Comparative Evaluation of Combinations of Extracts of *Euphorbia abyssinica* and *Coleus species* for Antifungal Effects

Abstract

Aim: Evaluation of Combinations of 50% methanol extracts of *Euphorbia abyssinica (Desert Candle)*, and *Coleus species* for antifungal activity using *Candida albicans, Trichophyton* mentagrophytes, *Microsporum gypseum* and *Epidermophyton floccossum* as test strains.

Study Design: The completely randomized block design, two way analysis of variance was used and Duncan's New Multiple Range Test, for mean separation.

Place and Duration of Study: The research was carried out in the Department of Microbiology, University of Nigeria Nsukka, Enugu State, Nigeria, between April 2011 and August 2012.

Methodology: The fungal strains used were species all collected from the University of Nigeria Teaching Hospital Enugu, Nigeria. Susceptibility testing was done using pour-plate method, while the checkerboard and Time kill assays were employed to evaluate the efficacy of the combinations.

Results: The individual plant extracts inhibited all the fungal strains tested at different concentrations. *Coleus species extracts* were more potent in activity than *Euphorbia abyssinica* extracts. The combination inhibited the test fungi for more than 14days. In the Time Kill assay, the combinations showed synergy on *E. Floccossum* only. It showed additive or antagonistic activity on the rest of the fungi tested. The Checkerboard combination showed synergy on *T. Mentagrophytes, M. gypseum, and E. foccossum. E. foccosum* was the most susceptible of the fungi tested while *C. albicans* was the least susceptible. The control drug voriconazole also inhibited all the fungi tested. The checkerboard assay was significantly more sensitive than the Time Kill assay (P=0.05) in this study.

Conclusion: The results authenticate the ethno-medicinal uses of these plants, suggesting that they can be used as alternative sources of agents for the treatment of resistant fungal infections.

Keywords: *Coleus species, Euphorbia abyssinica*, Combinations, antifungal effects, Checkerboard, Time kill, Cameroon, Nigeria.

1. Introduction

Fungal infections such as onychomycoses, disseminated infections associated with opportunistic pathogens like *C. albicans,* dermatophytosis, (invasion of keratinized tissues – skin, hair and nails – of humans and other animals) caused by three anamorphic fungal genera (*Epidermophyton,*

Microsporum and *Trichophyton*), have reportedly increased worldwide[1] and so have become a public health concern. Recently life-threatening and potentially fatal fungal infections have emerged in immune-compromised people [2] with increasing drug resistance recorded in several cases, which were previously susceptible to the normal synthetic antifungal agents.

The spread of antifungal drug resistance is equally becoming a public health challenge globally; and the situation has been exacerbated by global travel and distribution of food products, innate random mutations, acquisition of resistance genes from other microorganisms, widespread indiscriminate use of antimicrobials as pesticides or, in animal feed, as food preservatives, and for treatment of infected patients. To add to the problem of resistance, treatment failure, and toxicity, most synthetic drugs are unaffordable to most people in rural and less developed areas of the world. For the latter, their existence and survival history would be incomplete without a mention of the role plants as sources of food and/or medicines. Plants are naturally endowed with primary and secondary metabolites that are incidentally very important nutrients or medicines to man and livestock. Thus, as research reports on medicinal plants accumulate, there is gradual replacement of synthetic drugs (now notorious for failure in treatment of resistant pathogens and in general toxicity) from the pharmaceutical shops with herbal remedies.

Application of combinations of herbs to cure certain diseases is common in ethno-medicine and has formed the basis for experimentation on combinations of therapies as solution to extensive drug resistance by microbes [3]. Thus, multiple drug resistance (MDR) inhibitors or resistance modifying agents work synergistically to modify the resistance phenotype in microorganisms. The search for such compounds in plants can give a leeway to the treatment of drug resistant infections as alternative to overcoming the problem of resistance [4].

*Euphorbia abyssinica (*family *Euphorbiaceae)* and *Coleus species*, 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 [5] are examples of such plants with broad spectrum of antimicrobial activity.

The word *Coleus* was derived from the Greek word *Coleos*, meaning “sheath. First described by the Portuguese naturalist, João de Loureiro (1717-1791), *Coleus* are aromatic herbs, belonging to the genus *Plectranthus* and family *Lamiaceae (Labiatae)* [6]. The plant is called Osem antuoh in Kendem

(Cameroon) and traditionally, it is used by local population and herbalists to treat infectious diseases as infusions for systemic infections or applied topically on the skin to cure local infections [7].

In the present study, we focused on investigating the antifungal effects of combinations of extracts of *Euphorbia abyssinica* and *Coleus species*

2. Materials and methods

2.1. Collection and Preparation of Plant Extracts

The stem-bark of *Euphorbia abyssinica* and whole plants of *Coleus species* were collected from Kendem village in the southern Cameroon. The specimens were authenticated at the Department of Botany, and the research carried out in the Department of Microbiology in the University of Nigeria, Nsukka. The specimens were thoroughly rinsed under running tap water and then cut into tiny pieces and air-dried in the dark. They were pulverized in a mortar, the powder weighed and stored in plastic bags. The powdered materials were then extracted using the method described by [8].

2.2. Test Organisms

Test fungi used were obtained from the Department of Medical Microbiology, University of Nigeria Teaching Hospital Enugu, Nigeria. They were purified and their identity reaffirmed by standard mycological techniques.

2.3. Susceptibility Testing of Fungi by Pour-plate Method

The susceptibility testing of fungi was done using pour-plate method as described by [9]. A 2.0 mL amount of a 1000 mg/mL reconstituted plant extract was pipetted into sterile glass test tube containing 18mL of molten Sabouraud Dextrose Agar (SDA) at about 45°C. The mixture was swirled carefully for the contents and agar to homogenize, thereafter, 100µl of the standard fungal inoculum was seeded onto each tube. Again they were thoroughly mixed, then contents of each tube poured into a sterile Petri dish and allowed to set before incubating at 25-35°C. A culture plate without the extract served as the positive control for growth while another plate containing 2.0 mL of 16 µg/mL voriconazole as the negative control. As soon as growth was observed at the positive control plates the test plates were checked for growth daily and the period of inhibition of growth was recorded in days.

2.4. Checker Board Assay

The 50% methanol extracts were further evaluated in combination using the Checker Board assay method [5]. Solutions of the plant extracts were prepared, each in sabouraud 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 (fungi) and all the mixtures were incubated aerobically at 25 -35^{o}c observing daily for appearance of growth.

The MICs of the combinations were then recorded and the fractional inhibitory concentration (FIC), for each extract, was calculated as MIC of extract in the combination divided by MIC of single extract. FIC index was also calculated using the formula, FIC index = Σ FIC *Euphorbia* + FIC *Coleus*. FIC index value of 1 indicates additive interaction, < 1, synergy, >1<2, Indifference and >2, antagonism [5].

