# ACTIVITY OF *CHROMOLAENA ODORATA* ON ENTERIC AND SUPERFICIAL ETIOLOGIC BACTERIAL AGENTS

***LOVET T. KIGIGHA, [1] AND DOUYE VICTOR ZIGE [1]**

[1] DEPARTMENT OF BIOLOGICAL SCIENCES
NIGER DELTA UNIVERSITY WILBERFORCE ISLAND,
PMB 71, YENAGOA, BAYELSA STATE, NIGERIA
***CORRESPONDENCE** AUTHOR: LOVET T. KIGIGHA
EMAIL: *lovet_k@yahoo.com*
P.O. Box 578, YENAGOA, NIGERIA

## ABSTRACT

Ethanol and expressed extracts of the leaves of the plant *Chromolaena odorata* were prepared for testing their antibacterial activity using two hospital bacterial isolates viz. *Escherichia coli* and *Staphylococcus aureus* as indicator organisms representing the enteric and superficial pathogenic bacteria respectively. The broad-spectrum antibiotic Ampiclox was used in a 1: 1 (i.e. 1%) dilution to provide approximate bases for comparison of the antibacterial activity of the leave extracts. Results after 72h at ambient temperature for *E.coli* indicated that among the treatment groups, the antibacterial activity of 1% Ampiclox control was significantly higher than the other treatments (P = 0.001) ranging from 26.19 (± 2.77) to 9.43 (± 3.64) $mm^2$ while the expressed extract was least. Tests using *S.aureus* however showed that the ethanol extract had the highest inhibitory zone while the Ampiclox control was least, ranging from 10.47 (± 3.76) to 6.28 (± 1.82) $mm^2$. The use of the extracts of *C. odorata* in traditional medicine for superficial disease cases, such as wounds, burns, pile, sore throat etc. appear to have been justified. However, indications for enteric bacterial illnesses appear to be very low; in fact, the plant has been shown to have nutritional importance.

**Keywords:** Chromolaena odorata, susceptibility testing, antimicrobial herbs, Escherichia coli, Staphylococcus aureus, traditional medicine

{**Citation:** Lovet T. Kigigha, Douye Victor Zige. Activity of *Chromolaena Odorata* on enteric and superficial etiologic bacterial agents. American Journal of Research Communication, 2013, 1(11): 266-276} www.usa-journals.com, ISSN: 2325-4076.

## 1.0 INTRODUCTION

1.1 *Chromolaena odorata*

*Chromolaena odorata* King and Rob. (Syn. *Eupatorium odoratum* Linn.) is a known toxic weed that is widespread over many parts of the world including Nigeria. *Chromolaena odorata* is a species from the family of *Asteraceae*. The weed goes by many common names including Siam weed, devil weed, French weed, communist weed, hagonoy, co hoy etc. The native range of *Chromolaena* is in the Americas, extending from Florida (USA) to Northern Argentina. Away from its native range, *Chromolaena* is an important weed in tropical and subtropical areas extending from west, central and southern Africa to India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, southern China, Taiwan, Indonesia etc (Bani, 2002; Umukoro and Ashorobi,2006; Hung et al, 2011). Nevertheless, the plant has also been incriminated in the illness and death of cattle and goats in Karnataka, India.

This weed was probably introduced into Nigeria about 50 years ago and found along road-sides, waste and fallow lands. *Chromolaena odorata* was first identified in Central America and Vietnam and formerly called *Eupatorium odorata*. It is a diffused scrambling

shrub that is mainly a weed of plantation crops and pasture of southern Asia and West Africa. It forms a bush 3-7 metre in height when growing in the open (Nyananyo, 2006).

1.2 *Traditional uses of Chromolaena odorata*

The plant is locally called "*bienqua*" among the Ijaws in the Niger Delta region of Nigeria where it is believed to posses healing potentials for wounds and treatment of pile ailment (Egunjobi, 1969). A decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria. Other traditional medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic (Vital and Windell, 2009; Suksamrarn et al, 2004).

