

# 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 floccosum* 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. Floccosum* 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. foccosum*. *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*,

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

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

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

55 *Euphorbia abyssinica* (family *Euphorbiaceae*) and *Coleus species*, both commonly used in ethno  
56 medicine among the *Kendem* people of Cameroon to remedy common problems such as postpartum  
57 bleeding, itching, wounds, skin, and respiratory infections; also as antispasmodic, and anti-histamine,  
58 smooth muscle relaxant and contracting agent [5] are examples of such plants with broad spectrum of  
59 antimicrobial activity.

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

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

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

## 67 **2. Materials and methods**

### 68 **2.1. Collection and Preparation of Plant Extracts**

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

### 75 **2.2. Test Organisms**

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

### 79 **2.3. Susceptibility Testing of Fungi by Pour-plate Method**

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

### 90 **2.4. Checker Board Assay**

91 The 50% methanol extracts were further evaluated in combination using the Checker Board assay  
92 method [5]. Solutions of the plant extracts were prepared, each in sabouraud broth, and diluted using

93 the continuous variation model, that is, by serially reducing the concentration by 10% with broth down  
94 to concentrations below the MIC. Then 2.0mL of each dilutions of *Euphorbia abyssinica* was put into  
95 the tubes in the columns such that while the concentrations of the extract changed 10% serially from  
96 column to column, the concentration along each column remained the same. The solutions of *Coleus*  
97 *species* extract were similarly distributed into the tubes in the rows such that while the concentrations  
98 of the extract vary from one row to the next, the tubes in each row contained the same concentration  
99 of the *Coleus species* extract. Consequently each tube received a combination of the two extracts at  
100 different ratios. Each of the tubes was then inoculated with 0.1mL of the standardized microorganisms  
101 (fungi) and all the mixtures were incubated aerobically at 25 -35°C observing daily for appearance of  
102 growth.

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

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

### 113 **2.5. Time Kill Assay**

114 The effects of 50% methanol extracts of *Euphorbia abyssinica* and *Coleus species* were evaluated by  
115 a kinetic time kill assay using the macrobroth dilution technique as described in [5]. The extracts were  
116 reconstituted in 20% Dimethyl Sulfoxide (DMSO) and appropriately diluted to the required  
117 concentrations. The inoculum size was determined according to the type of fungus, (e.g.  $1 \times 10^6$  for  
118 *Candida albicans*; and  $1 \times 10^5$  for dermatophytes). About 1.00mL of the extract was added to 9 ml of  
119 Sabouraud dextrose broth, seeded with the appropriate concentrations of the test fungus to achieve  
120 concentrations equivalent to 0.5 x MIC, 1 x MIC, 2 x MIC, or 4 x MICs values. Two sets of control  
121 tubes were included for each experiment. One set was seeded with the organism in broth without  
122 extract, and the other set contained broth without organism and extract. The control drug voriconazole

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

130 In the interaction study, plant extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and then  
131 combined using the continuous variation method to obtain a concentration range which included the  
132 MIC obtained with the individual plant extracts as well as sub-inhibitory concentrations. Then 0.1mL of  
133 the standardized inoculum was put in to 9.9mLs of the diluted plant extracts. Inoculated tubes of  
134 Sabouraud Dextrose broth were included as positive controls, Tubes of Sabouraud Dextrose broth  
135 only were included as negative controls while other tubes containing the MICs of the plant extract  
136 alone were also included in the tests. A volume of 100µL from the tubes containing fungi without plant  
137 extract were withdrawn immediately after inoculation, serially diluted and seeded on the already  
138 prepared Sabouraud Dextrose agar plates to determine the zero-hour count. The tubes were  
139 incubated at 25- 35 ° C for > 48 hours, during which aliquots of 100µl were withdrawn at intervals of  
140 15 minutes, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours after inoculation, diluted and plated for  
141 colony counts.

142 The means of two separate tests counts were determined and expressed as Log<sub>10</sub>CFU. The  
143 interactions were considered synergistic if there were decreases of ≥2log<sub>10</sub>CFU/mL in colony counts  
144 after incubation periods by the combination compared to the most active single agent. Additivity or  
145 indifference was described as a < 2log<sub>10</sub>CFU/mL change in the average viable counts after the  
146 incubation periods for the combination, in comparison with the most active single drug. Antagonism  
147 was defined as a ≥2log<sub>10</sub> CFU/mL increases in colony counts after the incubation periods by the  
148 combinations compared to that of the most active single extract alone [9].

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

152

153 **3. Results**

154 **3.1. Antifungal Susceptibility Testing**

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

159 **3.2. Combined activity of *E. abyssinica* (E) and *Coleus species* (C) Extracts on Test Fungi**  
160 **using Checkerboard assay**

161  
162 In this assay, a curved deviation to the left of the additive line was an indication of synergy, while a  
163 curved deviation to the right of the additive line indicated antagonism. At different combinations of the  
164 extracts, the susceptibility of the fungal isolates was such that *E. floccosum* > *M. gypseum* > *T.*  
165 *mentagrophytes* > *Candida albicans*.

166 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,  
167 FIC C 0.8, FIC Index 0.9 (Fig.1)

168 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  
169 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).

170 For *E. floccosum* 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  
171 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).

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

173 **3.3. Combined activity of *E. abyssinica* (E) and *Coleus species* (C) Extracts on Test Fungi**  
174 **using Time Kill Assay.**

175  
176 In this assay, the interactions were considered synergistic if there were decreases of  $\geq 2\log_{10}$ CFU/mL,  
177 additivity or indifference was described as a  $< 2\log_{10}$ CFU/mL and antagonism was defined as a  
178  $\geq 2\log_{10}$  CFU/mL increases in colony counts after the incubation periods by the combinations  
179 compared to that of the most active single extract alone.

180 The antifungal effect of combinations of 50% methanol extract of *Coleus species* and *E. abyssinica*  
181 was estimated as the reduction of viable cell count following exposure to a given concentration of the  
182 extract combinations or control drug (voriconazole) over a period of time (0 hour, 6hours, 12hours,  
183 24hours and 48hours), the viable cell count being kept constant at  $1 \times 10^6$  for *Candida albicans* and  
184  $1 \times 10^5$  for the dermatophytes.

