Combinations of *Alchornea cordifolia*, *Cassytha filiformis* and *Pterocarpus santalinoides* in diarrhoeagenic bacterial infections

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**Abstract**

**Objectives:** This study examines the rationale, if any, behind combining the extracts from the fruits of *Alchornea cordifolia* and *Pterocarpus santalinoides* and aerial parts of *Cassytha filiformis* in the traditional treatment of diarrhoeagenic bacterial infections.

**Results:** Four diarrhoeagenic bacterial isolates: *Salmonella typhi*, *Shigellae dysenteriae*, *Escherichia coli* and *Staphylococcus aureus* were used and their antibiotic susceptibility screening showed that they were multi-antibiotic resistant. The extracts exhibited activity against all the test isolates with minimum inhibitory concentration values ranging from 3.125 to 12.5 mg/mL. From the checkerboard assay, the fractional inhibitory concentration indices showed that *C. filiformis* has antagonistic and indifference activities in combination with either *P. santalinoides* or *A. cordifolia*. This showed that the combination of extracts from the fruits of *A. cordifolia* and *P. santalinoides* and aerial parts of *C. filiformis* is counterproductive and invalidates any claim for positive results in the management of diarrhoeagenic bacterial infections.

**Keywords:** *Cassytha filiformis*, *Pterocarpus santalinoides*, *Alchornea cordifolia*, *Escherichia coli*, *Shigellae dysenteriae*, *Salmonella typhi*, *Staphylococcus aureus*

**Introduction**

Plants are age-long sources of medicine to mankind especially in developing world. Besides serving as source of food, plants have been a potent weapon for combating diseases. Both traditional healers and pharmaceutical researchers have continuously looked up to plants to provide cure to both infectious and non-infectious diseases. For instance, the root, stem, leaves and fruits of *Cassytha filiformis* are widely used for the treatment of ulcer, cancer, conjunctivitis, diarrhoea, erectile dysfunction, post-partum haemorrhage, GIT infection and urinary tract infections [1–3]. Plants have been particularly helpful in the fight against infectious diseases because of the evolution of microorganisms with extraordinary ability to render conventional antibiotics relatively ineffective.

The leaves of *Pterocarpus santalinoides* are used for treating diarrhoea of bacterial and non-bacterial origin [4], have hypolipidemic effect [5] and are rich in phytochemical compounds such as flavonoid and alkaloid as well as being very good sources of vitamins [6]. Eze et al. [7] have also reported that various parts of *Pteropus santalinoides* are utilised in folk medicine for the treatment of malaria.

Decoctions of *Alchornea cordifolia* fruit, leaf or stem bark are utilised for the treatment of venereal diseases, cough, and impotency [8]. They are also utilised by traditional medicine practitioners for the treatment of diarrhoea [8, 9].

Diarrhoea is one of the leading causes of death among children below 5 years [10, 11] and diarrhoeal diseases are especially dangerous among the immune-incompetent populations [12, 13]. It was estimated that, globally, over 500,000 children below 5 years died of acute diarrhoea in 2015 only [11, 14].
All over the developing world, especially in rural communities, diarrhoea accounts for most incidences of child morbidity and mortality [8]. Diarrhoea may be caused by both infectious microorganism and non-pathogenic agents. Enterotoxigenic *E. coli* and *Shigellae* spp. are amongst the leading causes of diarrhoea globally [15, 16]. Other bacteria implicated in diarrhoea condition are *Staphylococcus aureus* and *Salmonella typhi*. *S. aureus* produces enterotoxin, which causes inflammation of the ileum and diarrhoea [17, 18].

Since the discovery of antibiotics, they have been widely utilised for the treatment of infectious diseases because they have proven to be efficacious against dangerous pathogens. However, with the increasing incidences of antibiotic resistance amongst clinically important pathogens, clinicians may no longer completely depend on antibiotics for the treatment of all infectious diseases. Several studies have presented plants as alternative to conventional drugs for the treatment of infectious diseases [19–21]. Beside their efficacy against resistant strains, plants are acclaimed to have phyto compounds that have very little or no adverse effects compared to conventional drugs [22, 23]. Traditional healers have always combined several herbs in a bid to harness the therapeutic effects of more than one plant to cure diseases. Also, drug combinations have been particularly helpful in the fight against infectious diseases because of the evolution of pathogens with extraordinary abilities to render conventional antibiotics relatively ineffective. This study seeks to evaluate the in vitro interactions of the methanolic herbal extracts from the fruits of *A. cordifolia*, *P. santalinoides* and aerial parts of *C. filiformis*. The result is expected to either justify, or otherwise invalidate, the local use of the concoctions containing these plants’ parts in the management of childhood diarrhoea associated with the tested bacteria.