The fresh leaves and extract of *C. odorata* are a traditional herbal treatment in some developing countries for burns, soft tissue wounds and skin infections. A formulation prepared from the aqueous extract of the leaves has been licensed for clinical use in Vietnam (Ayyanar and Ignacimuthu 2009). In Nigeria the local use of the leaf extracts of *C. odorata* for sore throat and treatment of pile, burns and wounds have been documented (Egunjobi, 1969).

1.3 *Phytochemicals and chemotherapy*

From the petroleum ether and methanol extracts of *Chromolaena odorata* leaves the following were identified: steroids, triterpenes, alkaloids, flavonoids, tannins, diterpenes, and saponins. From the chloroform extract: steroids, alkaloids, flavonoids, saponins, tannins, and glycosides. The observed toxicity of *Chromolaena odorata* was probably due to the relative presence of the different toxic phytochemicals (Che Man, Nuha 2010; Prasad et al, 2005).

The clinical effects of *Chromolaena odorata* in which wound healing occurred, was attributed to the proliferation of fibroblast and endothelial cells Toan- Thang *et al* (2001). The polyphenolic extracts of the leaves showed antimicrobial activity against *Staphylococcus aureus* and *S. epidermidis* (Nurul et al 2006). In the work by Ngono *et al* (2006), ethanol extracts of *Chromolaena odorata* inhibited the *in vitro* growth of *Cryptococcus neoformans*, *Microsporium gypseum*, *Trichophyton*, *Mentogrophyles* and *Trichophyton rubrum.* While Okigbo and Ajalie (2005) had reported that leave extracts were inhibitory against four human pathogens viz. *Bacillus cereus*, *Staphylococcus aureus, E coli* and *Salmonella typhi*.

This study was undertaken to determine the antibacterial activity of *Chromolaena odorata* using various extraction methods on two human pathogens (viz. *Escherichia coli* and *Staphylococcus aureus* as indicators for enteric and superficial disease causing agents respectively). Ampiclox (a broad spectrum antibiotic) was used as control in order to compare the effectiveness of the herb especially in traditional medical practices.

### 1.4. *Nutritional value of Chromolaena odorata*

Analysis of the leaves of *Chromolaena odorata* by Nwinuka et al, 2009, indicated that the leaves contained Carbohydrate (1.10±1.14%), Protein (24.08±0.08%), Lipid (14.00±0.01%), Fiber (50.26±0.01%), Ash (10.98± 2.00%) and Moisture content of 5.65±0.02%. An energy content of 220.20 kcal was recorded. The leaves also constituted a rich source of mineral elements such as Ca, Na, K, Fe, Mn, Zn, Cu, P, and Mg.

### 1.5. *Antimicrobial susceptibility testing*

Antimicrobial screening is a process of evaluating substances that act against clinical isolates. Many techniques have been introduced in determining antimicrobial susceptibilities.

However, the two most commonly used screening methods are the broth dilution assay and the disk or agar well diffusion assay. (NCCLS, 2000; Prescott, 2006). In this study the Agar Diffusion technique was modified in which extract impregnated filter paper discs were used (Kigigha and Atuzie, 2012).

## 2.0 MATERIALS AND METHODS

### 2.1 *Sample collection*

The Niger Delta region which is rich in luxuriant growth of *Chromolaena odorata*, is located in Bayelsa State of Nigeria which is in the epicentre of the region. Green leaves of *Chromolaena odorata* were collected from the Niger Delta University Wilberforce Island, Amassoma, Bayelsa State. The plant was identified using outlines and pictures of medicinal plants from Nigeria by Nyananyo (2006).

### 2.3 *Preparation of extracts*

One hundred grams of Fresh leaves of *C. odorata* were weighed and rinsed three times in distilled and deionised water and ground into paste in a sterilized porcelain mortar. The ground sample was squeezed using sterilized muslin cloth and the expressed fluid decanted into a sterile conical flask and stored in a refrigerator at $5^0$C for use. A second batch of ground 100 g of fresh leaves was ground and the paste was soaked in 100 ml of 80% ethanol in a separating funnel (in a cold room at $5^0$C) and filtered. The ethanol extract was also stored in the refrigerator at $5^0$C until required for use. The extracts were not concentrated by heating as treatments were designed to be compared with the traditional procedure (Kigigha and Atuzie, 2012).

