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ANTIMICROBIAL ACTIVITIES OF THE ROOT EXTRACTS OF *Carpolobia lutea* (CATTLE STICK) ON SELECTED PATHOGENIC MICROORGANISMS OF CLINICAL ORIGIN

I.I. Anibijuwon, J.A. Abioye, I.D. Gbala,
Department of Microbiology, Faculty of Science, University of Ilorin, P.M.B 1515, Ilorin, Kwara State, Nigeria.

**Abstract**

Cattle stick, *Carpolobia lutea* root was assayed in-vitro for antimicrobial efficacy of its root extracts using ethanol, methanol and hot water as extraction solvents against selected multi-drug resistant clinical isolates – *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* using the agar well diffusion method. All the test organisms were more susceptible to the ethanolic extract, with *Candida albicans* having the widest range of inhibition (25.50mm) and *Pseudomonas aeruginosa* having the least range (17.50mm) at 250mg/ml. *Salmonella typhi* and *Candida albicans* were inhibited at a reduced MIC value of 50 × 10<sup>3</sup>µg/ml each but *Staphylococcus aureus* at 200 × 10<sup>3</sup>µg/ml. The methanolic extract next showed potency on all the tested organisms with *Pseudomonas aeruginosa* being the most susceptible (20.50mm) at 250 mg/ml and MIC value of 250 × 10<sup>3</sup>µg/ml and 50 × 10<sup>3</sup>µg/ml respectively on *Salmonella typhi* and *Candida albicans*. The hot water extract showed activity on all tested organisms at 250mg/ml except for *Escherichia coli* and *Candida albicans*. MIC value of 150 × 10<sup>3</sup>µg/ml was recorded for the hot water extract on *Salmonella typhi*. Ofloxacin showed higher potency to all the tested bacteria than the plant extract components. The order of susceptibility of the test organisms to the root extract of *Carpolobia lutea* was *S. typhi* > *C. albicans* > *S. aureus* > *P. aeruginosa* > *E. coli*. The root extract of the plant was found to hold a promising source of therapeutic agent that can be used to control infections resulting from these pathogens.

**KEYWORDS:** *Carpolobia lutea*, Antimicrobial efficacy, root extracts, clinical isolates, Ofloxacin

**Introduction**

Antimicrobials come from various sources – plants, animals, chemicals and microorganisms (Anibijuwon et al., 2010). Meanwhile, the great modern advances in chemotherapy have come from the accidental discovery of the synthesized products or excrete from microorganisms. These products or excreta were found toxic and harmful to other microorganisms and hence used in the cure of infectious diseases. However, microbial resistance to antibiotics have necessitated the need for continuous search for new and effective antimicrobial agents (Willey et al., 2008).

Antimicrobial resistance has been a serious problem facing the therapeutic utility of various sets of antimicrobials causing their potency to be constantly dwindling and falling, creating a requirement for the discovery of new antimicrobial drugs (Chopra et al., 2002). The problems associated with the development and spread of antibiotic resistance has been a major threat to the treatment of hospital and community-acquired infections.
Such resistance has generally been associated with the overuse and abuse of therapeutic agents, and with the acquisition and fusion of genetic elements encoded within microbial plasmids. The widespread inappropriate use and over-prescription of antibiotics in clinical medicine, animal husbandry and veterinary practice has been widely accepted to be the main cause of this problem of resistance (Gilbert and Moore, 2005). Several drug discovery strategies have emerged, including incremental improvements to existing antibiotics by chemical manipulation and the search for novel drug targets based on genomic approaches (Chopra et al., 2002). Medicinal plants have therefore been discovered to have their intrinsic ability to resist pathogenic microorganisms; the mechanisms of action and isolation of the active compounds in them are thus being investigated enabling the exploitation of medicinal plants for the treatment of microbial infections in human.

Cattle stick, *Carpolobia lutea* G. Don is known as cattle stick in English, (Ajiwhen and Bisong, 2013). It grows in tropical rainforest of Africa, Guinea savannah of Sierra Leone and Cameroon as dense overgrowth or evergreen shrub (a small tree) of 5m (≈15ft) high, from April to September (Lucky and Paul, 2008). *C. lutea* is cultivated wild and propagated by seed. The phytochemical screening of the root extract of *C. lutea* revealed the presence of saponins, terpenes, simple sugars, cardiac glycosides in large amount with some anthraquinones and flavonoids (Ajiwhen and Bisong, 2013). The ethyl acetate leaf extract of *C. lutea* has also been reported to contain cinnamic and coumaric acid derivatives (Ettebong and Nwafor, 2009; Ajiwhen and Bisong, 2013).

