

1 **Antifungal Effects of Combined Extracts of *Euphorbia abyssinica* and *Coleus species***

2
3 **Abstract**

4 Although, different plant extracts have frequently been used in folklore medicines to cure different
5 ailments, the hidden truth behind their activity and efficacy is still to be fully unravelled (unraveled).

6 **Aim:** To Evaluate (evaluate) the effects of combined 50% methanol extracts of *Euphorbia abyssinica*
7 (*Desert Candle*), and *Coleus species* on *Candida albicans*, *Trichophyton mentagrophytes*,
8 *Microsporum gypseum* and *Epidermophyton floccosum*.

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

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

13 **Methodology:** All the fungal strains used in the research were collected from the University of
14 Nigeria Teaching Hospital Enugu, Nigeria. Susceptibility testing was done using pour-plate method,
15 while the checkerboard and Time kill assays were employed to evaluate the efficacy of the
16 combinations.

17 **Results:** The individual plant extracts inhibited all the fungal strains tested at different
18 concentrations. *Coleus species* extracts proved to be more potent in activity than *Euphorbia*
19 *abyssinica* extracts. The combination^s inhibited the test fungi for more than two weeks. In the Time
20 Kill assay, the combinations showed synergy on *E. Floccosum* only. It showed additive or
21 antagonistic activity on the rest of the fungi tested. The Checkerboard combination^s showed synergy
22 on *T. Mentagrophytes*, *M. gypseum*, and *E. foccosum*. *E. foccosum* was the most susceptible of the
23 fungi tested while *C. albicans* was the least susceptible. The control drug voriconazole also inhibited
24 all the fungi tested. Significant antifungal activity (P=0.05) was observed in the checkerboard assay
25 than in the Time Kill assay.

26 **Conclusion:** The results justify the folklore claims that these plants have a wide range of curative
27 uses, suggesting that they can be used as alternative sources of agents for the treatment of resistant
28 fungal infections.

29 **Keywords:** *Coleus species*, *Euphorbia abyssinica* Combined, Extracts, Antifungal Effects,
30 Checkerboard, Time kill.
31

33 Introduction

34 Fungal infections such as onychomycoses, disseminated infections associated with opportunistic
35 pathogens like *C. albicans*, dermatophytosis, (invasion of keratinized tissues – skin, hair and nails –
36 of humans and other animals) caused by three anamorphic fungal genera (*Epidermophyton*,
37 *Microsporum* and *Trichophyton*), have reportedly increased worldwide [1], and so, have become a
38 public health concern. Recently life-threatening and potentially fatal fungal infections have emerged
39 in immune-compromised people [2] with increasing drug resistance recorded in several cases, which
40 were previously susceptible to the normal synthetic antifungal agents.

41 The spread of antifungal drug resistance is equally becoming a public health challenge globally [3, 4]
42 ; and the situation has been exacerbated by global travel and distribution of food products [5], innate
43 random mutations [6, 7], acquisition of resistance genes from other microorganisms [8, 9], wide
44 spread indiscriminate use of antimicrobials [10,11,12,13,5] as pesticides [4]; or, in animal feed
45 [14,15], as food preservatives [16, 17], and for treatment of infected patients. To add to the problem
46 of resistance, treatment failure, and toxicity [9], most synthetic drugs are unaffordable to most people
47 in rural and less developed areas of the world [18]. For the latter, their existence and survival history
48 would be incomplete without a mention of the role plants as sources of food and/or medicines [18,
49 19, 20]. Plants are naturally endowed with primary and secondary metabolites that are incidentally
50 very important nutrients or medicines to man and livestock [19, 21, 22]. Thus, as research reports on
51 medicinal plants accumulate, there is gradual replacement of synthetic drugs (now notorious for
52 failure in treatment of resistant pathogens and in general toxicity) [9] from the pharmaceutical shops
53 with herbal remedies.

54 Application of combinations of herbs to cure certain diseases is common in ethno-medicine and has
55 formed the basis for experimentation on combinations of therapies as solution to extensive drug
56 resistance by microbes [23]. Thus, multiple drug resistance (MDR) inhibitors or resistance modifying
57 agents work synergistically to modify the resistance phenotype in microorganisms [24]. The search
58 for such compounds in plants can give a leeway to the treatment of drug resistant infections as
59 alternative to overcoming the problem of resistance [25].

60 *Euphorbia abyssinica* and *Coleus species* are perfect examples of medicinal plants with a wide
61 range of activity against pathogenic microorganisms (microorganisms). The native population of
62 *Kendem* in Cameroon often use these two plants [20] to prevent loss of blood after childbirth [20],

63 treat cuts [20], pruritus [20], superficial infections of the body [20], and diseases of the air ways [20];
64 also as antispasmodic, and anti-histamine, as well as constricting and releasing tension from smooth
65 muscles [26,20].

66 *E. abyssinica* is an evergreen, cactus-like plant that has been classified in the family *Euphorbiaceae*
67 [20]. The word *Coleus* was originally coined from the word *Coleos* in Greek, which also is referred to
68 as “sheath” [27]. *Coleus species* belong to the genus *Plectranthus* or mint group of sweet smelling
69 fragrance plants that were formally classified in the *labiatae*, currently, the *lamiaceae* family of plants
70 [27]. Even though, the above original account of these plants was given by João de Loureiro in the
71 period between 1717-1791 [27], in other parts of the world, *Kendem* in Cameroon for example, it is
72 given different descriptions. There, it is described as *Osem antuoh*, meaning “Toad’s skin” [28].

73 In ethnomedicine, the traditional doctors in this locality use decoctions from the plant to treat
74 generalized systemic and or superficial skin diseases [28].

75 The purpose of this research therefore was to extract *Euphorbia abyssinica* and *Coleus species*
76 using a mixture of Ethanol (ethanol) and water (50/50%V) and evaluate the effects that the different
77 combinations of the extracts will have on some selected fungi strains.