2.4 *Test bacterial isolates*

*Staphylococcus aureus* and *Escherichia coli* which were isolated from hospital patients were obtained from the Medical Laboratory of the University of Port Harcourt Teaching Hospital (U. P. T. H) in Rivers state, Nigeria. Subculture was made in Nutrient Agar three times to purify the test bacterial isolates and each was maintained on nutrient agar slant at $5^0$C in a refrigerator. The isolates were then characterized with respect to their identity and Antibiotic susceptibility pattern.

2.5 *Impregnation of extracts and Ampiclox on filter paper discs*

The broad spectrum antibiotic (Ampiclox) was used as control to provide bases of comparison for the treatment groups. 500 mg of Ampiclox capsule was poured out and dissolved in 500 ml of distilled and deionised water in a conical flask to give a 1:1 dilution (i.e. 1 mg $ml^{-1}$ concentration). From this, 0.1 ml was used to impregnate in triplicates, sterilized paper discs (which were cut with a 10 mm borer). Also in triplicates, 0.1 ml of each of the extracts were impregnated on filter paper discs; all the discs were sun-dried and kept dry in separate Petri dishes in desiccators.

2.6 *Antibacterial susceptibility testing*

The ability of the leave extracts and Ampiclox control to inhibit the growth of the test bacterial species was determined using the Agar Diffusion Disk Technique. Nutrient agar plates were inoculated with each test organism in a confluent-growth using sterile swab. For each extract and the Ampiclox extract, two plates (one plate for each of the test organisms) were prepared. The impregnated discs were well spaced on the confluent-growth of the test organisms to prevent their zones of inhibition from over lapping. The inoculated nutrient agar

plates were allowed to dry for 10 min at room temperature in a fume chamber (~ 32$^{o}$ C) and then incubated for 72h at 37$^{o}$ C. The zone of inhibition was calculated as the difference between the area of the inhibition area and that of the impregnated disc (Kigigha and Atuzie, 2012). One way analysis of variance was carried out on the zones of inhibition using the Zigma Stat 32 Statistical Package.

## 3. RESULTS

Results after 72h at ambient temperature indicated that there were significant differences between the zones of inhibition of treatment groups (P = 0.001) see Fig. 1. Indication was that the Ampiclox control, had the highest zone of inhibition on *E. coli* and this was followed by the expressed extract; values ranging from 26.19 (± 2.8) to 7.33 (± 1.04) mm $^{2}$. Interestingly, inhibitory pattern for *S.aureus* however, indicated that the Ethanol extract showed the highest zone of inhibition, followed by the expressed extract while Ampiclox control was least; ranging from 10.47 (± 3.8) to 6.28 (± 1.8) mm $^{2}$.

In Table 1, the characteristics of the hospital test bacterial isolates were shown. Table 2 showed the antibiotic susceptibility patterns in which *E. coli* showed resistance to Gentamycin (GN), Tetracycline (TE), Norfloxacin (NB), Amoxycillin AX) and Cefuroxoine (CF); *S.aureus* was resistant to Clindamycin (CD), Cephaploxin (CX) and Cotrimozole (CW). Both *E. coli* and *S.aureus* were highly susceptible to Ciprofloxacin (CIP); while *S.aureus* showed further high susceptibility to Ofloxacin (Of), Erythromycin (E),Gentamycin (GN) and Augumentin (AU), *E. coli* was only partially resistant to Ofloxacin (Of), Ampicllin & Cloxacillin (AP) and Nitrofurantron (N).

