

An Evaluation of the antimicrobial potency of *Lasianthera africana* (BEAUV) and *Heinsia crinata* (G. Taylor) on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*

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ABSTRACT

Information on the potency of many African medical plants against microorganisms is scanty, and in the current wave of antimicrobial resistance against chemotherapeutic drugs, there is need to search for plants that could be resistance-free and affordable. The objective of this study was to investigate the antimicrobial effects of the extracts of *Lasianthera africana* (E1) and *Heinsia crinata* (E2) in combination with chloramphenicol, on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*. For this purpose, the dilution sensitivity and disc diffusion techniques were respectively applied in determining minimum inhibitory concentration (MIC) of the plant extracts, and the sensitivities of the organisms to the plant extracts and their combinations with chloramphenicol. *L. africana* and *H. crinata* showed very high antimicrobial activity against all the test organisms. In combination, the effect of E1 on *E. coli* and *S. typhi* was completely antagonized by that of E2, whereas additive effect on *S. aureus* and *C. albicans* was observed, indicating that the combination of E1 and E2 might be effective against gram positive pathogenic organisms. The combination of either plant extract with chloramphenicol produced synergistic effect on only *C. albicans*. The smaller MIC of E2 indicated greater effectivity than E1. It is concluded that the additive effect produced by the combination of the two plant extracts, and the synergic effect from the combination of any of the extracts with chloramphenicol, offer alternative therapy to gram positive bacterial infections and candidiasis respectively.

Keywords: Antimicrobial potency, *Lasianthera africana*, *Heinsia crinata*, chloramphenicol, microorganisms

Introduction

Traditionally, the people of south eastern Nigeria and other West African countries utilize the plants, *Lasianthera africana* (BEAUV) and *Heinsia crinata* (G.Taylor) for both food and therapeutic purposes. The two plants respectively called Editan and Atama in Efik local dialect of Nigeria, have for several hundred years been exploited by traditional herbalists for the treatment of various ailments, including typhoid fever, diarrhoea, candidiasis, etc. These plants can be used in various forms to treat some diseases, and this qualifies them to be called medicinal plants (Lewis and Elvin-Lewis, 1977). In the recent past, the medicinal status of *L. africana* has been established (Ebana *et al.*, 1995). Several other plants have also been used traditionally in the same manner. Since the end of World War 2 in 1945, orthodox medicine has overshadowed African traditional medicine, hence the over dependence on the industrialized nations for treatment and control of diseases in Africa. This is why scientific development of traditional medicine has eluded Africa. The traditional herbalists only use the plant without knowing the scientific basis for their activities. Also, there are few studies on African traditional plants including their phytochemical properties and safe doses of the plants in use (Gundiza, 1985; Ebana *et al.*, 1991; Kola *et al.*, 2002).

Only recently that there is an increasing concern and the need to source for locally available drugs because of un-affordability of conventional chemotherapeutic agents and clinical cost due to increased poverty in Africa. Apparently this situation has generated a few studies on the phytochemistry and the medicinal potency of some of the medicinal plants known to Africans. Besides, the current wave of antimicrobial resistance to chemotherapeutic drugs is of global concern (Levy, 1998). There is need, therefore, to search for such plants that could be resistance-free. It is equally for this reason that a lot of studies has been carried out on garlic (Jonkers *et al.*, 1999; Ross *et al.*, 2001; Eja *et al.*, 2007). Some plants, e.g., *Ocinum gratissimum* and *Eugenia uniflora* (LINN) have been reported to be rich in volatile oils which contain up to 75% thymol that has antimicrobial effect and has been popularly used in the treatment of diarrhoea and even ear infection, besides having antimicrobial properties against *S. aureus*, *Bacillus subtilis*, *E. coli* and *Shigella dysenteriae* (Fadeyi and Akpan, 1989; Etok and Ebana., 1996; Otung, 1998). Some studies on the phytochemical screening of medicinal plants identify alkaloids as one of the most essential active ingredients of some West African plants which have antimicrobial property (Fadeyi and Akpan, 1990; Hussain and Deeric, 1991). *L. africana* and *H. crinata* have high levels of alkaloids and cardiac glycosides which are mainly associated with antimicrobial activity among the phytochemical components of the

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medicinal plants (Hussain and Deeric, 1991; Otung, 1998). The chromatographic flow rates (RF) values of alkaloids and cardiac glycosides of *L. africana* and *H. crinata* have been reported to be respectively 0.679(E1) and 0.586 (E2) alkaloids compared to Digoxin (standard for alkaloids), besides 0.586 (E1) and 0.759 (E2) cardiac glycosides (Otung, 1998), where E1 and E2 represent *L. africana* and *H. crinata* respectively. Other phytochemical components present in *L. Africana* and *H. crinata* are anthranoids, anthraquinones, saponins and tannins, among others in trace concentrations (Otung, 1998).

