

## ANTIMICROBIAL EFFECTS OF THE LEAF EXTRACTS OF *CHROMOLAENA ORDORATA* (SIAM WEEDS) ON SOME HUMAN PATHOGENS

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### ABSTRACT

Antimicrobial effects of *Chromolaena odorata* (commonly called Siam weed), were tested on *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Different solvents such as methanol and chloroform were used for the leaf extraction. Test organism *Pseudomonas aeruginosa* and *Salmonella typhi* were susceptible to methanol extracts of *Chromolaena odorata* at concentration of 25, 50 and 100mg/ml with zone of inhibition of 15mm, 19mm and 22mm respectively for *Salmonella typhi* and 29mm, 32mm and 36mm for *Pseudomonas aeruginosa*, while the chloroform extract produced similar concentration of 18mm, 20mm and 22mm for *Salmonella typhi* and 19mm, 20mm and 21mm for *Pseudomonas aeruginosa*. The Minimum Inhibitory Concentration (MIC) of

methanol extract against *Salmonella typhi* and *Pseudomonas aeruginosa* were 12.5mg/ml and 3.12mg/ml respectively. The MIC of chloroform extract against *Salmonella typhi* and *Pseudomonas aeruginosa* were 6.25mg/ml and 6.25mg/ml. For the pathogen *Staphylococcus aureus* and *Candida albicans*, no MIC was carried out because there was no zone of inhibition seen on the plate with 100% concentration. This justifies the therapeutic use of *Chromolaena odorata*. This research work establishes a good support to the use of these plants in herbal remedies and for the development of new drugs.

**KEYWORDS:** Antimicrobial, Human pathogens. *Chromolaena odorata*, *Methanol*, *Chloroform*.

## INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al.*, 2000). Plants for decades have been a valuable source of natural products for maintaining human health, especially within -depth investigation for their natural therapeutic potentials. According to Santos, *et al.*, (1995) several varieties of drugs can be derived from medicinal plants. There is a continuous and urgent need to discover new antimicrobial compounds with diverse mechanisms of action and chemical structures that can be used against novel and re-emerging infectious diseases (Rojas, *et al.*, 2003). 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. 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 (Jansen, *et al.*, 1987) 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). Secondary metabolites (also called specialized metabolites) is a term for pathways and small molecule products of metabolism that are not absolutely required for the survival of the organism, many of which are antibiotics and pigments. Plants synthesize varieties of phytochemicals such as alkaloids, phenolics, terpenoids, glycosides etc.

*Chromolaena odorata* (Siam weeds) 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, cohoy 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 (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 according to (Nyananyo, 2006). 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 (Hung, *et al.*, 2011). A Concoction 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). The fresh leaves and extract of *Chromolaena. 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 *Chromolaena odorata* for sore throat and treatment of pile, burns and wounds have been documented (Anwanni and Atta, 2006). This research is aimed at determining the inhibitory activities of the extracts against *S.aureus*, *P. aeruginosa* *C. albicans* and *S. typhi*.

## MATERIALS AND METHODS

### Materials

All equipments, reagents and materials used for this project were obtained from BMD Laboratories Ahiake, Abia State, except the leaf *Chromolaena odorata* (siam weed) which was gotten from the forest reserve of National Root Crops Research Institute, Abia State, Nigeria.

### Sample Collection

Fresh leaves of *Chromolaena odorata* collected from the forest reserve of National Root Crops Research Institute, Abia State Nigeria. The leaves were collected in September, 2014 during the rainy season. The plant was identified using outlines and pictures of medicinal plants from Nigeria by Nyananyo (2006). They were confirmed and authenticated by Dr. I.C. Okwulehie of department of plant science and biotechnology, Michael Okpara University of Agriculture, Umudike Abia State, Nigeria. They were sorted to removed debris and later sun dried and stored to be used later.

**Preparation of Extracts**

One hundred grams (100g) of Fresh leaves of *C. odorata* was weighed and ground into powdery form using an electric blender. The ground extract were sieved to get a uniform powder that is very smooth.

