

Original Article

Microbiological Evaluation of Combinations of Extracts of *Euphorbia abyssinica* and *Coleus species* for Antibacterial Activity

Tarh, Jacqueline Ebob* and Iroegbu, Christian Ukwuoma

Department of Biological Sciences Cross River University of Technology Calabar, Nigeria

*Corresponding Author

Tarh, Jacqueline Ebob,
Department of Biological Sciences
Cross River University of Technology
Calabar, Nigeria
E-mail ID: jebobtarh@yahoo.com

Keywords:

Coleus species,
Euphorbia abyssinica,
Combinations antibacterial activity
Checkerboard,
Time kill,
Cameroon,
Nigeria.

Abstract

Antibacterial activity of 50% methanol extracts of *Coleus species* and *Euphorbia abyssinica* were investigated singly and in combination against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, using agar diffusion, macrobroth dilution, Checkerboard and Time kill assays. The inhibition zone diameters (IZDs) produced by the two plant extracts singly ranged from 17mm to 24mm, and those produced by the combination ranged from 17mm to 29mm. In the macrobroth dilution assay, the MICs obtained for the individual plant extracts ranged between 1.95-25mg/ml. In the Time Kill assay of the individual plant extracts, *S. aureus* was killed by 3.91mg/ml concentration of *E. abyssinica* and *Coleus species* alone, (at 1MIC and 2MIC), respectively. The combination killed it at 3.695mg/ml, indicative of synergy. *S. typhi* was killed by 12.5mg/ml and 6.25mg/ml (1MIC) concentration of *E. abyssinica* stem-bark and *Coleus species*, respectively. It was, also, killed by 6.26-, 6.88-, 7.51-, 8.14- and 8.75-mg/ml respective concentrations of different combinations of the two plant extracts. This is, also, indicative of synergy. *P. aeruginosa* was inhibited by 2MIC (25 mg/ml and 9.38mg/ml) of *E. abyssinica* stem-bark and *Coleus species* extracts, respectively, but a combination of the two plant extracts killed at 5.32mg/ml concentration, again indicating synergy. Some of the combinations tested using the checkerboard method showed synergy while others were indifferent or antagonistic after 24hours of exposure. The control drug, Gentamycin, was effective against all the test bacteria. A comparison of two methods revealed that the checkerboard assay was significantly more sensitive than the Time Kill assay ($P < 0.05$). The results authenticate the ethno-medicinal uses of these plants.

1. Introduction

Multiple drug resistance has become a public health problem, worldwide. A vast number of antimicrobial agents available for treatment of infections are becoming therapeutically useless as the number of bacteria becoming drug resistant are ever increasing; in addition, efficacious drugs are showing high toxicity while opportunistic pathogens, for which no anti-microbial drugs are available, are emerging [1,2]. The situation justifies the growing attention of researchers towards finding new antimicrobial agents and/or new treatment regimens with recourse towards evaluation of medicinal plants for biological activity [3].

All through history, man, has depended on plants, individually or in combination (the roots leaves, flowers, barks or whole plants) for food as well as on their decoctions for treatment of different ailments and complaints. Plants store secondary metabolites some of which are bioactive against pathogenic microorganisms while others possess physiological activity. These properties are being continuously unraveled as researches such as the one reported in this paper are being carried out. In folklore medicinal applications some plant are used singly and some in combination with other plants to prepare concoctions with broad spectrum activity against stubborn or persistent infections [4]. Extracts from such medicinal plants are widely being investigated both in crude and pure forms in search of unique bioactive metabolites with chemical properties and mechanisms of action that qualify them for use in combating multidrug resistance.

Coleus species are examples of such plants with a long term history of use in ayurvedic and folklore medicine due to their rich potentials as aromatic and kitchen edibles. They are perennial herbs with mint or regano-like flavor and odor [5,6]. The green *Coleus species* leaves (country borage), which are often used raw with butter for

stuffing bread, can also be used as substitute for seasoning meat dishes, for sage (*Salvia officinalis* L.), and in food products [1]. *Euphorbia abyssinica* (family *Euphorbiaceae*) and *Coleus species* (family *Labiatae* or *Lamiaceae*) are both commonly used in ethno medicine among the *Kendem* people of Cameroon to remedy common problems such as postpartum bleeding, itching, wounds, skin, and respiratory infections; also as antispasmodic, and anti-histamine, smooth muscle relaxant and contracting agent [6]. Other properties of *Coleus species* reported by other researches include anti-epileptic, anti-mutagenic, anti-tumorigenic and anti-genotoxic, anti-inflammatory and anti-tumor, diuretic, anti-oxidant and anti-microbial activities [7]. They have, also, been used as analgesic, in bile flow stimulation, treatment of chronic cough and asthma, headache, fever, dyspepsia, indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough and bronchitis [1,5]. This work set out to evaluate the effect combining extracts of these two plant extracts against some multidrug resistant bacterial strains.

