ABSTRACT: Phytochemical and antibacterial properties of water, methanol and ethanol extracts obtained from root and leaf of Calotropis procera were investigated. Antibacterial growth inhibition was determined using Ditch method against Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Staphylococcus aureus and Streptococcus pyrogenes. The phytochemical screening reveals the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides, balsams and volatile oil and steroids with higher amount in water extracts. The concentration of the phytochemical constituents were in the order of water > methanol > ethanol. Water extracts showed broad spectrum activity against the tested organisms at concentrations of 30, 60, 90 and 120 mg/ml. Methanol and ethanol extracts did not show a significant effect against the tested organisms at 120 mg/ml as compared with those of tetracycline. The result of this study validates the use of water extract of this species in ethnomedicine and could provide a lead in the isolation of antibacterial agents from water extracts of Calotropis procera.

Keywords: Calotropis procera, antibacterial, phytochemical, ethnomedicine

INTRODUCTION

Many research efforts have been directed towards the provision of empirical proofs to back up the use of plants species in trade and medicinal practices in recent years (Ojo et al., 2005). Many researchers have examined the effects of plants used traditionally by indigenous healers to support treatment of various diseases; scientific validations are being made globally to get evidence for traditionally reputed herbal plants. However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated. In many developed countries 70- 80% of the population have used some forms of alternative or complementary medicine e.g. acupuncture, while in most African communities 80% of the population depends on traditional medicine for primary health care (WHO 2005).

Calotropis procera (Ait) R. Br. (Sodom Apple) belongs to the family Asclepiadaceae. It occurs in most parts of the tropical world, in dry sandy and alkaline soils, in waste land and grows abundantly as a weed. It is a shrub which grows up to 5.5 m high, occasionally branchless to a height of 2.5 m. All parts of the plant exude white latex when cut or broken, leaf is large ovate, opposite, sessile, up to 30 cm long and 16 cm wide (Aliyu, 2006). Medicinally, the pungent sap latex is used to treat boils, infected wounds and other skin problems in people and to treat parasitic skin infestation in animals. It also yields ash for making gun powder; the latex is processed and used in treating vertigo, baldness, hair fall, tooth aches, intermittent fevers, rheumatoid /joints swellings and paralysis (Vohra, 2004). The whole plant when dried and consumed is a good tonic, antihelminthic and as an expectorant (Agharkar, 1991; Warrier et al., 1996). The dried root is used to cure bronchitis, asthma, leprosy, eczema and elephantiasis, hepatic and splenic enlargement and as eye tonic (Vohra, 2004). The milky juice and caustic flowers were considered to improve digestion, catarrh and increases appetite (Oudhia, 2001). The juice was also found to induce abortion in women and tanners use the milky juice to remove hair from the hides (Singh et al., 1996). The presence of alkaloids calotropin, calotaxein and uskerin has been reported and are stimulant to heart (Ashwari, 2009). It is also used by traditional medicine practitioners in Gwari communities for the treatment of ring worms (Kuta, 2008).

In North Western Nigeria, C. procera is used by local communities for the treatment of infectious diseases. There is the need to carry out scientific investigations to ascertain the authenticity of the claims on the medicinal properties of this species. The aim of this study is to evaluate the phytochemical and antibacterial properties of root and leaf extracts of C. procera.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh leaves and root of C. procera were collected from Tudun Wada ward of Gusau, Zamfara state. The species was identified and authenticated at the Herbarium Department of Biological Sciences, Usman Danfodiyo University, Sokoto, where the
The powdered plant parts were separately extracted with 95% ethanol, methanol and distilled water. The extracts were filtered using Whitman No. 1 filter paper and the solutions were concentrated using water bath. The extracts were stored in different containers labelled and kept in polythene bags before analysis. The methods of Trease and Evans (1989) and Sofowora (1982) were used for the phytochemical screening of the extracts.