It has been observed that not much work has been done on the antimicrobial efficacy of the root extract of the plant used in this study. Hence, the aim of this study is to screen the hot water, 95% methanolic and 95% ethanolic extract of the root of *C. lutea* for antimicrobial activity. The objective is to assess and reaffirm the antimicrobial efficacy of extracts of the root of *C. lutea* against selected pathogenic microorganisms of clinical origin.

**Materials and Methods**

**Plant Material**

The fresh roots of the plant were purchased from herb sellers at a market in Ilorin (Oja tuntun), Nigeria. Identification and authentication of the plant was done at the Plant Biology Department of the University of Ilorin. The roots were properly washed with distilled water and air dried, then pulverized (with a grinder) into powder. The powder was again blended with an electrical blender into fine powder, to further increase its surface area. The total weight of the powdered plant sample was taken to be 450g and stored at room temperature.

**Collection and Maintenance of Test Organisms**

Pure cultures of clinical isolates of selected pathogenic microorganisms were obtained from...
the Medical Microbiology laboratory of the University of Ilorin Teaching Hospital (U.I.T.H), Ilorin, Kwara State. The organisms obtained were \textit{Escherichia coli}, \textit{Salmonella typhi}, \textit{Pseudomonas aeruginosa}, \textit{Staphylococcus aureus} and \textit{Candida albicans} (fungus). The test organisms were inoculated in sterile agar slants and incubated. They were thereafter tested biochemically to confirm their purity before storing in a working refrigerator at 4°C as a stock culture. Subcultures of the refrigerated stocks were carried out at every 2 weeks interval to maintain the viability of the test organisms. It was also ensured that 24-hour-old cultures of the test organisms were used for the antimicrobial assay.

\textbf{Preparation of Plant Extracts}

The root of the plant was air-dried and pulverized into fine powder. It was then prepared for extraction by cold maceration in three different solvents: by 95\% ethanolic extraction (95\%EE), hot water extraction (HWE) and 95\% methanolic extraction (95\%MEE). 100g of powdered root sample were introduced into 400ml of each solvent in separate flasks, followed by vigorous shaking for five (5) minutes and thereafter left for 48hours on a rotating shaker. The resulting solutions were filtered using fine muslin cloth and the filtrates were left on the workbench to settle (decantation), the pellets were discarded and the supernatants were concentrated in a hot water bath then subjected to centrifugation at 2500 revolutions per minute (rpm) for five (5) minutes for clarification before they were then sterilised by membrane filtration using Millipore filter paper and thereafter stored in sterile bottles in a working refrigerator, appropriately labelled.

\textbf{Determination of pH of the Root Extracts of \textit{Carpolobia lutea}}

The pH of the extracts was determined by dipping a pH meter into conical flasks containing a portion of the extracts. The constant reading on the pH meter was taken as the pH of each of the antimicrobial substance in question. The pH readings were again taken three weeks after extraction.

\textbf{Sterility test of Plant Extract}

After sterilization, each of the extracts was checked for growth or contaminants by plating on both nutrient agar and Potato Dextrose agar and subsequently incubating at 37°C for 24hrs and 25°C for 48 hours respectively. The extracts were considered sterile when no visible growth was observed on the cultured plates.

\textbf{Incubation of Test Organisms}

All bacterial cultures were incubated at 37°C for 18-24 hours while the fungal isolate was incubated throughout at room temperature (25°C) for 48 hours.

\textbf{Antimicrobial assay of Extract of \textit{C. lutea} using Agar Well Diffusion Method}

Overnight culture plates of the test organisms were transferred to peptone solution, pH 7.2, and the turbidity was adjusted to 0.5 McFarland standards (10⁶cells/ml). They were then swabbed on sterile Mueller Hinton agar (for the bacteria) and on Potato Dextrose Agar (PDA, for the fungus) in Petri dishes using sterile cotton swab sticks. Ditches or wells were aseptically made on the plates with the aid of a 6mm diameter sterile cork borer. 1ml of the respective root extracts was then put into each appropriately labelled ditch/well using sterile micropipettes. The inoculated plates were left on the table for about 30 minutes for the extracts to diffuse into the agar. The plates were then incubated at 37°C for 18hrs (for the bacterial isolates) and at room temperature for 48 hours (for the fungal isolate). The diameter of the zones of inhibition around each well was then measured along two axes using a meter rule and the mean of the readings were taken as the final inhibition zone (Adetitun et al., 2013).