2. Materials and methods

2.1 Collection and Preparation of Plant Extracts

Whole plants of *Coleus species* and the stem-bark of *Euphorbia abyssinica* were collected from *Kendem* in the south-west region of Cameroon. Both plants were authenticated and voucher specimens deposited at the Department of Botany, University of Nigeria Nsukka. The specimens were, first, thoroughly rinsed under running tap water and then cut into tiny pieces air-dried in the dark. The dried material was then pulverized in a mortar, weighed and stored in plastic bags in the dark. Extraction of plant materials was done by the method described by Tarh *et al* [6].

2.2 Test Organisms

Test organisms used were drug-resistant strains of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained from the Department of Medical Microbiology, University of Nigeria Teaching Hospital, Enugu, Nigeria. Purification and reaffirmation of all bacterial strain identity were by standard bacteriological techniques [8].

2.3 Determination of Antimicrobial Activity of Plant Extracts

Susceptibility testing was carried out generally using the agar plate method and the minimum inhibitory concentrations (MICs) of the extracts, singly and in combinations, were determined using the macro broth dilution method according to the European Committee for Antimicrobial Sensitivity Testing [9].

A 1.0ml amount of an 18 hour culture of the bacterial strain, with bacterial cell concentration adjusted to 0.5 MacFarlane standards, was put into sterile tubes containing 19mls of molten nutrient agar (at 45°C) The test tubes were gently shaken to homogenize the bacterial cells into the agar. The content of the tubes were poured into sterile plates and allowed to stand on a flat and undisturbed surface for the agar to solidify. Wells of approximately 6mm diameter and 2.5mm depth were bored on the agar medium, using a sterile cork borer. Approximately 0.5ml of the reconstituted plant extracts, singly or in combination, at concentrations of 100mg/ml, was aspirated with a Finn-pipette into each of the holes. Control experiments consisted of 0.5ml of sterile water (negative) and 0.5ml of 10µg/ml-gentamycin suspension (positive). The plates were left to stand for one hour at room temperature to allow for diffusion of the extract to occur, then, they were incubated at 37°C for 18-24 hours. Zones of inhibitions were measured to the nearest mm; only extracts that showed measurable inhibition zone diameter (IZD) in the test were chosen for further evaluation.

2.4 Checker Board Assay

The 50% methanol extracts were further evaluated in combination using the Checker Board Eumked (1999)[10]. Solutions of the plant extracts were prepared, each in Nutrient broth, and diluted using the continuous variation model, that is, by serially reducing the concentration by 10% with broth down to concentrations below the MIC. Then 2.0ml of each dilutions of *Euphorbia abyssinica* was put into the tubes in the columns such that while the concentrations of the extract changed 10% serially from column to column, the concentration along each column remained the same. The solutions of *Coleus species* extract were similarly distributed into the tubes in the rows such that while the concentrations of the extract vary from one row to the next, the tubes in each row contained the same concentration of the *Coleus species* extract. Consequently each tube received a combination of the two extracts at different ratios. Each of the tubes was then inoculated with 0.1ml of the standardized microorganisms (bacteria) and all the mixtures were incubated aerobically at 37 °C for 24 hours.

The MICs of the combinations were then recorded and the fractional inhibitory concentration (FIC), for each extract, was calculated as MIC of mixed extract divided by MIC of single extract. FIC index was also calculated using the formula, FIC index = \sum FIC *Euphorbia* + FIC *Coleus* [11]. A FIC value of 1 indicates additive interaction, < 1, synergy, and >1, antagonism.

Interactions of antimicrobials in combination were also evaluated from Isobolgram data constructed using MIC data directly or calculating the FICs. These results were plotted as the first points which no growth occurred and this resulted in a plot or graph called an "isobole". If, the two compounds were additive, the results fell on a straight line between the x and y axes. Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line [12,13].