The Isobologram data generated from the results of the interactions of plant extracts in combination, using MIC data directly as well as the calculated FICs, were plotted as the first points which no growth occurred. This resulted in a plot or graph called an “isobole”. Any points which fell on a straight line between the x and y axes was considered as additive. A curved deviation to the left of the additive line, was an indication of synergy, while antagonism was indicated by a curved deviation to the right of the additive line [5].

2.5. Time Kill Assay

The effects of 50% methanol extracts of *Euphorbia abyssinica and Coleus species* were evaluated by a kinetic time kill assay using the macrobroth dilution technique as described in [5]. The extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and appropriately diluted to the required concentrations. The inoculum size was determined according to the type of fungus, (e.g. 1 x10^{6} for *Candida albicans*; and1 x10^{5} for dermatophytes). About 1.00mL of the extract was added to 9 ml of Sabouraud dextrose broth, seeded with the appropriate concentrations of the test fungus to achieve concentrations equivalent to 0.5 x MIC, 1 x MIC, 2 x MIC, or 4 x MICs values. 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 contained broth without organism and extract. The control drug voriconazole

was similarly diluted. All the fungal cultures were incubated at 35°C for ≥48 hours. Immediately after inoculation of the tubes, aliquots of 100 µL of the negative control tubes contents were taken, serially diluted in saline and seeded on nutrient agar plates to determine the zero hour counts. The same was done for the tubes which contained the test fungi after 0 hour, 6 hours, 12 hours, 24 hours and 48 hours, respectively. After incubation, the emergent colonies were counted and the mean count (CFU) of each test organism was determined and expressed as $\log_{10}$. The Minimum Lethal Concentrations (MLCs) of the extract were the lowest concentrations that gave 99.9% to 100% killing.

In the interaction study, plant extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and 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 standardized inoculum was put in to 9.9mLs of the diluted plant extracts. Inoculated tubes of Sabouraud Dextrose broth were included as positive controls, Tubes of Sabouraud Dextrose broth only were included as negative controls while other tubes containing the MICs of the plant extract alone were also included in the tests. A volume of 100µL from the tubes containing fungi without plant extract were withdrawn immediately after inoculation, serially diluted and seeded on the already prepared Sabouraud Dextrose agar plates to determine the zero-hour count. The tubes were incubated at 25- 35 ^{o} C for > 48 hours, during which aliquots of 100µl were withdrawn at intervals of 15 minutes, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours after inoculation, diluted and plated for colony counts.

The means of two separate tests counts were determined and expressed as Log_{10}CFU.The interactions were considered synergistic if there were decreases of ≥2$\log_{10}$CFU/mL in colony counts after incubation periods by the combination compared to the most active single agent. Additivity 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 ≥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 [9].

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. Antifungal Susceptibility Testing

From the number of days the inhibition of the fungi lasted in the Susceptibility testing, the 50% methanol extracts of the two plants exhibited inhibitory effects on all the test fungal strains. The number of days the combination of the extracts inhibited all the fungi tested were more than 14days (Table 1)

3.2. Combined activity of *E. abyssinica* (E) and *Coleus species* (C) Extracts on Test Fungi using Checkerboard assay

In this assay, a curved deviation to the left of the additive line was an indication of synergy, while a curved deviation to the right of the additive line indicated antagonism. At different combinations of the extracts, the susceptibility of the fungal isolates was such that *E. floccossum* > *M. gypseum* > *T. mentagrophytes* > *Candida albicans.*

For *T. mentagrophytes* synergy was seen at FIC E 0.2+ FIC C 0.7, FIC Index 0.9, and at FIC E 0.1, FIC C 0.8, FIC Index 0.9 (Fig.1)

For *M. gypseum*, it was observed at FIC E 0.8, FIC C 0.1, FIC Index 0.9, FIC E 0.7, FIC C 0.2, FIC Index 0.9, FIC E 0.4, FIC 0.5, FIC Index 0.9, FIC E 0.3, FIC C 0.6, FIC Index 0.9 (Fig. 2).

For *E. floccossum* synergy was seen at FIC E 0.6, FIC C 0.1, FIC Index 0.7, FIC E 0.6, FIC C 0.2, FIC Index 0.8, FIC E 0.6, FIC C 0.3, FIC Index 0.9 and at FIC E 0.1, FIC C 0.8, FIC Index 0.9 (Fig. 3).

The combinations showed either additive or antagonistic effects against *Candida albicans* (Fig. 4).

3.3. Combined activity of *E. abyssinica* (E) and *Coleus species* (C) Extracts on Test Fungi using Time Kill Assay.

In this assay, the interactions were considered synergistic if there were decreases of $\geq 2\log_{10}$CFU/mL, additivity or indifference was described as a $< 2\log_{10}$CFU/mL and 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.

The antifungal effect of combinations of 50% methanol extract of *Coleus species* and *E. abyssinica* was estimated as the reduction of viable cell count following exposure to a given concentration of the extract combinations or control drug (voriconazole) over a period of time (0 hour, 6hours, 12hours, 24hours and 48hours), the viable cell count being kept constant at $1x10^6$ for *Candida albicans* and $1x10^5$ for the dermatophytes.

The MIC and x2 MIC of 50% methanol extract of *Coleus species* (the more active single drug) reduced the viable fungal cell number of *Candida albicans* and *Trichophyton mentagrophytes* respectively to less than 0.05log10 in 48 hours (Fig. 5 & 6). The x2 MIC (15.6mg/mL) concentrations killed *M. gypseum* in 6hours and the control drug (voriconazole*)* cleared the cells in 48hours (Fig. 8). Combinations of the two plant extracts showed virtually just additive effects on all the three fungi species (Fig. 5, 6 & 7)).

The fungicidal activity of the 50% methanol extract of *Coleus species on E. floccosum* was such that 2MIC (1.96mg/mL) concentrations inhibited *E. floccosum* totally in 3hours. The 1MIC (0.98mg/mL) reduced the viable cell count to 0.97$\log_{10}$ and1µg/mL of the control drug inhibited the fungal cells in 48 hours (Fig. 8).

Compared to the most active single extract *(Coleus species),* all the combinations showed synergistic activities against *E. floccosum.* The initial cell proportion of 5.0 $\log_{10}$ CFU was reduced by a concentration of 1/8 (0.098E +0.78Cmg/mL = 0.878mg/mL) to 2.0 $\log_{10}$ in 48 hours (Synergy), 6/1 (0.59E +0.098Cmg.mL = 0.688mg/mL) and 6/2 concentration (0.59E+0.196C = 0.786mg/mL) concentration reduced the count to undetectable levels in 48 hours, a greater than 2$\log_{10}$ decrease in cell count (Synergy). The 6/3 (0.59E+0.29Cmg/mL 0.88mg/mL) inhibited the fungal growth reducing the viable cell count to 1.0 $\log_{10}$ in 24 hours and 0.3 $\log_{10}$ in 48 hours. This also indicated synergy (a greater than 2$\log_{10}$ decrease in the viable cell count), though the fungus was not completely inhibited within the time period of the experiment (Fig. 8).