185 The MIC and x2 MIC of 50% methanol extract of *Coleus species* (the more active single drug)  
 186 reduced the viable fungal cell number of *Candida albicans* and *Trichophyton mentagrophytes*  
 187 respectively to less than 0.05log<sub>10</sub> in 48 hours (Fig. 5 & 6). The x2 MIC (15.6mg/mL) concentrations  
 188 killed *M. gypseum* in 6hours and the control drug (voriconazole) cleared the cells in 48hours (Fig. 8).  
 189 Combinations of the two plant extracts showed virtually just additive effects on all the three fungi  
 190 species (Fig. 5, 6 & 7).

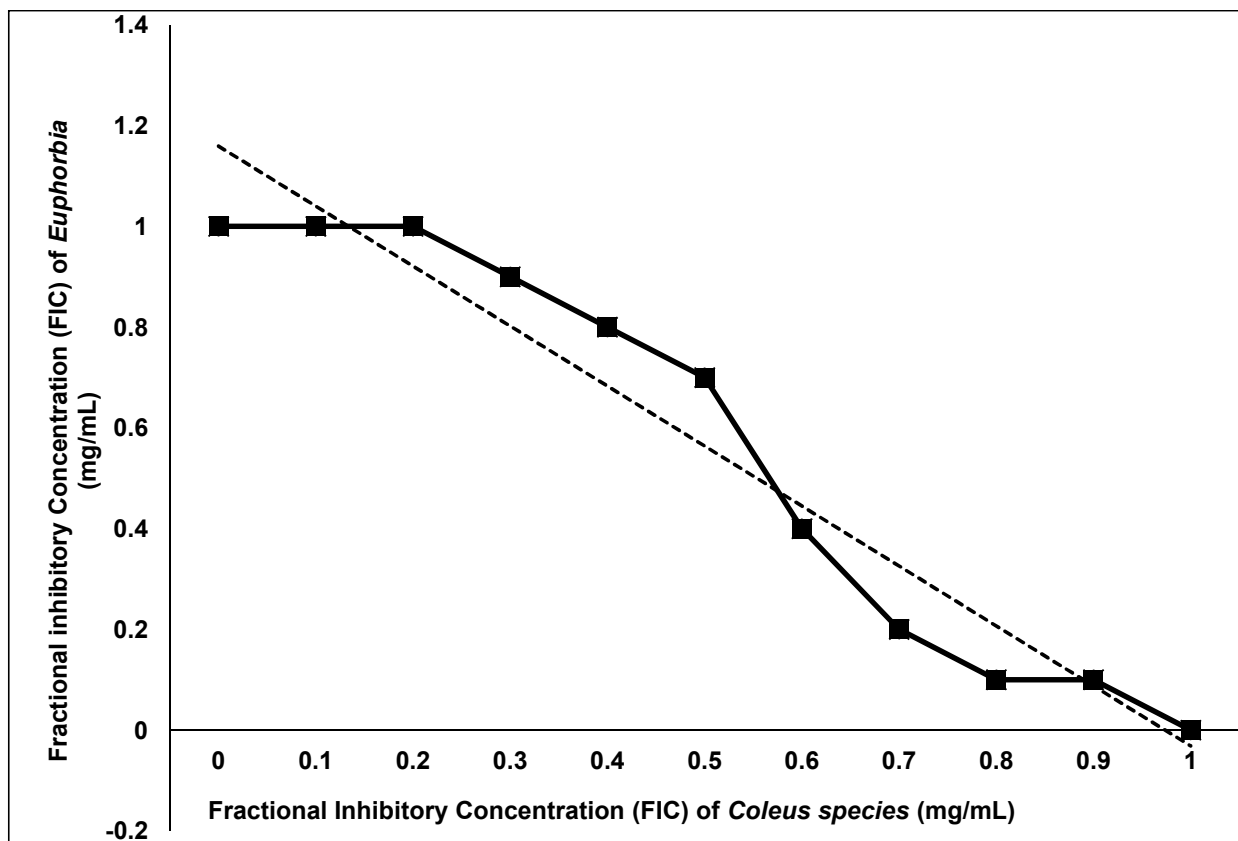
191 The fungicidal activity of the 50% methanol extract of *Coleus species* on *E. floccosum* was such that  
 192 2MIC (1.96mg/mL) concentrations inhibited *E. floccosum* totally in 3hours. The 1MIC (0.98mg/mL)  
 193 reduced the viable cell count to 0.97log<sub>10</sub> and 1µg/mL of the control drug inhibited the fungal cells in 48  
 194 hours (Fig. 8).

195 Compared to the most active single extract (*Coleus species*), all the combinations showed synergistic  
 196 activities against *E. floccosum*. The initial cell proportion of 5.0 log<sub>10</sub> CFU was reduced by a  
 197 concentration of 1/8 (0.098E +0.78Cmg/mL = 0.878mg/mL) to 2.0 log<sub>10</sub> in 48 hours (Synergy), 6/1  
 198 (0.59E +0.098Cmg.mL = 0.688mg/mL) and 6/2 concentration (0.59E+0.196C = 0.786mg/mL)  
 199 concentration reduced the count to undetectable levels in 48 hours, a greater than 2log<sub>10</sub> decrease in  
 200 cell count (Synergy). The 6/3 (0.59E+0.29Cmg/mL 0.88mg/mL) inhibited the fungal growth reducing  
 201 the viable cell count to 1.0 log<sub>10</sub> in 24 hours and 0.3 log<sub>10</sub> in 48 hours. This also indicated synergy (a  
 202 greater than 2log<sub>10</sub> decrease in the viable cell count), though the fungus was not completely inhibited  
 203 within the time period of the experiment (Fig. 8).

