IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. II (Nov. - Dec.2016), PP 49-52
www.iosrjournals.org

In-Vitro Antibacterial Effect of Crude Extract of Chromolaena Odorata Leaves on Wound Isolates

[*1]Nwachukwu, Ifeanyi; [2]Aliga, Chinanu; [3]Upabi, Chibueze F. [4]Ukogo, Ifeoma
[1-2] *Department of Biology/Microbiology, Abia State Polytechnic Aba, Abia State Nigeria.*
[3]*Department of Chemistry/Biochemistry, Abia State Polytechnic Aba, Abia State Nigeria.*
[4]*School of Nat. Science, Alvan Ikoku Fed. Colleg. of Education, Owerri, Imo State Nig.*

Abstract: *Medicinal plants have played an important role in prevention and control of diseases. The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional plant as potential sources of new antimicrobial agents. This study was carried out to determine the antibacterial effect of the crude extract of Chromolaena odorata leaves on some wound isolates (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella species). The result shows that the crude extract of Chromolaena odorata leaves was effective on the isolated bacteria, however the control drug Ciprofloxacin had greater inhibition on the tested organism than the plant extracts. The zone of inhibition of crude extract of Chromolaena odorata leaves on S. aureus ranged from 7mm - 18mm, Klebsiella species ranged from 6mm - 17mm, Pseudomonas aeruginosa 5mm -15mm and Escherichia coli 5mm-13mm while the control drug (ciprofloxacin) ranged from16mm-20mm. The minimum inhibitory concentration ranged from (25.0% to 50.0%).This research work establishes a good support to the use of this plant in herbal remedies and as base for development of new drugs against wound isolates.*

Keywords: *Antibacterial effect, Chromolaena odorata, Traditional plant, Crude extract, Medicinal plants.*

I. Introduction

In human society from time immemorial, medicinal plants have played an important role in prevention and control of diseases. The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional plant as potential sources of new antimicrobial agents (Douye *et al.,* 2013). Therefore, researchers are increasingly turning their attention to complementary medicine looking for new ways to develop better drugs against microbial infections (Benkeblia, 2004). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized as secondary metabolites of plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils as well as tannin (Saxena *et al.,* 1994). Medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Daniel, 1999). One of these valuable plants is *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. This weed was probably introduced into Nigeria about 50 years ago and found along road-sides, waste and fallow lands (Kigigha and Zige, 2013). *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 an open area (Nyananyo, 2006). *Chromolaena odorata* plants are used by traditional medicine practitioners for treatment of burns, wound healing, skin infections, post-natal wounds, and malarial (Nurul *et al.*, 2004). Fresh juice squeezed out from the leaves of *Chromolaena odorata* is used to stop bleeding. The decoction of the leaves and stems are reported to be effective against the treatment of skin disease like *Propionibacterium acnes* (Chakraborty *et al*., 2010). It is also used for healing wounds, effective against KI strain of *Plasmodium* which causes malaria, possess antigonorrheal, anti-inflammatory, anthelmintic, analgesic, antioxidant and antifungal activities. *Chromolaena odorata* is also an ornamental flower (Mbajiuka *et al*., 2014).

This common plant called Siam weed is known among the Ibos of the South-Eastern Nigeria as: 'Awolowo', 'Elizabeth', 'Independence leaf', 'Enugu plantation weed' and 'bienqua' or 'inenghiqua' among the Ijaws in south-south. Traditionally, fresh leaves or a decoction of *C. odorata* have been used throughout many tropical countries for the treatment of leech bite, soft tissue wounds and liver diseases (Alisi *et al.*, 2011). Although synthetic and semi synthetic antimicrobial drugs abound in various markets today, there is a need for continuous search for new ones to cope with the increased evolution of multiple antibacterial resistant strains of

organisms especially bacteria from wound infections (Hart and Kariuki, 1998). This study is therefore aimed at determining the in-vitro antibacterial effect of *Chromolaena odorata* leaves extracts on wound isolates

Fig 1: *Chromolaena odorata* leaves King and Rob.

II. Materials And Methods

Collection and Identification of Plant Materials

Fresh leaves of *Chromolaena odorata* were collected from Umuosu Nsulu in Isialangwa North L.G.A. of Abia State South Eastern Nigeria. It was identified and authenticated by a plant taxonomist in the Department of Biology/Microbiology Abia State Polytechnic, Aba.

Extraction of Plant Materials

Fresh leaves of *C. odorata* were macerated in a mortar and pestle and then expressed by means of pressure to obtain 100ml of the undiluted crude juice. This juice was filtered with watchman N0 1 filter papper to obtain a sterile juice and stored in an air-tight bottles at 4°C.