\textbf{Determination of Effect of Varying Concentration of the Plant Extract on the Selected Organisms}

Stock concentration of the extracts of \textit{Carpolobia lutea} (95\%EE, HWE and 95\%MEE) was diluted from 250mg/ml to 200mg/ml to 150mg/ml to 100mg/ml to 50mg/ml to 25mg/ml and finally to 5mg/ml with extraction solvent. Each of these concentrations was then introduced into ditches on the plates containing inoculum of broth cultures of each of the test organisms using the
Agar well Method. The plates were left on the table for about 30 minutes to allow the extracts to diffuse and subsequently incubated at the right temperatures for 18hrs for the bacterial isolates and 48hours for the fungal isolate. After incubation, clear zones of inhibition were checked and measured.

**Determination of Minimum Inhibitory Concentration (MIC) using Broth Inoculation Method**

Only about 0.2ml of the overnight broth cultures of the organisms that were found susceptible to the extracts of *Carpolobia lutea* were inoculated onto sterile liquid growth medium. The growth medium have been pre mixed with 1ml of each of the diluted extracts of *C. lutea* that were found potent in the previous test above (200 × 10³ µg/ml, 150 × 10³ µg/ml, 100 × 10³ µg/ml, 50 × 10³ µg/ml, 25 × 10³ µg/ml and 5 × 10³ µg/ml). A negative control was also setup to contain the liquid growth medium and the extract only (without test organism). The inoculated and control tubes were then incubated at 37ºC and at room temperature for 18 and 42 hours for both bacterial and fungal cultures accordingly. The tubes were observed for turbidity after incubation. The lowest concentration that shows no turbidity (indicating no growth of the test organism) was taken as the MIC (Adetitun *et al.*, 2013).

**Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)**

Samples from the tubes used in the MIC assay which did not show observable sign of turbidity (or growth) after incubation were streaked out on solidified nutrient agar/PDA plates using sterile cotton swab and incubated at 37/25ºC for 18/48 hours for the bacterial and fungal plates accordingly. The tubes were observed for turbidity after incubation. The lowest concentration that shows no turbidity (indicating no growth of the test organism) was taken as the MIC (Adetitun *et al.*, 2013).

**Antimicrobial Susceptibility Test of some Standardized Antibiotic Discs (ASTSA)**

The antibiotic susceptibility testing by a standardized disc method as described by Bauer *et al.*, (1965) was employed. The antibiotics used for this study had been prepared into kit containing multiple discs, each with small discs impregnated with different types of antibiotics. The antibiotics used and their corresponding concentrations are as follows: Gentamicin (10µg), Cotrimaxazole (25µg), Ofloxacin (30µg), Tetracycline (30µg), Amoxycilin (25µg), Notrofurantoin (300µg), Nalidixic acid (30µg) and Augmentin (30µg) for the gram-positive bacteria and Ceftazidine (30µg), Cefumoxime (30µg), Gentamicin (10µg), Ceftriaxone (30µg), Erythromycin (5µg), Cloxacilin (5µg), Ofloxacin (5µg) and Augmentin (30µg) for the gram-negative bacteria isolate.

The disc diffusion technique was used for the antibiotic sensitivity test. 18 hour-old broth cultures of the organisms were swabbed on sterile Mueller Hinton agar plates using sterile swab sticks. The multiple antibiotic discs were then placed on the agar surface and pressed using sterile forceps to ensure complete contact with agar. The bacterial isolate plates were incubated at 37ºC for 18hours and the fungus plate at 25ºC for 48hours. The zones of inhibition generated by the antibiotics were measured to the nearest millimetres (mm) (Adetitun *et al.*, 2013). This was intended for comparison of results with those obtained from the potent extracts.

**Results**

Figure 1 indicates the pH values obtained for the various stock extracts of the root of the plant, *Carpolobia lutea*, just few hours after their extraction and subsequently 3 weeks after the extraction to measure extracts’ potency rate. The results revealed a progressive increase in each extract’s pH value and this suggests that the extracts at the first week of extraction are more potent on the test organisms than those of the 3 weeks.
Figure 2 shows the effect of the varying concentrations of the crude extracts for the hot water (aqueous) extract (HWE), 95% Ethanol extract (95%EE) and for the 95% Methanolic extract (95%MEE). *S. typhi* and *C. albicans* were susceptible to the effect of varying concentrations of the 95% Ethanolic root extract of *C. lutea up to the least 5mg/ml concentration. *S. aureus* was only susceptible to the 200mg/ml concentration with a zone of 11.50mm while the other tests organisms namely: *P. aeruginosa* and *E. coli* remain resistant. There is a noted slight increase in the inhibition zone observed with *S. typhi* for the 50, 25 and 5mg/ml concentrations: 13.50mm, 16.50mm and 13.50mm, respectively. This is unexpected as higher concentrations of the extract have a lower zone of inhibition: 100 and 150mg/ml with inhibition zones of 12.50 and 10.00mm respectively. *S. typhi* and *C. albicans* were the susceptible test organisms to the lowered concentrations of the 95% Methanolic root extract of *C. lutea*; the other organisms are resistant to the lowered concentrations. *C. albicans* is susceptible to the extract up to 50mg/ml while *S. typhi* is susceptible up to 5mg/ml. The comparison of the three extracts as well as the antibiogram profile of the test organisms in Figure 2 showed that the Ethanolic extract was the most effective plant extract while ofloxacin was the most effective antibiotic.