204 **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			
	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>M. gypseum</i>	<i>E. floccosum</i>
		>7	>14	>14
Voriconazole(16µg/mL)	>14	>14	>14	>14

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

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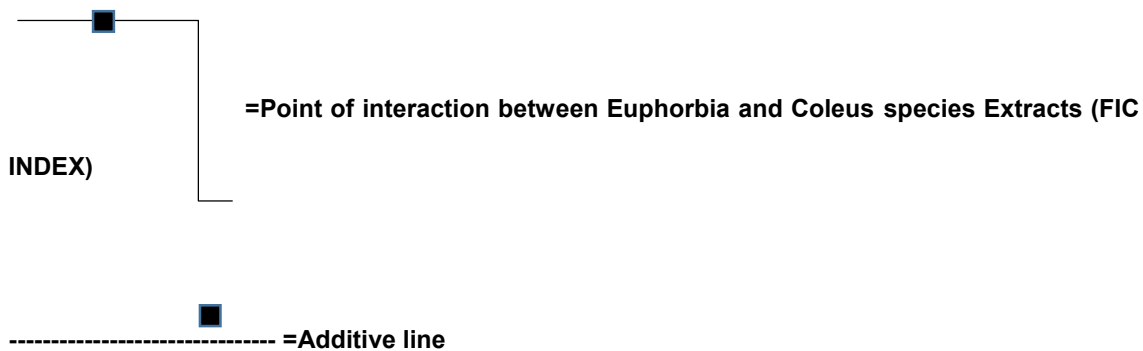
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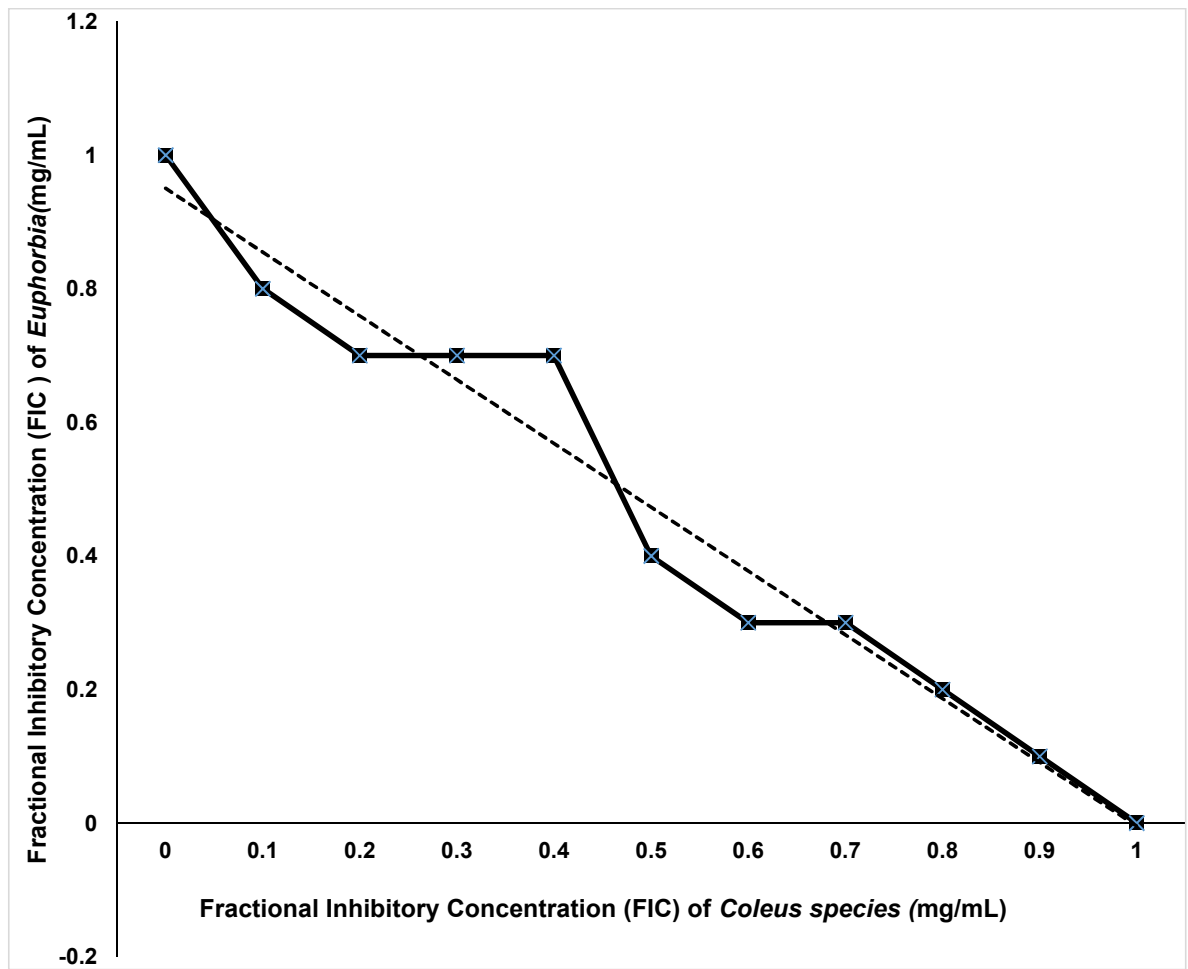


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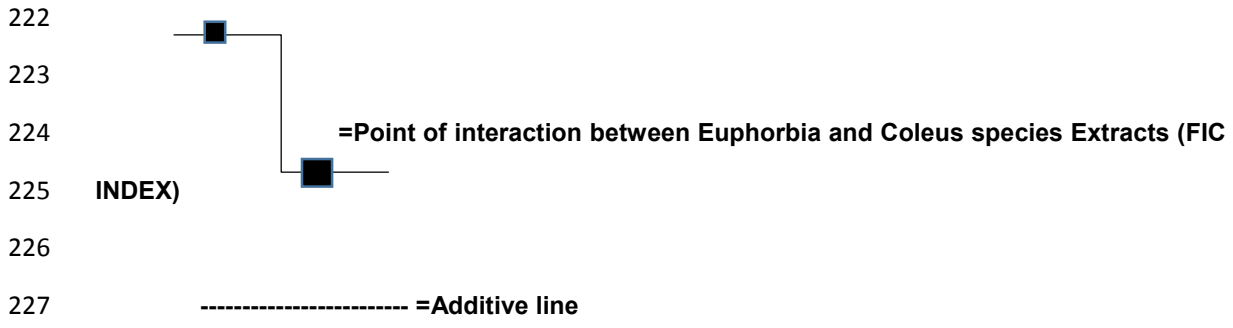
Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line.