**Methanol Extract Preparation**

50 grams of dried plant material was extracted with 300ml of methanol. It was kept for 24 hours and was occasionally shaken vigorously. After 24 hours, the sample was filtered using whatman No.1 filter paper and the filtrate was evaporated using a laboratory water bath at 60°C. The extract obtained was weighed and stored in airtight bottles and kept inside a refrigerator at 4°C until when needed.

**Chloroform Extract Preparation**

About 50 grams of the powder was extracted with 300mls of distilled water in a conical flask. It was kept for 24 hours and was occasionally shaken vigorously. After 24 hours, the sample was filtered using whatman No.1 filter paper and the filtrate was evaporated using a laboratory water bath at 60°C. The extract obtained was weighed and stored in airtight bottles and kept inside a refrigerator at 4°C until when needed.

**Preparation of Stock Solution of Extract**

This was prepared by dissolving 0.2g (200mg) of the extract in 2ml of DMSO to give 100mg/ml of stock solution. Further dilutions were made by transferring 0.2ml of the stock solution (100mg/ml) and diluted in 2ml of DMSO to yield 50mg/ml and 25mg/ml.

**Media Used**

The media used includes; Nutrient agar (NA) for isolation of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, and Sabouraud Dextrose Agar for isolation of *Candida albicans* Mueller-Hinton Agar (MHA) and Mueller Hinton Broth were used for agar discs diffusion for diameter zone of inhibition and minimum inhibitory concentration respectively.

**Media Preparation**

The media used for the isolation of Nutrient agar, Mueller Hinton Agar and Mueller Hinton Broth were prepared according to the manufacturer's direction and specification. They were

sterilized by autoclaving at 121°C at 15 psi for 15 minutes. They were dispensed into already labeled sterile petri plates.

### Collection of Test Bacterial Isolates

*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans* were obtained from stock cultures in Beacon & Guide Diagnostics Ltd (BMD Laboratories) Ahiaeke, Abia State Nigeria.

### Preparation of Paper Disc

Whatman No. 1 filter paper was cut into circular disc using a perforator, giving a diameter of 6mm. The disc was sterilized in glass petri dish in hot air oven at 140°C for 1 hour. 20 µl containing 250 µg, 500µg and 1000µg of the extracts were each impregnated on the discs. The paper disc were dried in hot air oven at 50°C and stored in inoculating chamber in a cool dry place.

### Paper Disc Diffusion Methods

Some colonies of test organisms isolated on Nutrient Agar plate were picked and suspended in sterile saline and adjusted to same turbidity to Macfarland turbidity standard tube No. 0.5. Using micropipette, 20µl of the suspension was dispensed into the Mueller Hinton Agar. It was spread about the agar surface using a swab stick to ensure effective spread. The moisture was allowed to disappear from agar surface before dropping test (containing *C. odorata* extracts) disc into agar. After ensuring that disc was firmly planted on the agar, it was incubated at 37°C for 24 hours.

### Determination of Minimum Inhibitory Concentration

This was carried out using the broth dilution method. Stock solution of extracts (100 mg/ml) was made by dissolving 0.2g of the extracts in 2ml of DMSO. 1ml of the stock was diluted in 0.95ml of Muller Hinton broth to get a concentration of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml and 1.56mg/ml, and then serial dilutions were carried out to obtain further dilutions of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml, 1.56mg/ml, and 0.78mg/ml. A suspension of the test organisms move from a sterile saline adjusted to same turbidity as Macfarland standard tube No. 0.5, was dispensed using micropipette into each of the tubes. A volume of 50µl (0.05ml) was dispensed. The tubes were incubated for 24 hours at 37°C.