The comparison between ethanol and methanol extracts of *C. lutea* and Ofloxacin showed a significance difference in the antibacterial activity of the plant extracts and the conventional antibiotic (Figure 3). The test organisms showed higher susceptibility to Ofloxacin than the extracts. From the results of the MIC and MBC, it was observed that none of the *C. lutea* root extracts has inhibitory or killing effect on *E. coli*. The Ethanolic root extract of *C. lutea*, however has only inhibitory effect on *S. aureus*, *S. typhi* and *C. albicans* at concentrations of 200 $\times 10^3$µg/ml, 50 $\times 10^3$µg/ml and 50 $\times 10^3$µg/ml accordingly. The Methanolic extract of the root of *C. lutea* also has only inhibitory effect at 50 $\times 10^3$µg/ml on *C. albicans* and at 250 $\times 10^3$µg/ml on *S. typhi* and *P. aeruginosa*. It (95%MEE) has no killing effect on the test organisms. The Hot water root extract of *C. lutea* has an inhibitory effect only on *S. typhi* at a concentration of 150 $\times 10^3$µg/ml. None of the extracts, at any concentration, has a killing effect on any of the test organisms.

![Figure 1: The pH values of *C. lutea* root extracts](image-url)
Table 1: Preliminary screening of crude root extracts of *C. lutea*

<table>
<thead>
<tr>
<th>S/N</th>
<th>Organisms</th>
<th>Hot water Extract</th>
<th>Ethanolic Extract</th>
<th>Methanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td><em>Salmonella typhi</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td><em>Candida albicans</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ potent - non. potent

Table 2: The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal/Fungicidal Concentration of the root extracts of *C. lutea*.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Minimum Inhibitory Concentration (× 10³ µg/ml)</th>
<th>Minimum Bactericidal Concentration (× 10³ µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extracts</td>
<td>Extracts</td>
</tr>
<tr>
<td></td>
<td>Ethanol Methanol Aqueous Ethanol Methanol Aqueous</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>200 - -</td>
<td>- - -</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>50 250 150</td>
<td>- - -</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>- 250 -</td>
<td>- - -</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>50 50 -</td>
<td>- - -</td>
</tr>
</tbody>
</table>

**Key:** (-) → No inhibitory/cidal effect.
Figure 2: A- Antibacterial activity of Hot water extract  
B- Antibacterial activity of Ethanol extract  
C- Antibacterial activity of Methanol extract  
D- Comparison of the extracts’ activity  
E- Antibiogram profile of test organisms
DISCUSSION
The result from this study has affirmed the prophylactic as well as possible therapeutic use of *C. lutea* root in bacterial and yeast infections. Both traditional and modern medicines are now sourced from medicinal plants (Yakubu and Akanji, 2014), it is therefore imperative to evaluate the antimicrobial potential of various plants. All the test organisms employed in the course of this research work have been identified to be clinically resistant forms that have frequently shown resistant to common antibiotics in use. Three different solvents have been used in the extraction process to maximise the chances of increased solubility and extraction of the active constituents that are present in the plant’s root. The order of susceptibility of the tested organisms, in general, to the plant extract used is *S. typhi* > *C. albicans* > *S. aureus* > *P. aeruginosa* > *E. coli*. Also, the order of potency of solvents used in the extraction is 95% Ethanol >95% Methanol > Hot aqueous (water). It is worthy to note that the potency of the extracts used have probably reduced on prolonged storage as indicated by their progressive increase in pH values (Fig. 1). Alkaline conditions tend to support bacterial growth and survival. This observation agrees with Alade and Irobi (1993) and James (2012) confirming that the antimicrobial activity of some plants significantly reduces with increase in pH. El-Mahmood (2009) has also posed that extracts of herbal medicines are subject to degradation and decomposition on storage. The hot water root extract of *C. lutea* has shown very low activity compared with Ethanol and Methanol root extract of the plant. This is probably due to the fact that water cannot bring about effective extraction of the bioactive components embedded in the root of the plant. This assertion coincides with the findings of other researchers like Brain and Turner (1975) and Adetitun et al., (2013). The activity it (HWE) has on *S. aureus* and *P. aeruginosa* occurs only at high concentration of 250mg/ml (stock concentration) while *S. typhi* that shows susceptibility better than any of the test organisms has low inhibition zone diameter (15.0mm). There is therefore the possibility of good performance of the hot aqueous (water) extract of the root of the plant at high concentrations of about 250mg/ml and above. The result of the experimental evaluation of MIC shows that hot water extraction, as less effective as it may seem, has inhibitory effect.