78 2. Materials and methods

79 2.1. Collection and Preparation of Plant Extracts

80 The stem-bark of *Euphorbia abyssinica* and whole plants of *Coleus species* were collected from
81 *Kendem* village in the southern Cameroon. The specimens were authenticated at the Department of
82 Botany, and the research carried out in the Department of Microbiology in the University of Nigeria,
83 *Nsukka*. The specimens were thoroughly rinsed under running tap water and then cut into tiny
84 pieces and air-dried in the dark. They were pulverized in a mortar, the powder weighed and stored in
85 plastic bags. The powdered materials were then extracted using the method described by *Tarh et al.*
86 [29].

87 2.2. Test Organisms

88 The test fungi used were obtained from the Department of Medical Microbiology, University of
89 Nigeria Teaching Hospital Enugu, Nigeria. They were subcultured, purified and their identity
90 reaffirmed by slide culture, staining and biochemical tests.

91 2.3. Susceptibility Testing of Fungi by Pour-plate Method

92 The susceptibility testing of fungi was done using pour-plate method as described by Tarh and
93 Iroegbu [30]. A 2.0 mL amount of a 1000 mg/mL reconstituted plant extract was pipetted into sterile
94 glass test tube containing 18 mL of molten Sabouraud Dextrose Agar (SDA) at about 45°C. The
95 mixture was swirled carefully for the contents and agar to homogenize, thereafter, 100 µL of the
96 standard fungal inoculums was seeded onto each tube. Again they were thoroughly mixed, then
97 contents of each tube poured into a sterile Petri dish and allowed to set before incubating at 25-
98 35°C. A culture plate without the extract served as the positive control for growth while another plate
99 containing 2.0 mL of 16 µg/mL voriconazole as the negative control. As soon as growth was
100 observed at the positive control plates the test plates were checked for growth daily and the period
101 of inhibition of growth was recorded in days.

102 2.4. Checker Board Assay

103 The 50% methanol extracts were further evaluated in combination using the Checker Board assay
104 method described by Tarh and Iroegbu, [20]. Solutions of the plant extracts were prepared, each in
105 sabouraud broth, and diluted using the continuous variation model, that is, by serially reducing the
106 concentration by 10% with broth down to concentrations below the MIC. Then 2.0 mL of each
107 dilutions of *Euphorbia abyssinica* was put into the tubes in the columns such that while the
108 concentrations of the extract changed 10% serially from column to column, the concentration along
109 each column remained the same. The solutions of *Coleus species* extract were similarly distributed
110 into the tubes in the rows such that while the concentrations of the extract vary from one row to the
111 next, the tubes in each row contained the same concentration of the *Coleus species* extract.
112 Consequently each tube received a combination of the two extracts at different ratios. Each of the
113 tubes was then inoculated with 0.1 mL of the standardized microorganisms (fungi) and all the
114 mixtures were incubated aerobically at 25 -35°C observing daily for appearance of growth.

115 The MICs of the combinations were then recorded and the fractional inhibitory concentration (FIC),
116 for each extract, was calculated as MIC of extract in the combination divided by MIC of single
117 extract. FIC index was also calculated using the formula, $FIC\ index = \sum FIC\ Euphorbia + FIC\ Coleus$.
118 FIC index value of 1 indicates additive interaction, < 1, synergy, >1 < 2, Indifference and >2,
119 antagonism [20].

120 The Isobologram data generated from the results of the interactions of plant extracts in combination,
121 using MIC data directly as well as the calculated FICs, were plotted as the first points which no

122 growth occurred. This resulted in a plot or graph called an “isobole”. Any points which fell on a
123 straight line between the x and y axes were considered as additive. A curved deviation to the left of
124 the additive line was an indication of synergy, while antagonism was indicated by a curved deviation
125 to the right of the additive line [20].

126 2.5. Time Kill Assay

127 The effects of 50% methanol extracts of *Euphorbia abyssinica* and *Coleus species* were evaluated
128 by a kinetic time kill assay using the macrobroth dilution technique as described by Tarh and
129 Iroegbu, [20]. The extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and appropriately
130 diluted to the required concentrations. The inoculum size was determined according to the type of
131 fungus, (e.g. 1×10^6 for *Candida albicans*; and 1×10^5 for dermatophytes). About 1.0 mL of the extract
132 was added to 9 ml of Sabouraud dextrose broth, seeded with the appropriate concentrations of the
133 test fungus to achieve concentrations equivalent to 0.5 x MIC, 1 x MIC, 2 x MIC, or 4 x MICs values.
134 Two sets of control tubes were included for each experiment. One set was seeded with the organism
135 in broth without extract, and the other set contained broth without organism and extract. The control
136 drug voriconazole was similarly diluted. All the fungal cultures were incubated at 35°C for ≥ 48
137 hours. Immediately after inoculation of the tubes, aliquots of 100 μ L of the negative control tubes
138 contents were taken, serially diluted in saline and seeded on nutrient agar plates to determine the
139 zero hour counts. The same was done for the tubes which contained the test fungi after 0 hour, 6
140 hours, 12 hours, 24 hours and 48 hours, respectively. After incubation, the emergent colonies were
141 counted and the mean count (colony formation unit, CFU) of each test organism was determined
142 and expressed as \log_{10} . The Minimum Lethal Concentrations (MLCs) of the extract were the lowest
143 concentrations that gave 99.9% to 100% killing.