Table 1: Inhibition of Pathogenic Fungal growth by Plant Extract Combination

Combination of Plant extracts (100mg/mL)	**Pathogenic fungi and the number of days the inhibition of growth lasted**			
	C. albicans	***T. mentagrophytes***	***M. gypseum***	***E. floccossum***
	>7	**>14**	**>14**	**>14**
Voriconazole(16µg/mL)	**>14**	**>14**	**>14**	**>14**

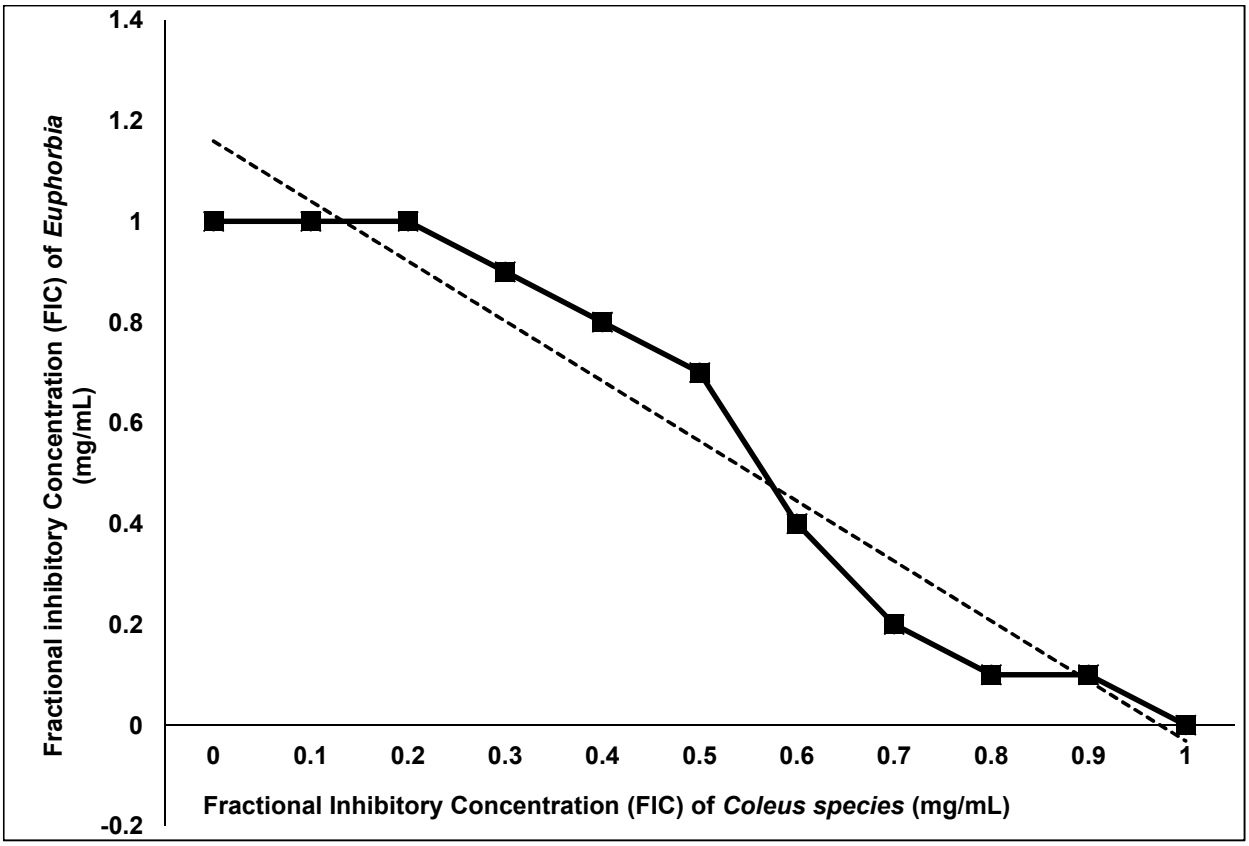


Figure 1: Isobole of Interaction between *E. abyssinica* and *Coleus species* against *T. mentagrophytes*

=Point of interaction between Euphorbia and Coleus species Extracts (FIC INDEX)

---------- =Additive line

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.

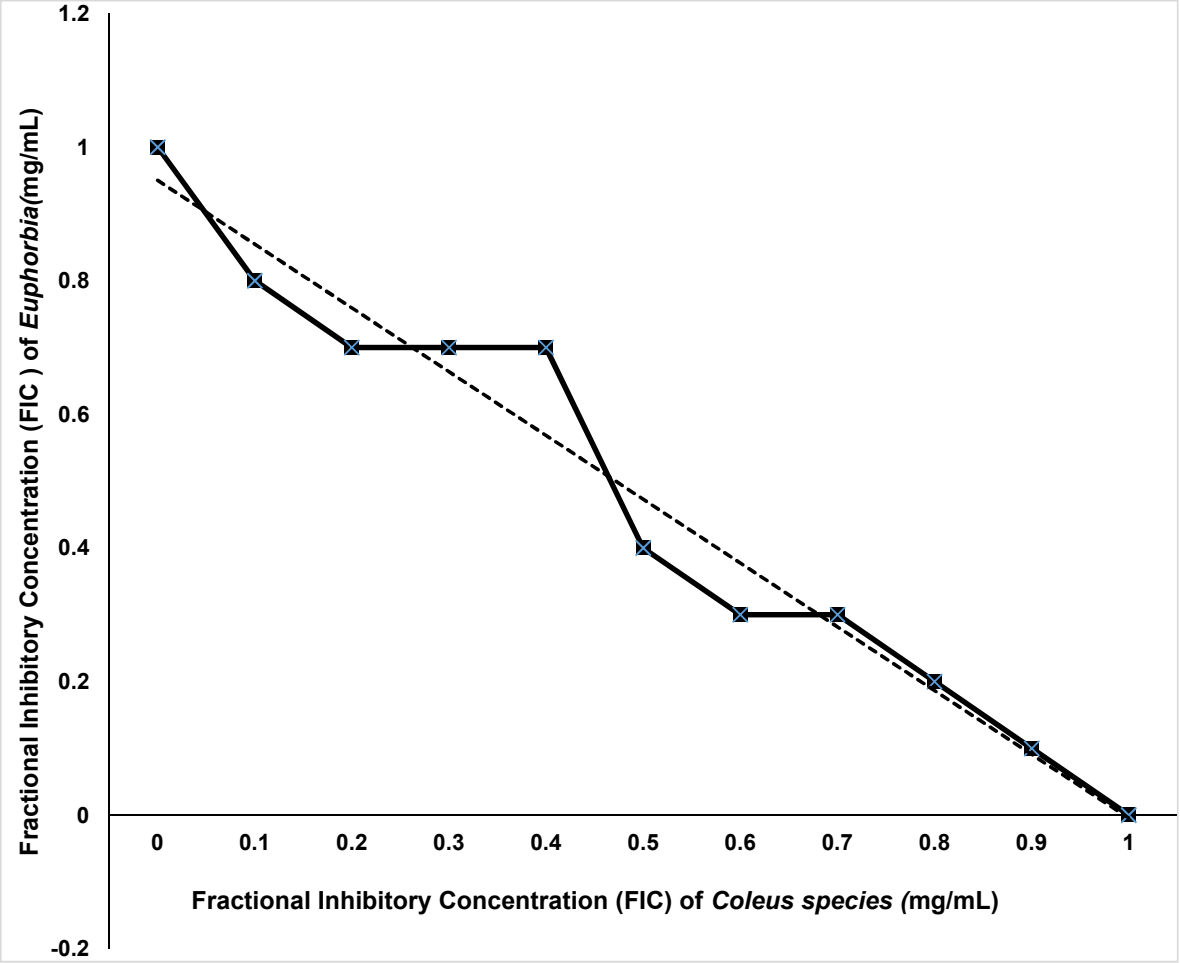


Figure 2: Isobole of Interaction between *E. abyssinica* and *Coleus species* against *M. gypseum*