**Main text**

**Test isolates**

All the isolates used for this study were obtained from previous stocks deposited in the laboratory of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. Isolates were originally from diarrhoeic stool of children below 5 years of age. Isolates were subcultured on Mannitol salt agar, Salmonella- Shigellae agar and MacConkey agar respectively and appropriate biochemical tests undertaken as described by Salami and Georgia [24].

Plant samples collection, preparation and extraction: the fruits of *A. cordifolia*, *P. santalinoides* and aerial parts of *C. filiformis* were harvested from a farmland at Nnewi, Nigeria. They were authenticated by a taxonomist: Okeke Philomena N of the Department of Botany, Nnamdi Azikiwe University, Awka and deposited in the departmental herbarium with the voucher codes: *A. cordifolia*—78D; *P. santalinoides*—52A; *C. filiformis*—124A. The samples were washed with clean water, air-dried under shade and pulverized. The methanolic extraction of the pulverized samples was carried out using a method described by Basri and Fan [25] with little modification. A 500 g of the pulverized samples were immersed in 2.5 L of methanol for 24 h with shaking. The resultant mixtures were filtered using muslin cloth, then with Whatman no. 1 filter. The filtrates were concentrated using rotary evaporator and further concentrated by heating in water bath. The crude extracts were stored in a refrigerator at temperature 4–8 °C until ready for use.

**Antibiotic susceptibility assay**

Test isolates were challenged with several antibiotics using disc diffusion method as described by Cheesbrough [26]. The test inocula were prepared with 24 h broth cultures and then adjusted with physiological saline until they are equivalent to McFarland 0.5 turbidity standard. The standardised inocula were applied on the surface of solidified sterile Mueller–Hinton agar in a Petri dish and allowed to stand. Commercial antibiotic discs were gently placed on the agar using sterilised forceps. The cultures were then incubated at 37 °C for 24 h. The zones of inhibition were measured using transparent plastic rule.

**Determination of minimum inhibitory concentration (MIC):** the MICs of the extracts were determined using agar well diffusion assay as described by Gamba et al. [27]. The extracts were prepared by dissolving 200 mg of the extracts in 2 mL of DMSO to obtain a concentration of 100 mg/mL. Dilutions of 50, 25, 12.5, 6.25 and 3.125 mg/mL were prepared from the 100 mg/mL stock solutions of the six extracts. Sterilised cork borer (6 mm in diameter) was used to make holes on solidified Muller Hinton agar containing the standardised pure culture of the test isolate. Each concentration of the extracts was carefully introduced into the holes and allowed to stand for 1 h before incubating at 37 °C for 24 h. The least concentration (measured in milligram) with zone of inhibition (measured in millimetre) is considered the MIC of the extract.

**Combined antimicrobial activities of the extracts**

(2 × MIC of individual significant activity against test isolates) were prepared according to a continuous variation checkerboard technique using the ratio 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0. The resulting solutions of these combined
extracts were further diluted in 2 fold dilution process to 5—serial dilutions to obtain the final concentrations used for the Checkerboard assay. Sterile molten Mueller–Hinton agar was inoculated with 0.1 mL fresh cultures of test isolates and various concentrations of the combined plant extracts. The procedure was carried out in triplicates. The FIC of all ratios of the combined extracts were determined and the FIC value for each extract was calculated using the formula:

\[
\text{FIC}_A = \frac{\text{Conc of } A \text{ in MIC } A + B}{\text{MIC of } A \text{ alone}}
\]

\[
\text{FIC}_B = \frac{\text{Conc of } B \text{ in MIC } A + B}{\text{MIC of } B \text{ alone}}
\]

FIC index = FIC\(_A + \text{FIC}_B\). The interpretations of FIC index according to Aiyegoro et al. [28] is as follow: FIC index < 1.0 means Synergism, = 1 means additivity, > 1 but less than 2 means indifference while ≥ 2 means antagonism.

**Results**

The antibiotic susceptibility assay confirmed the isolates to show multi-antibiotic resistant character (Additional file 1: Table S1), being resistant to more than two classes of antibiotic.

The extracts at various concentrations exerted antibacterial activity against all the test isolates. The least concentration that inhibited the growth of test isolates within 24 h was considered as the MIC. All the extracts have MIC ranges from 3.125 to 12.5 mg/mL (Table 1).

Tables 2 and 3 showed the fractional inhibitory concentrations of *C. filiformis* in combination with *A. cordifolia* and *P. santalinoides* respectively. The effect of the combinations are generally antagonistic or indifference. *C. filiformis* in combination with *A. cordifolia* against all the test isolates have FIC indices ranging from 1.04 to 2.84 while *C. filiformis* in combination with *P. santalinoides* have FIC indices ranging from 1.00 to 2.72 against all the test isolates.