144 In the interaction study, plant extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and
145 then combined using the continuous variation method to obtain a concentration range which
146 included the MIC obtained with the individual plant extracts as well as sub-inhibitory concentrations.
147 Then 0.1 mL of the standardized inoculums was put in to 9.9 mLs of the diluted plant extracts.
148 Inoculated tubes of Sabouraud Dextrose broth were included as positive controls, Tubes of
149 Sabouraud Dextrose broth only were included as negative controls while other tubes containing the
150 MICs of the plant extract alone were also included in the tests. A volume of 100 μ L from the tubes
151 containing fungi without plant extract were withdrawn immediately after inoculation, serially diluted

152 and seeded on the already prepared Sabouraud Dextrose agar plates to determine the zero-hour
153 count. The tubes were incubated at 25- 35°C for > 48 hours, during which aliquots of 100 µL were
154 withdrawn at intervals of 15 minutes, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours after inoculation,
155 diluted and plated for colony counts.

156 The means of two separate tests counts were determined and expressed as Log₁₀ CFU. The
157 interactions were considered synergistic if there were decreases of $\geq 2 \log_{10}$ CFU/mL in colony
158 counts after incubation periods by the combination compared to the most active single agent.
159 Additivity or indifference was described as a $< 2 \log_{10}$ CFU/mL change in the average viable counts
160 after the incubation periods for the combination, in comparison with the most active single drug.
161 Antagonism was defined as a $\geq 2 \log_{10}$ CFU/mL increases in colony counts after the incubation
162 periods by the combinations compared to that of the most active single extract alone [30]. All the
163 experiments were performed in quadruples and the data collected from four repeated experiments,
164 was analyzed using the Randomized Complete Block Design (Two-way analysis of variance).
165 Duncan's New Multiple Range Test was used to separate the means that were significantly different.

166

167 3. Results

168 3.1. Testing the Susceptibility of the fungi by Agar plate Method.

169 It was observed that there was considerable activity of the extract combinations against the fungi
170 tested. This was indicated by long periods of growth inhibition (above two weeks) observe with all
171 the fungal strains tested. (Table 1)

172 3.2. Checkerboard assay method of evaluating the antifungal effects of interactions between 173 *E. abyssinica* (E) and *Coleus species* (C) Extracts

174
175 In the study reported here, the susceptibility pattern seen with the fungi tested (test) showed that *E.*
176 *floccosum* was significantly inhibited than all the other fungi tested (test). This synergy was
177 observed in the isoboles as indented points away from the additive line to the left. *Candida albicans*
178 showed some significant level of antagonism to the various combinations tested (test). This was
179 seen as points of indentations distant away from the additive line to the right.

180 The Combined (combined) effect was synergistic against *T. mentagrophytes*. This was seen at
181 Fractional Inhibitory Concentrations (FIC) of Euphorbia 0.2 / FIC of Coleus 0.7 mg/mL with FIC Index
182 of 0.9 mg/mL, and at FIC of Ea 0.1 / FIC of Cs 0.8 mg/mL and FIC Index of 0.9 mg/mL (Fig.1)

183 The synergistic effects observed with *M. gypseum* were at four different combinations of *E.*
184 *abyssinica* (Ea) and *Coleus species* (Cs) extracts proportions viz; at FIC of Ea 0.8 / FIC of Cs 0.1
185 mg/mL, FIC Index 0.9 mg/mL, At FIC of Ea 0.7 / FIC of Cs 0.2 mg/mL, FIC Index 0.9 mg/mL, at FIC
186 of Ea 0.4 / FIC of Cs 0.5, FIC Index 0.9 mg/mL and at FIC of Ea 0.3 / FIC of Cs 0.6 mg/mL, FIC
187 Index 0.9 mg/mL respectively (Fig. 2).

188 The plant extract proportions that showed synergy against *E. floccosum* include (included): FIC Ea
189 of 0.6 / FIC of Cs 0.1 mg/mL, FIC Index 0.7 mg/mL, FIC of Ea 0.6 / FIC of Cs 0.2 mg/mL, FIC Index
190 0.8 mg/mL, FIC of Ea 0.6 / FIC of Cs 0.3 mg/mL, FIC Index 0.9 mg/mL and at FIC of Ea 0.1 / FIC of
191 Cs 0.8 mg/mL, FIC Index 0.9 mg/mL (Fig. 3).

192 **3.3. Time-kill assay method of evaluating the antifungal effects of interactions between *E.*** 193 ***abyssinica* (E) and *Coleus species* (C) Extracts**

194 In the assay method, the effect of interactions were compared to that of the most efficacious plant
195 extract singly. If the interactions were able to reduce the viable cell counts to more than 2 log₁₀
196 CFU/mL, this was accepted as synergistic but if there were increases in the viable cell counts which
197 were more than 2 log₁₀ CFU/mL, then this was antagonism.

198 The antifungal activity of combined hydro alcohol extracts of *Coleus species* and *E. abyssinica* was
199 evaluated by exposing the test fungi to various combined proportions of the extracts at different time
200 intervals, which included; 0 hour, 6 hours, 12 hours, 24 hours and 48 hours. The test fungi viable
201 cell counts were standardized to contain 1x10⁶ for the yeasts and 1x10⁵ for the moulds.

202 The more potent single plant extract observed was *Coleus species*.

203 The effect of *Coleus species* extract on *Candida albicans* and *Trichophyton mentagrophytes* cells,
204 showed that, the extract at MIC and at double the MIC concentrations decreased the cell counts to
205 about 0.05 log₁₀ by the 48 hours (Fig. 5 & 6). This same double MIC (15.6 mg/mL), killed *M.*
206 *gypseum* cells in 6 hours (Fig. 7).

207 However, when *Coleus species* and *E. abyssinica* extracts were combined, they exhibited no
208 synergistic interactions against *Candida albicans*, *Trichophyton mentagrophytes* and *M. gypseum*
209 (Fig. 5, 6 & 7).

