



Original Research Article

Enhancement of Therapeutic Success Through Herbal-Herbal Combination

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ABSTRACT

Keywords

Herbal-
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*Kigelia
africana*,
*Dialium
guineense*
and
*Euphorbia
kamerunica*

An important step in curtailing the development and spread of resistant strains of infectious agents is the use of herbal-herbal combinations. The present study evaluated the effect of combination of three African herbs on some clinical isolates. The herbs (*Kigelia africana*, *Dialium guineense* and *Euphorbia kamerunica*) were combined in equal volumes and concentrations to achieve a final concentration of 100mg/ml and tested against clinical isolates using the agar diffusion method. The combination of *D. guineense* and *E. kamerunica* against *Candida albicans* gave a greater zone of inhibition (30.0mm) than either of the plants. Synergistic relationship was equally noticed when all the three extracts were combined against *E coli* ATCC 25922 (18mm), also when *D. guineense* and *K. africana* were combined against *Pseudomonas aeruginosa*^a (18.0mm) and *Salmonella typhi* (18mm). The above findings show that a synergistic relationship exist between some of the plants extracts against some bacteria isolates and this justifies the use of combined herbs by the herbal practitioners. However, the assessment of the toxicity level of the combined herbs needs to be done in all cases of herbal combination.

Introduction

Continuous resistance of pathogens to antibiotics remains a major health problem among the nations of the world (Zhang *et al.*, 2006). Organisms like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis* and *Salmonella enterica* serovar Typhi have been reported to show increased resistance to antimicrobial agents. This and the appearance of new infectious agents have thus resulted in increased morbidity and mortality among the general populace (Karlowsky *et al.*,

2003; Sjolund-Karisson *et al.*, 2011). There is an urgent need then to research into novel antimicrobial agents that will stem the tide of continuous spread of resistant strains of pathogens with the attendant consequences. Many natural products have been investigated individually for their antimicrobial activities (Olajubu *et al.*, 2012; Deji-Agboola and Olajubu, 2010; Gibbons,2004). Different regions and cultures either use herbal products as single herb, combination of herbs or combination of herb(s) and drug(s) (Che *et al.*, 2013).

Herbal preparation can either be taken raw or processed. The use of alcohol to extract the active properties of the herb leads to the production of tinctures. The essences of the herb can be leached out with water producing aqueous extracts. Herbs can be formulated into capsules or tablets containing powdered form of the raw herb. Teas and Lozenges could be produced from raw herbs. Cowan (1999) also reported that herbs can be formulated into ointments, salves and rubs which are applied topically. Many combined orthodox medicine with herbs for treatment of various ailments, even though this might be dangerous. In a study conducted among hypertensive patients in LUTH, it was found that 85% of respondents used different herbs alongside the medicines given at the clinic (Amira and Okubadejo, 2007). When herbs are used in combination, the often intended or expected outcome is interaction that can result in additional therapeutic benefit. However, due to the presence of multiple components in the herbal products, the effects arising from herb-herb or herb-drug interactions are often unpredictable and complicated (Che *et al.*, 2013). Various reactions have been reported by the use of herb-drug combinations (Colalto, 2010; Fasinu *et al.*, 2012; Herman and Richter, 2012) just as herb-herb combination has been used and documented as a desirable therapeutic approach in many parts of the world especially in China.

The general believe that, there are many causes of a disease and an attempt to treat them all has given rise to combination of herbs. It is believed that each active component of a plant will be strengthened by the presence of another plant that has such active ingredient (synergism) or can aid its effectiveness in the body. Some plants serve as preservative to others when mixed together. Quite a number of herbs are believed to help the active ingredient in a

recipe get to the desired target i. e. aiding penetration, examples are *Zanthoxylum rubescens* root, *Aframomum melegueta* and *Tetrapluera tetraptera*. Some common ailments treatable with herbal preparations range from hypertension, dysentery, low sperm count, weak erection, coated tongues, pile, infertility, menstrual disorders, rheumatism, fevers of all types, gonorrhoea, chicken pox, small pox, convulsion, eye problems, burns, wounds and ulcers (Odugbemi,2006) Most Chinese medicine prescriptions contain more than one medicinal plant to form a multi-item concoction (O'Brien and Xue, 2003) Based on Chinese herbal medicine theory and practice, multi-item prescriptions are formulated deliberately according to six basic modes of her-herb interactions; namely, reinforcement, potentiation, restrain, detoxification, counteraction and toxicity.