=Point of interaction between Euphorbia and Coleus species Extracts (FIC INDEX)

---------- =Additive line

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.

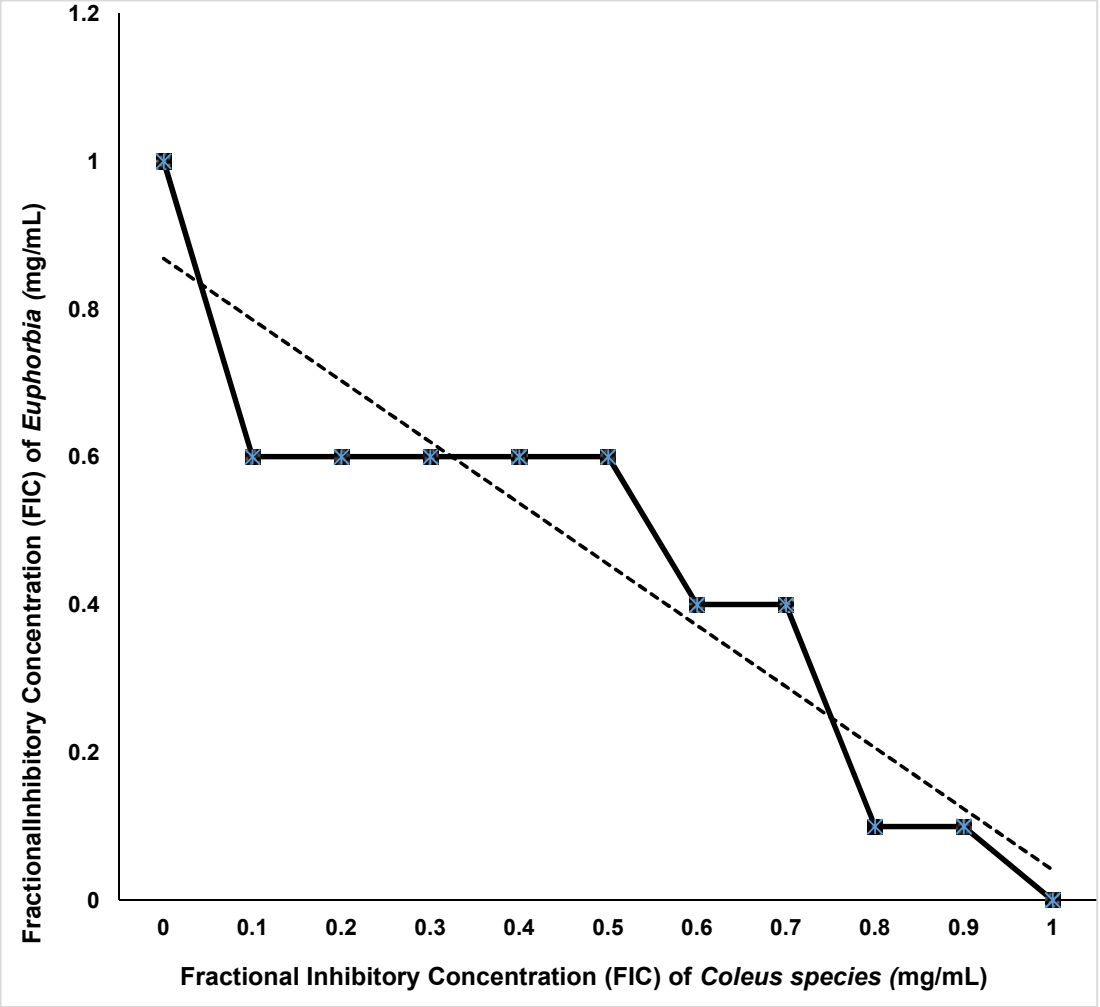


Figure 3: Isobole of Interaction between *E. abyssinica* and *Coleus species* against *E. floccosum*

= Point of interaction between Euphorbia and Coleus species Extracts (FIC INDEX)

---------- =Additive line

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.

