Antibacterial and synergistic efficacy of acetone extracts of *Garcinia kola* (Bitter kola) and *Buchholzia coriacea* (Wonderful kola)

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Abstract

This study investigated the antibacterial and synergistic efficacy of acetone extracts of seed of *Buchholzia coriacea* (wonderful kola) and *Garcinia kola* (bitter kola). Extraction was carried out using acetone, and Agar well diffusion sensitivity testing method was adopted. Results showed that the highest zone of inhibition was achieved when both kola was mixed at ratio of 1:1. The zone of inhibition of synergy extract of *Garcinia kola* nut and *Buchholzia coriacea* seed for *E. coli*, *Pseudomonas sp.*, *Staphylococcus aureus* and *Bacillus* sp. were 14.00 mm, 12.33 mm, 13.0 mm and 12.00 mm respectively. Furthermore, individual zones of inhibition for *E. coli*, *Pseudomonas sp.*, *Staphylococcus aureus* and *Bacillus* sp. were 11.33 mm, 9.67 mm, 13.67 mm and 11.00 mm respectively for *Buchholzia coriacea* and 13.00 mm, 11.00 mm, 13.0 mm and 12.67 mm respectively for *Garcinia kola*. Analysis of variance showed that there was no significant difference (*p* > 0.05) among the different isolate for the different extracts except for that of *Buchholzia coriacea*. This study showed that synergystic efficacy of bitter kola and wonderful kola produced superior antimicrobial effects. Again, the positive result for both gram negative bacteria (*E. coli*, *Pseudomonas sp.*) and gram positive bacteria (*Staphylococcus aureus* and *Bacillus* sp.) suggest that they could be used as broad spectrum antibiotics.

Keywords: *Garcinia kola*, *Buchholzia coriacea*, Antimicrobial activity, Bitter Kola, Wonderful kola

1. Introduction

Drug resistance, incidence of emerging and reemerging disease is a major challenge in the field of pharmaceutical microbiology despite its advancements [1-3]. According to Ezeigbo *et al.* [4], Ejikeugwu *et al.* [5], drug resistant or multi drug resistant strain is a major factor leading to search of new antimicrobial agents. Several plants have demonstrated antimicrobial properties [2, 3, 6, 7]. Basically, medicinal plants are plants whose tissues including roots, leaves, bark possess healing properties [2, 3, 6 – 8]. The use of plant for food/spices and herbal medicine against some disease is a long term practice [9]. Hence the field of herbal science is as old as human history.

According to world health organization, over 80% of global population rely on herbs for cure of several diseases especially individuals residing in developing countries [8, 10-14]. Under rural areas, Amole and Ilori [15] attributed the use of herbal remedies to inaccessibility of modern drugs and economic factor. Poverty and educational level are also contributing factor on the use of medicinal plants.

Plants have proven to be a credible source of new drugs [10, 16]. Globally, plants are used for treatment of specific disease condition [17]. Several plant species are abound in the world. Some medicinal plants are important to a particular locality and not to another. Several cola, sometimes spelled as kola, are also abound in the world.

*Buchholzia coriacea* (Wonderful kola), belonging to the family of Capparidaceae is an evergreen shrub, which is distributed in Cameroon, Central African Republic, Gabon, Congo, Angola, Nigeria, Ghana etc [9, 18, 19]. *Buchholzia coriacea* can grow up to 20 m tall with large, glossy, leathery leaves and conspicuous creamy white flower [20].

*Garcinia kola* often called bitter cola/ kola is another kola family that is highly distributed in West Africa including Sierra Leone and Nigeria [4]. *Garcinia kola* belongs to the Guttiferae family and thrives in forest. The plant can attain up 12 m height and produces fruit mostly between July to October of each year.

*Garcinia kola* have been proven to have several medicinal properties including antioxidant, antimicrobial, anti-inflammatory, hypocholesterolemic, antiviral, anti-diarrhoal, antiinflammatory, and antiendrogenic and anticoronary disease activities [10, 21]. On the specific parts, the seed can be used as a stimulant and for many other illnesses such as respiratory tract infection, gastritis, bronchitis, antiseptic, hepatitis and throat problems [4]. Authors have also reported that it has antibacterial potentials [4, 22, 23].

*Buchholzia coriacea* also has antimicrobial, anti-inflammatory, anthelmintic, anti-trypanosomal, hypoglycaemic and analgesic activities [9, 24]. It is also used against headache, sinusitis, bronchitis, ophthalmias, pleurisy, kidney pain and nasal congestion [9]. Specifically the seed is used to make decoctions against hyperthesion, diabetes, cold, cough, rheumatism, headache, catarrh and

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The seed also have antimicrobial properties [19, 20, 25]. In a review study, Erhirhie et al. [26] reported that wonderful kola is a potential therapy for several diseases including diarrhoea, malaria, rheumatism, ulcers, worm infection, asthma and cough, diabetes, hypertension, psychiatric disorders and impotence among others.

