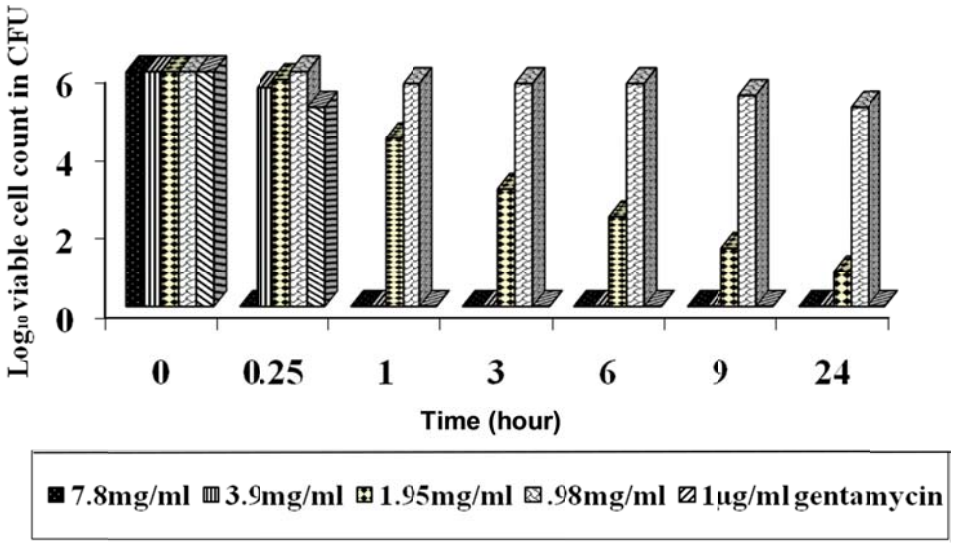

Figure 1. Effect of 50% methanolic extract of *Coleus* species (whole Plant) on viable cell count of *Staphylococcus aureus*.

of 50% methanol extract of *Coleus* species reduced the viable cell count to undetectable levels in15 min. A 2 MIC (9.38 mg/ml) concentration of the extract inhibited it in 9 h, while a 1 MIC (4.69 mg/ml) dosage reduced the viable cell count to $0.98\log_{10}$ in 24 h and the sub-inhibitory (0.5 MIC) concentrations had no effects on *P. aeruginosa.* At

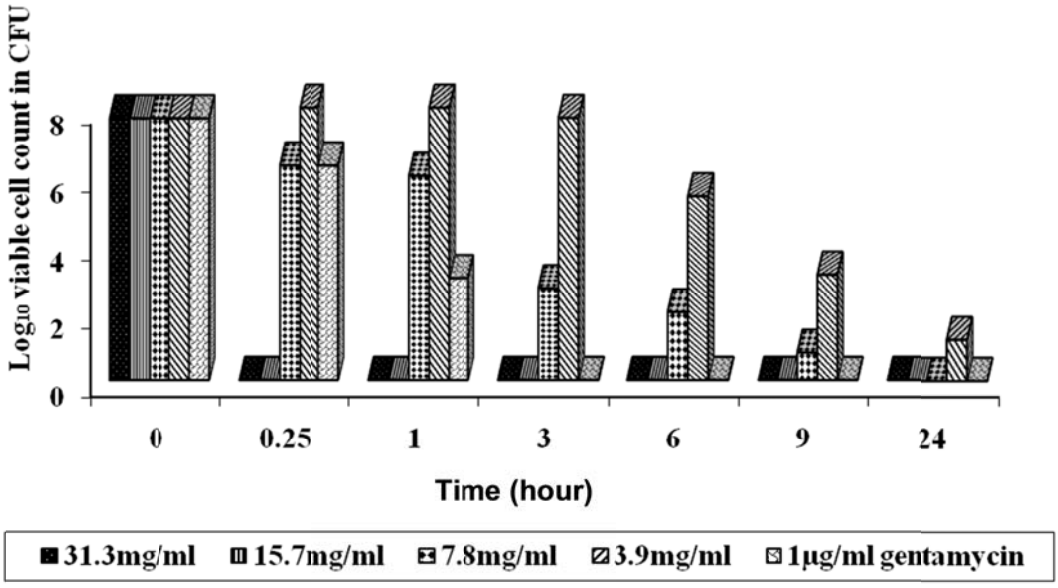

Figure 2. Effect of 50% methanolic extract of *Coleus* species (whole Plant) on viable cell count of *E. coli*.