2.5 Time Kill Assay

Time kill assay was done using the method described in Tarh et al[6]. In this method, plant extracts were reconstituted in 20%

Dimethyl Sulfoxide (DMSO) and diluted to MIC concentrations. These were then combined using the continuous variation method to obtain a concentration range which included the MIC obtained with the individual plant extracts as well as sub-inhibitory concentrations. Then 0.1ml of the (MacFarland) standardized inoculum was put in to 9.9mls of the diluted plant extracts. Tubes of nutrient broth only were included as positive controls while other tubes containing the MICs of the plant extract alone were also included in the tests. A volume of 100µl from the negative control tubes i.e. tubes containing bacteria without plant extract were withdrawn immediately after inoculation, to determine the zero-hour count. These were serially diluted and seeded on the nutrient agar. The tubes were incubated at 37°C for 24 hours, during which aliquots of 100µl were withdrawn at intervals of 15 minutes, 1 hour, 3 hours, 6 hours, 9 hours, 24 hours after inoculation, diluted and plated for colony counts.

The means of two separate tests counts were determined and expressed as Log₁₀CFU. The interactions were considered synergistic if there were decreases of $\geq 2\log_{10}$ CFU/ml in colony counts after 24 hours by the combination compared to the most active single agent. Additively or indifference was described as a $< 2\log_{10}$ CFU/ml change in the average viable counts after the incubation periods for the combination, in comparison with the most active single drug. Antagonism was defined as a $\geq 2\log_{10}$ CFU/ml increases in colony counts after the incubation periods by the combinations compared to that of the most active single extract alone [14].

The data analysis was done using the Randomised Complete Block Design (Two-way analysis of variance). Duncan's New Multiple Range Test was used to separate the means that were significantly different.

3. Results

3.1 Antimicrobial Susceptibility Testing

From the IZDs obtained in the Susceptibility testing, the 50% methanol extracts of the two plants exhibited inhibitory effects on all the bacteria strains tested. A combination of the extracts inhibited all the bacteria tested with IZDs Ranging from 22 (*P. aeruginosa*) to 27 (*S. typhi*) (Table 1)

3.2 Checkerboard assay of the Combined *E. abyssinica* (E) and *Coleus species* (C) Extracts on Test Bacteria

As previously stated, synergy was indicated by a curved deviation to the left of the additive line, while antagonism was a curved deviation to the right of the additive line. The checkerboard assay, of the combined extract preparations showed synergistic activities against *S. aureus*. Synergy was seen at MIC combinations of 3.5E + 0.195C and FIC index of 0.99 (Fig. 1). For *E. coli*, it was seen at MIC combinations of 7.5E+4.68C, FIC Index, 0.90 (Fig 2). For *Salmonella typhi*, synergy was seen at respective MIC combinations and FIC indices of 6.25E+0.63C; 0.60; 6.25E+1.89C, 0.80; 6.25E+ 2.52C, 0.90; 2.5E+4.38C, 0.90; and 1.25E+5.01C, 0.90 (Fig. 3). For *P. aeruginosa*, synergy was seen at MIC combinations of 2.5E+2.82C, FIC Index, 0.80; 1.25E+3.29C, FIC Index, 0.90 and 1.25E+ 3.79C, FIC Index, 0.90 (Fig. 3). The rest of the combinations showed additive and antagonistic effects against the bacteria (FIC indices >1<2).

3.3 Time-Kill (Inhibition) Assay

The antimicrobial activity of the combination of *Euphorbia abyssinica* stem bark and *Coleus species* extracts against *S. aureus* showed that the initial cell population of 6.0 log₁₀ CFU/ml was reduced by 9/1 combination (3.5E + 0.195Cmg/ml = 3.695mg/ml) of the extract to undetectable levels in 24 hours of contact time (Fig.5).

No antibacterial activity was shown against bacterial test suspension of *E. coli* in 24hours with all the combinations tested (Fig. 6). The initial population of test suspension of *Salmonella typhi* was reduced by 5/1 (6.25E+0.63C = 6.88mg/ml) combination to undetectable levels in 6 hours (Synergy). The 5/2 combinations ((6.25E+1.26C =

7.51mg/ml), 2/7 (2.5E+4.38Cmg/ml = 6.88mg/ml), and 1/8 combination (1.25E+5.01Cmg/ml =6.26mg/ml) reduced the bacterial count to undetectable levels in 3hours (synergy). The 5/3 and 5/4 combinations [(6.25E+1.89C = 8.14mg/ml) and (6.25E+2.5C = 8.75mg/ml)], reduced the population to undetectable level in 1 hour (Synergy) (Fig.7).

Pseudomonas aeruginosa was not affected by low concentrations (4.54 mg/ml and 5.04mg/ml) of the combination of the plant extracts (Fig. 8), but a higher concentration (5.32mg/ml) of the combination inhibited it after 24 hours of contact time (synergy).