Most of the study on the antimicrobial activities of both kola focused against both gram positive and gram negative bacteria. But information on the synergistic efficacy of both plant is deficient in literature. Hence, this study aimed at assessing the antibacterial and synergistic efficacy of acetone extract of wonderful kola and bitter kola.

2. Materials and methods

2.1. Sample procurement

Triplicate samples of the Wonder kola and Bitter kola were purchased from Swali market in Yenagoa, Bayelsa state, Nigeria and were transported to the laboratory of the Department of Biological Sciences, Faculty of Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State where the experimental set up was conducted.

2.2. Preparation of plant extracts

Both kola were first grounded using mortar and pestle. The samples were sun dried and blended to powder. About 40 g of dried kola were weighed and soaked in 100 mL of acetone. After 72 hr of soaking, it was filtered using muslin cloth and re-filtered using Whatman No.1 filter paper.

2.3. Source of the microorganisms for the experiment

The microorganisms used in this study were obtained from the stock culture in the Medical Microbiology unit, Federal Medical Centre, Yenagoa, Bayelsa state. The purity of the bacteria was checked using the scheme of Benson [27] and Cheesbrough [28]. Other confirmatory test for special microbes such as Staphylococcus aureus using Mannitol salt Agar; E. coli using Levine’s eosin Methylene Blue (EMB) Agar were carried out.

2.4. Experimental design

The Completely Randomized Design (CRD) experimental method was adopted for this study. In the sensitivity studies, each isolate was analysed in triplicate for the individual extracts. Furthermore, synergy of both type of kola was carried out at a ratio of 1:1.

2.5. Antibacterial susceptibility test

Agar well diffusion method previously described by Opoku and Akoto [29] with slight modification using the guide of Kigigha et al. [6, 7], Epidi et al. [2, 3] were adopted in this study. The test organisms were inoculated into prepared peptone water and incubated. After 24 hr of incubation, 0.2 mL of bacteria was aseptically inoculated into gelled sterile nutrient agar and then swabbed uniformly on the entire surface. Three wells of 6.0 mm diameter were made with sterile cock borer. Thereafter 0.3 mL of the concentrated extracted was dispensed into the wells. The plates were masked with tape to avoid shifting and contamination. All the plates were incubated at 37 °C for 24 hr and then, the resultant zone of inhibition was measured.

2.6. Statistical analysis

SPSS software version 20 was used to carry out the statistical analysis. The data were expressed as mean ± standard error. A one-way analysis of variance was used to show significant variation at $p = 0.05$. Multiple comparisons were carried out using Tukey Honestly Significant Difference to compare the means.
3. Results and Discussion

The zone of inhibition at varying concentrations for the acetone extracts of *Buchholzia coriacea* nut is presented in Table 1. The zone of inhibition for *E. coli*, *Pseudomonas sp*, concentration. In 95% concentration, *Pseudomonas* species were the source of the observed difference. Furthermore, there was no significant variation between the means of *Pseudomonas* and *Bacillus* sp (not effective at 90% of the extract), and between *Staphylococcus aureus* and *E. coli*.

### Table 1. Zones of Inhibition (mm) of acetone extracts of *Buchholzia coriacea* nut

<table>
<thead>
<tr>
<th>Isolates</th>
<th>100%</th>
<th>95%</th>
<th>90%</th>
<th>85%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>11.33±0.33ab</td>
<td>8.00±0.58b</td>
<td>7.33±0.33b</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>Pseudomonas sp</em></td>
<td>9.67±0.33a</td>
<td>7.67±0.67a</td>
<td>0.00±0.00a</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13.67±0.88b</td>
<td>10.33±0.033b</td>
<td>8.33±0.33b</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>Bacillus sp</em></td>
<td>11.00±0.58a</td>
<td>7.67±0.33a</td>
<td>0.00±0.00a</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SE; a and b represent significant difference (p < 0.05) according to Tukey HSD statistics.

### Table 2. Zones of Inhibition (mm) of acetone extracts of *Garcinia kola* nut

<table>
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<td>0.00±0.00a</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13.00±0.58a</td>
<td>10.00±0.58b</td>
<td>7.33±0.33b</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td><em>Bacillus sp</em></td>
<td>12.67±0.33a</td>
<td>8.67±0.33ab</td>
<td>0.00±0.00a</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SE; a and b represent significantly difference (p < 0.05) according to Tukey HSD statistics.