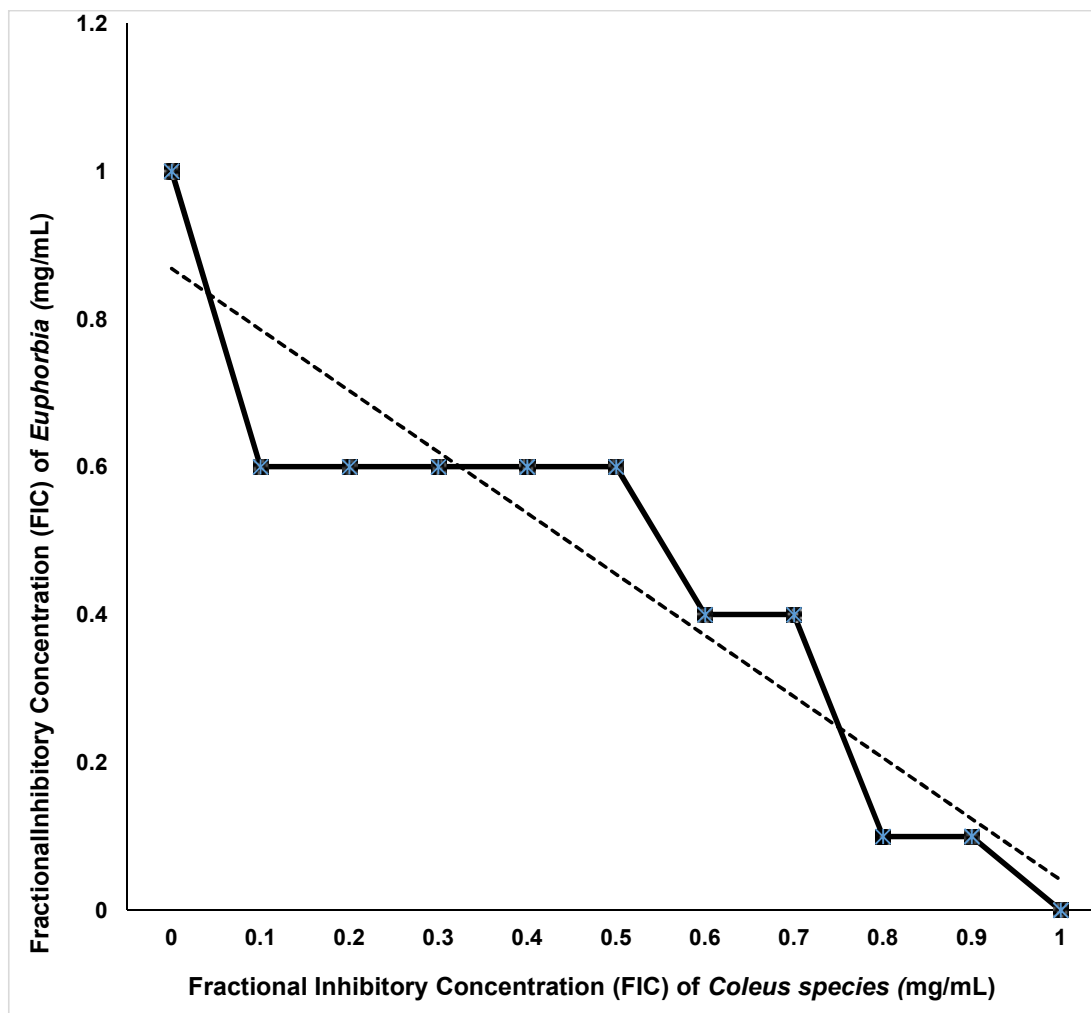
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221 **Figure 2: Isobole of Interaction between *E. abyssinica* and *Coleus species* against *M. gypseum***



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

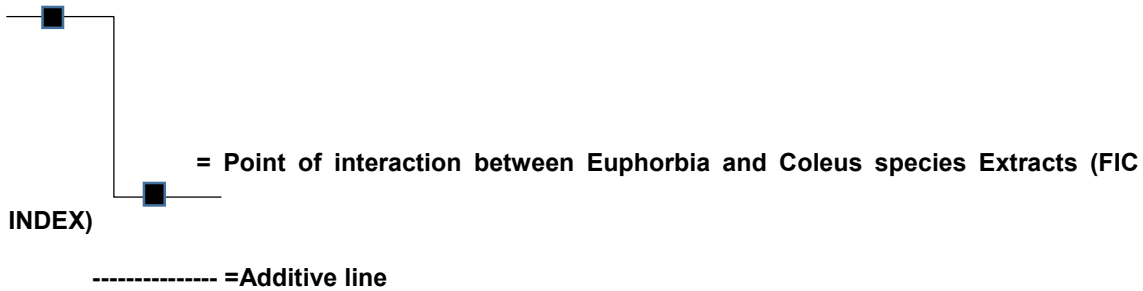
229 indicated by a curved deviation to the right of the additive line.