Table 1: Inhibition Zone Diameters (IZDs) in mm of the Plant Extracts on Bacteria.

50% Methanol extract of plant 100mg/ml	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
<i>E. abyssinica</i> stem bark	23 ±1.60	17±2.30	18±2.30	19±1.61
<i>Coleus species</i>	24±2.80	22±2.30	21±0.82	21±1.16
Combination (Euphorbia+Coleus)	26±1.60	22±0.00	27±0.00	22±0.00
Gentamycin (Control)(10µg/ml)	20±0.00	21 ±0.00	16±0.00	18±1.16

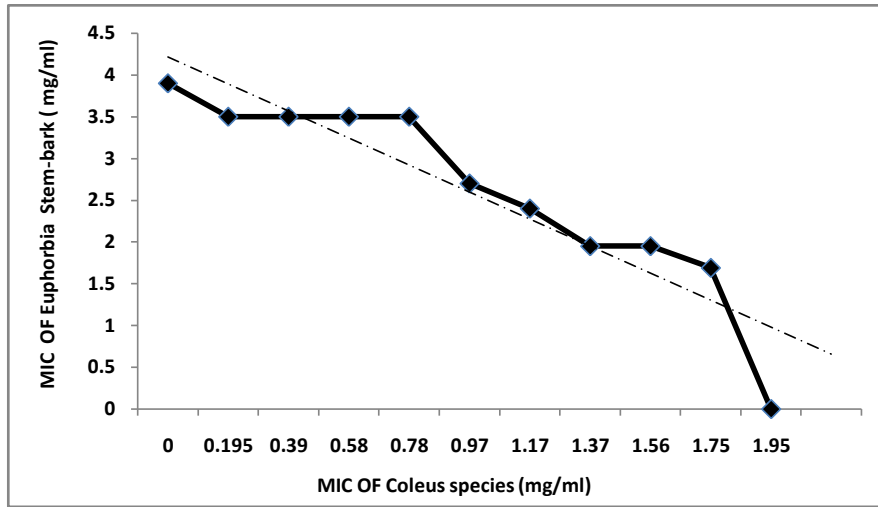


Figure 1: Checkerboard assay of *E. abyssinica* and *Coleus species* against *S. aureus*.

----- =Additive line

◆ = Point of interaction between Euphorbia and Coleus species Extracts

Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line.

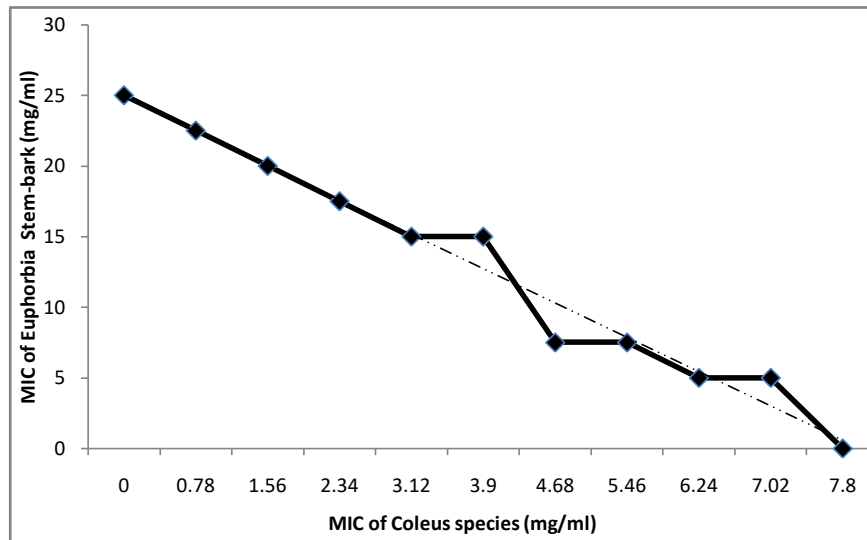
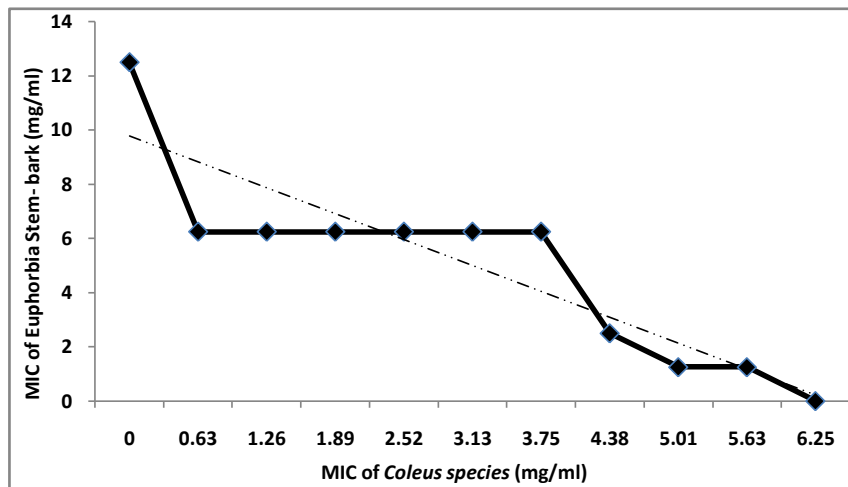


