ejpmr, 2016,3(9),540-544

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

IN-VITRO EVALUATION OF CO-ADMINISTRATION OF CHLOROFORM LEAF EXTRACT OF *CHROMOLAENA ODORATA* ON THE ANTIMICROBIAL ACTIVITY OF CLINDAMYCIN AND ITRACONAZOLE.

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Article Received on 19/07/2016 **Article Revised on 10/08/2016** **Article Accepted on 31/08/2016**

ABSTRACT

Objective: To investigate the interacting effects of co-administration of leaf extract of *Chromolaena odorata* on the antimicrobial activity of clindamycin as well as itraconazole, in-vitro. **Materials** and **Methods:** The chloroform extract of the dried leaves obtained by 48 h cold maceration was evaluated using agar cup diffusion technique; and checkerboard technique for evaluation of interaction between the two antimicrobial agents. **Results:** The extract showed inhibitory effect against *Staphylococcus aureus* and *Aspergillus niger* at concentrations of 5 mg/ml and 10 mg/ml respectively. Clindamycin had MIC of 0.003 mg/ml against *Staphylococcus aureus* while itraconazole had MIC of 0.0002 mg/ml against *Aspergillus niger*. Concomitant administration of extract and drugs revealed that some ratios showed additive properties, some other ratios were synergistic, while some showed indifferent activities against both isolates. **Conclusion:** Clindamycin and itraconazole exhibited synergistic interaction with *C. odorata* leaf extract against microbial infections. At a given ratio, the combination of *Chromolaena odorata* and clindamycin or itraconazole could be of possible clinical significance in the treatment of bacterial and fungal infections.

KEYWORDS: *Chromolaena odorata*, clindamycin, itraconazole, co-administration, in-vitro.

INTRODUCTION

The folkloric use of herbs in the treatment of several ailments by traditional healers whose patients also take prescription drugs is a growing trend[1]. Microbial infection is one of these ailments which have been acclaimed to be managed by herbal health practitioners. This is more significant in view of global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs obsolete in the treatment of infections[2]. *C. odorata* is among the numerous plants reported to attack microbial infections[3]. *C. odorata* formerly referred to as *Eupatorium odaratum*[3] is called and known as "Awolowo" or "Elizabeth" in Nigeria. It is found in waste places, roadsides and farmlands where it is considered more as a pest than a weed.

The plant has numerous ethnomedicinal uses both in animals and humans as a decoction of the leaves alone is valued as cough remedy; together with lemon and guava leaves, the decoction is used for the treatment of malaria; juice from the crushed leaves when applied to cut helps to stop bleeding. In limited quantity, fresh leaves of *C. odorata* have been used to enrich the fodder for domestic animals. Other medicinal application of *C. odorata* include antispasmodic, antidiarrhoeal, antihypertensive, diuretic and anti-inflammatory[3]. Lincomycin and clindamycin are the principal members of the lincosamides. However, lincomycin is no longer of choice nowadays in the light of the availability of clindamycin. Similar to that of the macrolides and chloramphenicol, lincosamides are bacteriostatic in which the mechanism of action involves reversible inhibition of protein synthesis[4]. Interaction of lincosamides with ganglion blockers leads to potentiation of effects[5]. Itraconazole, a systemic azole derivative is considerably more active than ketoconazole and other azoles, against Aspergilli[6]. Being a broad-spectrum antifungal, itraconazole exerts activity against candida species, dermatophytes, Cryptococcus, cladosporum and sporothrix[7,8]. It is known that interaction due to concurrent administration with rifampin decreases significantly the concentration of itraconazole in plasma[9]. It also reduces ergosterol synthesis by inhibition of fungal cytochrome P450 enzymes[9]. As noted, the interactions of lincosamide and itraconazole with some drugs potentiated their effect; consequently, this study investigated the in-vitro interactions between the extract of *C. odorata* and clindamycin as well as itraconazole against *S. aureus* and *A. niger*.

MATERIALS AND METHODS

Plant material

Fresh leaves of *C. odorata* were collected from Owerri, Nigeria and official identification was confirmed at the Department of Pharmacognosy, Madonna University, Elele, Nigeria, where Herbarium specimen was deposited. The leaves were sun-dried and reduced to powder by granulation. Two hundred grams of the powder was soaked in 1000 ml of chloroform for 48 h with occasional shaking. The chloroform extract was filtered and allowed to evaporate to dryness at room temperature.

Test micro-organisms

Isolates of *Staphylococcus aureus* (NCTC 6571) and *Aspergillus niger* (Lab stock) were obtained from Medical Laboratory Unit of Madonna University Teaching Hospitak, Elele, Nigeria. For maintenance and standardization, each of the isolated, characterized and purified test organisms were sub-cultured every week in 5 ml sterile nutrient agar slants. The agar slants containing *S. aureus* and *A. niger* were stored in a refrigerator at 4°C after 24 h incubation at 37°C for bacteria and after 48 h incubation at 25°C for fungi.