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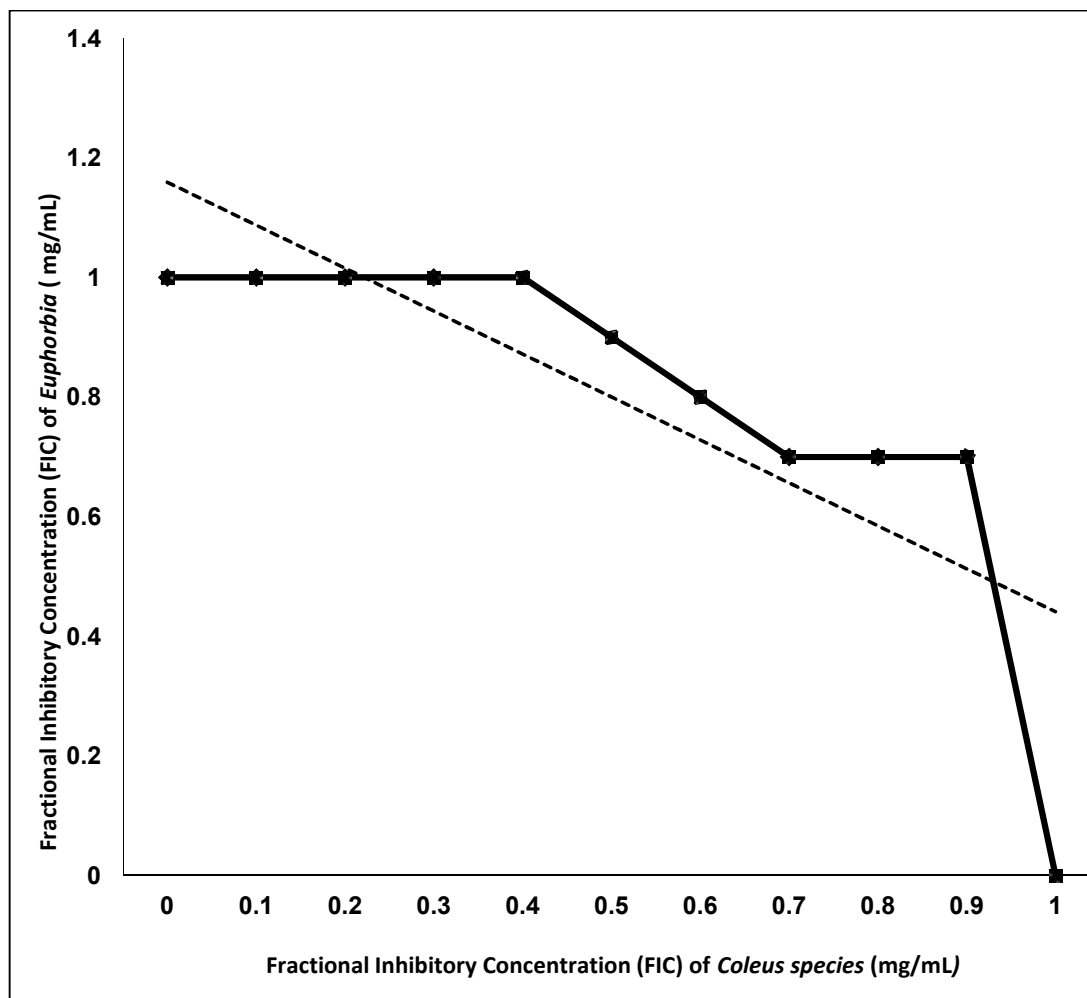
231 **Figure 3: Isobole of Interaction between *E. abyssinica* and *Coleus species* against *E.***  
 232 ***floccosum***

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

240 indicated by a curved deviation to the right of the additive line.



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

