Phytochemical and Antimicrobial Evaluation of Aqueous and Organic Extracts of *Calotropis procera* Ait Leaf and Latex

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**ABSTRACT**

The aqueous and ethanol extract of *Calotropis procera* leaf and latex were investigated. The leaves and latex from the plant were tested for antimicrobial activities. The bioactive constituents extracted from the leaf and latex were tested against pathogenic organisms (*Eschericia coli, Salmonella typhi, Bacillus subtilis, Candida albicans, Aspergillus niger*) using the Agar well diffusion method. The ethanolic latex extract showed significant activity against all the test organisms. The results revealed that ethanol is a more effective extractive solvent for antimicrobial activity of leaf and latex of *C. procera*. The ethanol extract of the latex gave the widest zone of inhibition (21mm) against *B. subtilis*. All the extracts inhibit the growth of all the organisms except *B. subtilis* of which the aqueous extract has no effect. The Minimum Inhibitory Concentration (MIC) for the latex extract was between 3 and 7.5 mg/ml for bacteria, and 5.0 to 7.0 mg/ml for fungi. For the leaf extract the MIC for bacteria was between 5.0 and 10.5 mg/ml and 11 and 15 mg/ml for fungi. The results also showed an increase in antimicrobial activity with increase in temperature. This study therefore revealed that *C. procera* latex extract demonstrated strong and better inhibitory activity on the test organisms than the leaf extract. These findings therefore provide an explanation for the traditional medicinal use of *C. procera* extracts.

**Keywords:** *Calotropis procera*, latex, bioactive, antimicrobial activity, minimum inhibitory concentration.

**Introduction**

Plants and their essential oils have been associated with antimicrobial activity since prehistoric times (Shelef, 1983; Chang 1995). Plants have been endowed with innate ability to synthesize aromatic substances such as phenols and their derivatives (Geissman, 1963), these secondary metabolites have therapeutic tendencies. Due to the potential health benefit of plants of medicinal value (Gomez – Flores et al., 2008), medicinal plant extracts in recent times, have been developed and proposed for use in food as natural antimicrobials (Hsieh et al., 2001). Some of their metabolites have been successfully used in the treatment and prevention of infectious diseases, cancer or in stimulating the immune system. The medicinal value attributed to plants is a function of the bioactive phytochemical constituents that produce definite physiological action on the human body (Akimoladun et al., 2007). The most important of these plants bioactive chemical constituents are flavoids, alkaloids, tannins and phenolic compounds (Okwu, 2001; Edeoga et al., 2005). Phytochemicals are natural bioactive compounds of secondary metabolite found in plants that work with nutrients and fibres to act as a defence system against disease. These phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah et al., 2009) and

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many more such as flavonoids, tannins, etc. The biological activities reported to have been associated with plants and plant derived compounds can be categorized according to the disease area (Butler, 2005), which include anticancer activity, Nervous system Activation/suppression, cardiovascular/metabolic, antimicrobial activity immunomodulating activity, anti-inflammatory activity.

*Calotropis procera* is a species of flowering plant which belongs to the family asclepiaadaceae. It is widely distributed in West Africa and other parts of the tropics (Irvine, 1961). It is commonly known as Apple of Sodom. The English name is milk weed. In Western part of Nigeria, it is called *Bomubomu* by the Yorubas, the Hausas call it *tumfafia*, in Sudan it is called *Oshar*. It is called *calotrops* in Italian, *pomme de sodome* in French and *kisher* in Arabic. Parrotta (2001) reported that the secretions from the root back of *C. procera* are used traditionally in India for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms. In Nigeria, it has been used traditionally to treat diseases like fever, eczema, diarrhoea, leprosy, ringworm, cough, asthma and convulsion (Odugbemi, 2006).

This paper provides basic information on the antimicrobial activities of *C. procera* leaf and latex against some bacterial and fungal pathogens and the effect of temperature on the effectiveness of the plant extract.

**Materials and Methods**

**Collection of plant samples**

Fresh leaves and latex of *Calotropis procera* were collected in Agbado, Ifo Local Government Area (6049’N, 3012’E) of Ogun State in Nigeria. This plant was characterized and authenticated at the Department of Crop Science Technology of the Federal University of Technology, Owerri. The leaves were hand plucked aseptically and cleaned for debris using tap water and then rinsed in sterile distilled water. The leaves were air-dried in the shade at room temperature. The dried leaves were blended using a domestic electric blender, powdered samples were stored in airtight glass containers protected from sunlight for subsequent extraction and further bioassay.