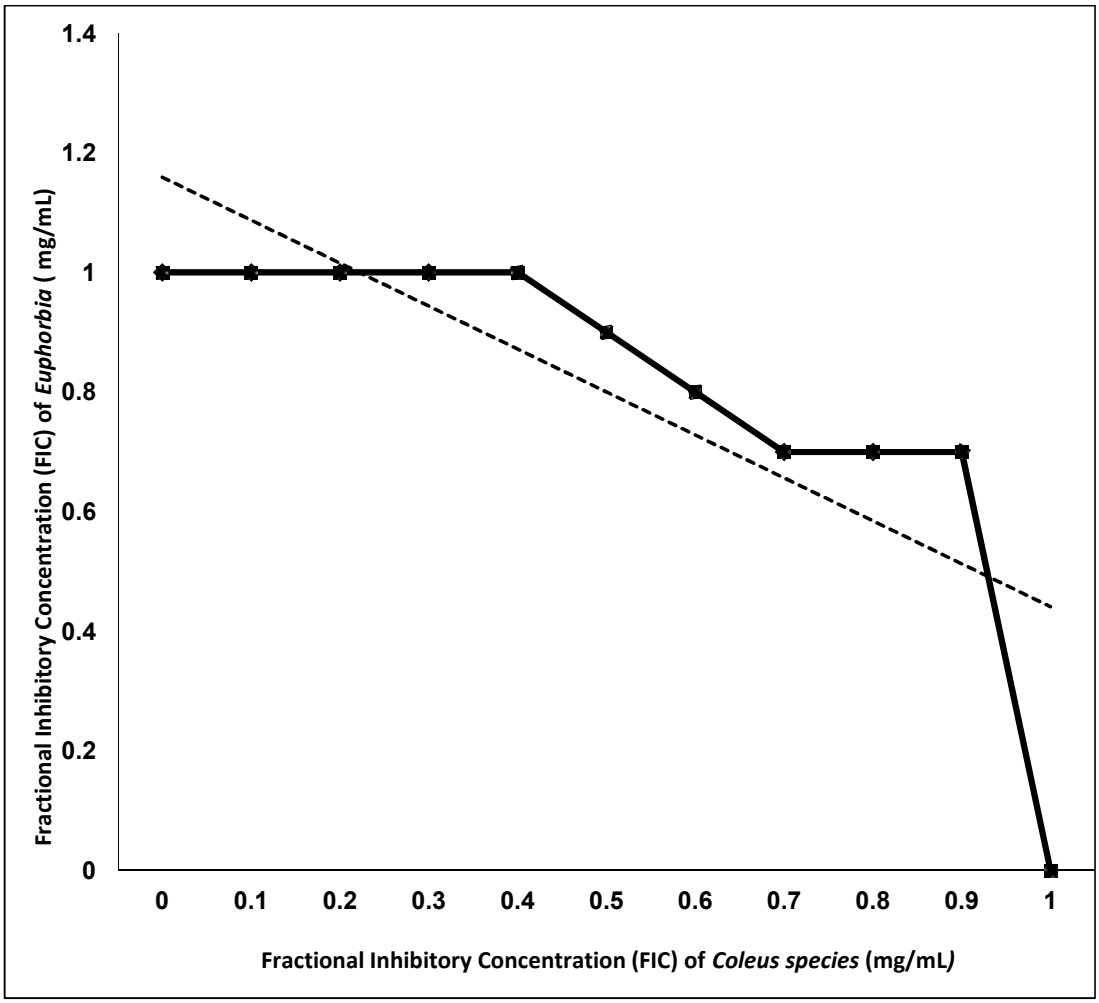


Figure 4: Isobole of Interaction between *E. abyssinica* and *Coleus species* against *Candida albicans*.

= Point of interaction between Euphorbia and Coleus species Extracts (FIC INDEX)

---------- =Additive line

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.

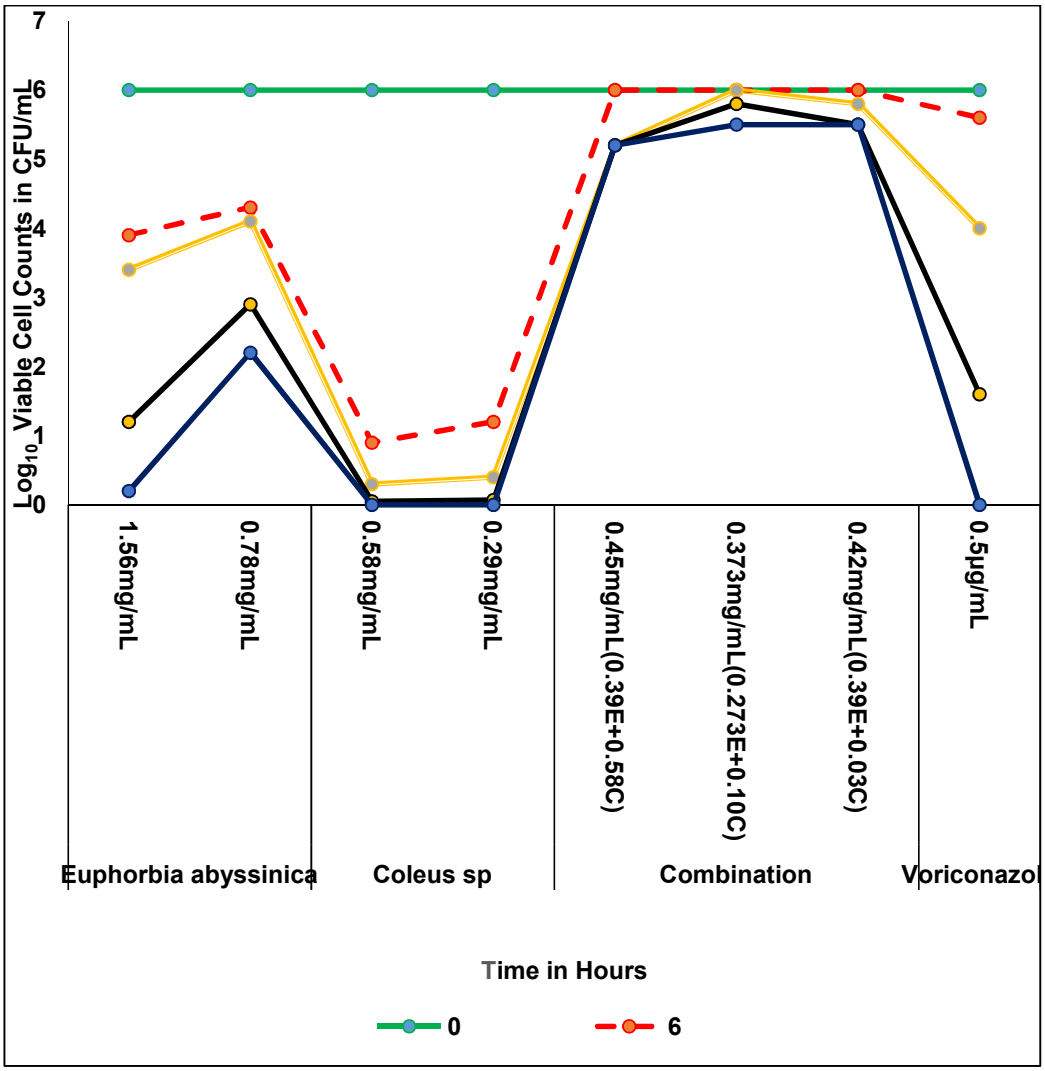


Figure 5: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus species*

(Whole Plant) on the viable cell count of *Candida albicans*; E=*Euphorbia abyssinica*, C=*Coleus species*

Synergy is defined as a ≥2log$_{10}$ CFU/mL decrease in viable cell count, Antagonism is a ≥2log$_{10}$CFU/mL increase in viable cell counts after 24 hours by the combination compared to the most active single agent.