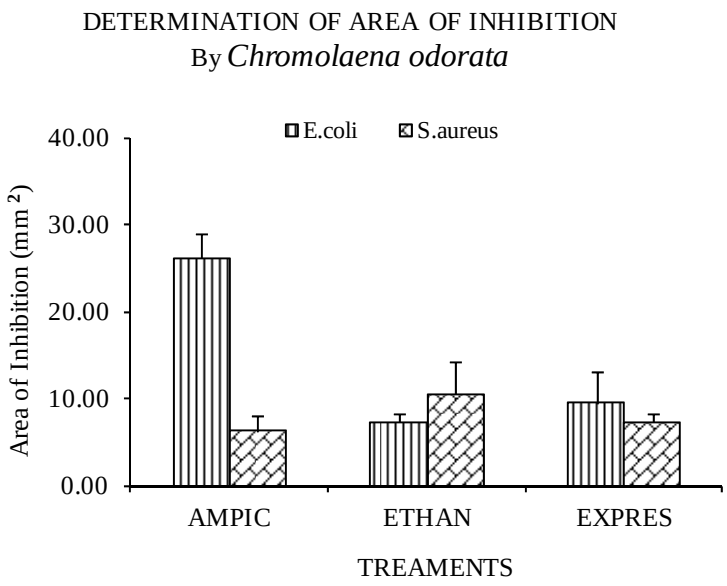


**Figure 1. The relationship between the treatments and Ampiclox on the test organisms (P = 0.001) Mean = (± SE); n = 3.**

**Table 1: Morphological and Biochemical Characterization of Isolates**

| Tests & assessments | *Staphylococcus aureus* | *Escherichia coli* |
|---|---|---|
| Colony morphology | cocci | rod |
| Grams' Reaction | + ve | -ve |
| Growth MSA | + ve | NT |
| Coagulase (Tube & Slide) | + ve | NT |
| Catalase activity | + ve | -ve |
| Sugar utilization | | |
| Lactose | NT | AG |
| Mannose | NT | AG |
| Glucose | NT | AG |
| Sucrose | NT | AG |
| EMB | NT | AG |
| CLED | NT | AG |

+ve = Positive; -ve = negative; NT = not tested; EMB = Eosin Methylene Blue; C.L.E.D = Cystine Lactose Electrolyte Deficient; AG = acid and gas.

**Table 2: Antibiotic susceptibility test results on bacterial Isolates**

| Antibiotics | *S.aureus* | *E.coli* |
|---|---|---|
| Ofloxacin (Of) | +++ | ++ |
| Erythromycin (E) | +++ | NT |
| Ciprofloxacin (CIP) | +++ | +++ |
| Clindamycin (CD) | R | NT |
| Gentamycin (GN) | +++ | R |
| Cephaploxin (CX) | R | NT |
| Cotrimozole (CW) | R | NT |
| Ampicllin & Cloxacillin (AP) | ++ | ++ |
| Floxapen (FX) | + | NT |
| Augumentin (AU) | +++ | NT |
| Nitrofurantron (N) | NT | ++ |
| Tetracycline (TE) | NT | R |
| Norfloxacin (NB) | NT | R |
| Amoxycillin AX) | NT | R |
| Chloramphenicol (C) | NT | + |
| Cefuroxoine (CF) | NT | R |

R = resistant; NT = not tested; += Low, ++ = high; +++, = very high degree of susceptibility.

## 4.0 DISCUSION

The local use of the leaf extracts of *C. odorata* for sore throat and treatment of pile, burns and wounds have been indicated (Egunjobi, 1969). There are many factors that could influence the potency of medicinal plants, these include the age of plant, extracting solvent, method of extraction and even the time of harvesting the plant materials ((Qasem and Abu-Blan (1996), Okigbo and Okigbo and Emoghene (2004)). The present study however appear to indicate that the expressed and ethanol extracts were significantly less inhibitory on *E.coli* when compared with 1% Ampiclox. This would imply a very low antibacterial effect against the enteric pathogenic bacteria forms. Nwinuka *et al*, 2009, also observed that the methanolic extracts of *C. odorata* showed positive inhibition of *Bacillus subtilis, Klebsiella Pneumoniae, Staphylococcus aureus,* which are associated with superficial infections and indicated a

negative inhibition for *Pseudomonas pyrogenes* and *Escherichia coli* which are enteric forms. The need to carry out further in-depth and more precise studies in order to capitalize on the possible use of *C. odorata* for some medicinal preparations is recommended.