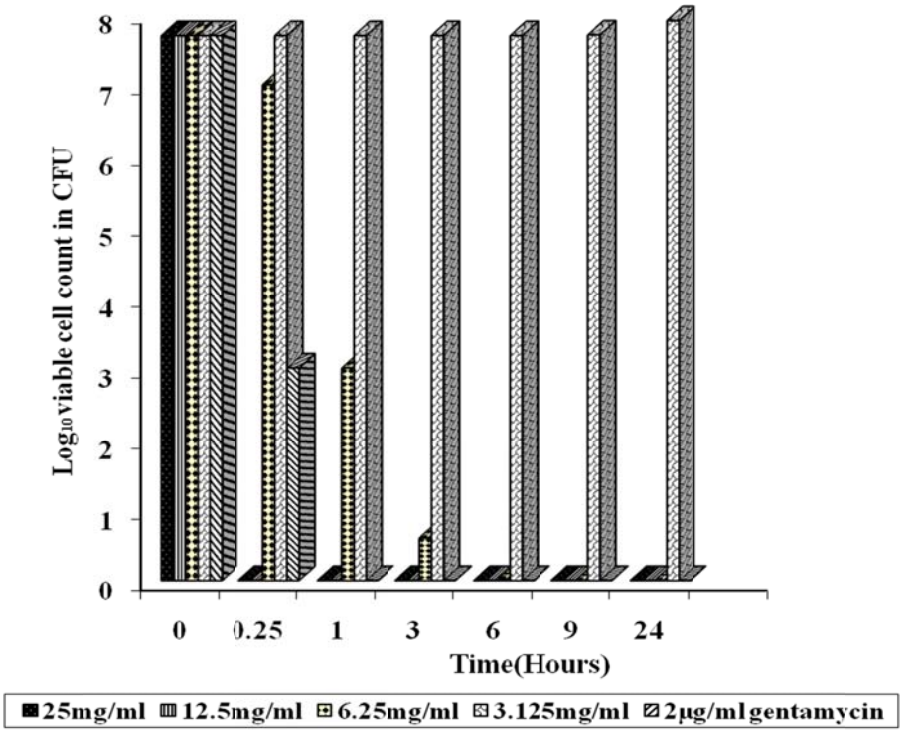

Figure 3. Effect of 50% methanolic extract of *Coleus* species (Whole Plant) on viable cell count of *Salmonella typhi*.

4 µg/ml of the control drug (gentamycin), *P. aeruginosa* was inhibited in 3 h (Figure 4). The Minimum Lethal Concentration (MLC) (of the 50%-methanol extract) was considered to be 9.38 mg/ml for *P. aeruginosa.*

DISCUSSION

The large zones of inhibition produced by the plant extracts against the test bacteria, especially those by the

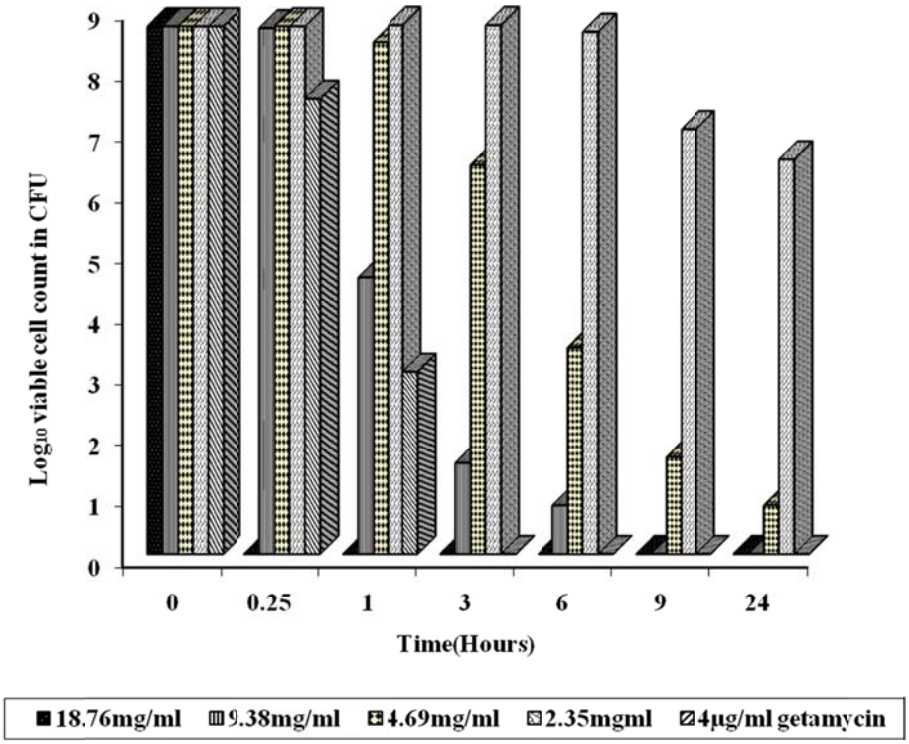

Figure 4. Effect of 50% methanolic extract of *Coleus* species (Whole Plant) on viable cell count of *Pseudomonas aeruginosa*.