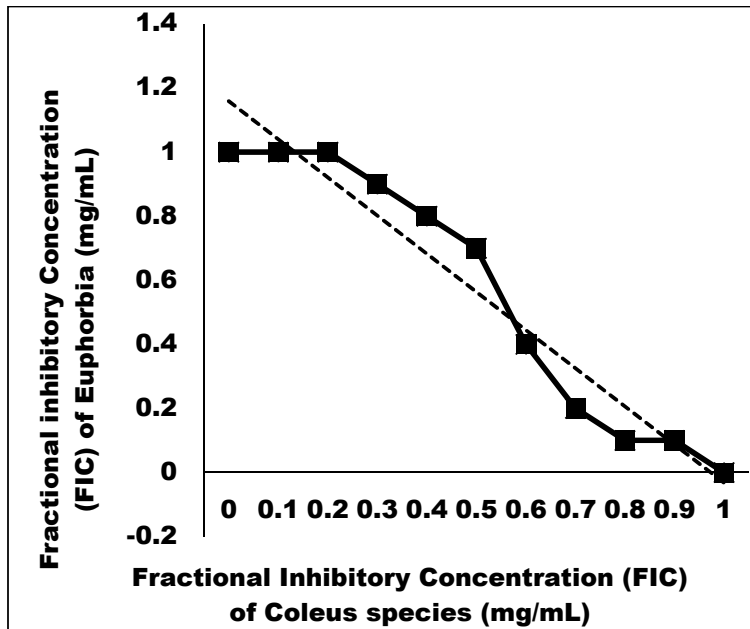
210 In 48 hours, *Coleus species* (the more active of the plant extracts) at MIC of 0.98 mg/mL, decreased
211 *E. floccosum* viable cell counts from 1x10⁵ CFU to 0.97 log₁₀. However, by doubling the MIC to 1.96
212 mg/mL, the fungicidal effect became prominent against *E. floccosum* cells, which were all inhibited in
213 3hours. The 1 µg/mL of the control drug inhibited the fungal cells in 48 hours (Fig. 8).

215 On combining the two plant extracts, and comparing the activity observed with *Coleus species*
 216 extract alone, the interactions showed synergistic effects against *E. floccosum*.
 217 This was detected by the effects observed with the following interactions; Adding 1:8 proportions i.e
 218 0.098 mg/mL of Ea with 0.78 mg/mL of Cs to yield 0.878 mg/mL of these extracts decreased the cell
 219 counts from 1×10^5 to $2.0 \log_{10}$ CFU in 48 hours. In the same trend, combining 0.59 mg/mL of Ea with
 220 0.098 mg/mL of Cs to give 0.688mg/mL and 0.59 mg/mL of Ea with 0.196 mg/mL of Cs to get 0.786
 221 mg/mL i.e. 6:1 and 6:2 combinations respectively, eradicated the viable cells within 48 hours.
 222 However, combining 6:3 proportions i.e. 0.59 mg/mL of Ea and 0.29 mg/mL of Cs to get 0.88 mg/mL,
 223 dropped the cell count to $1.0 \log_{10}$ and to $0.3 \log_{10}$ in 24 hours and 48 hours respectively. The cell
 224 counts were all in this case reduced beyond $2 \log_{10}$, signifying synergy (Fig. 8).

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226 **Table 1: Duration of Fungal Growth Inhibition in Weeks by 100 mg/mL of Combined Extracts**
 227 **of *Euphorbia abyssinica* and *Coleus species***

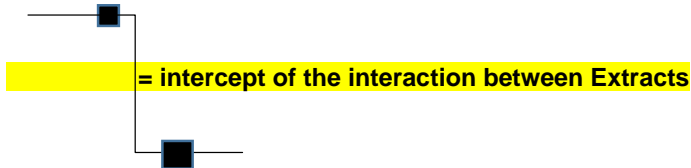
	Fungal species / growth inhibition in weeks			
	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>M. gypseum</i>	<i>E. floccosum</i>
Plant Extract Combination	>1	>2	>2	>2
Voriconazole 16µg/mL	>2	>2	>2	>2



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230 **Figure 1: The response effect of *T. mentagrophytes* to Combined Extracts of *E. abyssinica***
 231 **and *Coleus species*.**

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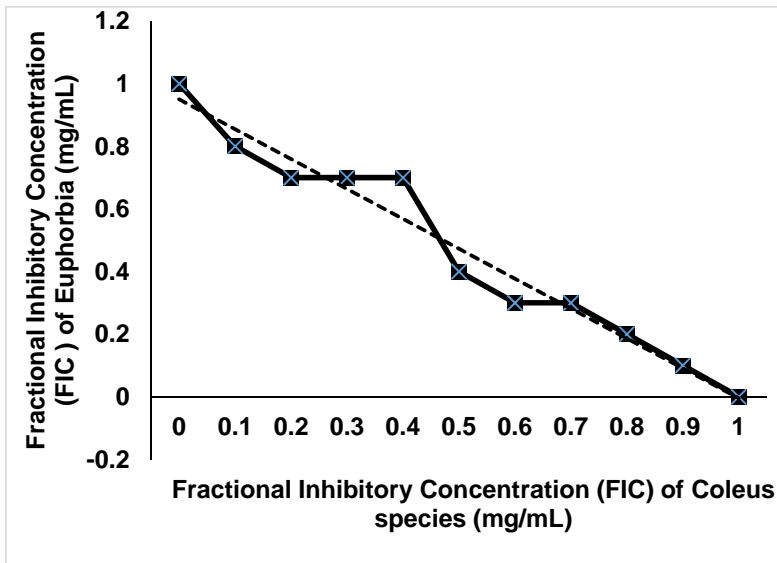
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Synergy was observed in the isoboles as indented points away from the additive line to the left while antagonism was seen as indentations distant away from the additive line to the right



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242 **Figure 2: The response effect of *M. gypseum* to Combined Extracts of *E. abyssinica* and**
243 ***Coleus species*.**