**REFERENCES**

AKAH, P. A., GAMANIEL, K. S., SAMSON, A. AND C. O. WAMBEBE. (1997). Evaluation of Nigerian traditional medicine: effects of Gakani, an herbal anti-asthmatic drug. *J. Ethnopharmacol.* 55: 87-92.

AYYANAR M, AND IGNACIMUTHU S (2009). Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. *International Journal of Applied Research in Natural Products* 2009; 2(3): 29-42.

BANI G. Status and management of Chromolaena odorata in congo. Proceedings of the fifth international workshop on biological control and management of Chromolaena odorata, ARC-PPRI 2002; 71-73.

EGUNJOBI, J.K. (1969). Some common weeds of western Nigeria. *Bull. Res. Div. Min. Agr. Nat. Resources, Western state Nigeria.*

HUNG T M, CUONG T D, DANG N H, ZHU S, LONG P Q, KOMATSU K AND MIN B S 2011. Flavonoid Glycosides from Chromolaena odorata Leaves and Their in Vitro Cytotoxic Activity. *Chem. Pharm. Bull*. 59(1): 129-131.

NCCLS (National Committee for Clinical Laboratory Standard) (2000). Methods for Dilution antimicrobial susceptibility tests of Bacteria that grow aerobically. Approved standard M 7-A5 and informational supplement M 100-510. NCCLS Wayne, PA, U.S.A.

NURUL .A.H., MAMATI.A.S, EFFENDI A. W. M. HUSSIN., Z. M AND SAYED. M.Z.H. (2006). Extract of the leaves of *Chromolaena odorata* on gram-positive bacteria Pp51 *journal of ethnopharmacology*.

NWINUKA, N, NWILOH, B AND J ERESAMA (2009). Nutritional and potential medicinal value of chromolaena odorata leaves. *Int. J. Tropical Agric. and Food Systems* : Vol 3, No 2

NYANANYO. B.L (2006) Plants From The Niger Delta. Onyoma research publication. Port-Harcourt, Rivers State Nigeria. p85.

NGONO N, A.; EBELLE E, R.; NDIFOR, F. BIYITI, L.;AMVAM ZOLLO, P.H. AND BOUCHET, P. (2006)Antifungal Activity of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) of Cameroon *international of ethnopharmcognosy.*

OKIGBO, R.N. AND A.O. EMOGHENE. (2004).Antifungal activity of leaf extracts of some Plant species on *Mycosphaerella fijensis morelet*, the casual organism of black sigatoka disease in banana (Musa acumr acuminata KMITL. *science J.* 4.20-31.

OKIGBO, R. N AND A. N. AJALIE (2005). Inhibition of some human pathogens with tropical plants extracts (*Chromolaena odorata* and *Citrus aurantifolia* and some antibiotic). *Int. J. Mol. Med. Adv. Sc.* 1 (1) pp 34-40.

OKIGBO, R.N AND O.D. OMODAMIRO (2004). Antimicrobial effects of leaf extracts on pigeon pea (*Cajanus cajan* (L.) Mill.) on some human pathogens. J. Herbs, species medi. Plants.

PRESCOTT, L.M., J.P. HARLEY AND D.A KLEIN (1996). Microbiology. 3rd edition. McGraw Hill. New York.

QASEM, J.R. AND ABU-BLAN, H.A. (1996). Fungicidal activity of some common weed extracts against different plant pathogenic fungi; *Journal phytopathol* 144:157-161.

TOAN-THANG,. P. LINGZHI W. PATRICK S. RENEE J. G. SUI-YUNG. C AND LEE, S. T (2001). Phenolic Compounds of Chromolaena odorata Protects Cultured Skin Cells from Oxidative Skin Cells from Damage: Implication for Cutaneous Wound healing", *Boil. Pharm. Bull, vol.24, 1373-1379.*

UMUKORO S AND ASHOROBI R B 2006. Evaluation of the anti-inflammatory and membrane stabilizing effects of Eupatorium odoratum. International Journal of Pharmacology 2006; 2(5): 509-512.

Vital P G and Windell L R. 2009. Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. *Journal of Medicinal Plants Research*; 3(7): 511-518.