These serve as guiding principles when multi-item concoctions are prescribed in order to enhance the safe and effective use of the herbs (Che *et al.*, 2013). Reports of document studies on herbal-herbal combination for the treatment of infectious agent are scanty. This study therefore aimed at investigating three Nigerian medicinal plants'(*Kigelia africana*, *Dialium guineenses*, *Euphorbia kamerunica*) combination effects against some selected clinical isolates. These plants have been studied individually for their antimicrobial activity (Olajubu *et al.*, 2012)

Materials and Methods

Plants collection and authentication

Euphorbia kamerunica (Pax) stem was obtained in a traditional healer's herbal garden at Ikenne-Remo, while *Dialium guineense* (Willd) stem bark and *Kigelia*

africana (Benth) leaves were donated by an herbal practitioner at Ode-Lemo, all in Ogun State, Nigeria between March and April, 2007. The plants were examined and identified by Prof. Oluwalana S.A of college of Environmental Resource Management, Department of Forestry and Wild life, UNAAB. Voucher specimens of these plants were submitted at Forest Research Institute of Nigeria (FRIN) Ibadan, where Forest Herbarium Ibadan (FHI) numbers were given as 108008,108009 and 108010 for *Euphorbia kamerunica*, *Dialium guineense* and *Kigelia africana* respectively.

Extracts' preparation

The plant extracts were prepared using a modified method of Rojas *et al* (2006). Fifty grams (50g) of each air dried, powdered plants were soaked in 1L of Ethanol (50% v/v) for 72hours. Each mixture was refluxed, agitated at 200rpm for 1hour and filtered using Whatman No 1 filter paper. The filtrates were placed in vacuum oven at 40°C and dried for 1 week to obtain dry extracts. All crude extracts were stored at 4°C until ready for use.

Sources of test organisms used

The organisms used in this study consisted of those isolated from clinical specimens (urine, wound swabs, stool, blood and sputum) sent to the Department of Medical Microbiology, Olabisi Onabanjo University Teaching Hospital, Sagamu and National Institute of Medical Research (NIMR), Lagos for culture and susceptibility tests. The standard organisms used were obtained from NIMR, Yaba, Lagos.

Preparation of bacteria inoculums

A modified method of McFarland (1907) was used. Uniform suspensions of overnight

pure cultures of the test organisms were made in peptone water. The turbidity of the suspensions was then adjusted until they matched 0.5 Mc Farland turbidity standard. This was prepared by adding 0.05ml of BaCl₂ (1% w/v) to 9.95ml of H₂SO₄ (1% v/v). This produced a suspension of approximately 1.5×10^8 cells/ml. The optical densities of the 0.5 McFarland turbidity standard and the organism suspensions were compared using Unico 2100 spectrophotometer at 520nm wavelength and adjustment were made by either further diluting the organism suspension or adding more suspension from the stock.

Agar well diffusion method

The method of Shahidi and Rashidi (2004) was used. Freshly prepared Muller-Hinton (MH) agar plates were surface-dried in an oven at 45°C for 30mins. Heated Blood Agar (HBA) was used for *Streptococcus pneumoniae* culture and Saboraud Dextrose Agar (SDA) for all the fungi isolates. The agar plates were seeded with 2.0mls of the bacteria preparation. Excess of the suspension was drained off on a pad of filter paper. Wells of 6.0mm diameter were bored in the MH and HBA culture media with sterile cork borer. A concentration of 100mg/ml of each extract combination were prepared and 0.2ml of each combination was used to fill each well. The plates were incubated at 37°C for 24hours. The diameter of inhibition zones were then measured with vernier callipers in millimeter (mm). All samples were tested in duplicate and the average is recorded as the mean inhibition zone in millimeter. Inhibition zones of 10mm diameter and above were considered as sensitive. Gentamycin at 1µg/ml and ethanol (50%) served as positive and negative controls respectively.