In carrying out this study, the aim was to evaluate in vitro the antimicrobial potency of *L. africana* and *H. crinata* by investigating the sensitivities of known pathogenic bacteria, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*, to the plant leaf extracts, individually and in combination with themselves and with a known antibiotic.

Materials and methods

Sources of test organisms and plants

Known cultures of *E. coli* (OD15), *S. typhi* (WD11), *S. aureus* (WD20) and *C. albicans* (OD2) were obtained from the Department of Medical Microbiology and Parasitology Laboratory of the University of Calabar Teaching Hospital (UCTH), Calabar. The cultures were preserved in agar slants until they were used. The test plants were collected from homes in Calabar city since they are edible and domesticated. They were carried to the herbarium in the Botany Department of the University of Calabar, for identification as *L. africana* (BEAUV) and *H. crinata* (G. Taylor).

Preparation of the plants extracts

After the leaves of the plants were air-dried and grounded in a mortar (Mukhtar and Tukur, 2000), the crude extracts of the leaves were prepared using standard procedures (Fatope *et al.*, 1999; Mukhtar and Huda, 2005). This involved soaking 50 g of the powdered extract in 95% ethanol for 48 h at room temperature to allow for maximum extraction of the components. This was followed by evaporation of the filtrate using a rotary evaporator (STUARC SCIENTIFIC, ENGLAND). The residue was retained as the crude extract for each of the test plants and stored in reagent bottles and maintained in the freezer until it was used.

Preparation of extract concentrations for the determination of zones of inhibition

The crude extract (10 mg) was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to obtain a concentration of 10 mg/mL. When 0.1 mL of this solution was dissolved in 9.9 mL DMSO, a solution of concentration 1 mg/mL was obtained. By incorporating 1mL of this final solution into 9 mL of DMSO, a final concentration of 100 µg/mL was obtained.

To test the combined extracts, equal volumes (0.1: 0.1) were mixed and the mixture was tested along with the individual extracts separately.

Preparation of extract concentrations for minimum inhibitory concentration (MIC) test

The crude extract (100 mg) was dissolved in one mL of DMSO to make a concentration of 100 mg/mL and labeled solution 1 (Adoum *et al.*, 1997). When solution 1 was dissolved in 0.5 mL of DMSO, a concentration of 50 mg/mL was obtained, and this was labeled solution 2. Further, solution 2 was dissolved in 0.5 mL of DMSO to obtain a concentration of 25 mg/mL referred to as solution 3. Solution 4 was obtained by dissolving solution 3 in 0.5 mL of DMSO to give a concentration of 12.5 mg/mL. This process was continued to obtain further concentrations 6.25, 3.12, 1.56, 0.78, 0.39 and 0 mg/mL corresponding to solutions 4 to 9 respectively.

By incorporating 1.0 mL of each of solutions 1 to 9 into 9mL Mueller-Hinton broth, final concentrations of 5000, 2500, 1250, 625, 312,156, 78, 39 and 0 mg/mL were obtained for minimum inhibitory concentration (MIC) test.

Preparation of the concentration of a broad-spectrum antibiotic, chloramphenicol, used for test in combination with test plants

The drug used was chloramphenicol 250 mg (Clarion medicals Ltd., Lagos). Chloramphenicol was selected because it is a drug of choice against *S. typhi* and other gram negative enteric bacterial pathogens, and *S. aureus*, a gram positive bacterium (Prescott *et al.*, 2005). Since *C. albicans* also forms part of the microbial flora of the gastrointestinal tract, it was desirable to test the effect of the combination of chloramphenicol with the local herbs on the bacterial pathogens alongside *C. albicans*. 250 mg of the powdered chloramphenicol was dissolved in deionized water and DMSO as solubilizing agent and made up to a volume of 25.0 mL at room temperature (Mukhtar and Huda, 2005). This gave a concentration of 10 mg/mL. Further dilutions as with the extracts were made to obtain a solution with a concentration of 1 g/mL. By mixing 1.0 mL of the solution with 9.0 mL of DMSO, a final concentration of 100 µg/mL was obtained. To test the extracts combined with chloramphenicol, equal volumes of extracts and chloramphenicol (0.1: 0.1) were mixed and the mixture was tested along with the individual extracts and chloramphenicol separately.