Preparation of Drug solution

A stock solution of chromolaena leaf extract was prepared on each occasion by weighing 5 g of extract and dissolving in 50 ml of dimethylsulphoxide (DMSO) to obtain a concentration of 10 mg/ml. Also, appropriate amounts of clindamycin (500 mg) and itraconazole (100 mg) were dissolved in distilled water to obtain a stock solution of 0.02 mg/ml and 0.0016 mg/ml respectively.

Antimicrobial screening

Agar cup diffusion method[10] was employed to determine the activity of the extract on *Staphylococcus aureus* (NCTC 6571) and *Aspergillus niger* (Lab. Stock). Nutrient agar (NA, pH 7.4, oxoid) was prepared according to manufacturer's specifications. Sabouraud dextrose agar (SDA, pH 5.4, oxoid) containing 0.4 % chloramphenicol was also prepared according to manufacturer's specifications. Other materials used are as obtained from the supplier without further processing including DMSO and chloroform (M&B Chemicals, UK). After seeding nutrient agar and sabouraud dextrose agar plates with *S. aureus* and *A. niger* respectively, wells were bored on the plates using a sterile cork borer (6 mm). Ten-fold serial dilutions of the stock solutions of each drug in distilled water was prepared and introduced into each of these wells and placed on the bench for 30 minutes to allow for pre-diffusion. Thereafter, the nutrient agar plates incubated at 37°C for 24 h while the sabouraud dextrose agar plates were incubated at 25°C for 48 h. After the incubation period, the inhibition zone diameters (IZD) surrounding each well were measured and the MIC estimated.

Interaction studies

This is in accordance with the continuous variation checkerboard protocol[11]. Stock solutions of *C. odarata* (10 mg/ml) and clindamycin (0.02 mg/ml) were freshly prepared for their combined effect against *S. aureus*. The two agents were mixed in varying ratios ranging from 0:10 clindamycin: *C. odarata* to 10:0 of the same agents. Each of the eleven combinations was serially diluted 2-fold with distilled water. The test isolate (*S. aureus*) was seeded into sterilized molten nutrient agar and allowed to set. Holes (6 mm) were bored in the nutrient agar plates and each of the dilutions introduced into the holes and incubated at 37°C for 24 h. The IZD were measured and used to estimate the MIC of extract and drug alone in the various combination[10]. For *C. odorata* and itraconazole against *A. niger*, the same procedure was adopted but the culture medium was sabouraud dextrose agar and incubation was at 25°C for 48 h. The combined effect of extract with clindamycin and itraconazole against *S. aureus* and *A. niger* respectively was determined after estimating the fractional inhibitory concentration (FIC) index thus:

$$\text{FIC index} = \frac{A1}{A} + \frac{B1}{B}$$

Where A1 and B1 are the concentrations of the *C. odorata* and clindamycin/itraconazole in combination producing the combined MIC; while A and B are the concentrations of *C. odorata* and clindamycin/itraconazole that produced MIC when acting alone.

RESULTS

The outcome of the MIC of chloroform extract of *C. odorata*, clindamycin and itraconazole against the test micro-organisms are as shown in Table 1. Test isolates of *S. aureus* and *A. niger* were inhibited by leaf extract of *C. odorata* MIC, 10 mg/ml and 5 mg/ml respectively. Also, *S. aureus* was susceptible to clindamycin (MIC, 0.005 mg/ml) while *A. niger* was susceptible to itraconazole (MIC, 0.0002 mg/ml). The combined effects of *C. odorata* leaf extract and clindamycin against *S. aureus* are as shown in Table II; while the combined effects of *C. odorata* leaf extract and itraconazole against *A. niger* are presented in Table III. From Table II, five out of the nine drug combination ratios (clindamycin: *C. odarata)* exhibited synergism against *S. aureus*. Ratio 6:4 (clindamycin:C. odorata) exhibited the greatest degree of synergy while 3:7 exhibited the least. One drug combination (4:6) exhibited additivity; while ratios 7:3, 8:2, and 5:5 produced indifference effects. From Table III, synergistic effect was obtained for 4 ratios thus 9:1, 8:2, 7:3, and 5:5 with the ratio 5:5 (itraconazole: *C. odorata*) producing the most synergistic effect. The rest of the ratios produced indifference effect.

Table I: MIC of the Chloroform extracts of *C. odorata*, clindamycin and itraconazole against the test micro-organisms.

