THE IN-VITRO EFFECT OF CORCHORUS OLITORIUS (LINN.) ON THE ANTIBACTERIAL ACTIVITIES OF FIVE ANTIBIOTICS.

Ashidi Joseph Senu¹, Efuntoye Moses Olusola², Odunbaku Omobola Afolashade¹ and Biliaminu Sekinat Ajoke¹

¹Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, PMB 2002, Ago-Iwoye.
²Department of Microbiology Olabisi Onabanjo University, PMB 2002, Ago-Iwoye.

Corresponding author’s E-mail: ashidisenu@yahoo.com , +2348056953078

Accepted 28th November 2012

This study evaluated the antimicrobial effect of the ethanol extracts of Corchorus olitorius leaf at 150, 100, 50, 25 mg/mL on eight organisms by Kirby-Bauer disc diffusion method. At 100 mg/mL and below, all the bacteria were resistant to the extract except Shigella dysenteria which was susceptible at the concentration of 100 mg/mL. At the concentration of 150 mg/ml all the bacteria were susceptible except Bacillus subtilis.

It also described the in-vitro interaction of the extract and five antibiotics namely, ciprofloxacin, gentamycin, streptomycin, erythromycin and ampicillin/cloxacilin mixture at concentrations of 150 and 100 mg/mL which were active and sub-active respectively on Methicillin sensitive Staphylococcus aureus and Methicillin resistant Staphylococcus aureus (MRSA). The extract synergized the antibacterial potential of ciprofloxacin and ampicillin/cloxacilin mixture and antagonized gentamycin, streptomycin and erythromycin on S. aureus.

On the other hand, the extract synergized the activities of streptomycin and ciprofloxacin and antagonized the activities of gentamycin, erythromycin and ampicillin/cloxacilin mixture on MRSA.

This suggests that simultaneous administration of antibiotics to patients who eats C. olitorius regularly need be re appraised in view of possible synergism and antagonism.

Keywords: Corchorus olitorus, ethanol extract, antibacterial activity, five antibiotics, synergism.

Introduction

Indigenous vegetables play important roles in human diets. They provide substantial amount of important nutrients for human health because they are particularly important sources of micronutrients and vitamins. Corchorus olitorus is a very popular vegetable especially in South-Western Nigeria with some medicinal properties reported in literature (Pall et al., 2006; Parekch et al., 2006; Zakaria et al., 2006; Tindall, 1983). C. olitorius is made into a common mucilaginous soup or sauce in some ethnic groups in West Africa as cooking traditions. It is also a popular dish in the northern provinces of the Philippines, also known as Salyut. The leaves are rich in beta carotene, iron, calcium and vitamin C. The plant has an antioxidant activity with a significant tocopherol equivalent Vitamin E (Ayodele, 2005).

Recently aqueous extracts of the seeds of C. olitorius were reported to possess peripheral and central anti ionoceptive, anti-inflammatory and anti-pyretic activities (Zakaria et al., 2006). The seeds are used as a purgative and have been found to contain cardenolide on preliminary analysis (Gupta et al., 2003), while the methanol extracts of the seeds have been reported to possess a broad spectrum of antibacterial activity (Pall et al., 2006).

The leaves are used in the treatment of chronic cystitis, gonorrhea, dysuria and toothache (Hillocks, 1998). A cold infusion is used as a tonic to restore the appetite and strength (Sharaf and Negm, 2005). The
leaves have also been found to suppress elevation of post prandial blood glucose levels in rats and humans (Innami et al., 2005). Meanwhile, incidents of epidemics due to drug resistant microorganisms are now a common global problem posing enormous public health concerns.

Plants have been found useful to man, not only as food or as sources of raw materials for industrial purposes, but also as sources of medicaments (Azoro, 2004; Erturk et al., 2006). Many investigation have been conducted into the antimicrobial effects of various plants (Musa and Nuh, 2000; Sibanda and Okoh, 2007; Nimsha et al., 2010).

It is also speculated that inhibition of drug efflux and alterations in membrane permeability could be effected by combined interaction between plant extracts and antibiotics (Zhao et al., 2001).

The present work therefore aims at determining the antibacterial activity of *C. olitorius* on some microorganisms which are clinically important pathogens with high rate of resistance to conventional drugs. The in-vitro interactions between the plant extract and some antibiotics on the bacteria *methicillin sensitive Staphylococcus aureus* (MSSA) and *methicillin resistant Staphylococcus aureus* (MRSA) were also evaluated.