Sensitivity test

To determine the effect of the extracts individually, combined with themselves and with chloramphenicol on the test organisms, a disc diffusion technique using the Kirby- Bauer method was applied in testing pure cultures of the test organisms for their antimicrobial sensitivities based on zones of inhibition on agar plates

(Cheesbrough *et al.*, 1999; Prescott *et al.*, 2005). In this method, punched circular discs from filter paper (Whatman No.1) that were sterilized in a hot air oven for 1 h were impregnated with 0.1 mL of each of the plant extracts. They were air-dried for a few minutes, and transferred aseptically onto the surface of previously prepared Mueller-Hinton agar plates. This followed incubation at 37 °C for 24 h, following which the plates were observed for zones of inhibition.

To test the combined extracts, or extracts combined with chloramphenicol, individual concentrations of the extracts or chloramphenicol were both mixed in equal volumes as earlier described, before impregnating the discs with the combinations. The combinations were tested along with the individual extracts or chloramphenicol separately.

To determine the minimum inhibitory concentration (MIC), a standard inoculum was first prepared by transferring a portion of the pure culture of each isolate into tryptone soya broth (Oxoid CM129) that was incubated at room temperature overnight. The overnight broth culture (0.1 mL) was diluted with 1 mL of distilled water in the ratio of 1:100 to form the standard inoculum (Adoum *et al.*, 1997) following which the dilution susceptibility test technique (Prescott *et al.*, 2005) was applied. This involved inoculating the previously prepared Mueller-Hinton broth containing various concentrations of the extracts of the plants with the standard inoculum. This was done for each of the test organisms followed by incubation at 37 °C for 16 to 20 h. At the end of incubation, the presence or absence of growth for each concentration was recorded. The lowest concentration of the extracts resulting in no growth after 16 to 20 h of incubation was taken as the minimum inhibitory concentration (MIC). The same treatment was given to the combination of the extracts of the two plants and those of the individual extracts and chloramphenicol in the appropriate volume ratio.

Statistical analysis

Differences, if any, in the effectivities of the test plants, singly, in combination with each other and with chloramphenicol, were determined using the statistical method, analysis of variance (ANOVA) (Bailey, 1981; Miller and Miller, 1986).

Results

The effect of the extracts of *L. Africana* (E1) and *H. crinata* (E2) on the test organisms are shown in Table 1. Both E1 and E2 showed appreciable zones of inhibition (≥ 15 mm) indicating reasonably good effectivity of each of the plants on test organisms. In the combination of E1 and E2, the effect of E1 on *E.coli* and *S. typhi* was completely antagonized or masked by that of E2 which was not disturbed and remained the same as it was when uncombined. However, the combination of E1 and E2 produced an enhanced or additive effect on *S. aureus* and *C. albicans*.

Table 1: Effect of ethanolic extracts of *L. africana* (E1) and *H. crinata* (E2) on the test organisms

Test organisms	Zone size (mm)		
	E1	E2	E1+E2
<i>Escherichia coli</i>	15.5±0.42 ⁺	19.8±0.73	19.8±0.73
<i>Salmonella typhi</i>	19.2±0.39	24.3±0.39	24.3±0.39
<i>Staphylococcus aureus</i>	14.9±0.08	19.3±0.08	29.6±0.40
<i>Candida albicans</i>	17.1±0.09	20.3±0.25	25.7±0.06

The figures represent means and standard deviations of triplicate zones of inhibition obtained from 100µg/mL. + =Zones of inhibition ≥ 15 mm that indicating very high sensitivity of test organisms.

The combination of E1 and CAF or E2 and CAF appeared to produce little additive or enhanced effect on all organisms except *C. albicans* for which there was synergistic effect on the organisms as shown in Table 2. However, there was significant difference ($p < 0.05$) between the plants extracts and their combinations with chloramphenicol, and between the test organisms with respect to their sensitivities to the extracts singly and in combination.

Table 3 shows the minimum inhibitory concentrations (MIC) of *L. africana* (E1) and *H. crinata* (E2), singly and in combination on the test organisms. There was significant difference ($p < 0.05$) between the plants extracts (E1, E2 and E1+E2) with respect to minimum inhibitory concentrations (MIC). E2 showed advantage over E1 (which had higher MIC) whereas there was no significant difference ($p > 0.05$) between E2 and E1+E2. There was significant difference ($p < 0.05$) between the test organisms. Both *E. coli* and *S. typhi* were more susceptible to E1 and E2 than *S. aureus* and *C. albicans*.

