

Susceptibility of Bacterial Isolates of Wound Infections to *Chromolaena odorata*

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Abstract

Hot ethanolic extract, cold ethanolic extract and hot aqueous extract of *Chromolaena odorata* were assayed for in vitro antibacterial activity using agar diffusion method. The extracts produced measurable zones of inhibition against *Staphylococcus aureus*, *Proteus vulgaris* and *Escherichia coli*. However, some organisms such as *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Klebsiella species* were found to be resistant to the extracts. Potency of the extracts depends on the solvent used for the extraction and method of extraction. Hot ethanolic extraction was the most effective which has its highest activity at low pH. The minimum inhibitory concentration of the extracts ranged from 15 mg/ml to 50mg/ml. Hot ethanolic was found to be bacteriocidal at 25 mg/ml on *E. coli*.

Key words: *Chromolaena odorata*, ethanolic extracts, bacteriocidal, bacteriostatic.

Introduction

A wound is an abrasion in the skin and the exposure of subcutaneous tissue following the loss of skin integrity which provide moist, warm and nutritious environment that is conducive for microbial colonization and proliferation (Duguid and Colle, 1975). Wounds disrupt the protective barrier of the skin and provide a portal of entry through which microorganisms can enter the circulatory system and deep body tissue.

Wound and other open lesions are liable to infection with a multiplicity of organism from the body surface or environment. Infection occurs when one or more of the contaminants evade the cleaning effect of the host's defenses, replicate in large

number, attack and harm to the host and may best be described as colonization (Simchen *et al.*, 1991). Wound infection may be endogenous or exogenous. Endogenous infection or auto-infection is caused by organism that has been living a commensal existence in the patient's body. On abdominal surgical wound for instance, may become infected from the large bowel after an operation involving incision of the colon. While exogenous infections are spread from person to person, this may occur after accident or intentional trauma of the skin or other tissue which is also called surgical or post-operative sepsis. Exogenous infection involves an effected person providing source from which other patient may acquire the organism.

There are various ways by which wound may become infected by different types of microorganisms. It may be directly from the patient, a member of the operating room staff or from other person in the hospital ward. Wound may be infected outside hospital ward via exposure to dust carrying infecting organism either in vegetative form or in form of spores. In fresh wound, bacteria have little time to multiply and there is practically no evidence of inflammatory tissue response. Hence, with few exceptions, bacteria are regarded as contaminants. But after a few hours, however, if sign of inflammation or other tissue response appear, then the bacteria must no longer be considered as contaminant but as infecting bacteria (Topley and Wilson, 1988).

The commonest pyogenic bacteria often associated with infected wounds are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pneumococcus sp* and coliform bacilli, such as *Escherichia coli*, *Proteus sp*, *Pseudomonas aeruginosa* and other enteric bacilli. Aerobic organisms particularly *Clostridium perfringes* and other species of bacteriodes and aerobic cocci are present (Klein *et al.* 1995). *Pseudomonas aeruginosa* causes a wide variety of septic infections in man and other vertebrates (Hare and Wilits, 1962).

Among species much less commonly encountered in wound infections are *Pasteurella multocida* in animal bites, *Corynebacterium diphtheria* and *Bacillus anthracis* in malignant pustules of skin. In chronic infection that are slow to heal and in pus showing no other microbes, the possibility of infection with *Mycobacterium tuberculosis* and other Mycobacteria is there (Colle *et al.*, 1963).

Despite the fact that antibiotics are inhibiting to pathogenic microorganisms, Russell and Hugo (1983) reported that a large number of antibiotics that have been

discovered are now useless in the treatment of infections due to selective toxicity apart from the problem of microbial resistance. In view of this and other problems associated with the use of antibiotics, scientists have reverted to the initial form of chemotherapy which was by the use of natural plant and plant products. These plants are usually regarded as medicinal plants (Owonubi, 1988).