**Materials and Methods**

**Plant collection and authentication**

The plant, *C. olitorius* were purchased in Agolwoye market, Ogun State, Nigeria. After collection, they were authenticated by Dr. J.S Ashidi of Plant Science and Applied Zoology Department, Olabisi Onabanjo University and were taken to the laboratories for further processing.

**Preparation of plant extract**

Fresh leaves of *C. olitorius* were air-dried after which they were milled into coarse powder using a kitchen blender (National MX-795N, UK). Out of it, 400g of the plant powder was soaked in 2.5 L of ethanol. The set up was allowed to stand for five days with regular vigorous shaking six times a day and then filtered on the last day. The filtrate was concentrated to dryness using rotary evaporator (Buchi Rotavapor R-210). The dried extract was kept in a desiccator pending use.

**Test Microorganisms**

Eight human pathogenic bacteria species used in this study were obtained from the National Institute of Medical Research, Yaba, Lagos, Nigeria. They are; *Methicillin Sensitive Staphylococcus aureus* (MSSA), *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Shigella dysenteria*, *Salmonella typhi*, and *Methicillin Resistant Staphylococcus aureus* (MRSA).

**Antibacterial activity determination**

The antibacterial activity was determined using the Kirby-Bauer agar disc diffusion method (Brown, 2005). Muller Hinton agar (25 mL) (Sigma-Aldrich, St Louis, MO, USA) plate was poured and allowed to set. Then, 10 µL of the bacterial culture was diluted to 0.5 McFarland standards with saline and spread over the surface of the agar using a spreader. A preparation of 300 mg/mL of the extract was first made in 50% ethanol. From this stock, serial dilutions were made to obtain concentrations of 150, 100, 50 and 25 µg/mL. The serial dilutions reduced the amount of ethanol in the highest concentration tested to 25%.

Sterile Filter paper discs (6 mm) were soaked with 25 µL of 150, 100, 50 and 25 µg/mL of the extracts respectively. They were placed in predetermined positions on the plates. After one hour the plates were incubated for 24hr at 37°C. All the experiments were carried out in triplicates and the mean values of the zones of inhibition recorded.

**Evaluations of drug-crude extract interaction**

The assessment of the interaction between the extracts of *C. olitorius* and standard antibiotics followed the same method described above. However, the sub-active and active concentrations (100 and 150 mg/mL respectively) were loaded on the separate pre-loaded standard concentrations of the antibiotics. Standard antibiotics drugs were used as positive control. The plates were incubated at 37°C for 18 hours. The inhibition zone was measured and expressed in millimeter. Antibacterial activity was recorded if the inhibition zone was greater than 7 mm (Hammer et al., 1999). The assay was done in three replicates and the mean value and standard deviation were recorded.

**Results**

The results of the antimicrobial activity of the ethanol extracts of *C. olitorius* against the eight test organisms are shown in Table 1. Generally the antibacterial activity was exhibited at the highest concentration (150 mg/mL) with the highest activity recorded for *S. dysenteriae* followed by *S. typhi, P. mirabilis*, MRSA, *K. pneumonia, E. coli* and *S. aureus* (MSSA) in that order.
Table 1: Antibacterial potential of ethanol extract of *C. olitorius* leaves

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition zones in millimeters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150 mg/mL</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>8.67 ± 1.15</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>10.67 ± 2.08</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>11.33 ± 0.58</td>
</tr>
<tr>
<td><em>S. dysenteria</em></td>
<td>13</td>
</tr>
<tr>
<td>MSSA</td>
<td>8.0 ± 1.0</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>9.67 ± 0.58</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>0</td>
</tr>
<tr>
<td>MRSA</td>
<td>10 ± 0</td>
</tr>
</tbody>
</table>

The *in-vitro* interactions between the plant extract and some antimicrobial agents against MSSA and MRSA are shown in Table 2. The highest synergistic effect was recorded between the plant extract and streptomycin against MRSA. The plant extract combined with ampicillin/cloxacillin mixture also exhibited synergistic effect on the MSSA strain tested. There was a moderate synergy between the extract and ciprofloxacin against the two tested strains. All other interactions were antagonistic.