**Collection and preparation of latex extracts**

The method of crude latex collection described by Singhal and Kumar (2009) and its slight modification according to Nenaah and Ahmed (2011) was employed. Latex from the young leaves close to the tip of branches was collected as the leaves were plucked from the branches. The Latex was collected into a sterile bottle. The bottle was shaken slightly during collection to avoid coagulation. This was air-dried under shade. Petroleum ether was then added to the dried latex and filtered, so as to remove chlorophyll pigments and rubber materials that could be present. The volume of the filtrate was reduced under vacuum and the obtained extract were air-dried at room temperature. Extraction of bioactive components of the extract were carried out using distilled water and ethanol, and the obtained latex extract were dried and stored at 4°C until bioassayed.

**Test organisms**

The aqueous and ethanol extracts were tested for antibacterial and antifungal activities. The bacterial isolates (*B. subtilis, E. coli* and *S. typhi*) and fungal isolates (*C. albicans, A. niger*) were obtained from the Federal Institute of Industrial Research (FIIRO), Oshodi, Lagos State, Nigeria.

**Preparation of extracts**

The method described by Akerele *et al.*, (2008) was used to extract the bioactive components from the powdered samples with slight modification. Briefly, to 10 g portion of the leaf sample, one hundred milliliters (100 ml) each of Ethanol and water were added in separate sterile conical flask and allowed to soak at room temperature for 24 h. The soaked sample were periodically shaken to ensure complete extraction. The extracts were then filtered using filter paper (Whatman no1), and the filtrates concentrates are vacuo at 40°C using a rotary evaporator (*Akerele et al.* 2008; Doughari and Manzara 2008 and Nenaah and Ahmed, 2011). The residue obtained were dried and stored at 4°C until bioassayed.
**Determination of phytochemical constituents**
The method(s) described by Krishnaiah et al. (2009) and Mikail (2010) was used for the determination of tannins, alkaloids, flavonoids, glycosides and saponin.

**Antibacterial activity bioassay**
The extracts were evaluated for antibacterial activity against *B. subtilis*, *E. coli* and *S. typhi*, using the methods of Ogbulie et al., (2007a); Doughari and Manzara, (2008).

**Determination of Minimum Inhibitory Concentration (MIC)**
Determination of the Minimum Inhibitory Concentration (MIC) was carried out as described by Doughari and Okafor (2008).

**Antifungal activity**
Antifungal activity of the test extracts were evaluated by the agar well diffusion method against *C. albicans* and *A. niger* described by Afolayan and Ashafa (2009) with slight modification. Briefly, to a prepared potato dextrose agar, about 500 µl of the actively growing *C. albicans* and *A. niger* previously diluted to approximately 10⁵ colony forming unit per milliliter (cfu/ml) were inoculate. Five wells of 5 mm bored on the plates 0.5 ml of the extracts were then introduced in the well. The plates were then incubated for 48 h at 25°C, after which the inhibition zone obtained around the well was measured. Controls were plates containing microorganisms without the extracts. The experimenters were carried out in triplicates under aseptic conditions.

**Effect of temperature on antibacterial activity of extracts**
The effect of temperature on antibacterial activity of extracts was determined according to (Doughari and Okafor, 2008). Five milliliters of 60 mg/ml of ethanol extracts were constituted in test tubes and treated for 1 hour at 4°C in the refrigerator and at 30°C, 60°C and 100°C in water bath, and further tested for antibacterial activity.

**Results and Discussion**
The result of the qualitative phytochemical study of *C. procera* revealed the presence of alkaloids, flavonoids, saponins and tannins (Table 1). Phytochemicals have been reported to inhibit bacteria growth (Mungole et al., 2010). Sodipo et al., (1991), reported that tannins prevent the development of microorganisms by making useful proteins unavailable to the organism and facilitate the precipitation of microbial protein. Plants rich in tannins have been found to be astringent in nature and are used for treating intestinal disorder (Mungole et al., 2010). Saponin known to have expectorant activity were observed in the plant. Ogbulie et al., (2007a) and Banso (2009) in separate reports revealed that phytochemicals exhibit antimicrobial activity against a number of microorganisms. Plants containing phytochemicals have been found to have anti-cancer properties (Nanasombat and Teckchuen, 2009), anti-arthritic (Woode et al., 2008), anti-malaria (Oyewole et al., 2008), antioxidant (Veeru et al., 2009), immunomodulatory (Sudha et al., 2010). Plant extracts have been reported to be active against both gram positive and gram negative bacteria (Ashafa and Afolayan, 2009).