50%-methanol extract are indicative of the potency of the bioactive components of the plant against all the test organisms. The susceptibility of the organisms to the plant extracts varied according to the strains and species of the organisms. This was supported by previous works by Karou et al. (2006) and that by Banso and Mann (2006) who observed that microorganisms varied widely in their degree of susceptibility. Sonia et al. (2011), also, observed large zones of inhibition when they tested extracts of *Coleus forskohlii* against strains of *S. aureus, P. fluorescens, Sericea, Kelebsiella pneumonia* and *B. pumilus. K. pneumonia* and *Bacillus pumilus.* The 50%-methanol extract showed better activity than the rest of the extracts. The absolute methanol extracts were also more active than the aqueous extract. The low activity of the aqueous extracts suggests that some of the active principles in these plants were not soluble in water. However, that 50%-methanol extracts showed higher activity than absolute methanol extracts means that more constituents dissolved in 50%-methanol; in other words, dilution with water improved solubility of constituents which would otherwise not dissolve in pure alcohol.

There was no significant difference between the results obtained by the macro broth dilution method and that obtained by the agar dilution method. But it was observed that the broth dilution method is more sensitive than the agar well diffusion method. Diffusion of the crude extracts is usually slower than that of the pure antimicrobial agent in the solid medium. The influence of antibacterial test methods on the results obtained from the susceptibility assays of different organisms to antimicrobial agents has also been reported by Guilherm et al. (2007).

The MIC and MBC values of the crude extracts obtained for the entire tests were high (0.14-100 mg/ml) compared with the MIC values frequently recorded for conventional antibiotics (0.01-10 µg/ml);but the results obtained here are similar to those presented by Adesokan et al. (2007). El-Mahmood (2009) explained that the observed difference between the MIC values obtained with plant extracts and synthetic antibotics is because synthetic antibiotics are in the pure form while crude plant extracts contain some impure substances that may not have any antimicrobial activity and yet contribute to the calculated concentrations. The MBC values obtained in this study were either the same or slightly higher than the MIC values, similar to the results of Karou et al. (2006).

The crude extracts of *Coleus* species inhibited the growth of such recalcitrant microorganisms as *P. aeruginosa* which is usually resistant to many antibiotics. Pseudomonas strains usually display above average resistance to most antibacterial agents, because, they have virulence factors from a restrictive outer membrane barrier and trans-envelope multidrug resistance efflux pumps (Winstanley et al., 1997), which are responsible for a significant level of resistance to antibiotics in

pathogenic bacteria (Kumar and Schweizer, 2005). The MICs obtained for the Gram-negative strains were about four fold higher than those obtained for *S. aureus*, a Gram-positive strain. Extracts of *C. aromaticus* have severally been reported to inhibit *Staphylococcus sp.* and other pathogenic Gram positive bacteria as well as Gram negatives (Jasmine and Selvi, 2013; Malini et al., 2013). However, the low MICs and MBCs observed for some of the extracts against various test microorganisms confirm that certain phytochemical constituents of the extracts have antimicrobial potentials that could change the notion that Pseudomonas, for example, is notoriously drug resistant. Some of these phytochemical compounds include including Saponins and polyphenols which have been severally reported to be bioactive against microorganisms (Sato et al., 2004; Cushnie and Lamb, 2005). Some of these phenols have been shown to exert their antibacterial actions through membrane perturbbations, which when coupled with the action of β-lactams on the transpeptidation of cell membrane could lead to an enhanced antimicrobial effect of the combination (Esimone et al., 2006). Others have been observed to enhance the activities of some antimicrobial compounds by inhibiting multidrug-resistance (MDR) efflux systems in bacteria (Tegos et al., 2002), for example, 5-methoxyhydnocarpin, an inhibitor of the NorA efflux pump of *S. aureus,* has been isolated from *Berberis fremontii (*Stermitz et al., 2000).

The effect of 50%-methanol extract of *Coleus* species on the viable cell counts of the bacteria, varied with the concentration of the extract, duration of exposure and test organism. The 100% killing of the viable cells recorded at different concentrations of the extracts and time is unusual for crude plant extracts which have generally been reported to spare some viable cells even at the highest drug concentration (Ibrahim et al., 1998). It was also observed that sub-inhibitory concentrations of some of the extracts killed some of the microorganisms. In terms of spectrum of activity, 50%-methanol extract of *Coleus* species showed a spectrum of activity that was comparable to the control drug, Gentamycin, and even better in some. However, the control drug was tested in microgram concentrations while the plant extracts were tested in milligram concentrations. Therefore, actual comparison between the control drugs and the extracts would await isolation, purification and determination of molar concentrations of the pure active ingredients of these plant extracts.

Conclusion

These results indicate that the extracts have promise for use in treatment of the traditionally identified diseases provided they are used in the lethal concentrations; absorbed and remain bioactive for up to the time they can achieve complete killing of the etiologic agents. Therefore, actual record of this plant as conventional broad spectrum antibacterial agent would await isolation, purification and determination of molar concentrations of the pure active ingredients.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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