Antifungal screening of the plant extracts Preparation of yeast inoculum

The procedure used was the same with that adopted for bacteria. However, Saboraud Dextrose Agar was used and the plates were incubated at 28°C for 48h.

Assessment of the effect of combination of plant extracts on microorganisms

Any two of the three crude extracts were combined in equal parts at random and another combination contained all the three extracts in equal parts to achieve a concentration of 100mg/ml. These combinations were used for antimicrobial test using agar diffusion method.

Results and Discussion

The combination of the plant extracts did produce a significant difference from the individual activity of the plants as shown in Table 1 and plates 1-4. Outstandingly, the combination of *D. guineense* and *E. kamerunica* against *Candida albicans* gave a greater zone of inhibition (30.0mm) than either of the plants. Synergistic relationship was equally noticed when all the three extracts were combined against *E coli* ATCC 25922, also when *D. guineense* and *K. africana* were combined against *Pseudomonas aeruginosa*^a (18.0mm) and *Salmonella typhi* (18mm). The combination of *K. africana* and *E. kamerunica* manifested enhanced activity against *Escherichia coli*^a (12.0mm) and *S. aureus* ATCC 25923. (21.0mm) than it was for individual plant extracts.

Herbal preparations are often taken as an individual herb or as complex herbal formulation in which synergism is expected. In this study, enhance activity was noticed in the combination of the three plant extracts against *E. coli* ATCC 25922 and

Streptococcus pneumoniae while combination of *D. guineense* and *K. africana* gave wider zones of inhibition against *S. aureus* and *Shigella flexneri*.

Staphylococcus aureus ATCC 25923 and *Pseudomonas aeruginosa* were better inhibited with the combination of *K. africana* and *E. kamerunica* than the individual plants. *Candida albicans* was more sensitive to the combination of *D. guineense* and *E. kamerunica*. The above findings show that a synergistic relationship exist between some of the plants extracts against some bacteria isolates and this justifies the use of combined herbs by the herbal and orthodox practitioners (Lyon *et al.*, 2001) Combination therapies are currently being employed for the treatment of critical diseases, such as cancer, acquired immunodeficiency syndrome (AIDS), malaria and pulmonary tuberculosis. In order to achieve enhanced therapeutic effects. The modern approach of combination therapy is a renewal of what was advocated in Chinese medicine that started thousands of years ago on the use of herb-herb combination for improvement of therapeutic outcome (Che *et al.*, 2013). Herbal combination is now being advocated as a measure against development of resistant strains of bacteria just as the combined therapy for malaria treatment. This method is now being adopted in the treatment of Osteoarthritis pain (Adams, 2014), irritable bowel syndrome with diarrhea (Ko *et al.*, 2011), hypertrophic scar formation after burn injury (Muangman *et al.*, 2011) and clinically important bacteria (Olajuyigbe and Afolayan). However, there are few cases of additive relationship in which the effect of combination was equal to that of individual plant extract as demonstrated by the combination of *D. guineense* and *K. africana* against *Salmonella typhi*.