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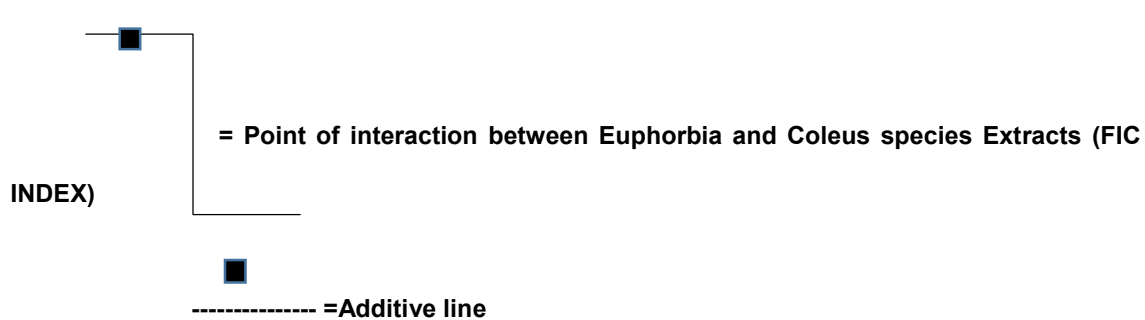
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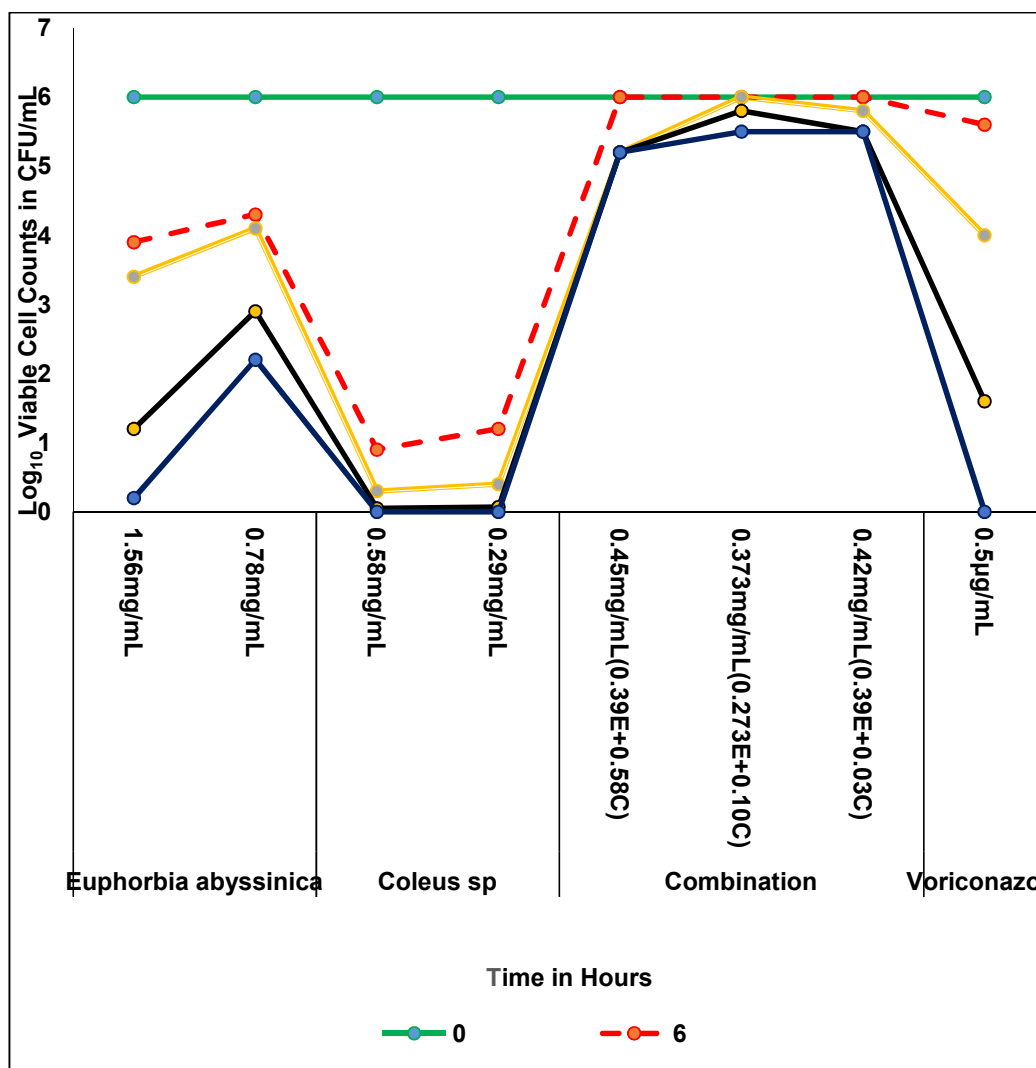
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Synergy was indicated by a curved deviation to the left of the additive line, while antagonism was indicated by a curved deviation to the right of the additive line.