## RESULTS

*Chromolaena odorata* was tested against four (4) organisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans*) using the disc agar diffusion test. Zone of inhibition (mm) of methanol plant extract against the test organisms in the disc agar diffusion test is shown in Table 1. Zone of inhibition (mm) of chloroform plant extract against the test organisms in the disc agar diffusion test is shown in Table 2. Zone of inhibition (mm) produced by the antibiotics used as controls in the disc agar diffusion test is shown in Table 3. The minimum inhibitory concentration of methanol plant extract against susceptible organism is shown in Table 4. The minimum inhibitory concentration of chloroform plant extract against susceptible organism is shown in Table 5 at various concentration of the extract.

**Table 1: Zone Of Inhibition (mm) Of Methanol Extract Of The Test Organisms.**

PATHOGENS	CONCENTRATIONS OF EXTRACT (mg/ml)		
	25	50	100
<i>Staphylococcus aureus</i>	0.0	0.0	0.0
<i>Salmonella typhi</i>	12.5	15	18.5
<i>Pseudomonas aeruginosa</i>	19	25	30
<i>Candida albicans</i>	0.0	0.0	0.0

Note: Zone of inhibition = (mm)

Concentration = (mg/ml)

**Table 2: Zone Of Inhibition (mm) Of Chloroform Extract of The Test Organisms.**

PATHOGENS	CONCENTRATIONS OF EXTRACT(mg/ml)		
	25	50	100
<i>Staphylococcus aureus</i>	0.0	0.0	0.0
<i>Salmonella typhi</i>	16	18.5	21
<i>Pseudomonas aeruginosa</i>	16	18.5	20.5
<i>Candida albicans</i>	0.0	0.0	0.0

Note: Zone of inhibition = (mm)

Concentration = (mg/ml)

**Table 3: Zone Of Inhibition (mm) Produced By The Antibiotics Used As Controls**

<b>PATHOGENS</b>	<b>GENTAMYCIN (mm)</b>	<b>CHLORAMFENICOL (mm)</b>
<i>Staphylococcus aureus</i>	20	39
<i>Salmonella typhi</i>	32	37
<i>Pseudomonas aeruginosa</i>	38	49
<i>Candida albicans</i>	32	0.0

**Table 4: Minimum Inhibitory Concentration Of Methanol Plant Extract Against Susceptible Organisms.**

<b>Pathogens</b>	<b>100</b>	<b>50</b>	<b>25 (mg/ml)</b>	<b>12.5</b>	<b>6.25 (mg/ml)</b>	<b>3.12</b>	<b>1.56</b>	<b>MIC (methanol)</b>
<i>Staphylococcus aureus</i>	–	–	–	–	–	–	–	–
<i>Salmonella typhi</i>	22	19	15	13	–	–	–	12.5
<i>Pseudomonas aeruginosa</i>	36	32	29	22	16	13	–	3.12
<i>Candida albicans</i>	–	–	–	–	–	–	–	–

Note: Zone of inhibition = (mm)

Concentration = (mg/ml)

