Studies on antimicrobial effects of chromolaena odorata on pathogenic bacteria isolates

Mbata C. A.¹, Nyenke C. U.¹, Obi-Thomas J. N.², Isoma C. J.¹, Adewoye M. O.¹

¹Department of Medical Laboratory Sciences, Rivers State University of Science and Technology, Nkpolu Oroworukwo, Port Harcourt, Rivers State, Nigeria.
²Department of Microbiology, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.

*Corresponding Author: Mbata C. A
Email id: alfrrose1@yahoo.com

ABSTRACT
The study was conducted to investigate the antimicrobial effects of Chromolaena odorata leaf on some pathogenic bacteria isolates that infect humans. The bacteria isolates include: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus species. The disc diffusion method was employed to determine the inhibitory effects of the leaf extract on the tests organisms. Aqueous and ethanol extract of the leaf were used at concentration of 80g/250ml and also diluted at concentration of 1:10, 1:100, 1:1000 and 1:10000. They were impregnated into a sterile filter paper disc. Ethanol alone was also impregnated into the sterile filter paper disc and used as control. The zones of inhibition for ethanol extract (80g/250ml) of the leaf on the selected clinical isolates showed zones of inhibition of 15mm for Staphylococcus aureus, 12mm for Escherichia coli, 15mm for Pseudomonas aeruginosa and 15mm for Proteus Specie. The Zone of inhibition obtained for diluted ethanol extract (80g/250ml) of the leaf at 1:10 showed Staphylococcus aureus 12mm, Escherichia coli 11mm Pseudomonas aeruginosa 12mm and Proteus Spp. 8mm. With Ethanol alone the inhibition rate varied form 0.4mm – 0.8mm, and an out rightly resistant at 1:1000 and 1:10000 with only 1:100 showing inhibition of 0.5mm for Proteus alone.

There was outright resistant by all organisms with the aqueous extract of the leaf. From the investigation carried out it can be seen that Chromolaena odorata leaf is a potential antimicrobial agent and effective only when used in organic solvent at a higher concentration. Ethanol alone has shown insignificant sensitivity for the organisms under investigation.

Keywords: Chromolaena odorata, Awolowo, Inhibition, Solvent, Antimicrobial.

INTRODUCTION
Chromolaena odorata (Siam weed) is a fast-growing perennial and invasive weed native to South and Central America. It is a known Toxic weed that is widespread in many parts of the world, including Nigeria. It is a specie from the family Asteraceae. The weed goes by many common names such as devil weed, communist weed, hagonoy, Awolowo leaf etc. Away from its native range, Chromolaena odorata is an important weed in tropical areas extending from west central and Southern Africa to India, Sri Lanka, Bangladesh, Cambodia, China, Taiwan and Indonesia (Bani, 2002., Umukoro and Ashorobi, 2006 and Hung et
Chromolaena odorata is an aggressive competitor that occupies different types of lands where it forms dense strands that prevent the establishment of other flora. It is a menace in plantations and other ecosystems. It suppresses young plantations, agricultural crops and smother vegetation as it possesses allelopathic potentialities and growth inhibitors (Ambica and Jayachandra, 2003) [1]. The economic value of Chromolaena odorata is low.

However, Chromolaena odorata is a perennial shrub and it has become a serious pest in the humid tropics of South East Asia, African and Pacific Islands. It spreads rapidly in lands used for forestry, pasture and plantation crops such as rubber, coffees, coconut, cocoa and cashew. The plant can be poisonous to livestock as it has exceptionally high level of nitrate in the leaves and young shoots; the cattle feeding on these die of tissue anoxia (Sajise et al; 2005) [2].

The plant of Chromolaena odorata is hairy and glandular and the leaves give off a pungent, aromatic odour when crushed (Lalith, 2009). The seed of the plant regenerate from the roots and under favourable conditions the plant can grow more than 3cm a day (Lalith, 2009) [7].

Chromolaena odorata is sometimes grown as a medicinal and ornamental plant. It is used as a traditional medicine in Indonesia. The young leaves are crushed, and the resulting liquid used to treat skin wounds (Iwu, 2000) [6]. It is also used as anti-diarrhea, anti-plasmatic, anti-hypertensive, anti-inflammatory, diuretic tonic, anti-pyretic and heart tonic (Vital and Windell, 2009) [11]. In some countries the fresh leaf extract are use for treatment of burns, soft tissue wounds and skin infections. (Ayyanaf and Ignacinuthu 2009) [5]. In Southern parts of Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding as an anticoagulant. The juice of the crushed leaves is applied to cuts to arrest bleeding: According to Iwu (2000) [6]. The macerated leaves are usually applied to swollen part of the body to relieve inflammation amongst the rural population in Southern parts of Nigeria.

The Micro-organisms under investigation against Chromolaena odorata namely, Staphylococcus aureus, Esherichia coli Pseudomonal aeruginosa and Proteus Spp are potential human pathogens. These organisms exist in nature and have posed serious threat to man. The aim of the study is to determine the antimicrobial activity of Chromolaena odorata leaf on common pathogenic bacteria that exist in nature.