255

256 **Figure 5: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus***  
 257 ***species***  
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259 **(Whole Plant) on the viable cell count of *Candida albicans*; E=*Euphorbia abyssinica*,**  
 260 **C=*Coleus species***  
 261  
 262  
 263

264 Synergy is defined as a  $\geq 2\log_{10}$  CFU/mL decrease in viable cell count, Antagonism is a  
 265  $\geq 2\log_{10}$ CFU/mL increase in viable cell counts after 24 hours by the combination compared to the most  
 266 active single agent.

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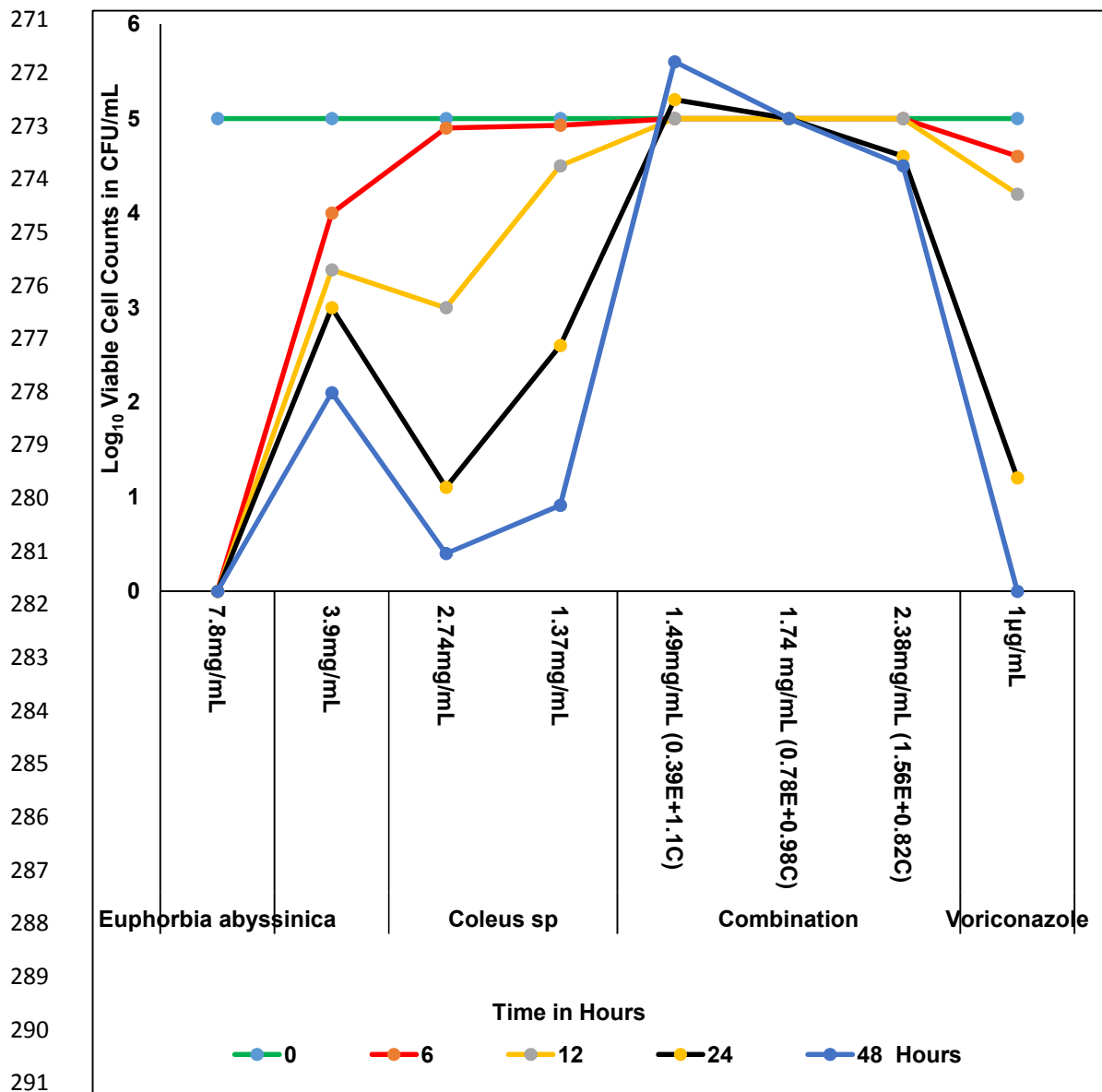
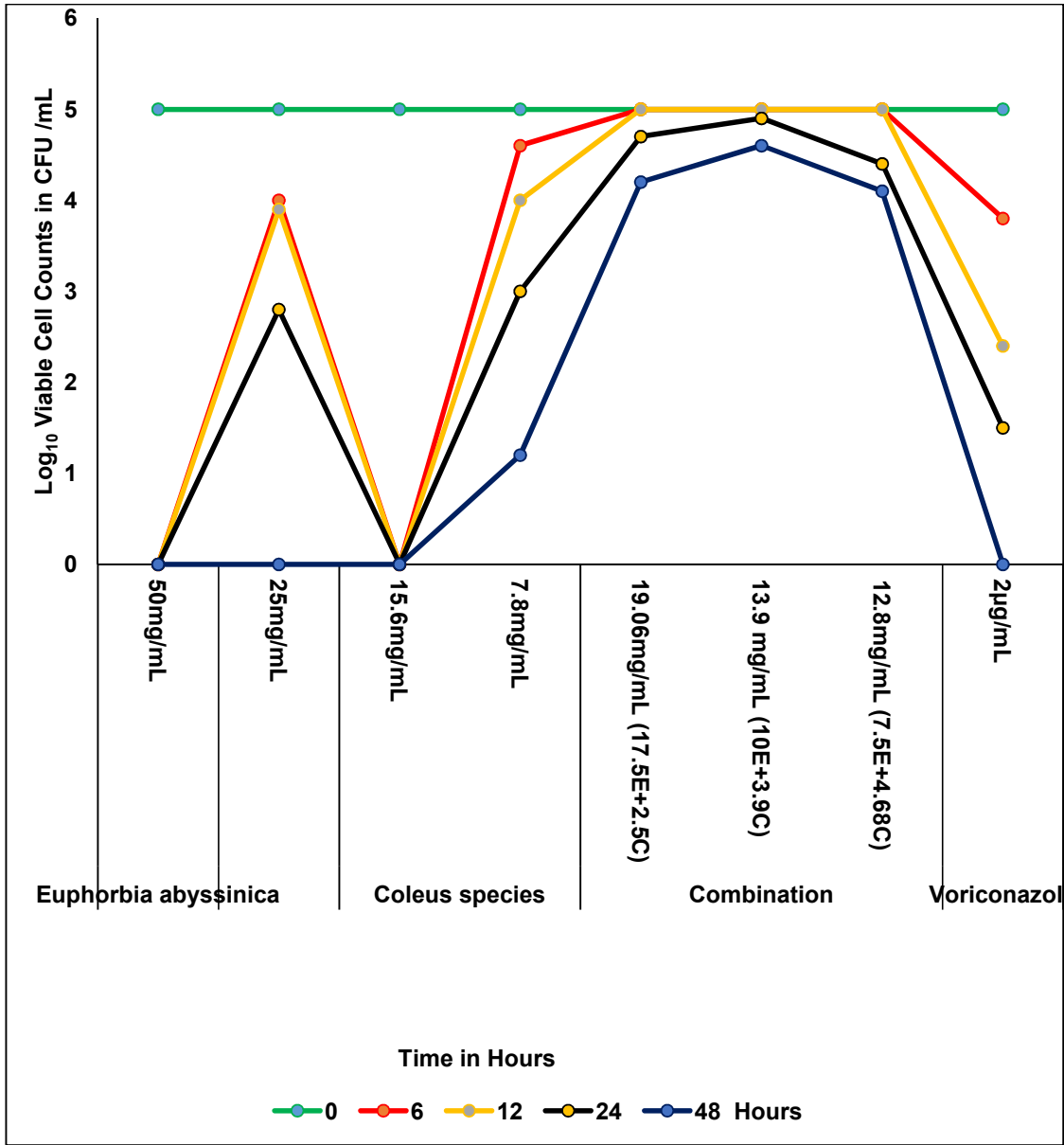