**Discussion**

Resistance to conventional antibiotics is the biggest hurdle to overcome in the treatment and eradication of infectious diseases. As the resistant strains spread across vulnerable populations (immunocompromised patients and children), finding other antibiotics that are both efficacious and have minimal adverse effects becomes more challenging. The isolates were resistant to more than 2 antibiotic classes, which is in line with the work of Usha et al. [29] that concluded that most bacteria of clinical importance are resistant to one or more antibiotics.

Herbal medicine have been considered as alternative to treating both infectious and non-infectious diseases because of their proven efficacy in traditional medicine practice [30–32]. All the methanolic extracts used for this study showed significant activities against all the test isolates even at low concentrations. However, when two or more of the herbal extracts were combined, the interaction results showed indifference or antagonism. It may be opined from this study that the bioactive principles in one of these extracts either suppresses the effects of the others or produced an outright cancellation of effect at the combination ratios. Similar negative results were also obtained when Adonu et al. [1] evaluated the effects of *C. filiformis* and *Cleistopholis patens* against *Pseudomonas aeruginosa* and *Escherichia coli* [2].

Drugs interactions showing indifferent or autonomy means that the combination is equipotent with the most potent of the drugs used alone while antagonism occurs when one therapeutic agent cancels out or just weakens the effect of another therapeutic agent(s).

The essence of drug combinations is to achieve a higher therapeutic effect than when one drug is used singly. This was not achieved when *C. filiformis* was combined with either *A. cordifolia* or *P. santalinoides*. The usefulness of combined therapeutic agents had been proven in several studies [33, 34]. For instance, the triple antibiotic paste (containing equal amounts of minocycline, ciprofloxacin, and metronidazole) is very effective in eliminating wide range of bacteria causing teeth decay [35]. Combination

| Test isolates                  | Staphylococcus aureus | Salmonella spp. | Shigellae dysenteriae | Escherichia coli |
|-------------------------------|-----------------------|-----------------|-----------------------|------------------|
| Herbal extracts (mg/mL)       |                       |                 |                       |                  |
| *Alchornea cordifolia*        | 12.5                  | 6.25            | 6.25                  | 12.5             |
| *Cassytha filiformis*         | 6.25                  | 3.125           | 6.25                  | 6.25             |
| *Pterocarpus santalinoides*   | 12.5                  | 12.5            | 6.25                  | 12.5             |
| Metronidazole 50 µg           | 5.00                  | 6.00            | 4.00                  | 5.00             |
| Tetracycline 30 µg            | 9.00                  | 9.00            | 9.00                  | 8.00             |
| DMSO (2.5% v/v)               | 0.00                  | 0.00            | 0.00                  | 0.00             |

Metronidazole 50 µg and Tetracycline 30 µg were the positive controls while DMSO (2.5% v/v) was the negative control.
of tetracycline and metronidazole was found to be effective against multi-drug resistant diarrhoegenic bacteria [36].

Polyherbal medication interactions had been reported to cause a wide range of effects ranging from useful to untoward effects [37–41]. Possible reasons for the
harmful effects may include lack of standardization [42], lack of observance of Good Manufacturing Practice and lack of adequate studies to establish efficacy and combinatorial ratios before marketing of the products.

In conclusion, whereas the use of herbal medicine for the treatment of infectious diseases is gaining global acceptance, indiscriminate combination of various plants extracts is strongly discouraged. This study has shown that combining various plants’ extracts do not necessarily yield a higher therapeutic effect. Therefore, there should be scientifically established bases before two or more plant parts are combined for any therapeutic purpose.

**Limitations**
First, our study relied on previous reports, morphological and biochemical characteristics of the isolates for identification and confirmation.

Secondly, the MICs of the extracts were in milligram quantities signifying low potency compared to conventional antibiotics used in orthodox medicine.

Thirdly, because those plants were used by traditional doctors in diarrhoea cases, we did not bother conducting toxicity test to verify their level of safety.

These limitations notwithstanding, this study offers scientific information on why there should be evidence to justify any therapeutic combinations before embarking on it. Even herbs should not be combined arbitrarily.

**Supplementary information**
Supplementary information accompanies this paper at https://doi.org/10.1186/s13104-019-4687-0.

**Additional file 1: Table S1.** The susceptibility pattern of the test isolates to conventional antibiotics. This data suggest the multi-drug resistant nature of the isolates used in the study.

**Abbreviations**
MIC: minimum inhibitory concentration; FIC: fractional inhibitory concentration; CF: methanolic *Cassuya filiformis* extract; PS: methanolic *Pterocarpus santalinoides* extract; AC: methanolic *Alchornea cordifolia* extract; GIT: gastro-intestinal tract.

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**Authors’ contributions**
ANO and IBE conceptualized and designed; ANO and MO prepared the manuscript. MO did laboratory investigations and data acquisition. All authors read and approved the final manuscript.

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**Availability of data and materials**
All the data needed in this work are provided in the manuscript and as additional file.

**Ethics approval and consent to participate**
Not applicable.

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Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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