Antibacterial Activities of Extracts of Cinnamomum Cassia and Xylopia aethiopica on Clinical Pathogens.

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Abstract

The study was conducted to investigate the antibacterial activities of the mixture of extracts of Cinnamomum cassia and Xylopia aethiopica against seven clinical bacterial isolates Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae and Pseudomonas aeruginosa using the Agar well diffusion method. In addition, the possible synergistic effects when associated with antibiotics were studied. The preliminary phytochemical screening revealed the presence of cardiac glycoside, flavonoids, phlobatannins, tannins, saponin and steroids in varying concentrations. The combined ethanol extracts of S. cassia and X. aethiopica at all concentrations showed no antibacterial activity against S. aureus, P. aeruginosa, K. pneumoniae, S. faecalis and E. coli. However, at a concentration of 50mg/ml - 150mg/ml the extracts inhibited the growth of S. dysenteriae and B. subtilis. The combined ethanol extracts of S. cassia and water extract of X. aethiopica only had moderate antibacterial activity against S. dysenteriae and B. subtilis. The antibiotics in this study had low to very high inhibitory effects on the bacteria species tested except Ampicillin that had no effect on P. aeruginosa. The combined effect of ethanol extracts of S. cassia and X. aethiopica showed no synergistic effect on all tested organisms; the combined effect of ethanol extract of S. cassia and X. aethiopica also showed no synergistic effect on all tested organisms.

Keywords: Cinnamomum cassia, Xylopia aethiopica, antibiotics, pathogens and Synergism.

1. Introduction

Cinnamon (Cinnamomum cassia) is from the family Lauraceae and is widely grown in tropics. The inner bark is a pungent, sweet, hot herb that stimulates the circulatory system, improves digestion, relieves spasms and vomiting, and controls infections. (Ayfer and Ozlem, 2003). It is also used for colds, influenza, fevers, arthritic and rheumatic complaints. Other medicinal and health benefits of cinnamon include lowering of cholesterol, reduces blood sugar, reduces heart diseases, fighting of cancer among others (Cralg, 1999; Vangalapati et al., 2012). The essential oil and aqueous preparation of Cinnamomum cassia barks have also been reported to possess great antimicrobial properties (Nassia and Perween, 2006).

Xylopia aethiopica (Dunal) belongs to the family Annonaceae, it is a valuable medicinal plant that is widely distributed in the West African. Almost every morphological part of the plant is used in traditional medicine for managing various ailments such as skin infections, candidiasis, dyspepsia, cough and fever (Ghana Herbal Pharmacopoeia, 1992; Mshana et al., 2000). The essential oil as well as the crude extracts (both alcoholic and aqueous) of the plant have been shown to have antimicrobial property against a wide range of Gram positive and Gram negative bacteria, and Candida albicans (Thomas, 1989; Tatsadjieu et al., 2003; Asekun and Adeniyi, 2004; Okigbo et al., 2005).

Various parts of the plant have been traditionally employed in different therapeutic preparations. Sometimes, a combination of X. aethiopica with other plant types or a combination of different parts of X. aethiopica is used to achieve the desired effects (Fall et al., 2003; Ogunkunle and Ladejobi, 2006).

This study was carried out to determine the synergistic antibacterial potencies of ethanol and aqueous extracts of both plants, powdered bark of C. cassia and X. aethiopica., the synergism between the plant extracts and antimicrobial drugs was also investigated.

2. Materials and Methods

2.1 Collection and Identification of Plant Samples

The fruits of X. aethiopica and bark of Cinnamon (Cinnamomum cassia) were collected from Ago-Iwoye and Sagamu (Ogun State), identified and authenticated in the Elikaf herbarium of the Department of Plant Science in Olabisi Onabanjo University, Ago-Iwoye, Ogun State. The fruits of X. aethiopica and bark of Cinnamon (C. cassia) were ground into powdered form and kept in separate airtight containers.

2.2 Extraction method

500g of the powdered bark of cinnamon was macerated in 1litre ethanol; 300g of the powdered fruit of X. aethiopica was also macerated in 1litre of a 99.7% ethanol while 200g of X. aethiopica was macerated in 1litre of sterile water.
2.3 Test Microorganisms
The test organisms used in this study: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Shigella dysenteriae*, and *Streptococcus faecalis* obtained from Nigeria Institute of Medical Research Centre (NIMR), Lagos, Nigeria. The bacterial isolates were first sub cultured on a nutrient agar and incubated at 37°C for 24hrs.

2.4 Preliminary Phytochemical Screening
Phytochemical screening for major constituents was undertaken using qualitative methods in accordance with Trease and Evans (1989) and Sofowora (1993). The plant materials were screened for the presence of flavonoids, phenol, terpenoids, phlobatannins, anthraquinones, cardiac glycoside, tannins, saponin, steroids and alkaloids.