244 = intercept of the interaction between Extracts

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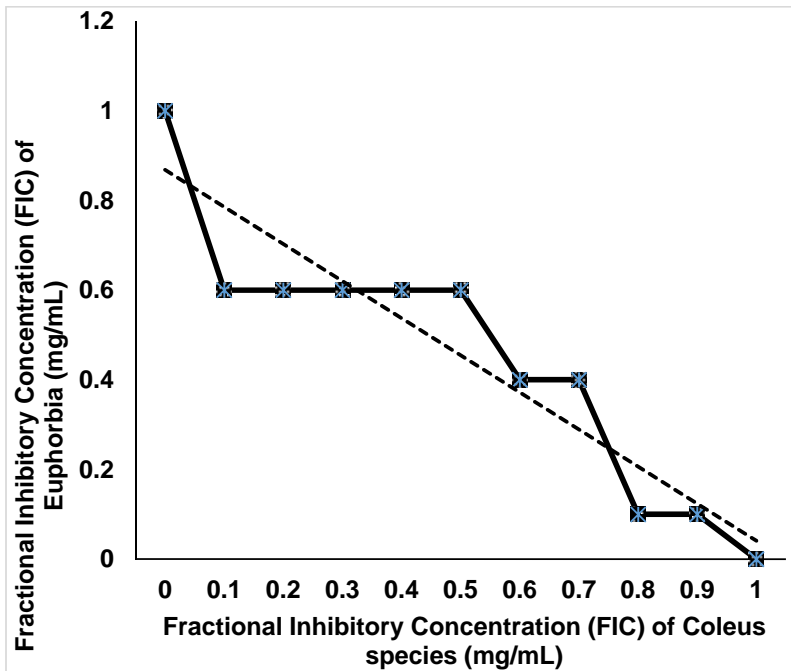
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247 ----- = Combined effect not better than the more active single plant extract

248 Synergy was observed in the isoboles as indentations away from the additive line to the left while

249 antagonism was seen as indentations distant away from the additive line to the right

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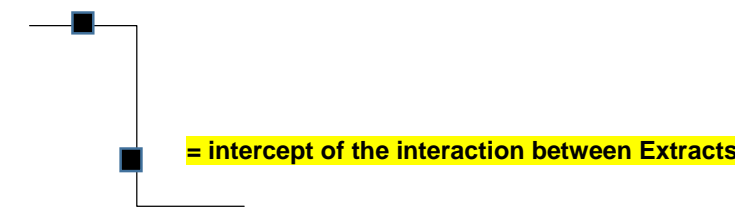
252 **Figure 3: The response effect of *E. floccosum* to Combined Extracts of *E. abyssinica* and**
 253 ***Coleus species*.**

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----- = Combined effect not better than the more active single plant extract

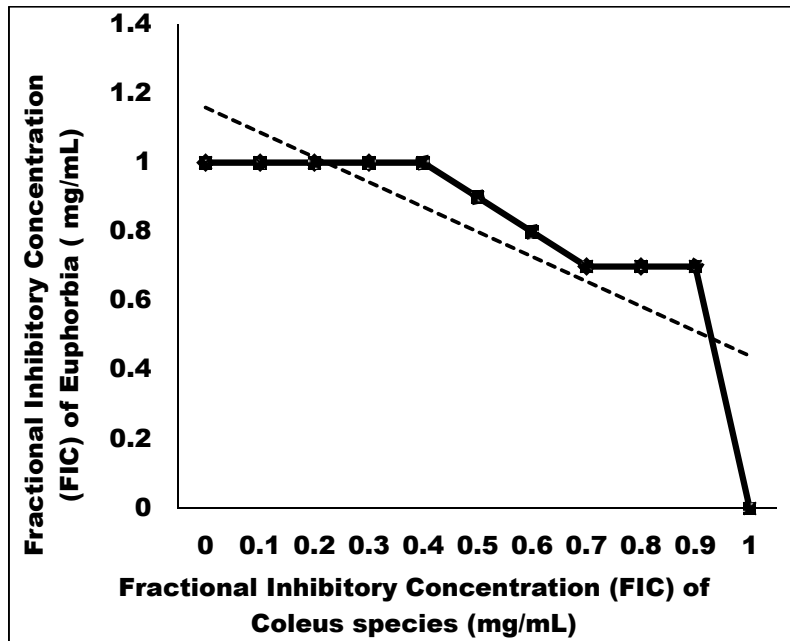
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Synergy was observed in the isoboles as indented points away from the additive line to the left while

260

antagonism was seen as indentations distant away from the additive line to the right

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263 **Figure 4: The response effect of *Candida albicans* to Combined Extracts of *E. abyssinica* and**
 264 ***Coleus species*.**

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= intercept of the interaction between Extracts

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----- = Combined effect not better than the more active single plant extract

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Synergy was observed in the isoboles as indented points away from the additive line to the left while

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antagonism was seen as indentations distant away from the additive line to the right