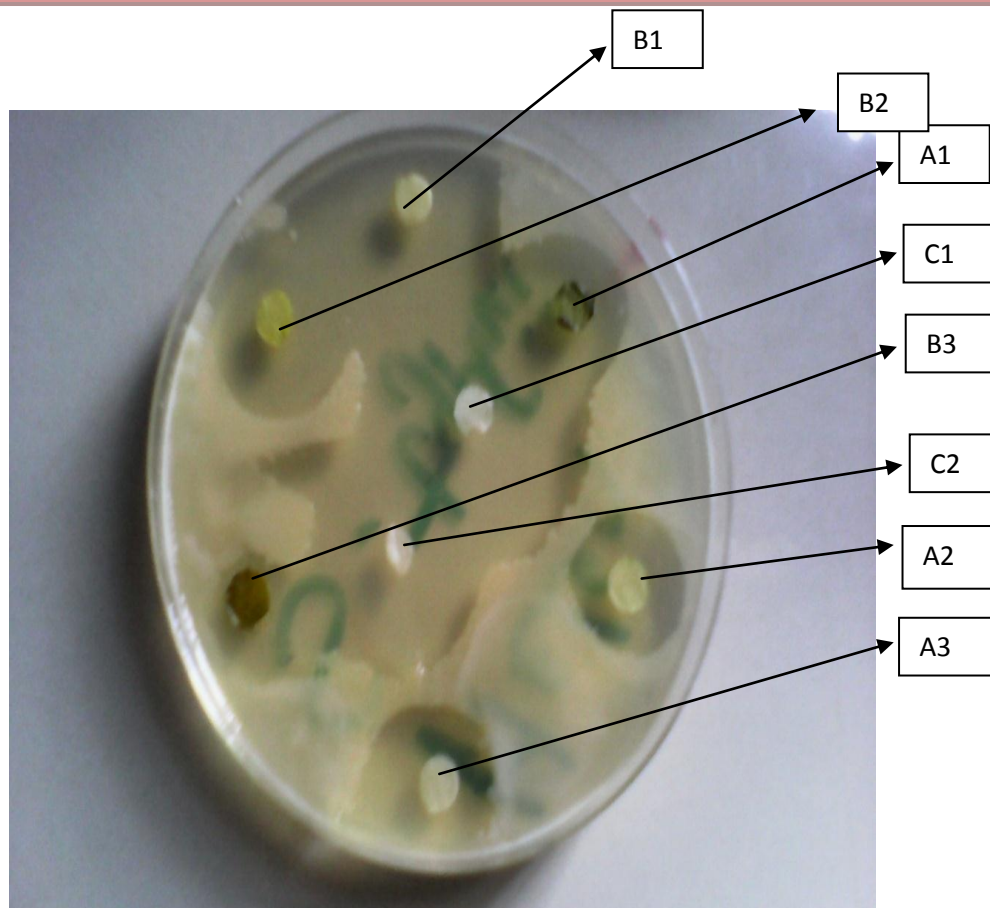
**Table 5: The Minimum Inhibitory Concentration Of Chloroform Plant Extract Against Susceptible Organisms.**

<b>Pathogens</b>	<b>100</b>	<b>50</b>	<b>25 (mg/ml)</b>	<b>12.5</b>	<b>6.25 (mg/ml)</b>	<b>3.12</b>	<b>1.56</b>	<b>MIC (chloroform)</b>
<i>Staphylococcus aureus</i>	–	–	–	–	–	–	–	–
<i>Salmonella typhi</i>	24	20	18	16	12	–	–	6.25
<i>Pseudomonas aeruginosa</i>	22	20	19	17	15	–	–	6.25
<i>Candida albicans</i>	–	–	–	–	–	–	–	–

Note: Zone of inhibition = (mm)

Concentration = (mg/ml)