2.5 Combined Effects of the Plant Extracts on the Test Organisms:
Antibacterial effect of the combined plant extracts was determined by the agar well diffusion technique. An overnight bacterial culture was obtained by sub culturing from the steady agar slope and inoculating into 5ml of sterile nutrient broth. The inoculated broth culture was incubated into sterile nutrient agar in petri-dishes using flood method. A sterile cork borer of 6mm diameter was used to bore wells into the seeded solidified agar and 2drops each of 25mg/ml, 50mg/ml, 100mg/ml and 150mg/ml (in which half of both *C. cassia* and *X. aethiopica* extracts were measured to give each concentration) of plants extracts were pipetted into the wells including the controls i.e. 70% of ethanol and water in separate wells. The plates were left in order to allow proper pre-diffusion of the plant extracts and controls into the medium after which the plate were incubated at 37°C for 24hrs. The zones of inhibition were then measured and recorded.

2.6 Effect of Combination of Plant Extracts and Antibiotics on Test Organisms
The cultured bacteria was poured and spread on the nutrient agar using flood method. A sterile cock borer of 6mm diameter was used to bore wells into each media. The mixture of different antibiotic drugs (Erythromycin, Ampicillin, Ciprofloxacin and Gentamicin) at the same concentration each (6.25mg/ml) and plant extracts were introduced into the wells. Plates were incubated at 37°C for 24hrs and the zone of inhibition were measured and recorded.

3.0 Results
The phytochemical constituents of cinnamon bark powder revealed the presence of cardiac glycoside, flavonoids, phenol, phlobatannins, anthraquinones, tannins, saponin, steroids and alkaloids and absence of terpenoids while the fruit of X. showed the presence of cardiac glycoside, flavonoids, phlobatannins, tannins and steroids but absence of phenol, terpenoids, anthraquinones and alkaloids (Table 1).

### Table 1: Phytochemical constituents of cinnamon bark powder and fruits of *X aethiopica*

<table>
<thead>
<tr>
<th>Phytochemical Tests</th>
<th>Cinnamom spices</th>
<th><em>Xylopia aethiopica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac glycoside</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Phenol</td>
<td>++++</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Saponin</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

- = absent, +++ = present, ++++ = strongly present

Table 2 shows the combined effect of extracts of *C. senna* and *X. aethiopica* on tested organisms, the ethanol extract of plants had no inhibitory effect on *P. aeruginosa*, *K. pneumoniae*, *S. faecalis* and *E. coli*. However, at 50mg/ml and above; the growth of *S. dysenteriae* and *B. subtilis* were inhibited. The combined effect of ethanol extracts of cinnamon and aqueous extract of X. showed antibacterial activity at 100mg/ml and 150mg/ml against *B. subtilis* and *E. coli* while no zone of inhibition was recorded for other tested organisms.
Table 2: Combined effect of extracts of *C. cassia* and *X. aethiopica* on tested organisms

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Conc. mg/ml</th>
<th><em>S. aureus</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>K. pneumonia</em></th>
<th><em>B. subtilis</em></th>
<th><em>S. dysenteriae</em></th>
<th><em>S. faecalis</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination of ethanol extract of cinnamon and ethanol extract of <em>X. aethiopica</em></td>
<td>25</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
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<td></td>
<td>50</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>20</td>
<td>17</td>
<td>6.00</td>
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<td></td>
<td>100</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>20</td>
<td>17</td>
<td>6.00</td>
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<td></td>
<td>150</td>
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<td>6.00</td>
<td>20</td>
<td>17</td>
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<td>Ethanol</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
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<td>15</td>
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<td></td>
<td>25</td>
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<tr>
<td></td>
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<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>15</td>
<td>6.00</td>
<td>6.00</td>
<td>10</td>
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<tr>
<td>Water</td>
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<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
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<td>6.00</td>
</tr>
</tbody>
</table>

Size of cork borer: 6.00mm

Table 3 is the combined effect of extracts of Cinnamon, *X. aethiopica* and antibiotics on tested organisms. The antibacterial effect of antibiotics was positive against all test organisms except Ampicillin that had no activity against *P. aeruginosa*. The combined effect of ethanol extracts of *C. senna*, *X. aethiopica* and antibiotics showed greater zone of inhibition against all test organisms except, for Ampicillin that recorded lower zones of inhibition against *B. subtilis* and *S. dysenteriae*. The table also showed the combined effect of ethanol extract of cinnamon, aqueous extract of *X. aethiopica* and antibiotics; Erythromycin and the extracts did not have effect on *S. faecalis*, *B. subtilis*, and *K. pneumoniae*. Ampicillin and the extracts did not have effect on *P. aeruginosa*.