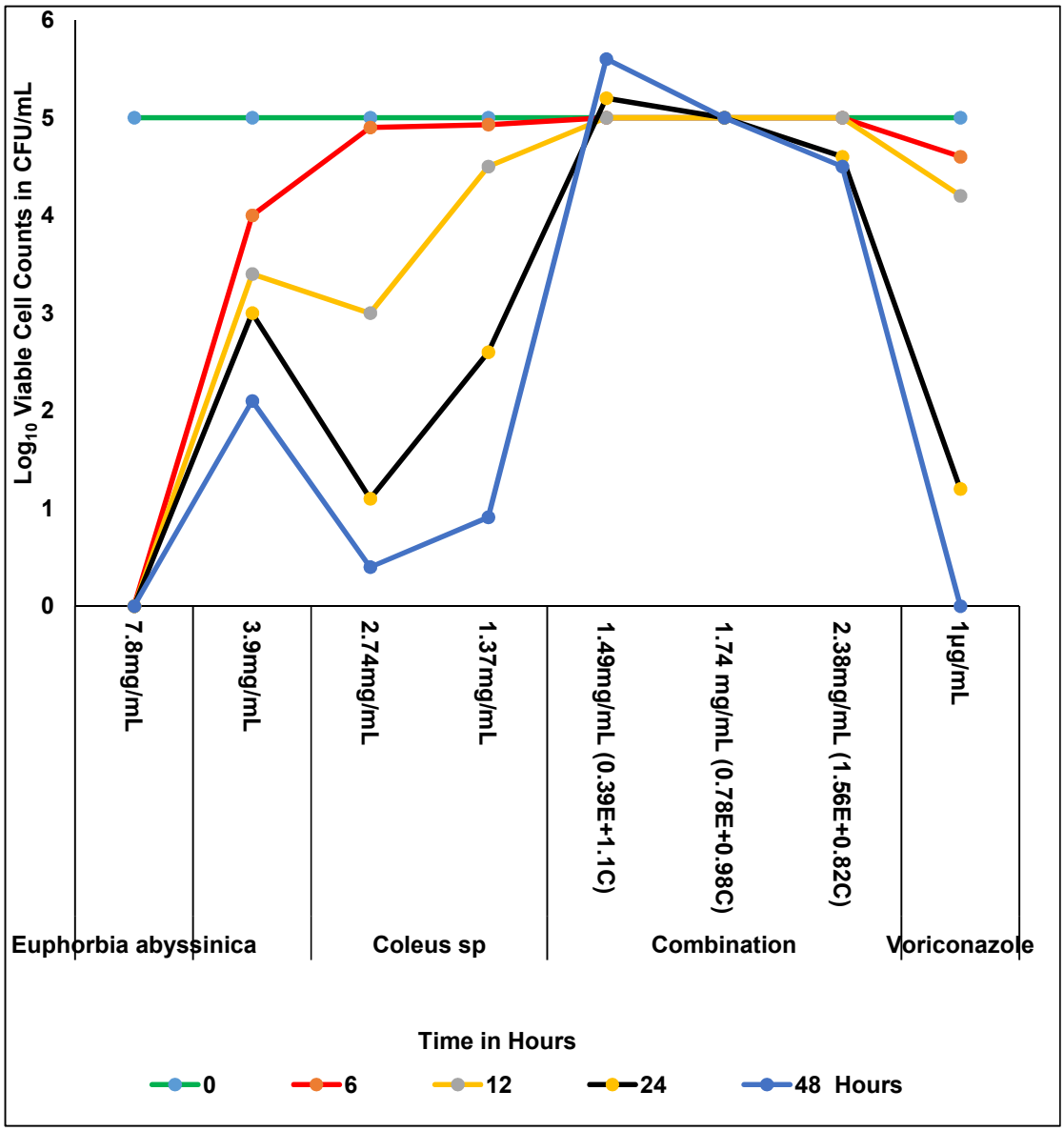
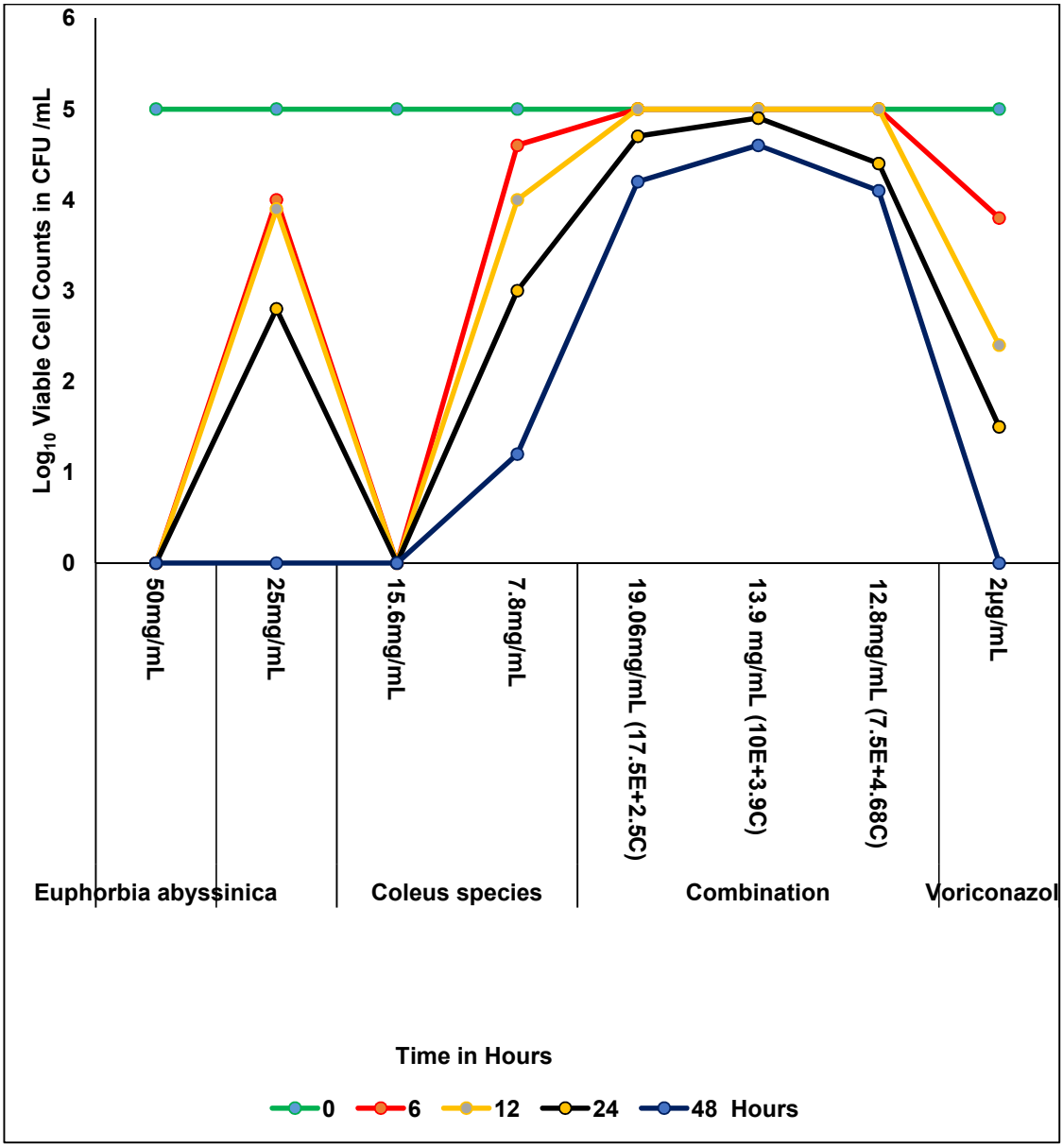


Figure 6: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus species*
(Whole Plant) on the viable cell count of *Trichophyton mentagrophytes*;
E=*Euphorbia abyssinica*, C=*Coleus species*

303



304

305
306

Figure 7: Effect of the combination of 50% methanol extracts of *E. abyssinica*and *Coleus species* (Whole Plant) on the viable cell count of *Microsporum gypseum*; E=*Euphorbia abyssinica*, C=*Coleus species*