Figure 2: Checkerboard assay of *E. abyssinica* and *Coleus species* against *E. coli*

----- =Additive line

◆ = Point of interaction between Euphorbia and Coleus species Extracts

Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line



----- =Additive line

◆ = Point of interaction between *Euphorbia* and *Coleus* species Extracts

Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line

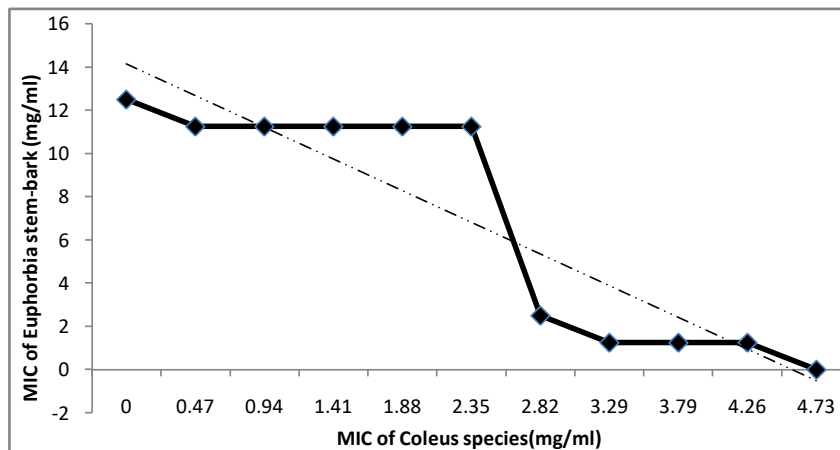


Figure 4: Checkerboard assay of *E. abyssinica* and *Coleus* species against *P. aeruginosa*

----- =Additive line

◆ = Point of interaction between *Euphorbia* and *Coleus* species Extracts

Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line

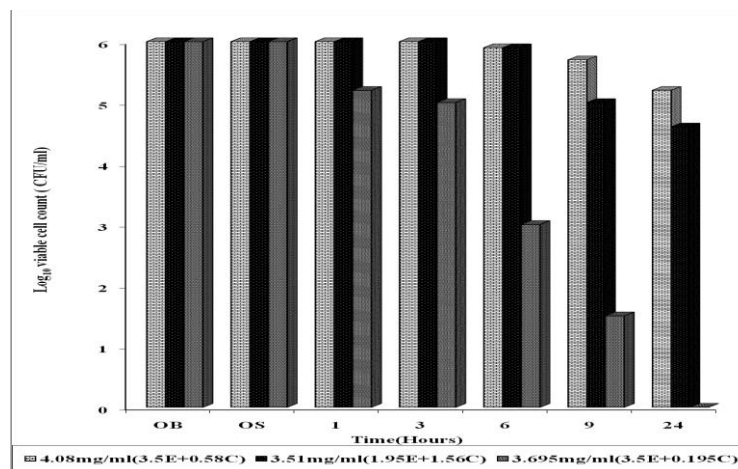


Figure 5: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus* species (Whole Plant) on the viable cell count of *Staphylococcus aureus*

KEY: OB= Time of commencement of the experiment. (Zero time); OS=15 minutes after the commencement of the experiment; E=*Euphorbia abyssinica*, C=*Coleus* species