Source of Test Organisms

Samples from wound infections were collected from patients in Abia State Teaching Hospital Aba using sterile swap stick and the samples were preserved with normal saline. Within 2 hours of collection, the samples were analyzed microbiologically. The specimens were inoculated on nutrient agar, Eosine Methylene Blue (EMB) agar and Mac-Conkey agar and incubated at 37^0C for 24 hours. After which the plates were observed for morphological characteristics and the growths were sub-cultured on fresh media for identification. Organisms were identified using Gram staining and biochemical test (catalase coagulase, Indole, citrate, oxidase, methyl red) as well as sugar fermentation test (Cheesebrough, 2008).

Antibacterial Sensitivity Testing

The antimicrobial sensitivity test was carried out using the methods modified by Isu and Onyeagba (2002). Twenty milliliter of molten sterile nutrient agar were poured into different petri dishes. After solidification overnight, cultures of bacteria were introduced into the surface of the sterile nutrient agar plate and a sterile glass spreader was used for even distribution. Holes were made aseptically with a sterile cork borer of 5 mm in diameter and 0.2 ml of different concentrations of the crude extracts were introduced into the well. The extracts were allowed to diffuse into the medium for 1 hour. The bacteria plates were incubated for 24 hours at 37°C. The plates containing the controls were incubated also. The plates were later examined for zones of inhibition, which indicated the degree of susceptibility of the test organisms.

Determination of Minimum Inhibition Concentration (MIC)

Minimum inhibitory concentration of *Chromolaena odorata* extract was determined using agar-well techniques as described by Ezeigbo *et al*., (2016). Media plates containing varying concentrations of 6.25% - 50% of the extracts respectively were incubated at 37°C for 24 hours. The lowest concentration of the various extracts that inhibited the bacteria growth was taken as the minimum inhibitory concentration (MIC).

III. Results

Table 1: Colonial, Morphological and Biochemical characteristics of the bacteria isolates

Colonial morphology	Microscopy	Gram stain	Motility	Oxidase	Citrate	Coagulase	Catalase	lactose	Glucose	Maltose	Isolated organism
Smooth and circular	short rods	-	+	-	-	-	+	AG	AG	AG	*Escherichia coli*
smooth On nutrient agar	Rods	-	-	-	+	-	+	AG	A	AG	*Klebsiella* species
Colonies round and smooth cream to white colour	Small scattered rods	-	-	+	-	-	+	A	AG	A	*Pseudomona aeruginosa*
Colony smooth and small	Cocci in cluster	+	-	-	-	+	+	A	AG	A	*Staphylococcus aureus*

Table 2: Antibacterial effect of crude extract of *Chromolaena odorata* leaves on wound isolates

Wound isolates	**Control (ciprofloxacin)**	**Concentrations (mg/ml) / zones of inhibitions (mm)**				
		100	**75**	**50**	**25**	**12.5**
Escherichia coli	18	13	10	5	nil	Nil
Staphylococcus. aureus	20	18	15	10	7	Nil
Pseudomonas aeruginosa	17	15	12	5	nil	Nil
Klebsiella species	16	17	13	10	6	Nil

Table 3*: Minimum inhibitory concentration (MIC) of Crude extract of Chromolaena odorata leaves on wound isolates*

Wound isolates	MIC (%)
Escherichia coli	50
Staphylococcus aureus	25
Pseudomonas aeruginosa	50
Klebsiella species	25

IV. Discussion

The result of this analysis showed that the leaves of *Chromolaena odorata* crude extract exhibited potential activity against the test organisms with the zones of inhibition ranging between 5.0mm -18.0mm (Table 1). This study revealed that leaves of *Chromolaena odorata* crude extract has antibacterial effect on the wound isolates tested. This result is in agreement with the findings of Nurul *et al.*, (2004); Douye *et al.*, (2013); Mbajiuka *et al*., (2014) and Srisuda *et al*., (2016). The findings also correlate with the work of Olukoya (1986) which shows that plants contain substances that are antimicrobial. Also recent findings by Kigigha and Zige (2013) on the antibacterial efficacy of *C. odorata* against *E. coli* correspond with this finding.

The wound isolates tested were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* sp. The plant extract was more effective on *S. aureus* (7mm - 18mm) followed by *Klebsiella* (6mm - 17mm) and the least was *Escherichia coli* (5mm-13mm). However, the control drug (ciprofloxacin) exhibited greater zones of inhibition than the plant extract (16mm-20mm). The minimum inhibitory concentration (MIC) of crude extract of *Chromolaena odorata* leaves on the test organisms is shown on Table 2. The result of minimum inhibitory concentration (MIC) ranged from 25-50%. The minimum inhibitory concentrations (MIC) of *Staphylococcus aureus* and *Klebsiella* species were 25% respectively while *Pseudomonas* and *Escherichia coli* were 50% respectively.