Figure 6: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus species*

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

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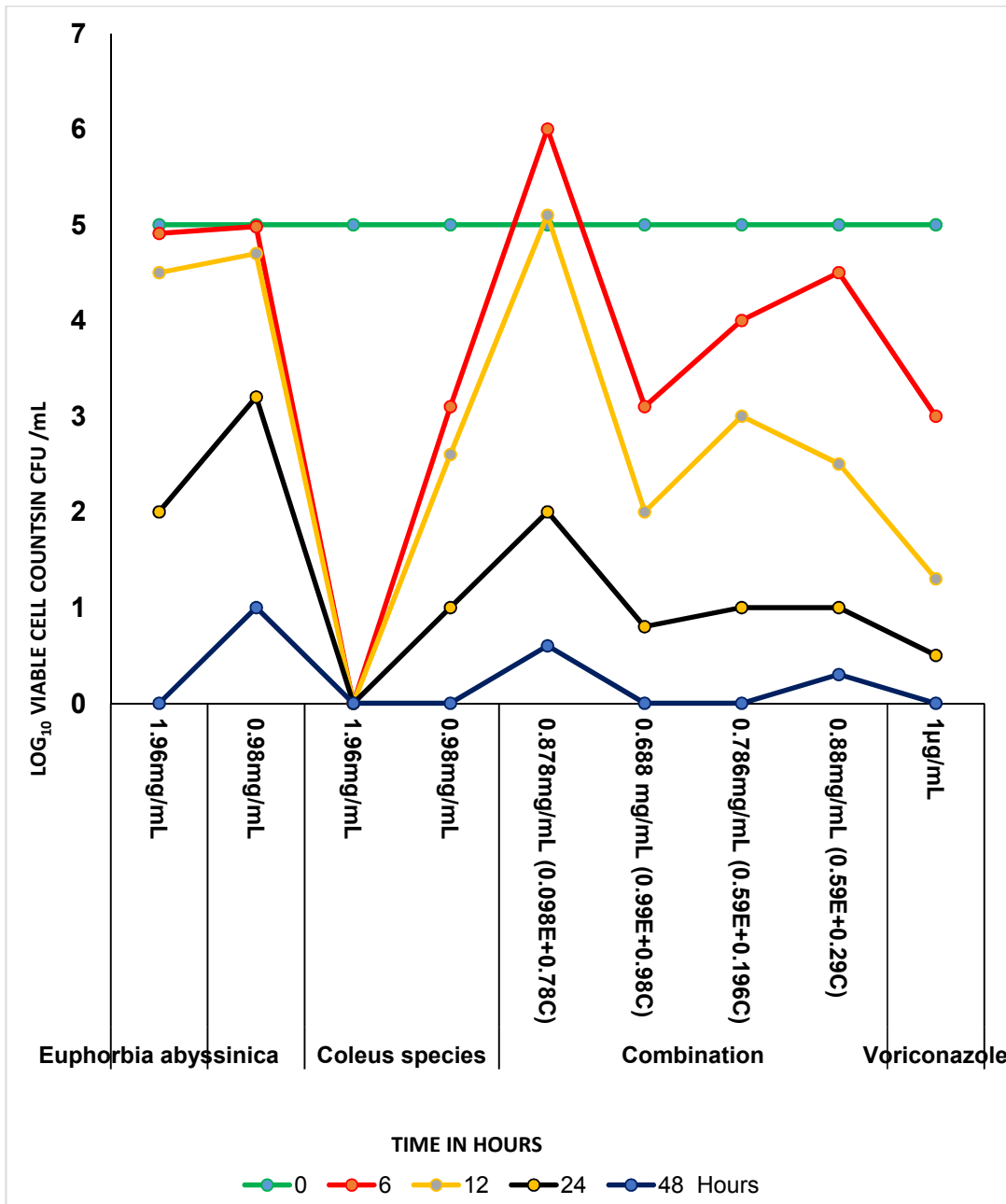
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**Figure 7: Effect of the combination of 50% methanol extracts of *E. abyssinica* and *Coleus species* (Whole Plant) on the viable cell count of *Microsporium gypseum*; E=*Euphorbia abyssinica*, C=*Coleus species***



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318 **Figure 8: Effect of the combination of 50% methanol extracts of *E. abyssinica* and**  
319 ***Coleus species* (Whole Plant) on the viable cell count of *E. floccosum*; E=*Euphorbia***  
320 ***abyssinica*, C=*Coleus species***  
321

#### 322 **4. DISCUSSION**

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

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

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

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



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

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

359 In this study, it was observed that in the Agar diffusion susceptibility method, the combination of the  
360 plant extracts produced a synergistic effect on the fungal pathogens tested. However, predominant  
361 indifference and antagonism was observed with most of the combination ratios used in the  
362 Checkerboard and Kinetic Time kill Assays [5] observed that these two methods of evaluating  
363 antimicrobial interactions rely on predetermination of the MICs of the component drugs or extracts e.g.  
364 drug A and drug B singly, hence, it heavily relies on the accuracy of MIC values and its utility is  
365 affected by day to day variations in this parameter which sometimes exceeds the predetermined MIC.  
366 The interaction study based on the checkerboard assay, showed that *E. abyssinica* and *Coleus*  
367 *species* in combination have a promising antifungal activity since synergism was observed with some  
368 of the combinatorial ratios. This method, which depends on the fractional inhibitory concentrations  
369 (FIC) of both extracts showed synergy with *T. mentagrophytes* and *M. gypseum* at FIC indices of 0.9,  
370 respectively. Synergy was observed with *E. floccosum* at FIC indices of 0.7, 0.8 and 0.9. For  
371 *Candida albicans*, the combined concentrations of the extracts did not show lethal effects on the  
372 viable cell count after 24hours of exposure. This signified indifference ( $FIC\ index >1 < 2$ ) to *Candida*  
373 *albicans*.

374 A comparison of the reduction in viable cell count of each test fungal strain when treated with  
375 combinations of the two extracts to the value obtained by exposing the strain to each extract singly,  
376 using the kinetic time kill assay, showed that a vast majority of the combinations were indifferent.  
377 ( $>1 < 2\log_{10}$  increase in viable cell count in 48hours). Some of the extract combinations were found to  
378 exhibit synergy on the fungi tested. In the case of *E. floccosum* a greater than  $2\log_{10}$  decrease in the

379 viable cell count was observed within 48hours, thus showing that the combinations tested were  
380 synergistic. Combined concentrations of 0.688mg/mL-0.786mg/mL) concentration reduced the count  
381 to undetectable levels in 48 hours. These combinations showed better effects because they reduced  
382 the fungal viable cell count to undetectable levels at lower concentrations. Concentrations of  
383 0.878mg/mL- 0.88mg/mL reduced the viable cell counts to 2.0 log<sub>10</sub> and 0.3 log<sub>10</sub> in 48 hours,  
384 respectively, but they are invariably higher concentrations of the plant extracts which could exhibit  
385 undesirable toxic effects.

386 This research has also confirmed the broad spectrum activity of *E. abyssinica* and *Coleus species* as  
387 both yeasts and dermatophytes were inhibited by the plant extracts both singly and in combination.  
388 Consequently, no significant difference (P=0.05) was observed in the susceptibility pattern of the  
389 strains tested. Similar bioactivity has been reported for alcohol extracts of other *Coleus species*  
390 [13;9].

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

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

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

## 413 5. Conclusion

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

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423 **commercial, or**  
424 **not-for-profit sectors.**

## 425 CONSENT

426 It is not applicable.

427

## 428 ETHICAL APPROVAL

429 It is not applicable.

430

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