Fig 3: Comparison of Ofloxacin with ethanol and methanol extracts of the root of *C. lutea*
Candida albicans showed the most susceptibility to the 95% Ethanol root extract of C. lutea followed by S. typhi. Candida albicans was also observed to be very susceptible in the work of Nwidu et al. (2012). S. typhi showed a progressive decrease in susceptibility from the concentrations of 250mg/ml to 100mg/ml but with a deviation at the 50mg/ml and 25mg/ml concentrations where an increase in susceptibility was observed before finally dropping back at 5mg/ml. This observation can be said to have resulted from the synergistic activity of both the bioactive components of the extract and the solvent used (95% Ethanol) in the dilution process. Ethanol, which has a sterilizing property, must have contributed to the activity of the extract leading to sudden rise in potency of the extract at those specified concentrations. The extract in itself is losing its inherent potency with time, marked by its increase in pH and long storage time –the more the increase in pH of some plant extract; the less potent they become (Alade and Irobi, 1993; James, 2012; El-Mahmood, 2009). In almost similar trend of the 95% ethanolic extract, Candida albicans and S. typhi showed increased susceptibility to the lower concentrations of the Methanolic extract than the other test organisms. However, P. aeruginosa has the highest inhibition zone diameter for the Methanolic extract of the root. In the MIC studies, the Hot Water extract was noted to inhibit the growth of S. typhi alone at a concentration of 150 × 10³µg/ml. Other test organisms have shown resistance to the HWE. The Methanolic extract was also observed to possess inhibitory effect on both S. typhi and P. aeruginosa at 250 × 10³µg/ml and on C. albicans at 50 × 10³µg/ml. The Ethanolic extract has shown antimicrobial (inhibitory) activity to three of the test organisms: S. aureus, S. typhi and C. albicans with P. aeruginosa and E. coli resistant. None of the extracts showed cidal/killing effect on any of the test organisms however, in the MBC assay of the Methanolic root extract on C. albicans at 50 × 10³µg/ml, there was very weak yeast growth. This signifies the possibility of complete eradication of the fungus on prolonged use of the plant extract in the treatment of the yeast (especially candidal) infections. Nwidu et al. (2012) also observed a good fungistatic activity of the root extracts against C. albicans but with n-hexane and chloroform fractions more potent than the ethanolic fraction.

The behaviour of some of the tested organisms, especially E. coli and P. aeruginosa, showing resistance to one or more of the root extracts of the plant may be due to elaboration of enzymes that possibly destroy or inactivate some of the bioactive phytoconstituents in the root of the plant. In addition, the complex nature of the cell envelope of gram-negative bacteria has been observed to retard or prevent the passage of many antimicrobial agents through the cell wall (El-Mahmood, 2009). Also, the ability of Carpolobia lutea root extracts to inhibit the growth of both the gram-positive and gram-negative bacteria (and fungus) shows that it has a broad spectrum of activity and hence, the possibility of its use in the formulation of newer broad spectrum antimicrobial substances.

**CONCLUSION**

The outcome of this research showed that the root of C. lutea contains certain active compounds that have actually demonstrated potency and possible use in the control of microbial infections. The plant was shown to have inhibitory effect on the test organisms especially when these active ingredients are extracted using ethanol. This however supports the local and traditional use of the plant in the control of intestinal disorders and also explains why, at times, traditional healers use the herb alongside with alcohol for increased efficiency. The methanolic extract of the root of the plant have also been demonstrated to have good effect on Pseudomonas aeruginosa and suggests its use in the possible control of nosocomial (hospital-acquired) infections associated with P. aeruginosa. The results obtained have also indicated a good chance of use of the root extracts of C. lutea (especially ethanolic and methanolic extract), in the control of yeast infections (Candida). It is believed that with careful isolation, further purification, toxicity tests and other pharmacological evaluations of the active
antimicrobial constituents of the root of *C. lutea*, a potent chemotherapeutic drug with broad spectrum of activity and stability (to resistance) can be produced.

REFERENCES