Table 2: Antibacterial activity against MRSA and MSSA of 5 antibiotics

<table>
<thead>
<tr>
<th>Antibiotics (µg/mL)</th>
<th>Bacteria</th>
<th>Inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>MRSA</td>
<td>10.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>12.5 ± 1.5</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>MRSA</td>
<td>27.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>31.5 ± 0.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>MRSA</td>
<td>12.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>22.0 ± 10.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>MRSA</td>
<td>22.5 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>20.0 ± 0</td>
</tr>
<tr>
<td>Ampicillin/cloxacillin mixture</td>
<td>MRSA</td>
<td>15.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>11.50 ± 2.2</td>
</tr>
</tbody>
</table>

KEYS: A Standard antibiotics of 10 µg/mL, B standard antibiotics with 100 mg/mL of plant extract, C standard antibiotic with 150mg/mL of plant extract, Means ± STDEV, * = synergistic ** Antagonistic

**Discussion**

The results obtained in this study revealed that ethanol extract of *C. olitorius* possesses antibacterial activity. The extract possessed antibacterial activity at very high concentrations (150 and 100 mg/mL). This may mean that the active ingredient of *C. olitorius* is present in very low amount, thus requiring the use of large amount of the crude extract. In this study, there was virtually no activity recorded for *B. subtilis*. This result was at
variance to that of Ramadevi and Ganapaty (2011) where their methanol extract showed antibacterial activity against *B. subtilis* at 100 mg/ml. The part of plant used by Ramadevi and Ganapaty (2011) and the solvent employed in extraction could have contributed to the differences observed. The polarity of solvents used in extraction has been reported to play an important role in the exhibition of antibacterial activity of plant extracts (Parakch et al., 2006). In a related study, Zakaria et al., (2006) reported the antibacterial activity of extract of *C. olitorius* plant against *B. cereus* whereas no activity was observed in the related species in our study. We reported antibacterial activity of the extracts against *P. mirabilis*, *E. coli* and *Shigella dysenteriae*, at concentration below 150 mg/mL, Zakaria et al. (2006) found no activity against *Proteus vulgaris*, *E. coli* and *Shigella flexneri* at all the concentrations used in their study. It was surprising that in our study no activity was recorded for the methanol extract at concentration of 25-100 mg/mL against most organisms studied, whereas Adegoke and Adebayo-Tayo (2009) reported some activity at 62.5mg/mL for all the organisms they investigated. Although the chemical constituents of *C. olitorius* was not investigated in the present study, the phytochemical screening of the extract of the plant has shown the presence of hydrocyanin, cardiac glycosides, tannins, flavonoids, anthraquinones and saponins (Adegoke and Adebayo-Tayo, 2009). These classes of secondary metabolites are known to possess antimicrobial activities and may thus be responsible for the antimicrobial activity of the plant extract under study (Ogunleye and Ibitoye, 2003). The extract did not have a measurable activity against *B. subtilis*. Therefore it may not be of value in treating opportunistic infections associated with the organism. However, the demonstration of antibacterial activity by the plant suggest that it could be effective and suitable for the treatment of *S. dysenteriae* infection, since its extract showed particularly high activity against the organism more than the other organisms tested. The potency of the extract against *S. typhi*, which causes typhoid, a public health problem in developing countries, also suggests the provision of alternative solution to the infection. The results of these findings thus strengthen the belief that the plant, a vegetable consumed in Western Nigeria, may have prophylactic effect against enteric fever and gastroenteritis (Adegoke and Adebayo-Tayo, 2009).

The synergistic study of the plant extract with some antibiotics against MSSA and MRSA revealed that the plant extract when combined with ciprofloxacin showed marginal synergistic effect on the two organisms studied. The combination of the plant extract with gentamicin and erythromycin however showed antagonistic effect against these two organisms. Interestingly, while the combination of plant extract with streptomycin exhibited synergistic effect on MRSA, it showed antagonistic effect on MSSA strain used. The reverse was however the case when the plant extract was combined with ampicillin/cloxacilin mixture for these two organisms. Several investigators have reported the synergistic effects of different plants extracts and antibiotics on strains of MSSA and MRSA (Esimone et al., 2006; Braga et al., 2005; Yang et al. 2005). Our results were consistent with these in-vitro studies, although there were differences in the antibiotics used and the plants investigated. Plant extracts are known to contain different phytochemicals which have different mechanisms by which they inhibit microorganisms. Consequently the combination of the plant extracts with antibiotics which act on different target sites could lead to a synergistic effect (Esimone et al., 2006). This may be the case in the plant extract-ciprofloxacin interaction in this study. It should be necessary to identify the active compound in the plant extract in order to understand the mode of action and the mechanism of synergy.

**Acknowledgment**

The authors are grateful to King’s College London for equipment support to Dr. JS Ashidi.

**REFERENCES**