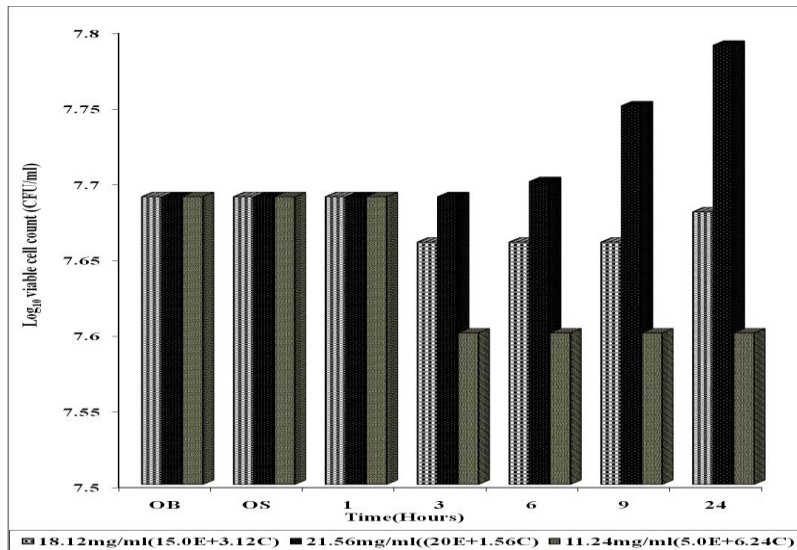


Figure 6: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus* species (Whole Plant) on the viable cell count of *E. coli*

KEY: OB= Time of commencement of the experiment. (Zero time); OS=15 minutes after the commencement of the experiment; E=*Euphorbia abyssinica*, C=*Coleus* species

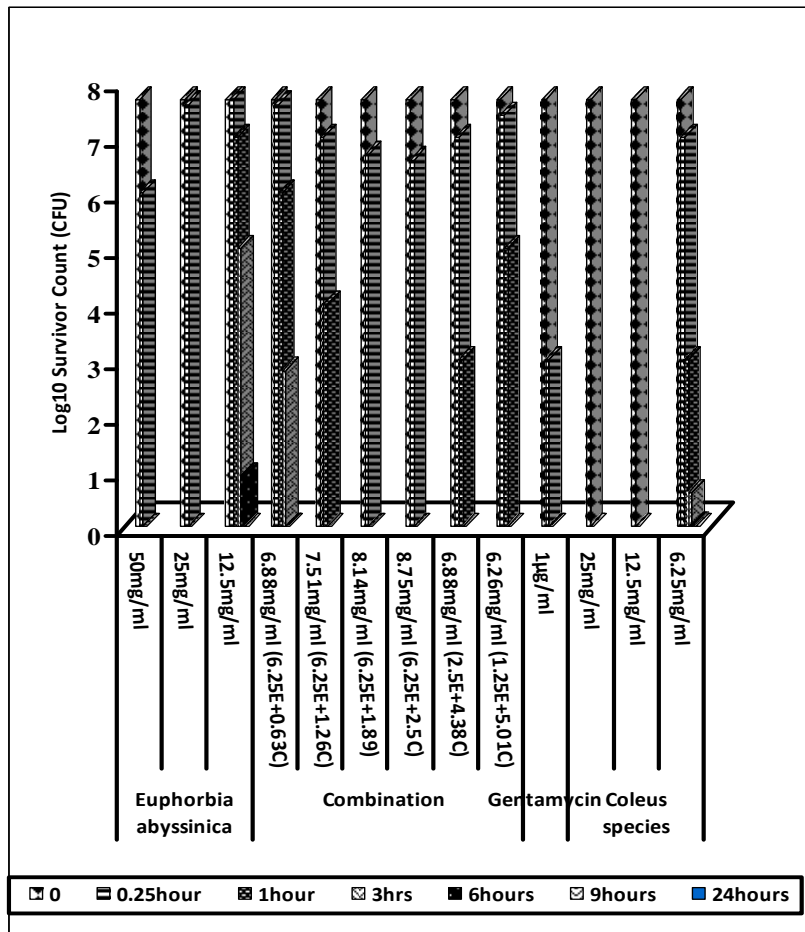


Figure 7: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus* species (Whole Plant) on the viable cell count of *S. typhi*

KEY: OB= Time of commencement of the experiment. (Zero time); OS= 15 minutes after the commencement of the experiment; E=*Euphorbia abyssinica*, C=*Coleus* species