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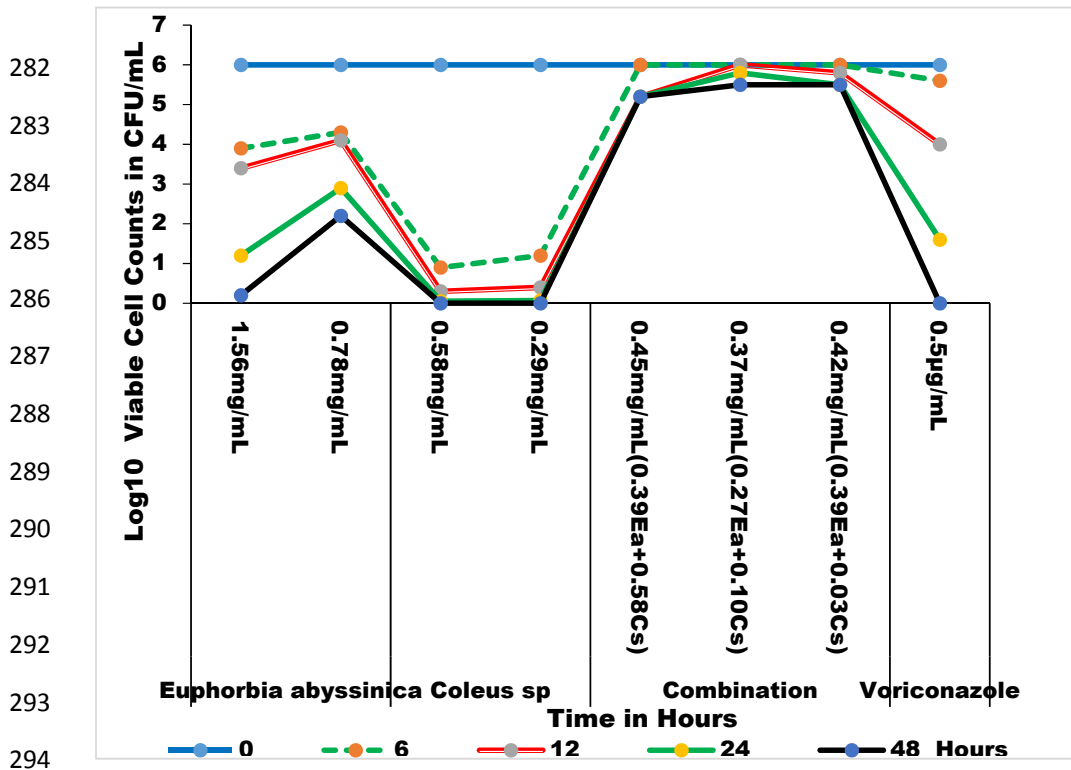
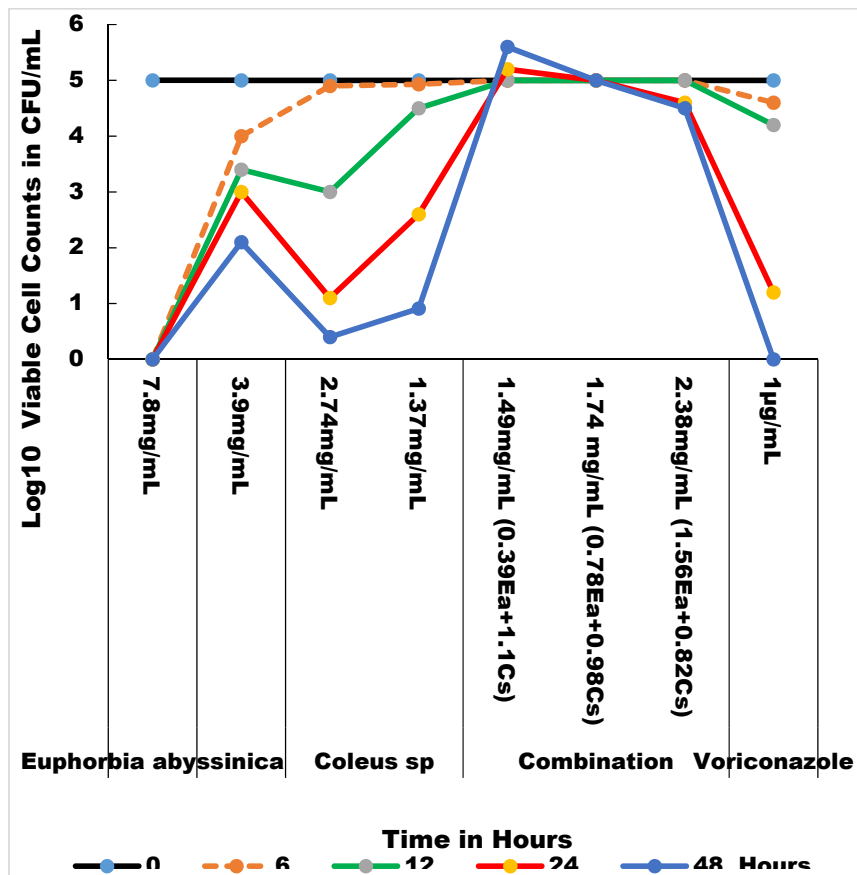


Figure 5: Effect of Time on the Reduction of Viable Cell Counts of *Candida albicans* by the combined extracts; Ea=*Euphorbia abyssinica*, Cs=*Coleus species*

Interactions that reduce the number of viable cells above 2 log₁₀ CFU/mL, were accepted as synergistic but if there were increases in the viable cell numbers which were more than 2 log₁₀ CFU/mL, then this was antagonism.



