

Full Length Research Paper

Evaluation of *in vitro* antimicrobial effect of combinations of erythromycin and *Euphorbia hirta* leaf extract against *Staphylococcus aureus*

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The *in vitro* combined effects of erythromycin and methanol extract of leaves of *Euphorbia hirta* against clinical isolates of *Staphylococcus aureus* were investigated using the Checkerboard technique. The organism was susceptible to the extract with MIC of 25 mg/ml, while erythromycin had MIC of 0.005 mg/ml. The research aims to investigate the possible interaction that may exist when standardize herbal drugs are combined with synthetic antimicrobials. Previously, stock solutions of both the extract and erythromycin were prepared in appropriate volume of dimethylsulphoxide and water respectively to get a final concentration of 50 mg/ml each. Minimum inhibitory concentration was determined by method of serial dilution while interaction study was carried out according to the continuous variation checkerboard method. Upon combination, some ratios showed synergistic (9:1, 8:2, 7:3, 6:4, 3:7, 2,8, 1:9) while others indicated indifference (5:5, 4:6) activities against the isolates. These results indicate that some combinations of the extract with erythromycin could be synergistic in activity for some ratio combinations and indifferent for some others.

Key words: *In vitro*, erythromycin, *Euphorbia hirta*, *staphylococcus aureus*, MIC checkerboard.

INTRODUCTION

Several reasons have been advanced to justify the use of combination of two or more antibiotic treatment (Esimone et al., 2006; Ibezim et al., 2006).

For many years now; combination of two or more antibiotics has been recognized as an important method for, at least, delaying the emergence of bacterial resistance (Chambers, 2006). Besides, antibiotic combinations may also produce desirable synergistic effects in the treatment of bacterial infections (Zinner, 1981).

Two very distinct traditional methods of testing *in vitro* antibiotic interaction are the Checkerboard technique and the Time killing curve method (Eliopoulos et al., 1988). The checkerboard method will be used in this research. erythromycin is usually bacteriostatic, but can be bactericidal in high concentrations against very susceptible organisms. The antibiotic is most effective *in vitro* against aerobic gram-positive cocci and bacilli (Goodman and

Gilman, 2001).

Euphorbia hirta belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries (Sofowora, 1982). *E. hirta* has various phytochemicals embedded in the plant parts.

The leaves are found to contain triterpenoids, sterols, alkaloids, glycosides and tannin (Anozie, 1991). In Nigeria, extracts of the plant are used as eardrops and in the treatment of boils, sore and promoting wound healing (Igoli et al., 2005).

In this study, the interaction between erythromycin and methanol extract of leaves of *E. hirta* has been investigated using Checkerboard method. The results of this research could provide rational basis for the use of standardized herbal drugs in combination therapy of prevailing diseases.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves of *E. hirta* were collected from Nsukka, Enugu State,

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Nigeria. The plant was authenticated by Mr. A. O. Ozioko of Bio-resources Development and Conservation Programme, Aku Road, Nsukka. Specimen vouchers were kept in the department of Pharmacognosy, University of Nigeria, Nsukka with number; E.hcco: 002

Sample preparation and extraction procedures

The fresh leaves were air dried for one week and ground into fine powder using a mechanical grinder. 25 g of the fine powder was macerated with 375 ml of methanol in a conical flask. This was covered and shaken every 30 min for 6 h, and then allowed to stand for 48 h.

The solution was subsequently shaken and filtered using Whatman filter paper. The filtrate was evaporated to dryness with the aid of a rotatory evaporator (Model type 349/2, Corning Ltd). The extract was then stored below ambient temperature.

Preparation of extract/drug stock solution

The stock solution of *E. hirta* leaf extract was prepared on each occasion by careful weighing and dissolving in suitable volume of Dimethylsulphoxide (DMSO) to get a concentration of 50 mg/ml. A tablet of erythromycin was dissolved in appropriate volume of water to get 50 mg/ml of stock solution.

Culture media

The media employed for the study was: Nutrient agar.