The potency of the plant extracts depends on the solvent used. This may be due to the degree of solubility of the bioactive constituents. It has been documented that different solvents have diverse solubility capacities for different phytochemicals (Takazawa et al, 1982; Nenaah and Ahmed 2011).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Cardiac glycoside</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. procera</em> leaf</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>C. procera</em> latex</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

The experimenters were carried out in triplicates under aseptic conditions.
This report revealed that the ethanolic extract was more effective than the aqueous extract. This supports the earlier findings of Ogbulie et al. (2007b) that ethanol is the best solvent for the extraction of plant bioactive principles of medicinal importance. The result showed that there is no significant difference in the antimicrobial activity of the leaf water extract and the leaf ethanol extract (Table 2). However, the antimicrobial activity of the latex extract revealed that the ethanolic extract of the latex showed the highest antibiotic activity against \textit{B. subtilis} (21 mm) compared to \textit{E. coli} (16 mm) and \textit{A. niger} (16 mm). The antimicrobial activity demonstrated by the leaf and latex extracts against some bacteria pathogens could be due to some bioactive constituents of the extract. The MIC value of \textit{C. procera} latex extract was 3.0 mg/ml for ethanolic extract against \textit{E. coli} and 5.0 mg/ml for aqueous extract against \textit{S. typhi} (Table 3). This means that ethanol extract of \textit{C. procera} latex will prove to be more effective against microbial pathogens. The inhibitory effect of \textit{C. procera} on the test organisms was more pronounced in the latex than the leaf (Table 2). The ethanolic extract of \textit{C. procera} latex demonstrated a wider zone of inhibition when compared to the water extract of the same latex. The bacteriocidal activity of \textit{C. procera} latex could be due to the presence of Calactin, mudarin and calotropain which are the active component of the latex (Parotta 2001; Kareem et al., 2008). The overall antibacterial activity of \textit{C. procera} confirms the earlier studies of Kareem et al., (2008) and Nenaah and Ahmed (2011). However, it contradicts the earlier report of Mossa et al., (1991) that \textit{C. procera} extract is devoid of any antibacterial and antifungal activity.

Table 2: Antimicrobial activity (mm) of ethanolic extract of the plant samples

<table>
<thead>
<tr>
<th></th>
<th>\textit{E. coli}</th>
<th>\textit{S. typhi}</th>
<th>\textit{B. subtilis}</th>
<th>\textit{C. albicans}</th>
<th>\textit{A. niger}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Water</td>
<td>Ethanol</td>
<td>Water</td>
<td>Ethanol</td>
</tr>
<tr>
<td>1</td>
<td>\textit{C. procera} (leaf)</td>
<td>10</td>
<td>10</td>
<td>0.95</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>\textit{C. procera} (latex)</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 3: Minimum inhibitory concentration of aqueous and ethanol extract of \textit{C. procera}

<table>
<thead>
<tr>
<th>Plant samples</th>
<th>\textit{E. coli}</th>
<th>\textit{S. typhi}</th>
<th>\textit{B. subtilis}</th>
<th>\textit{C. albicans}</th>
<th>\textit{A. niger}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous Extract</td>
<td>Ethanol Extract</td>
<td>Aqueous Extract</td>
<td>Ethanol Extract</td>
<td>Aqueous Extract</td>
</tr>
<tr>
<td>\textit{C. procera} (leaf)</td>
<td>10.0</td>
<td>6.0</td>
<td>10.5</td>
<td>5.0</td>
<td>ND</td>
</tr>
<tr>
<td>\textit{C. procera} (latex)</td>
<td>7.5</td>
<td>3.0</td>
<td>5.0</td>
<td>4.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

ND = Not Detected

The overall antimicrobial activity of \textit{C. procera} latex and leaf extracts increases with increase in temperature (Figure 1 and Figure 2) with the latex extract exhibiting greater effect. Further investigations therefore need to be carried out on the best or optimal temperature upon which phytochemicals could act best against pathogenic organisms.
Fig. 1: Effect of temperature on the leaf extract of *C. procera* against test organisms

Fig. 2: Effect of temperature on the latex extract of *C. procera* against test organisms

**Conclusion**

The results of the present study have shown that the aqueous and ethanol extracts of the leaf and latex of *C. procera* inhibit the growth of some bacteria and fungi test isolates with an inhibitory ability that increases with temperature. It could therefore be inferred that the plant leaf and latex contain bioactive constituents which can effectively inhibit the growth of some microorganisms. This lends credence to the traditional use of this plant as a medicinal plant. However, further studies need to be carried out on the toxicity, antioxidant and the immunomodulatory activities of *C. procera*.

**References**