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317 **Figure 6: Effect of Time on the Reduction of Viable Cell Counts of *Trichophyton***
 318 ***mentagrophytes* by the combined extracts;**
 319 **Ea=*Euphorbia abyssinica*, Cs=*Coleus species***
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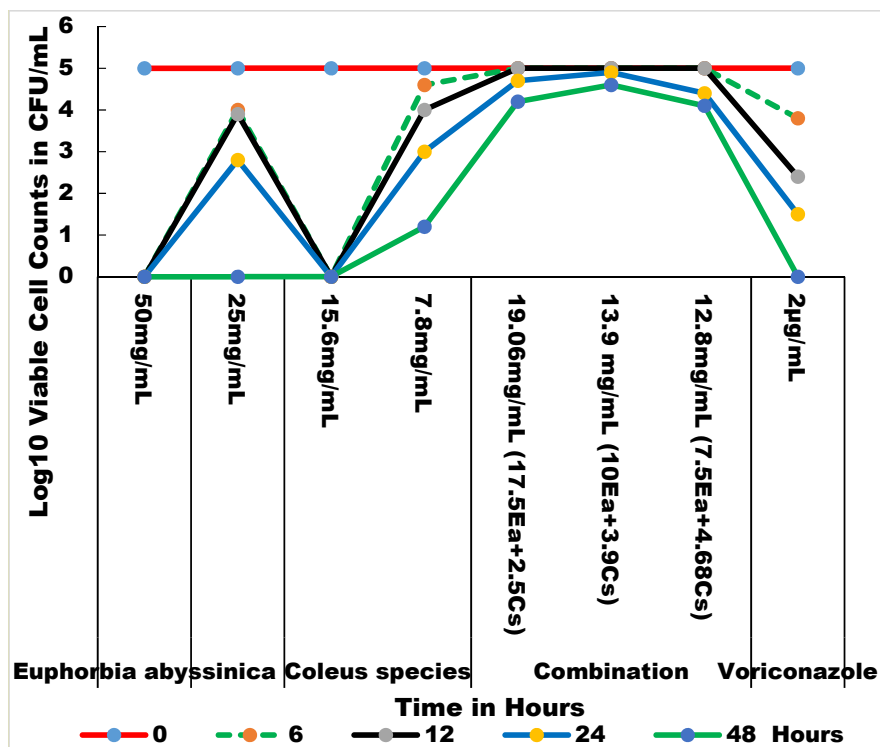
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329 **Figure 7: Effect of Time on the Reduction of Viable Cell Counts of *Microsporium gypseum* by**
 330 **the combined extracts;**
 331 **Ea=*Euphorbia abyssinica*, Cs=*Coleus species***

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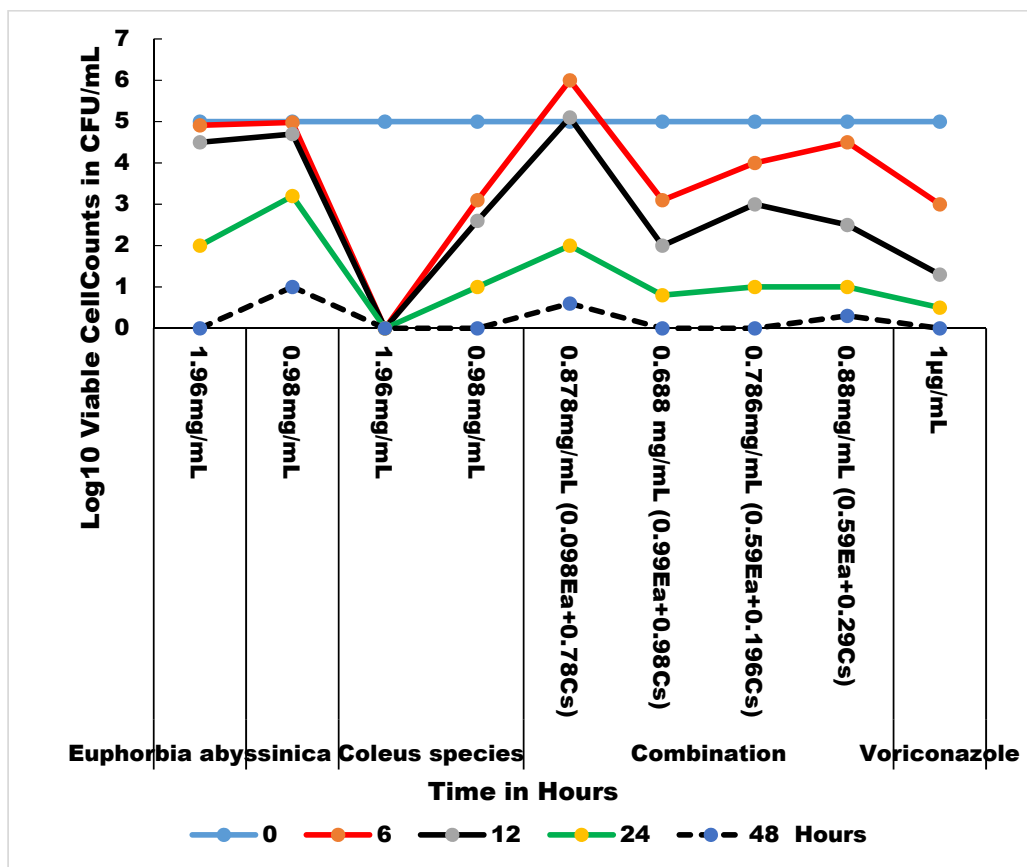
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340 **Figure 8: Effect of Time on the Reduction of Viable Cell Counts of *E. floccosum* by the**
 341 **combined extracts;**
 342 **Ea=*Euphorbia abyssinica*, Cs=*Coleus species***

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4. DISCUSSION

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In ethno medicine, plant extracts are often used in different combinations, whose quantifications are still problematic till today. However, they still remain the preferred method of treatment in most local and under developed areas of the world where the orthodox drugs are noted easily available [30]. Understanding the hidden truth behind the unusual potentials and efficacies of these plant extracts, was the aim of the study reported here. The Kinetic Time Kill and Checkerboard assay were used to evaluate the antifungal activity of single components as well as the interactions of different proportions of *E. abyssinica* and *Coleus species* extracts. This was to determine whether combinations of the two plant extracts, after interaction, could produce an effect that could be synergistic, antagonistic or additive against the fungi tested. An additive, effect is observed when the combined effect is not more than the individual effect of the most active plant extract. In such a case

356 even lower concentrations than those obtained with the individual extracts alone equally yield the
357 same effect [31].

358 For drug interactions which result in synergistic effects (agonists), the concentration of each drug in
359 the combinations may not necessarily need to be up to that obtained when the drugs are used
360 singly. If reduced quantities of the each interacting drug or of a particular component alone can
361 react, they will still produce the required synergy. At times, the observed effect with some drug
362 interactions may indicate that the activities of the reactant (s) have decreased and this effect is
363 termed sub-additive. [31].