Antibacterial Screening
The microorganisms used for antibacterial screening, were Staphylococcus aureus. Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli and Streptococcus pyogenes, obtained from Microbiology Department, Usmanu Danfodiyo University Teaching Hospital, Sokoto. The well plate diffusion method was used to determine the growth inhibition of bacteria by plant extracts as described by Mohammad and Dabai (2008). The nutrient agar (Muller Hinton) plates were prepared and seeded with the test organisms. Four holes of 6.0 mm diameter each were made on to the plates with a sterile cork borer and filled with 30, 60, 90 and 120 mg/ml extracts. The inoculated plates were allowed to congeal for 30 min to allow pre diffusion time and then incubated at 37°C for 24 hrs. The plates were examined for zones of inhibition (Cheesbrough, 2001). The diameter of such zone of inhibition was measured using a transparent meter ruler and the value was recorded and expressed to the nearest millimeter.

RESULTS AND DISCUSSION
The results of phytochemical screening of water, methanol and ethanol root and leaf extracts of C. procera revealed the presence of alkaloids, flavonoids, saponins, tannin and glycosides (Table 1). The concentrations of the various classes of secondary metabolite vary amongst the extracts evaluated. The concentrations of the constituents are in order of water > methanol > ethanol. The presence of these components in this species is an indication that it may have some medicinal potential. This is due to the fact that each of the components identified has one therapeutic usage or another. For instance, plants rich in saponins have immune boosting and anti inflammatory properties (Kenner and Requena, 1996). Similarly, tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors (Hassan et al., 2005; Aliero et al., 2008; Yesmin et al., 2008).

The antibacterial activity of C. procera water, methanol and ethanol extracts obtained from the root and leaves on the test organisms exhibited antibacterial activity (Figures 1-6). Generally, Salmonella typhi and Streptococcus pyogenes were observed to be the most susceptible organisms, this result is similar to that of Yesmin et al. (2008). Similarly, Staphylococcus aureus is least susceptible in this study and same observation was reported by Aliero et al. (2008). The methanol extracts of C. procera root did not show appreciable activity against, S. pyogenes and S. aureus (Figure 4).

Table 1: Phytochemical constituents of root and leaf extracts of C. procera

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Water Root</th>
<th>Water Leaf</th>
<th>Methanol Root</th>
<th>Methanol Leaf</th>
<th>Ethanol Root</th>
<th>Ethanol Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Saponins Glycosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Balsams</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile Oils</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: +++ = present in high amount, ++ = moderately present, + = Trace amounts, - = absent
The ethanol extracts of *C. procera* root showed no activity on *S. aureus*, *Salmonella typhi* and *E. coli* (Figure 6). A similar observation was also reported by Yesmin et al. (2008) on *C. procera* growing in India. Likewise, water extracts obtained from the root did not show activity on all the test organisms except against *Pseudomonas aeruginosa* (Figure 2). This lack of activity was also observed by Usman et al. (2005) on leaf extracts of *Celtis integrifolia*. Similarly, methanol, ethanol and water extracts of *C. procera* showed significant antibacterial activity against both the Gram positive and Gram negative bacterial strains (Yesmin et al., 2008). Zone of inhibition of tetracycline on the five test organism is presented in Table 2. *Salmonella typhi* showed the highest inhibition with 22 mm followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 20 mm each. The result of this study justifies the use of water extracts of *C. procera* in ethnomedicine for the treatment of infectious diseases caused by susceptible bacterial species.

**Table 2:** Zone of inhibition (mm) by synthesized standard antibiotic drug (Tetracycline) on the five Test organisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyrogenes</em></td>
<td>18</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>22</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>20</td>
</tr>
</tbody>
</table>

Note: Diameter of zone of inhibition is 6 mm; any zone of inhibition greater than 6 mm implies activity.

**Figure 1:** Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of water extracts of *C. procera* leaf.

**Figure 2:** Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of water extract of *C. procera* root.
Figure 3: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of methanol extract of C. procera leaf.

Figure 4: Diameter size of inhibition zone on the growth of different bacterial species due to application of different concentration of methanol extract of C. procera root.

Figure 5: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of ethanol extract of C. procera leaf.
**Figure 6:** Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of ethanol extract of *C. procera* root.

**REFERENCES**


the leaf extracts of *Celtis integriifolia* (Lam). "Journal of Tropical Biosciences, 5(2): 72-75"