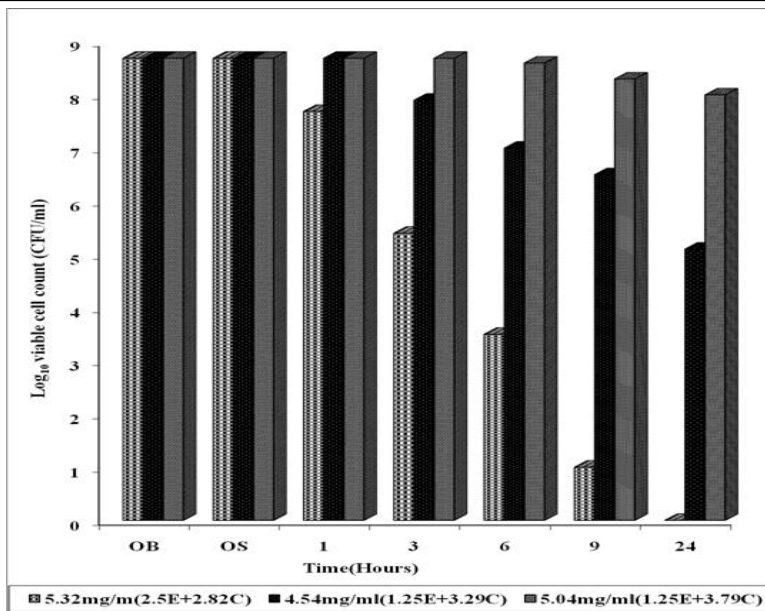


Figure 8: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus* species (Whole Plant) on the viable cell count of *P. aeruginosa*

KEY: OB= Time of commencement of the experiment. (Zero time); OS= 15 minutes after the commencement of the experiment; E=*Euphorbia abyssinica*, C=*Coleus* species

4. Discussion

The checkerboard and time kill methods of evaluating antimicrobial interactions rely on the predetermination of the MICs of the component drugs or extracts e.g drug A and drug B singly, hence, it heavily relies on the accuracy of MIC values and its utility is affected by day to day variations in this parameter which sometimes exceeds the predetermined MIC.

Based on the checkerboard assay, some of the extract combinations were found to exhibit synergy on the bacteria tested; but a vast majority of the combinations were either indifferent or antagonistic.

The time kill assay compared the reduction in viable cell count of each test bacterial strain when treated with mixture of extracts to the value obtained by exposing the strain to each extract singly. Synergistic effect was observed with *S. aureus* within 24 hours of exposure to a combination of 1.95mg/ml of *E. abyssinica* extract and 1.56 mg/ml of *Coleus* extract (3.51mg/ml combined concentration) with a reduction of >2 log₁₀. For *E. coli* a combined concentration of 11.24mg/ml of the extracts did not show lethal effects, rather there was an increase in the viable cell count after 24hours of exposure. This signified indifference (>1<2log₁₀ increase in viable cell count in 24 hours) to *E. coli*. In the case of *S. typhi* the cell were completely killed within 1-6 hours, thus showing that the combinations tested were synergistically quick acting and bactericidal against this strain (>2log₁₀ decrease in viable cell count). Combined concentrations of 6.85-6.88mg/ml showed better effects because they reduced the bacterial viable cell count to undetectable levels at shorter time interval (3 hours). Concentrations of 8.14-8.75mg/ml achieved the same effects in one hour, but they are invariably higher concentrations of the plant extracts which could exhibit undesirable toxic effects.

Sub-inhibitory concentrations of *Euphorbia abyssinica* and high concentrations of *Coleus* species killed *P. aeruginosa* in 24hours. Shiney et al [15] reported similar observations with combinations of alcoholic extracts of *Coleus aromaticus* and *Leucas aspera* on *E.coli*, *S. typhi*, and *Shigella*. Synergy against *P. aeruginosa* is significant because of the widely reported natural resistance of this organism to several antibiotics.

Synergy was detected against various Gram-negative and Gram positive organisms at varying concentrations of the combined

extracts and exposure intervals. The synergy detected in this study was not specific to any species of organisms (Gram- positive or -negative). Consequently, there was no significant difference (P>0.05) in the susceptibility pattern of the bacterial strains tested. This is similar to the observations of Senthilkumar et al [3] with extracts of *Coleus forskohlii* and *Tecoma pogostemon patchouli* on five bacterial species (*S.aureus*, *S. typhi*, *Klebsiella pneumonia*, *S.epidermidis* and *Vibrio parahaemolyticus*).

Comparing the checkerboard and time kill, the results of this experiment showed that the time kill assay was significantly less (P>0.05) sensitive, than the checkerboard assay.

Previous investigators have noted that the activities of this kind might result from several constituents of the plant extract and not just one active compound [16]. Crude extracts of plants in general would contain a mixture of bioactive compounds which can either enhance or antagonize the activities of one another. If drugs in combination are antagonistic fractionation of the extracts would be required to separate the antagonizing constituents and achieve better efficacies. Besides, antagonism would allow apparent resistance to develop and spread; the less effective constituents would allow weakly resistant clones to survive and be transmitted [13]. Certain drug combinations may show antagonism in-vitro without exhibiting same in vivo. Thus, evaluation of minimal lethal concentrations (MLC) and 50% lethal dose (LD₅₀) may be more reliable than MIC for calculating FICs because they would better represent the sensitive microbial population in plasma and the therapeutic doses [13]. In conclusion, use of combinations of the extracts of the investigated plants and others may provide solution to drug resistance by the susceptible bacterial strains and particularly, the notorious *Pseudomonas* species.