Table 3: Combined effect of extracts of *C. cassia*, *X aethiopica* and antibiotics on tested organisms

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Antibiotics</th>
<th><em>S. aureus</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>K. pneumonia</em></th>
<th><em>B. subtilis</em></th>
<th><em>S. dysenteriae</em></th>
<th><em>S. faecalis</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of <em>C. cassia</em> + Antibiotics</td>
<td>Erythromycin</td>
<td>11</td>
<td>15</td>
<td>6.00</td>
<td>6.00</td>
<td>14</td>
<td>0.00</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>35</td>
<td>35</td>
<td>30</td>
<td>35</td>
<td>12</td>
<td>30</td>
<td>35</td>
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<td></td>
<td>Ampicillin</td>
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<td>6.00</td>
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<td>12</td>
<td>11</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>25</td>
<td>33</td>
<td>20</td>
<td>20</td>
<td>15</td>
<td>25</td>
<td>22</td>
</tr>
</tbody>
</table>

Size of cork borer: 6.00mm

4.0 Discussion

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the
combinations of secondary products present in the plant.

In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids which are capable of producing definite physiological action on body. (Joshi et al., 2009). Plants with anti-bacterial effect are rich in polyphenolic substances such as tannins, alkaloids, steroids and polyphenolic acids. These phenolic substances as well as the alkaloids in plants have been listed as the most important bioactive constituents of natural products (Edeoga et al., 2005) which are useful supplements used for the maintenance of human health (Kumar et al., 2005) and sometimes with remarkable therapeutic potentials.

The presence of these metabolites suggests great potential for C. cassia and X. aethiopica as a source of useful phytomedicine. The presence of Tannins shows it is an astringent, which help in wound healing and anti-parasitic. Alkaloids are known to possess anti-malaria property (Ronan et al., 2009). Plant is a good source of anti-malaria for which it is already traditionally being used for. The presence of Phenols contributes to the development of flavor, taste and palatability, as well as the defense system of plants (Tarnai et al., 1994), including their antimicrobial activity (Ejechi et al., 1998). Literature search reveals that spices are able to prevent or retard the growth of microorganisms (Nychas, 1995). The mechanisms responsible for the antimicrobial activity of phenol include adsorption and disruption of microbial membranes, interaction with enzymes and substrate coupled with metal ion deprivation (Fattouch et al., 2007). The presence of Cardiac glycosides shows their use in the treatment of congestive heart failure and cardiac arrhythmia. The presence of Flavonoid compounds exhibit inhibitory effects against multiple viruses and other microorganisms. The presence of tannins, saponins, anthraquinones, alkaloids and phenols reported by Okogun (1986) implicated these and other chemicals in the curative values of many Nigerian medicinal plants in use. Thus, from the overall results obtained, it is evident that the two plants screened possess anti-microbial agents active against some pathogenic organisms associated with skin infections. They therefore justify their popular use by local herbalist in the treatment of skin diseases.

It has been reported by Ezeifeaka et al., (2004) that the ethanol extract of X aethiopica leaves had activity against S. aureus, E. coli and P. aeruginosa but the aqueous extract of the same had no effect on all the bacteria except S. aureus while in this study the synergistic antibacterial effect of the mixture of ethanol extract of cinnamon and ethanal extract of X. aethiopica also showed activity against S. aureus but had no effect against E. coli and P. aeruginosa but the synergistic antibacterial effect of the mixture of ethanal extract of cinnamon and aqueous extract of Xylopia showed activity against E. coli and had no effect against S. aureus and P. aeruginosa.

This work also agrees with the work of Ezeifeaka et al., (2004) who concluded that the ethanol extracts of the X. aethiopica plant parts showed more inhibitory effects than the aqueous extracts which indicated that the active ingredients of the plants were better extracted with ethanal than water. This observation was also seen in the extracts of C. cassia, in that the synergistic antibacterial effect of the mixture of ethanal extract of cinnamon and ethanal extract of X. aethiopica showed more inhibitory effects than the synergistic antibacterial effect of the mixture of ethanal extract of cinnamon and aqueous extract of X. aethiopica.

The ability of plants extracts of C cassia and X. aethiopica to inhibit microorganisms explains why they are used in folk medicines in Nigeria, Ghana and other tropical countries (G.H.P., 2007).

This study agrees with Nweze and Onyishi (2009) who reported that P. aeruginosa showed resistance to Ampicillin while the other conventional antibiotics Gentamicin and Ciprofloxacan had inhibitory effects on the same organism.

The synergistic antibacterial effect of the mixture of ethanal extract of cinnamon and ethanal extract of X. aethiopica with antibiotics showed more antibacterial activity than the synergistic antibacterial effect of the mixture of ethanal extract of cinnamon and ethanal extract of X. aethiopica without antibiotics. Also the synergistic antibacterial effect of the mixture of the ethanal extract of cinnamon and aqueous extract of X. aethiopica with antibiotics showed more antibacterial activity than the ethanal extract of cinnamon and aqueous extract of X. aethiopica without antibiotics.

5.0 Conclusion
This work has justified the ethnobotanical uses of both plant extracts due to their chemical constituents and also encourages the combination of drugs with the plant extracts since synergism in this study produced higher inhibitory effect on the organisms tested.

REFERENCES
Kumar, R.S., Sivakumara, T, Sunderem, R.S, Gupta, M., Murugesh, K., Rajeshwa, Y.,