Test microorganisms

Clinical isolates of *staphylococcus aureus* were obtained from the Department of Pharmaceutical Microbiology, University of Nigeria, Nsukka.

Sterilization of materials

The Petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven (Ov-335, Hareus) at 170 °C for 1 h at each occasion. Solution of the extract and culture media were autoclaved at 121 °C for 15 min.

Preparation of culture media

All culture media were formulated according to manufacturers' specification. Basically for nutrient agar, this involves appropriate weighing of nutrient agar, distributing into bijou bottles (in 20 ml) and then sterilization using autoclave at 121 °C, 151 b/sq. inch for 15 min; then allowed to cool to 45 °C before pouring into the agar plate. The pH of the agar medium was maintained at 7.4.

Maintenance and standardization of test organisms

The organism (*S. aureus*) was maintained by weekly sub culturing on nutrient agar slant. Before each experiment, the organism was activated by successive sub culturing and incubation. Standardization of the test microorganism was according to previously reported method (Chinwuba, 1991; NCCLS, 1990).

Sensitivity of test microorganism

The sensitivity of the test microorganism to the methanol extract and erythromycin was evaluated by determining the minimum inhibitory concentration (MIC) of both using the two fold broth dilution technique previously described (NCCLS, 1990; Esimone, 1999).

Evaluation of combined effects of *E. hirta* methanol extract and erythromycin

Stock solutions of *E. hirta* (50 mg/ml) and erythromycin (50 mg/ml) prepared in double -strength nutrient broth and autoclaved at 121 °C for 15 min were employed. Varying proportions of the extract (Euph) and erythromycin (Eryth) were prepared according to the continuous variation checkerboard method previously described by NCCLS, 1990.

Each proportion of the herbal extract/ erythromycin combination was serially diluted (2 fold), inoculated with 0.1 ml of 10⁶ cfu/ ml culture of test microorganism and then incubated for 24 h at 37 °C. Interaction was assessed algebraically by determining the fractional inhibitory concentration (FIC) indices according to the equations below:

$$FIC_{index} = FIC_{Extract} + FIC_{Erythromycin} \dots \dots \dots (1)$$

$$FIC_{Extract} \text{ (fractional inhibitory concentration of Extract)} \\ = \frac{\text{MIC of Extract in combination with Erythromycin}}{\text{MIC of Extract alone}} \dots \dots \dots (2)$$

$$FIC_{Erythromycin} \text{ (fractional inhibitory concentration of Erythromycin)} \\ = \frac{\text{MIC of Erythromycin in combination with Extract}}{\text{MIC of Erythromycin alone}} \dots \dots \dots (3)$$

RESULTS

Combined drug use is occasionally recommended to prevent resistance emerging during treatment and to achieve higher efficacy in the treatment of infections and diseases. The combination is hoped to achieve a desirable synergistic effect in this study.

Results of the systematic and scientific evaluation of the *in vitro* effects of *E. hirta* leaf extract and erythromycin have been presented in this paper (Table 1). FIC_{index} values < 1 were considered as synergy and the degree of synergy increases as the value tends towards zero. FIC_{index} values of 1 indicate additivity, values greater than 1, but less than 2 represent indifference while values greater than 2 show antagonism (Chinwuba, 1991; Esimone et al., 1999). Based on these, synergistic effect was obtained by combination of erythromycin and *E. hirta* against *S. aureus* in the ratios (9:1, 8:2, 7:3, 6:4, 3:7, 2:8, 1:9) while others (5:5, 4:6,) showed indifference.

DISCUSSION

The results reveal that *E. hirta* extract has promising antibacterial effects. A plausible mechanism of action could

Table 1. Combined activity of *E. hirta* leaf extract and erythromycin against *S. aureus*.