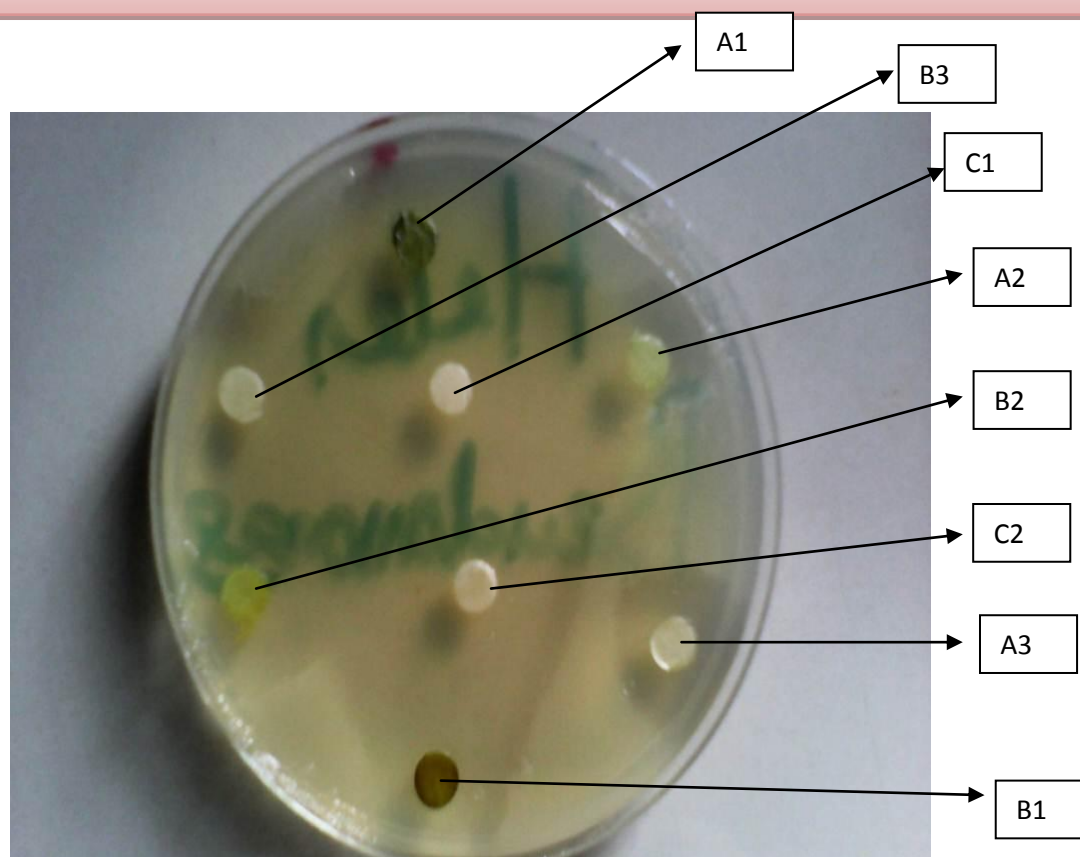


**PLATE 1: Zone of Inhibition (Mm) of the *Methanol and Chloroform extract of Chromolaena odorata* On *Salmonella typhi*.**

#### NOTE

- A1 = Methanol 100%
- A2 = Methanol 50%
- A3 = Methanol 25%
- B1 = Chloroform 100%
- B2 = Chloroform 50 %
- B3 = Chloroform 25%
- C1 = Chloramphenicol%
- C2 = Gentamycin%





**PLATE 2: Zone of Inhibition (Mm) of the Methanol and Chloroform extract of *Chromolaena odorata* On *Pseudomonas aeruginosa*.**

#### NOTE

- A1 = Methanol 100 %
- A2 = Methanol 50%
- A3 = Methanol 25%
- B1 = Chloroform 100%
- B2 = Chloroform 50%
- B3 = Chloroform 25%
- C1 = Chloramphenicol%
- C2 = Gentamycin. %

#### DISCUSSION

The local use of the leaf extracts of *Chromolaena odorata* for sore throat and treatment of pile, burns, skin infection, diabetes, malaria, rheumatism, insomnia and wound dressing have been indicated. It grows widely in Nigeria especially in the south and west. Likewise in other parts of West Africa. 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 *Chromolaena odorata* is well known in complementary medical practice in treatment of several ailments. For an antibiotic agent to be considered as safe for humans, it must have the ability to destroy pathogen, while relatively non-toxic, chemically stable and be able to reach the part of host organism in which infection persists. The methanol extracts contain alkaloids, coumarins and tannins. Coumarins and tannins have antibacterial and antihelminthic properties (Hedberg *et al.*, 1983).

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 Emoghene (2004). The methanol and chloroform extracts of *Chromolaena odorata* showed positive inhibition of *Salmonella typhi*, *Pseudomonas aeruginosa* and indicated a negative inhibition for *Staphylococcus aureus*, and *Candida albicans*.

## CONCLUSION

The results obtained with these plant extracts continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic population in the treatment of diseases caused by pathogenic bacteria. Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. There is need for further exploration of plant-derived antimicrobials. Also further studies will be needed to purify the bioactive compounds of the methanol and chloroform plants extract, and their mode of action should be further investigated.

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