The susceptibility of *Chromolaena odorata* leaves extracts to these wound isolates explains their use in native medicine for the treatment of wound infection and other bacterial infections. The Gram positive bacterial (*S. aureus)* was more sensitive to the crude extracts of *C. odorata* than the Gram negative bacterial (*E. coli, Pseudomonas aeruginosa* and *Klebsiella* specie). This agrees with the observation made by some researchers that plant extracts show considerable activity against Gram positive bacteria than Gram negative bacteria (Nostro *et al.*, 2000).

The use of plant extracts to treat diseases has stood the test of time (Anwannil and Atta, 2006). According to Suck (1989), more than 75% pure compounds derived from higher plants are used in modern medicine and *C. odorata* is well known in complementary medical practice in treatment of several ailments.

V. Conclusion

This study has shown that *C. odorata* is a potential source of new drug for treating wound infections and other clinical pathogens. The anti-bacterial activity of the extract could be enhanced if the components are purified. The study also indicates that leaf extracts of *Chromolaena odorata* possesses antibacterial activity which can be explored further in the field of human medicine. Therefore further research should be carried out to know the effect of different solvents in extracting the bioactive constituent of *C. odorata*.

References

[1]. Alisi, C. S., Nwaogu, L. A., Ibegbulem, C. O. and Ujowundu, C. U. (2011). Antimicrobial Action of Methanol Extract of *Chromolaena Odorata*-Linn is Logistic and Exerted by Inhibition of Dehydrogenase Enzymes. *Journal of research in Biology*, 3: 209-216.

[2]. Anwannil, H. G. and Atta, R. (2006). Trends in ethnopharmacology. *J. Ethnopharmacol*, 100: 43 – 49

[3]. Azoro, C. (2000). Antibacterial activity of crude extract of Azadirachita indica on *Salmonella typhi*. *World Journal of Biotechnology*, 3:347-351

[4]. Bamba, D., Bessiere, J. M., Marion, L., Pelissier, Y. and Fouraste, I. (1993). Essential oil of *Eupatorium odoratum*. *Plant Med.*, 59: 184-185.

[5]. Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and Garlic (*Allium sativum*). *Lebensmwiss u-Technol*., 37: 263-268.

[6]. Cheesbrough, M. (2008).District Laboratory practices in tropical Countries. Volume 2. Cambridge University Press, UK. Pp. 35-70

[7]. Daniel, M. (1999). Impediments preventing India becoming a herbal giant. *Curr.Sci*., 87: 275-276.

[8]. Ezeigbo, O. R., Awomukwu, D. A. and Ezeigbo, I. C. (2016).The Antimicrobial and Phytochemical Analysis of the Leaves of *Aspilia africana* on Clinical Isolates. *European Journal of Medicinal Plants*, 15(2): 1-6.

[9]. Isu, R. N. and Onyeagba, R.A. (2002). Basic principles in Microbiology 2nd Ed. Fasm Communication, Okigwe. Pp 134-143.

[10]. Kigigha, L. T. and Zige, D. V. (2013). Activity of *Chromolaena odorata* on enteric and superficial etiologic bacterial agents, *American Journal of Research Communication*., 1(11): 266-276.

[11]. Mbajiuka, C. S., Obeagu, E. I., Chude, C. N. and I. E. (2014). Antimicrobial effects of *Chromolaena odorata* on some human pathogens *Int.J.Curr.Microbiol.App.Sci*, 3(3): 1006-1012.

[12]. Nostro, A., Germane, M. P., Angelo, V. and Marino, A. (2000). Extraction methods, and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl. Microbiol*, 30: 379-384.

[13]. Nurul, H. A. K., Mamat, A. S., Effendy, A. W. M., Hussin, Z. M., Iskandar, C. T. N. F., Hassan, L., Dhaliwal, G. K., Yusoff, R., Omar, A. R. and Khan, M. A. K. G. (2004). The antimicrobial effect of *Chromolaena odorata* extract on Grampositive bacteria. Animal health: a breakpoint in economic development? The 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress, 23-27 August 2004, Petaling Jaya, Malaysia. pp. 342-343

[14]. Oyagade, J. O., Awwotoye, I. T., Adewunmi, A. and Thorpe, H. T. (1999).Antimicrobial activity of some Nigerian medicinal plants: Screening for antibacterial activity. *Bio. Res. Comm.*, 11(3):183-197.

[15]. Saxena, G., McCutcheon, A. R., Farmer, S., Towers, G. H. N. and Hancock, R. E. W. (1994). Antimicrobial constituents of *Rhus glabra*. *J. Ethanol. Pharm.*, 42: 95-99.

[16]. Srisuda, H. S. T., Piyaporn, W., Niwat, K. and Sukhumaporn, K. (2016). Antimicrobial Activity of *Chromolaena odorata* Extracts against Bacterial Human Skin Infection. *Modern Applied Science*, 10 (2): 159-171.

[17]. Suck, D. (1989). Higher plants as a source of drugs. 2nd edition, Macmillan publishing Company limited, London. Pp. 15 – 65.