MATERIALS AND METHODS

Study Area

The study was carried out in the Diagnostic Laboratory of Braithwaite Memorial Hospital Port Harcourt.

Plant Collection

The leaves of Chromolaena odorata were collected from Rivers State University Farm. The leaves were cleansed and dried in hot air oven at 100ºc for 2 weeks. The leaves were ground into fine particles with mortar and pestle. About 80gram of the grinded powder was dispensed into 250ml of 70% ethanol and allowed to ripen for 7 days at room temperate. Also 80g of the grinded powder was dispensed into 250ml of water and allowed to stand.

Test Organisms

Bacterial Isolates obtained from clinical samples such as urine, wound swabs, high vagina swab and stool samples were obtained and identified by Grams and Biochemical methods and stored on nutrient agar plants of 4ºC before use.

Preparation of antibiotic disc

Whatman filter paper was punched into circular shapes using a perforator and placed in a bijou bottle and sterilized at 121ºC for 15 minutes.

Dilution of extract

Nine milliliters (9mls) of ethanol and distilled water were delivered to 8 tests tubes in a row, 4 each for ethanol and water. Serial dilutions were made from the stock by transferring 1ml from 80g/250ml tube and mixed thoroughly and transferred to the next tube in the concentrations of 1:10, 1:100, 1:1000 and 1:10,000. About 0.01ml of the diluted aqueous and ethanol extract were placed on the Punched disc.

Thereafter well dried nutrient agar plates were seeded by streaking the required organisms separately throughout the entire surface of the plate. This was followed by transferring the Chromolaena odorata impregnated disc onto the surface of the inoculated nutrient agar plates with
the aid of a flamed forceps. Also an ethanol inoculated disc was also placed on the streaked plate that contains the test organism as control.
Finally the inoculated plates with the disc were incubated at 37°C. After 24 hours, plates showing clear zones of inhibition were noted and the diameter measured.

**Statistical Analysis**

Data generated was analyzed using the SPSS 11 statistical software and the Chi-Square Method. Data was expressed as percentages. A p-value of ≤ 0.05 was considered statistically significant.

**RESULT**

The study revealed anti-microbial activities of *Chromolaena odorata*. The inhibitory effect of the ethanol stock (80g/250ml) grounded extract of the leaf on the test organisms showed *Staphylococcus aureus* 15mm, *Escherichia coli* 10mm, *Pseudomonas aeruginosa* 15mm and *Proteus sp.* 15mm. The aqueous stock of leaf showed to inhibitory effect against all the organisms. Ethanol alone showed minimal (insignificant) inhibitory effect of 0.4 – 0.8mm as shown in table 1.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Zone of Inhibition (mm)</th>
<th>Ethanol Extract (Stock)</th>
<th>Diluted Ethanol Extract</th>
<th>Ethanol Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>15</td>
<td>12</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12</td>
<td>11</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>15</td>
<td>12</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>Proteus sp.</em></td>
<td>15</td>
<td>8</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Comparative antimicrobial sensitivity of extracts of leaf of *Chromolaena odorata*.

Diluted Ethanol extract showed Zones of Inhibition at 1:10 with *Staphylococcus aureus* showing 12mm, *Escherichia coli* 11mm, *Pseudomonas aeruginosa* 12mm and *Proteus species* 8mm. At 1:100 dilution only *Proteus species* showed inhibition of 0.5mm while others showed no zone of inhibition as showed in table 2 and figure 1.

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Neat 1:10(mm)</th>
<th>1:100(mm)</th>
<th>1:1000(mm)</th>
<th>1:10,000(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>15</td>
<td>12</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12</td>
<td>11</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>15</td>
<td>12</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td><em>Proteus Specie</em></td>
<td>15</td>
<td>8</td>
<td>0.5</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Table 2: Zone of Inhibition in relation to dilution
DISCUSSION

The study has revealed that *Chromolaena odorata* has anti-microbial action on Pathogenic clinical isolates. The inhibitory effect of the stock (80g/250ml) in ethanol grounded extract of *Chromolaena odorata leaf* on the entire organisms showed that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus* all exhibited high level of susceptibility with inhibitory zones ranging from 12 -15mm. The susceptibility of these organisms to the extract explains their use in the native medicine for the treatment of infections especially wound. This suggests that the extract of the plant is broad spectrum in nature. This of course correlates with the previous studies that the plant contains substances that are antimicrobial (Olukoya *et al.*, 1986) Aqueous extract of *Chromolaena odorata* showed weak activity against test organisms. This may be as a result of loss of the plants active principle when drying or the inability of the solvent to dissolve some of the active principle of the plant (Ellof, 1998). Ethanol alone showed no inhibition this has shown that it is not a good antimicrobial agent.

CONCLUSION

*Chromolaena odorata* has the potential of a good antibiotic on common bacterial isolates that cause infection to man. This has come to put to bear that antibiotics can be produced from the extract which could be beneficial to man consequently; pharmaceutical companies should avail themselves of this study and incorporate the leaf in most of the antimicrobial agents in use.

REFERENCES


[4]. Ellof, J. N. Which extract should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethno Pharmacology, 60, 1998, 1-5.


Source of Support: Nil. Conflict of Interest: None declared.