### Table 3. Zones of Inhibition (mm) of acetone extracts of mixture of *Garcinia kola* nut and *Buchholzia coriacea* nut

<table>
<thead>
<tr>
<th>Isolates</th>
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<th>95%</th>
<th>90%</th>
<th>85%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>14.00±0.58a</td>
<td>11.00±0.58a</td>
<td>8.33±0.33a</td>
<td>0.00±0.00</td>
</tr>
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<td><em>Pseudomonas sp</em></td>
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<td><em>Staphylococcus aureus</em></td>
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<td><em>Bacillus sp</em></td>
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<td>9.00±0.58a</td>
<td>7.33±0.33a</td>
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Data is expressed as mean ± SE; a and b represent significantly difference (p < 0.05) according to Tukey HSD statistics.

*Staphylococcus aureus* and *Bacillus* spp was 11.33 mm, 9.67 mm, 13.67 mm and 11.00 mm respectively (100%), 8.00 mm, 7.67 mm, 10.33 mm and 10.33 mm respectively (95%) and 7.33 mm, 0.00 mm, 8.33 mm and 0.00 mm respectively (90%). At 85%, the extract was not effective against the selected pathogens under study. Basically, there was no significant variation between the means of *Pseudomonas* and *Bacillus* sp, and between *Staphylococcus aureus* and *E. coli*.

Table 2 presents the zone of inhibition at varying concentrations for the acetone extracts of *Garcinia kola* nut. The zone of inhibition for *E. coli*, *Pseudomonas sp*, *Staphylococcus aureus* and *Bacillus sp* was 13.00 mm, 11.00 mm, 13.0 mm and 12.67 mm respectively (100%), 9.76 mm, 7.67 mm, 10.00 mm and 8.67 mm respectively (95%), and 7.67 mm, 0.00 mm, 7.33 mm and 0.00 mm respectively (90%). At 85%, the extract was not effective against the selected pathogens under study. Basically, there was a significant difference (p < 0.05) among the different microbes for each concentration apart from 100%

*Garcinia kola* nut and *Buchholzia coriacea* nut have antimicrobial potentials. This is in consonance with the report of Okigbo and Mmeka [23], Osadebe et al. [30], Ejikengwu et al. [5], Ghamba et al. [22], Ibrahim and Fagbohun [18], Ezekiel and Onyeoziri [19], Nwachukwu et al. [25], Umeokoli et al. [9]. The antimicrobial and other therapeutic potential could be due to the presence of phytochemicals or bioactive constituents [2, 3, 6, 7, 31, 32]. Authors have variously reported that both *Garcinia kola* nut and *Buchholzia coriacea* contain phytochemical
The significant variation ($p < 0.05$) among the different bacteria investigated could be due to differences in the metabolism and physiology of the different organisms under study [2, 3, 7]. The zone of inhibition decreased with the antimicrobial properties of both nuts. For instance, the concentration of tannin could contribute to the metabolism and physiology of the different organisms under investigation could be due to differences in the parts, environmental conditions including habitats, bacteria investigated could be due to differences in their bioactive constituents. For instance, the concentration of tannin could contribute to the antimicrobial properties of both nuts.

The synergy of both nuts had superior efficacy against the microbes under study. These trends have been reported by Epidi [35]. Several solvents have been used to extract plant materials for antimicrobial activities. Some of the common solvents include methanol, ethanol, water, acetone, chloroform. Slight variation exists among the antimicrobial potential of microbes extracted with different solvents [2, 3, 7, 36]. The variation could be due to the polarity of the solvents. Furthermore, Kigigha et al. [7], Okigbo and Mmeka [23] is with the opinion that moisture content, plant parts, environmental conditions including habitats, temperature, pH etc. and the concentration of the plant extracts affects the therapeutic potential.

The zone of inhibition of this study is lower than the values previously reported by Mbata et al. [20], Ezekiel and Onyeoziri [19], Nwachukwu et al. [25], but had some similarity with the study of Ibrahim and Fagboun [18], Kigigha et al. [6, 7], Epidi et al. [2, 3], Epidi [35], Osadebe et al. [30], Ejikeuwu et al. [5], Ezeigbo et al. [4] on some plants. The variation that exists could be due to the choice of microbes, bioactive contents of the plants, concentration used for the sensitivity testing, physical constituents of the plants among others factors.

4. Conclusion

*Garcinia kola* and *Buchholzia coriacea* nuts are widely consumed, and there have been several claims by traditional health practitioners about their medicinal potentials for the cure of different ailments. This study investigated the efficacy of acetone extracts of seeds/nut of *Garcinia kola* and *Buchholzia coriacea* against some common pathogens including *E. coli*, *Pseudomonas* sp, *Bacillus* sp and *Staphylococcus aureus*. The study found that both extracts have antibacterial potential against the tested isolates, but the synergistic effects have superior effects. The zone of inhibition for all the microbial isolates suggests that the extracts can be used as broad spectrum antibiotics i.e. against gram positive and gram negative organisms.

References