Discussion

Results obtained in this study indicate that *L. africana* and *H. crinata* have very high antimicrobial activity (zone of inhibition ≥ 15 mm) against the test organisms, *E. coli*, *S. typhi*, *S. aureus* and *Candida albicans*. Of course, it has been reported that *L. africana* and *H. crinata* have high levels of alkaloids and cardiac glycosides besides anthranoids, anthraquinones, saponins and tannins which are mainly associated with antimicrobial activity (Fadeyi and Akpan, 1990; Hussain and Deeric, 1991; Otung, 1998). The combination of the extracts of the two plants revealed that the effect of *L. africana* on *E. coli* and *S. typhi* was completely antagonized (or masked) by that of *H. crinata*, the effect of which remained the same as it was when uncombined. However, the combination produced an enhanced (or additive) effect on *S. aureus* and *C. albicans*. This implies that the combination of the extracts of the two plants may be effective for the treatment of infections from gram positive organisms and

Table 2: Effect of extracts of *L. africana* (E1), *H. crinata* (E2) and chloramphenicol (CAF) on the test organisms

Test organisms	Zone of inhibition (mm)				
	E1	E2	CAF	E1+CAF	E2+CAF
<i>Escherichia coli</i>	15.5±0.42	19.8±0.73	27.4±0.20	30.20±0.17	30.60±0.17
<i>Salmonella typhi</i>	19.2±0.39	24.3±0.39	35.5±0.04	35.50±0.02	38.60±0.08
<i>Staphylococcus aureus</i>	14.9±0.08	19.3±0.08	20.3±0.06	25.50±0.02	25.50±0.32
<i>Candida albicans</i>	17.1±0.09	20.3±0.25	0.00	19.20±0.05	22.40±0.05

The figures represent means and standard deviations of triplicate zones of inhibition obtained from 100µg/mL of extracts and chloramphenicol

Table 3: The minimum inhibitory concentration (MIC) of *L. africana* (E1) and *H. crinata* (E2), singly and in combination on the test organisms

Test organisms	MIC (µg)		
	E1	E2	E1 E2
<i>Escherichia coli</i>	6.25	3.12	3.12
<i>Salmonella typhi</i>	12.50	6.25	6.25
<i>Staphylococcus aureus</i>	50	6.25	3.12
<i>Candida albicans</i>	50	50	25

Figures represent means of two readings obtained from 5000µg/mL.

may not be effective for the treatment of infections from gram negative organisms.

Little additive effects on all the organisms were observed when *L. africana* and chloramphenicol, or *H. crinata* and chloramphenicol were combined. Synergism was observed on *C. albicans*. The factors responsible for the synergistic effect were not known during the study especially as *C. albicans* is a fungus. However, since *C. albicans* is gram positive (Cheesbrough, 1991), the mode of action of the plant extracts might be similar to that of chloramphenicol on gram positive bacteria. Elsewhere, synergistic effect was observed for the combination of garlic and omeprazole against *Helicobacter pylori* and none or even antagonistic effect was observed between garlic and amoxicillin, clarithromycin or metronidazole (Jonkers *et al.*, 1999). This indicates that synergism between a medical plant and a chemotherapeutic agent may be selective. That is, there may be synergism between one plant and a particular chemotherapeutic agent, and may not be so in another combination. There was antagonism observed between the two test plants in their combined effect on *E. coli* and *S. typhi* as exhibited by the MIC of the plant extracts. The combined MIC of the plant extracts showed neither antagonism nor synergism on *S. aureus* and *C. albicans*. Significant differences ($P < 0.05$) in the MIC of the plant extracts (E1, E2 and E1+E2) was observed. Least significant difference (LSD) test showed that there was significant difference ($P < 0.05$, 0.01) between E1 and E2 and between E1 and E1E2, while there was no significant

difference ($p > 0.05$) between E2 and E1+E2. Thus, *H. crinata* (E2) with smaller MIC against the test organisms was more active than *L. africana* (E1).

We conclude that *L. africana* (E1) and *H. crinata* (E2) might individually be very effective against *C. albicans* infections. Chloramphenicol alone has no effect on *C. albicans*, but its combination with either E1 or E2 might produce a synergistic effect on *C. albicans* infections and on gram positive bacterial infections. There is need for further research in this aspect. Antagonism among herbs should be further studied to assist traditional herbalists who always combine them in treatment.

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