Extract/Drugs	*Staphylococcus aureus*	*Aspergillus niger*
C. odorata	10.0	5.0
clindamycin	0.005	----
itraconazole	----	0.0002

Table II: The combined effect of the chloroform extract of *C. odorata* and clindamycin against *S. aureus.*

Combination Ratio (Clind: Chrom)	MIC of Clind (mg/ml)	MIC of Chrom (mg/ml)	FIC Clind	FIC Chrom	FIC Index	Activity Index	Effect
10:0	0.005	-	-	-	-	-	-
9:1	0.0045	0.25	0.90	0.025	0.925	-0.034	Syn
8:2	0.008	1.00	1.60	0.100	1.70	0.230	Ind
7:3	0.0014	3.00	2.80	0.300	3.10	0.491	Ant
6:4	0.003	1.00	0.60	0.100	0.70	-0.154	Syn
5:5	0.0010	0.625	2.00	0.063	2.063	0.314	Ant
4:6	0.004	3.00	0.70	0.300	1.00	-0.000	Add
3:7	0.003	3.50	0.60	0.350	0.95	-0.022	Syn
2:8	0.002	4.00	0.40	0.400	0.80	-0.096	Syn
1:9	0.001	9.00	0.20	0.700	0.90	-0.046	Syn
0:10	-	10.0	-	-	-	-	

Clind= Clindamycin, FLC= Fractional inhibitory concentration, MIC= Minimium inhibitory concentration ,Chron= Chromolaena odorata (Aqueous extract), Syn= Synergistic, Add=Additivity
Ant= Antagonism, Ind=Indifference.

Table III: The combined effect of the chloroform Extract of *C. odorata* and Itraconazole against *Aspergillus niger*.

Combination Ratio (Itra: Chrom)	MIC of Itra (mg/ml)	MIC of Chrom (mg/ml)	FIC Itra	FIC Chrom	FIC Index	Activity Index	Effect
10.0	0.0002	-	-	-	-	-	-
9.1	0.0018	0.125	0.90	0.025	0.925	-0.033	Syn
8.2	0.00016	0.250	0.80	0.050	0.850	-0.070	Syn
7.3	0.00014	1.500	0.70	0.250	0.950	-0.022	Syn
6.4	0.00024	0.500	1.20	0.100	1.300	-0.144	Ind
5.5	0.00010	0.625	0.50	0.125	0.625	-0.204	Syn
4.6	0.00032	6.000	1.60	1.200	2.800	0.447	Ind
3.7	0.00048	7.000	2.40	1.400	3.800	0.579	Ind
2.8	0.00032	8.000	1.60	0.90	3.200	0.505	Ind
1.9	0.00008	4.500	0.40	1.60	1.300	0.113	Ind
0.10	-	5.000	-	-	-	-	

Itra= Itraconzole, FLC= Fractional inhibitory concentration, MIC= Minimium inhibitory concentration, Chrom= Chromolaena odorata (Aqueous extract), Syn= Synnergistic, Ant= Antagonism, Ind= indifference.

DISCUSSION

The result showed that *S. aureus* was susceptible to clindamycin while *A. niger* was susceptible to itraconazole. This is in agreement with documented reports that lincosamides are effective against Gram-positive cocci[12] as well as the effectiveness of azoles against fungi[13]. Five out of nine drug combination ratios (clindamycin: *C. odorata*) exhibited synergism against *S. aureus*. This confirms the report that in the evaluation of interaction between two antimicrobial agents by checkerboard method, values of FIC index less than one indicates synergism, the degree of synergy increasing as the value turn towards zero[14]. A negative value for activity index also indicates synergism[11]. A keen look at ratio 6:4 that gave the highest degree of synergy, revealed that the combination reduces the MIC of clindamycin and *C. odorata* by 1.67 and 10 times respectively. This finding implied that both agents

modify the activity of each other against *S. aureus* and that clindamycin modifies the activity of *C. odorata* extract to a much larger extent than *C. odorata* extract does to it. Hence, clindamycin could be said to sensitize *S. aureus* to the actions of *C. odorata* extract. In the drug ratio that showed the greatest synergy (5:5), the MIC of itraconazole and *C. odorata* extract were reduced by 20 and 8 times respectively. The implication is that *C. odorata* extract modifies the activity of itraconazole to a much larger extent than itraconazole does to it.

CONCLUSION

It can be inferred that both clindamycin and itraconazole exhibited synergistic interaction with *C. odorata* leaf extract against *S. aureus* and *A. niger* respectively. *C. odorata* possess a stronger antibacterial activity than antifungal activity.

Conflict of interest: No conflict of interest declared.

Source of support: Nil

ACKNOWLEDGEMENT

The author is grateful to Dr. I. N. Nnatuanya of the Department of Medical Laboratory Sciences, Madonna University, Elele, Nigeria, for his technical assistance.

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