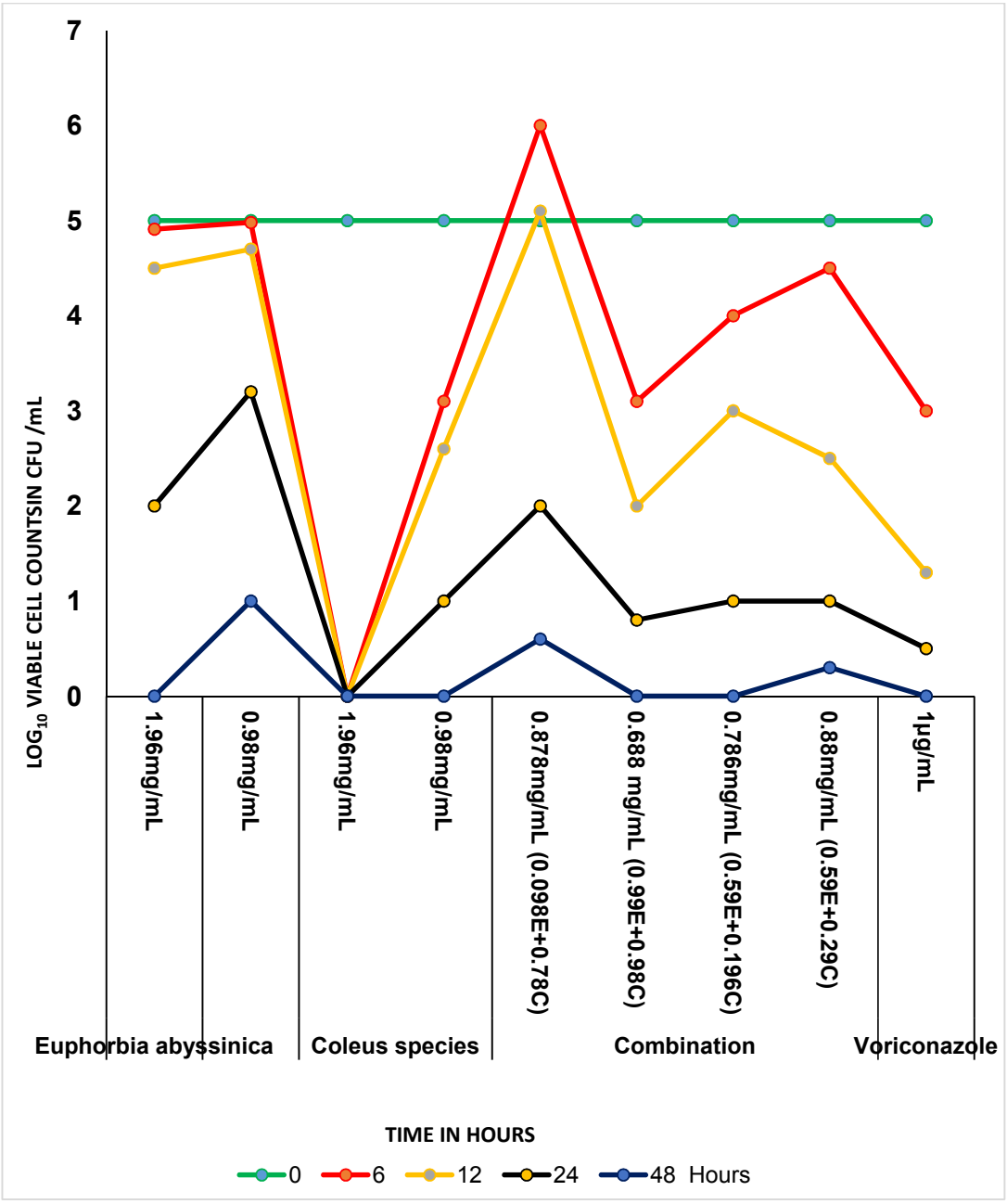


Figure 8: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus species* (Whole Plant) on the viable cell count of *E. floccossum*; E=*Euphorbia abyssinica*, C=*Coleus species*

4. DISCUSSION

Although many medicinal plant extracts are used in various combinations in folklore medicine; the proportions are hardly quantified. The research reported here was aimed at understanding the mysteries behind the unusual antimicrobial powers of such medicinal plants and their combinations using *E. abyssinica* and *Coleus species as examples.* Novel test methods such as Kinetic Time Kill and Checkerboard assay were used to test the effects and interactions of these plant extracts singly and in combination.

The objective of this study was to provide information on methods for assaying the effects of two or more plant extracts. The question being addressed was whether the two plant extracts, acting together, produce additive effect, synergistic effect or sub-additive effect. When a combination of drugs exhibits additivity, less amounts of each drug than applied singly can be used to achieve the same response. If the combination shows synergy, then only a small amount of one or both drugs can be used together to obtain the desired effect. In some cases, however, combination of drugs may act as though the potencies of either or both are reduced. Such an interaction is described as sub-additive.

There have been many descriptions of approaches used to determine and quantify such drug interactions. In 1957 Loewe used a methodology expressed graphically and produced a curve called an isobole i.e. a plot in rectangular coordinates with axes that represent the doses of drug A and drug B. The points that constitute the isobole are, therefore, doses that represent the amount of each drug expected to yield an effect of specified magnitude when the two compounds are administered together [10].

Even if two drugs are not administered together, it is clear that the administration of even a single drug places it in potential contact with a myriad of other chemicals already present in the system. Therefore, a quantitative knowledge of drug combination pharmacology is important—in all experiments aimed at studying mechanisms of action of combinations of two agonists that produce a common effect through mechanisms that are not obviously related to a common receptor, i.e., situations in which the presence of one does not affect the receptor binding of the other. This kind of

agonist joint action was termed “similar and independent” by Bliss [11]. The model of joint action, therefore, is derived only from the potency and efficacy information contained in each drug’s dose-effect data. An important first question is whether the two drugs produce an effect whose magnitude is consistent with the individual dose-effect relations, or the combination effect is exaggerated. This is because two drugs that produce overtly similar effects will sometimes produce exaggerated or diminished effects when used concurrently.

Coleus species extracts were significantly more potent (P=0.05) than *Euphorbia abyssinica* extract. A contributory factor could be that the *Coleus species* was used as a whole plant. Whole plant extracts, also referred to as full-spectrum plant extracts, contain the entire chemical profile available in the flowers present in the final medicinal form [12].

In this study, it was observed that in the Agar diffusion susceptibility method, the combination of the plant extracts produced a synergistic effect on the fungal pathogens tested. However, predominant indifference and antagonism was observed with most of the combination ratios used in the Checkerboard and Kinetic Time kill Assays [5] observed that these two methods of evaluating antimicrobial interactions rely on 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.