Ratio of drug combination Eryth : Euph	MIC Eryth (mg/ml)	MIC Euph (mg/ml)	FIC Eryth	FIC Euph	FIC Index	Inference
10 : 0	0.005	-	-	-	-	-
9 : 1	0.0045	1.25	0.9	0.05	0.95	Syn
8 : 2	0.004	2.5	0.8	0.1	0.9	Syn
7 : 3	0.0035	3.75	0.7	0.15	0.85	Syn
6 : 4	0.003	5.0	0.6	0.2	0.8	Syn
5 : 5	0.005	12.5	1.0	0.5	1.5	IND
4 : 6	0.004	15.0	0.8	0.6	1.4	IND
3 : 7	0.0015	8.75	0.3	0.35	0.65	Syn
2 : 8	0.001	10.0	0.2	0.4	0.6	Syn
1 : 9	0.005	11.25	0.1	0.45	0.55	Syn
0 : 10	-	25	-	-	-	-

MIC = Minimum inhibitory concentration; Eryth = erythromycin; Euph = *E. hirta*; FIC = fractional inhibitory concentration; Syn = synergism; IND = indifference.

could be suggested that the *E. hirta* leaf extract potentiated the activity of erythromycin, giving rise to synergism.

The results of these *in vitro* tests indicate that the combination of *E. hirta* leaf extract and erythromycin at a given ratio has a possible clinical significance in the treatment of bacterial infection caused by *S. aureus*. Unguided and indiscriminate combination may result to an effect or outcome which has no clinical significance.

Moreover, this herbal extract is widely available, cheap and quite safe. It also has mild side effects of nausea and vomiting.

In conclusion, it may be stated there is a favorable interaction between *E. hirta* leaf extract and erythromycin against *S. aureus* in some given combination ratios.

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REFERENCES

- Anozie VC (1991). God's healing power in Plants. Clin. Pharm. Herb Medium. 7: 23-25.
 Chambers HF (2006). General principles of antimicrobial therapy. In Goodman and Gilman's pharmacological Basis of Therapeutics, Bruton LL (e+d), 11th Ed., McGraw Hill: USA. 1102 – 1104

- Chinwuba GN, Chiori Go, Ghobashy AA, OkorE VC (1991). Determination of Synergy of antibiotic combination by overlay inoculum susceptibility Research. 41:14 –150
 Eliopoulos GM, Eliopoulos CT (1988). Antibiotic combinations: should they be tested? Clin. Microbiol. Rev. 1: 139–156.
 Esimone CO, Adikwu MU, Uzuegbu DB, udeogaranya PO (1999). The effect of ethylenediaminetetraacetic acid on the antimicrobial properties of Benzoic acid and cetrime J. pharm. Res. Dev. 4(1): 1-8.
 Esimone CO, Iroha IR, CO Ude IG, Adikwu MU (2006). *In vitro* interaction of ampicillin with ciprofloxacin or spiramycin as determined by the Decimal assay for additivity technique. Niger. J. Health Biomed. Sci. 5(1): 12–16.
 Goodman and Gilman's (2001). The Pharmacological basis of therapeutics. Tenth Edition Library of Congress Cataloging-in-Publication Limited. pp. 1250-1251.
 Ibezim EC, Esimone CO, Okorie O, Obodo CE, Nnamani PO, Brown SA, Onyishi IV (2006). A study of the *in vitro* interaction of cotrimoxazole and ampicillin using the checkerboard method. Afr. J. Biotechnol. 5(13): 1284–1288.
 Igoli JO, Ogaji TA, Tor-Anyiin, Igoli NP (2005). Traditional medicine practice amongst the Igede People of Nigeria. Part 11, Afr J Tradit. Complement Altern. Med. 2(2): 134-152.
 National Committee for Clinical Laboratory standards. Performance standards For Antimicrobial Disc susceptibility test 4th ed., approved Document M2 – A4 (NCCLS) villanova p. 1990.
 Sofowora EA (1982). Medicinal Plants and Traditional Medicine in Africa, John Wiley and Sons, Chichester. p. 198.
 Zinner SN, Klastersky J, Gaya BC, Riff JC (1981). *In vivo* and *in vitro* studies of three antibiotic combinations against gram negative bacteria and *Staphylococcus aureus*. Antimicrob. Agents chemother. 20: 463–469.