364 Many different methods have been used by different authors to evaluate and represent the effects of
365 drug combinations; and an example of such representation is the isobole, a curve produced by
366 Loewe in 1957. He plotted a graph using the doses of two drugs, one on the 'Y' and the other as the
367 'X' axes. The individual drug concentrations that could interact, when given in combination, to
368 produce an effect (synergy, antagonism, etc) were seen on the rectangular plot as points which he
369 called the "isobole" [31].

370 However, when *in-vivo*, drugs, regardless of the fact that they are administered in combination, or
371 singly, may be encountered by the plasma proteins and other natural components, present in the
372 human system. The evaluation of the activity and effects of the dose of each agonist, gives better
373 information, especially about those agonists which use different modes of action with different
374 receptors to produce synergy. These types of agonists, termed "similar and independent" by Bliss
375 [32], do not interfere with each other since their binding sites are independent of one another.

376 Studies of this sought try to address some doubts which may arise about the response produced by
377 ion of two agonists; whether their interactions will be additive, synergistic or antagonistic when
378 compared to the single drugs effects.

379 In this study, *Coleus species* extracts, one of the single components used in the interaction study,
380 showed a significantly level of activity ($P=0.05$) than the second counterpart (*Euphorbia abyssinica*
381 extract). A contributory factor could be that the *Coleus species* was used as a full-spectrum plant
382 extracts, which means that the entire chemical profile available in the flowers and all other parts
383 together with the roots is present in the final medicinal form [33].

384 In comparing the methods used in the study, the results indicated that the Agar diffusion, method
385 produced the best response than the Kinetic Time kill and the Checkerboard assays. This was

386 indicated by the synergistic effects produced by the combined plant extracts against the fungal
387 species tested. Tarh and Iroegbu [20] observed that the Kinetic Time kill and the Checkerboard
388 assays are dependent on predetermined MICs of the single extracts, and this could at times not be
389 hundred percent reliable, due to the fact that MIC values can be affected by confounding, bias,
390 inaccuracy and lack of precision in the variables used.

391 In the checkerboard assay the antifungal activity observed with interaction between *E. abyssinica*
392 and *Coleus species* extracts indicated that the two plant extracts are agonists' *in-vitro*. The fractional
393 inhibitory concentrations (FIC) of both extracts indicated that there was synergy against *T.*
394 *mentagrophytes* and *M. gypseum* at FIC indices of 0.9 mg/mL, respectively. This was also seen
395 against *E. floccossum* at FIC indices of 0.7, 0.8 and 0.9 mg/mL. The effect of the plant extracts at
396 different combinations was indifferent against *Candida albicans* (*C. albicans*).

397 In the Kinetic Time kill assay, Synergy was significantly observed against *E. floccossum* with more
398 than 2 log₁₀ reduction in the number of viable cells counted within 48hours. Interacting lower
399 concentrations of 0.688 mg/mL and 0.786 mg/mL, killed the cells in 48 hours, while higher
400 concentrations of 0.878 mg/mL and 0.880 mg/mL decreased the cell counts to 2.0 log₁₀ and 0.3 log₁₀
401 in 48 hours, respectively. The lower combinations showed better effects because at higher
402 concentrations, the plant extracts could present some unwanted adverse side effects.

403 However, in comparison, the Checkerboard assay showed a more significant sensitivity pattern
404 (P=0.05) in this study than the Time kill Assay. The plant extract combinations inhibited the growth of
405 the three molds tested, but the effects against the yeast *C. albicans* were the reverse because, no
406 synergy was observed at all the combinations tested.

407 The response effects observed between the above plant extracts interactions and the fungi tested,
408 could have resulted from so many factors, both environmental, human as well as the innate changes
409 exerted in the kinetics of one drug by the other. The observation of a diminished effect or inactivity
410 *in-vitro* is not a confirmation that the same scenario will be observed when the drug is administered
411 internally. This is so because some components of the body may play some roles when the drug
412 gets into the system. These interactions between the tissues and the drugs may also cause changes
413 that may affect the activity and effects of others that use the same receptor type [34]. e.g Calcium,
414 magnesium and aluminum ions, which are components of some antacids can calcify and crystallize
415 metal-tetracycline and render it less absorbable [34]. Drugs that are taken orally, pass through the

416 digestive tract and are encountered by digestive enzymes prior to their absorption in to the blood.
417 This condition may cause a vast amount of the drug to be lost through the quick metabolic activity of
418 the hepatic system – the so-called “first pass effect” [34]. Competitive Inhibition can also occur
419 amongst the drugs because some of them extensively bound to plasma proteins and, therefore,
420 competition for binding sites, on the receptor, may result in an inadequate serum concentration, of
421 the antibiotic being reached, with consequent failure of therapy [34].

422 Toxicity test which evaluates the lethal dose (LD50) may present a better picture of the drug effect *in*
423 *vivo*. Better still, the quanta dose-effect or dose-response curve that displays the percent of animals
424 that respond to the drug i.e the hyperbolic curve described by the equation $E = E_{max}D/(D + C)$ where
425 E is the effect, D is dose and C the constant, which is equal to the dose needed for a half-maximal
426 response, a measure of drug potency, often denoted as ED_{50} or D_{50} [31] can be used.

427 The wide range of antimicrobial activity observed by other researchers with these two plants has
428 also been confirmed in this research work. Extracts of *E. abyssinica* and *Coleus species* in
429 combination, were able to inhibit the growth of both yeasts and molds. There was no observable
430 significant difference ($P=0.05$) in the response pattern seen with the different fungal strains used in
431 the study. There have been reports of the same pattern of antimicrobial effects of alcohol extracts of
432 *Coleus species* by Jay, [35] and Tarh and Iroegbu, [30].

433

434 5. Conclusion

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

443 CONSENT

444 It is not applicable.

445

446 ETHICAL APPROVAL

447 It is not applicable.

448

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