The interaction study based on the checkerboard assay, showed that *E. abyssinica* and *Coleus species* in combination have a promising antifungal activity since synergism was observed with some of the combinatorial ratios. This method, which depends on the fractional inhibitory concentrations (FIC) of both extracts showed synergy with *T. mentagrophytes* and *M. gypseum* at FIC indices of 0.9, respectively. Synergy was observed with *E. floccossum* at FIC indices of 0.7, 0.8 and 0.9. For *Candida albicans*, the combined concentrations of the extracts did not show lethal effects on the viable cell count after 24hours of exposure. This signified indifference (FIC index >1<2) to *Candida albicans.*

A comparison of the reduction in viable cell count of each test fungal strain when treated with combinations of the two extracts to the value obtained by exposing the strain to each extract singly, using the kinetic time kill assay, showed that a vast majority of the combinations were indifferent. (>1<2log10 increase in viable cell count in 48hours). Some of the extract combinations were found to exhibit synergy on the fungi tested. In the case of *E. floccossum* a greater than $2\log_{10}$ decrease in the

viable cell count was observed within 48hours, thus showing that the combinations tested were synergistic. Combined concentrations of 0.688mg/mL-0.786mg/mL) concentration reduced the count to undetectable levels in 48 hours. These combinations showed better effects because they reduced the fungal viable cell count to undetectable levels at lower concentrations. Concentrations of 0.878mg/mL- 0.88mg/mL reduced the viable cell counts to 2.0 $\log_{10}$ and 0.3 $\log_{10}$ in 48 hours, respectively, but they are invariably higher concentrations of the plant extracts which could exhibit undesirable toxic effects.

This research has also confirmed the broad spectrum activity of *E. abyssinica* and *Coleus species* as both yeasts and dermatophytes were inhibited by the plant extracts both singly and in combination. Consequently, no significant difference (P=0.05) was observed in the susceptibility pattern of the strains tested. Similar bioactivity has been reported for alcohol extracts of other *Coleus species* [13;9].

Comparing Checkerboard and Kinetic Time kill Assays, it was observed that the Checkerboard assay was significantly more sensitive (P=0.05) than the Time kill Assay. Synergy was seen on three out of the four fungal pathogens tested and these were all dermatophytes. The yeast (*C. albicans)* showed either additive or antagonistic response to all the combinations tested.

The interactions observed above could have been the result of a change in the kinetics of one drug by another. Antagonism *in-vitro* may not necessarily be antagonism *in vivo*, because some important interactions occur, however, by a change in the sensitivity of a tissue to the actions of a drug. Drugs which modify the function of sympathetic nerve terminals can change the pharmacological effects of other drugs acting on the system [14]. Some drugs may interact chemically in the gut, leading to impaired absorption of both drugs e.g Calcium, magnesium and aluminium ions contained in antacid preparations cause the formation of a metal-tetracycline chelate which is poorly absorbed. Drugs which are absorbed from the gut are exposed to drug-metabolizing enzymes in the hepatic cells before they enter the systemic circulation. This results in a considerable loss of drugs that are metabolized rapidly on first passage through the liver – the so-called “first pass effect”. A number of drugs are extensively bound to plasma proteins and, therefore, competition for binding sites can be a problem. These interactions may result in an inadequate serum concentration of the antibiotic being reached, with consequent failure of therapy [14].

Toxicity test which evaluates the lethal dose (LD50) may present a better picture of the drug effect *in vivo*. Better still, the quantal dose-effect or dose-response curve that displays the percent of animals that respond to the drug i.e the hyperbolic curve described by the equation $E = E_{max}D/(D + C)$ where E is the effect, D is dose and C the constant, which is equal to the dose needed for a half-maximal response, a measure of drug potency, often denoted as ED_{50} or D_{50} [10] can be used.

5. Conclusion

In this study, the interaction combinations observed with combinations of 50% methanol extracts of *E. abyssinica* and *Coleus species* against the fungi tested, showed that the plant extracts inhibited all the fungi tested though not at all the combinations. This provides novel information about the antifungal potential of *E. abyssinica* and *Coleus species* against drug resistant pathogens. It remains to be determined if the effects and interactions observed with the crude extracts used in this study would be reproduced with purified plant extracts or indeed with the isolated active ingredients. Further investigations on the mechanism of synergistic action of these plants are necessary if they must be considered as alternative sources of broad spectrum drugs for antifungal therapy.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

REFERENCES

[1] Shivakumar SP, and Vidyasagar GM. J. Appl. Pharmaceut. Sc. Antifungal screening of 61 folkloric medicinal plant extracts against dermatophytic fungi *Trichophyton rubrum* 5 (05); 2015: 038-044

[2] Shanmugam K, Gnanaprakasam AR and Manivachagam C. Antifungal activity of different crude extracts of leaves of *Madhuca indica.* Int. J. Nat. Prod. Res. 2014; 4(3): 88-95

[3] Dry RJ Mi Yang and Saez-Rodriguez J. Looking beyond the cancer cell for effective drug combinations. Gen. Med. (2016) 8:125

[4] Sanjeev R, Bhavya K2, Muntaj Sk, Basumata G, Rajesh M, Sanjeev R. Synergistic Effect of Some Medicinal Plants and Antibiotics Against Few Pathogenic Bacteria *Int. J. Biol. & Pharmaceut. Res.* 2012; 3(8): 1000-1004.

[5] Tarh JE and Iroegbu, CU. Microbiological Evaluation of Combinations of Extracts of *Euphorbia abyssinica* and *Coleus species* for Antibacterial Activity Indian E-J. Pharmaceut. Sc. 2017a; 03[01]

[6] Mariya Paul AR and Suresh KD. On the High value Medicinal plant, *Coleus forskohlii Briq. Hygeia.J.D.Med*. 2013; 5(1):69-78.

[7] Biqiku L, Lupidi G, Petrelli D and Vitali LA. Antimicrobial Activity of Single and Combined Extracts of Medicinal Plants from Cameroon**.** IOSR-JPBS 2016; 11 (4) Ver. IV: 86-90.

[8] Tarh JE Okafor JI and. Iroegbu, CU. Evaluation of Extracts of *Coleus* Species For Antibacterial Activity. *African Journal of Biotechnology*, 2015; 14(2): 125-132.

[9] Tarh JE and Iroegbu, CU. Evaluation of Anti-fungal Activity of Coleus Species Extracts, 2017b; Int. J. Curr. Res. Biosci. Plant Biol, 4(1), 131-138.

[10] Tallarida RJ. Drug Combinations: Tests and Analysis with Isoboles. Curr Protoc Pharmacol, 2016; 72: (9) 19.1–9.

[11] Bliss CI. The toxicity of poisons applied jointly. *Ann Appl Biol,* 1939; 26**:**585–615.

[12] Saraswati JKK and Avinash KN**.** Analytical Techniques for Phytochemicals Screening and Bioactivities of Some *Coleus*. Species: A Review. J. Pharm. Sci. & Res. 2016; 8(4), 227-237

[13] Jay Whole Plant Cannabis Extracts: The Important Combination of THC and CBD September 27, 2016 https://www.yerba.org.

[14] Richens A. Drug interactions and lethal drug. *J. Clin. Pathol.* 1975; s3-9: 94-98