Table.1 Combination Effects of the Extracts on Test Micro-organisms

S/No	Organism	Extracts combination/ Inhibition zone (mm)			
		DEK	DE	DK	KE
1	<i>Staphylococcus aureus</i> ^a	14.0	12.0	17.0	11.0
2	<i>Staphylococcus aureus</i> ^b	12.0	10.0	12.0	10.0
3	<i>Staphylococcus aureus</i> ^c	16.0	10.0	17.0	12.0
4	<i>Escherichia coli</i> ^a	10.0	8.0	10.0	12.0
5	<i>Escherichia coli</i> ^b	10.0	10.0	8.0	8.0
6	<i>Proteus mirabilis</i>	10.0	8.0	8.0	0.0
7	<i>Pseudomonas aeruginosa</i> ^a	10.0	12.0	18.0	0.0
8	<i>Pseudomonas aeruginosa</i> ^b	8.0	10.0	10.0	8.0
9	<i>Klebsiella pneumoniae</i> .	8.0	8.0	12.0	8.0
10	<i>Streptococcus pneumoniae</i>	13.0	13.0	8.0	8.0
11	<i>EH E. coli</i>	13.0	8.0	8.0	10.0
12	<i>Salmonella typhi</i>	12.0	15.0	18.0	10.0
13	<i>Shigella flexineri</i>	12.0	10.0	17.0	8.0
14	<i>Bacillus subtilis</i>	13.0	12.0	12.0	10.0
15	<i>Candida albicans</i>	15.0	30.0	10.0	10.0
16	<i>Aspergillus niger</i>	0.0	0.0	0.0	0.0
17	<i>Penicillium notatum</i>	0.0	0.0	0.0	0.0
18	<i>Epidermophyton floccosum</i>	0.0	0.0	0.0	0.0
19	<i>Staph aureus ATCC 25923</i>	17.0	13.0	16.0	21.0
20	<i>E. coli ATCC 25922</i>	18.0	12.0	10.0	10.0

Legend: DEK- *D. guineense* + *E. kamerunica* + *K. Africana*; DE – *D. guineense* + *E. kamerunica*
 DK – *D. guineense* + *K. Africana*; KE – *K. africana* + *E. kamerunica*

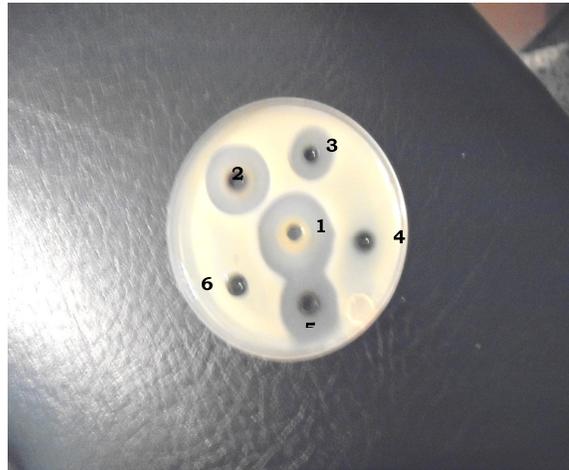
Plate.1 *S. aureus*^a Susceptibility Pattern with Combined Plant Extracts.



Legend:

1. DK combination – 17.0mm
2. Control drug (Gentamycin at 10µg/ml) – 23.0mm
3. Control solvent (ethanol) – 0.0mm
4. DE combination – 12.0mm
5. DK combination – 17.0mm

Plate.2 *S. aureus* ATCC 25923 susceptibility pattern with combined plant extracts.



- Legend:**
1. KE combination – 21.0mm ;
 2. DEK combination – 17.0mm
 3. DE combination – 13.0mm
 4. (Ethanol) control solvent – 0.0mm
 5. DK combination – 16.0mm
 6. Control solvent (ethanol) repeat – 0.0mm

Plate.3 Multi-drug resistant *Pseudomonas aeruginosa* showed susceptibility to combined extracts



- Legend:**
1. DK combination – 18.0mm
 2. *D. guineense* alone – 14.0mm
 3. Control solvent (ethanol) – 0.0mm
 4. KE combination – 0.0mm
 5. DE combination – 12.0mm
 6. Control solvent (repeat) – 0.0mm

In these cases, the use of single herb is advocated while the other could be preserved for future use or combined with other herbs where synergism will be the outcome. The use of single herb here will equally save cost and further preserve our wild life. Antagonistic relationship was noticed in combination of the three plant extracts against *Bacillus subtilis* and *Proteus mirabilis* hence, use of single plant extract will be most preferred in such cases. Further studies are on-going to check for the toxicity of these herbs in their combined states.

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