References

- [1] Subhas C. M, Harsha, R., Dinesha, R., Thammanna, G. S. S. Antibacterial Activity of *Coleus aromaticus* Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2010; 2 (3): 63-66.
- [2] De Britto, J. A. Steena, R. S. & Mary. S.R. Antibacterial Activity of Selected Species of *Lamiaceae* against Human Pathogens. *Indian Journal of Natural Products and Resources*, 2012; 3 (3):334-342.

- [3] Senthilkumar, C. S. Kumar. M. S., & Pandian, M. R. *In Vitro* Antibacterial Activity of Crude Leaf Extracts From Tecoma Stans (L) Juss. Et Kunth, *Coleus Forskohlii* and *Pogostemon Patchouli* Against Human Pathogenic Bacteria. *International Journal of Pharmacy and Technological Research*, 2010; 2 (1): 438-442.
- [4] Okoli, A. S. and Iroegbu, C. U. Evaluation of extracts of *Anthocleista djalonenis*, *Nauclea latifolia* and *Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcal urethritis. *Journal of Ethnopharmacology*, 2004; 92: 135-144.
- [5] Annadurai, G. Malini, M. Abirami, G and Hemalatha, V. Antimicrobial activity of Ethanolic and Aqueous Extracts of medicinal plants against waste water pathogens. *International Journal of Research in Pure and Applied Microbiology* 2013; 3(2): 40-42
- [6] Tarh, J. E., Okafor, J. I., and. Iroegbu, C. U. Evaluation of Extracts of *Coleus* Species For Antibacterial Activity. *African Journal of Biotechnology*, 2015; 14(2): 125-132.
- [7] Jasmine, R. & Selvi S. J. Analysis of Antibacterial Activity of The Bioactive Constituents From The Acetone Extract of *Coleus Aromaticus* (Benth) Leaves by Gas Chromatography –Mass Spectrometry. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 2 (5): 3088-3093.
- [8] Cheesbrough, M (2006). District Laboratory Practice in Tropical Countries, Part 2; second edition. Cambridge, Cambridge University Press
- [9] European Committee for Antimicrobial Susceptibility Testing (EUCAST). Determination of MICs of antibacterial agents by agar dilution. *Clinical Microbiology and Infection* 2000; 6(9)509-515.
- [10] Eumkeb, G. (1999). Investigation of the Effects of Antifolates on *E. coli* 1810 (PhD Dissertation). The Robert Gordon University, United Kingdom
- [11] Mandal, S., Mandal, M. D., Pal, N.K. Evaluation of Combination effect of Ciprofloxacin and Cefazolin against *Salmonella enterica* serovar typhi isolates by in vitro methods. *Culicuture Medical Journal* 2004; 2(2): e2
- [12] Jee Young, R., You Sun, M., Seung Hee, J., Kyue Yun, L., Su Yun, L., Chang Sub, S. and Won Bong, P. Antomicrobial activities of combined extract of Aloe vera with propolis against oral pathogens. *Journal of Korean Society of Food Science and Nutriition*, 2002; 3:899-904.
- [13] Quinton, L.F. Pemida, S.A, David, C.W. Modified Fixed Ratio Isobologram method for studying *in vitro* interaction between Atovaquone and Proquanil or Dihydroartemisinin against Drug-resistant strains of *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy*, 2004; 48(11):4091-4102.
- [14] Pankey, G., Ascraft, D., Pantel, N. *In Vitro* Synergy of Daptomycin plus Riphampin against *Enterococcus faecium* Resistant to both Linezolid and Vancomycin. *Antimicrobial Agents and Chemotherapy*, 2005; 49(12):5166-5168.
- [15] Shiney, B. R., Ganesh, P. and Suresh, K. Pphytochemical Screening of *Coleus aromaticus* and *Leucas aspera* and their Antibacterial Activity Against Enteric Pathogens. *International Journal of Pharmaceutical and Biological Archives*, 2012; 3 (1):162-166.
- [16] Essawi, T. and Srour, M. Screening of Some Palestinian Medicinal Plants Activity, *Journal of Ethnopharmacology*, 2000; 70:343-349.