Medicinal plant is defined as any plant which one or more of its organs contain substance that can be used for therapeutic purpose or which can be used as precursors for the synthesis of useful drugs (Sofowora, 1982). There are thousand species of medicinal plants used globally for the cure of different infections. These plants are used as antimicrobial agents and several works has been carried out by scientists to find out its scientific basis (Omotayo, 1998). Some of these plants include: *Anacardium occidentale*, *Pilostigma recticulatum*, *Anogeissus leiocarpa*, *Enantia chlorantha*, *Senna occidentalis* and *Azadirachta indica*.

The use of medicinal plants predates the introduction of antibiotics and other modern drugs in the African continent. Herbal medicine has been shown to be effective and over 60% of the Nigerian population depend on traditional medicine for their health care needs (Ghani *et al.*, 1989). Traditional medicine practitioner in Nigeria use a variety of herbal preparations to treat different kinds of ailments including typhoid fever, paratyphoid fever, dysentery, malaria, diarrhoea and wound infections. In recent past, attention has been directed to medicinal research to substantiate the claims of cure made by the traditional healers and thus provide a scientific basis for their efficiency (Olukoya *et al.*, 1993).

Plants synthesize a large variety of chemical substances which include alkaloids, tannin, saponin, steroid, phlobatannin, flavonoid, cardiac glycoside and a host of other chemical compounds referred to as secondary metabolites. These compounds are of no importance to plant's own life (Sofowora, 1993). Many of these metabolites have prominent effects on the animal systems and microbial cells (Oyagade *et al.*, 1999).

Some of these plant metabolites possess important therapeutic properties which can be and have been utilized in the treatment of human and other animal diseases worldwide. For example, the roots of *Zanthoxylum* *sp.* which were used for cleaning teeth and for treatment of toothache, urinary problem and throat infection contain chemical with

anaesthetic, anti-tumor, anti-sickling and antimicrobial properties (Oyagade et al., 1999). *Chromolaena odorata* formerly called *Eupatorium odoratum* and also known as Siam weed is a shrub, which has diffuse stem branches. Leaves are ovate and acute to the stem. The flowers are usually terminal. A study by Bhat *et al.*, (1985) indicated that the leaves are used for the treatment of yellow fever and also used to protect an injury against infection by crushing between the palm and the soft crushed leaves are used to cover the wound. This is commonly practiced in the Western part of Nigeria where the leaves are known as “ewe Akintola”. The use of medicinal plants is commonly associated with traditional medicine. This is defined as the total combination of knowledge and practices whether explicable or not used in preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observations handed down from generation to generation verbally or in writing.

It is important and expedient to analyze the effectiveness of antibiotics in common use today. This is because a large part of the population will be affected by their short-coming. Many local medicinal plants are being researched into. This can help to reinstate them since they have been proven in some cases to be curative to a wide variety of infections. They are readily available, cheap and are believed to have fewer side effects since they come from natural sources (Owonubi, 1988). For as long as man’s existence depends on the control of the pathogenic microbial populations, he must continue to monitor the effectiveness of the antibiotics he uses especially in the light of the limitations of these drugs.

Materials and Methods

Collection of Clinical Isolates

Pure cultures of clinical isolates of *Escherichia coli*, *Klebsiella sp*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella sp* and *Proteus vulgaris* were collected from Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria. The isolates were preserved by inoculating onto sterile agar slants, and were kept in a refrigerator which serves as stock culture for all the tests carried out.

Collection of *Chromolaena odorata* plant

Fresh leaves of *Chromolaena odorata* were collected from Danialu, near

Agbabiaka area, Ilorin, Kwara State, Nigeria. Identification of the plant was done at Herbarium unit of Department of Plant Biology, Faculty of Science, University of Ilorin, Ilorin. The leaves were air-dried for two weeks until it was able to withstand heat without boiling. The dried leaves were grounded into powder and stored wrapped with aluminium foil until used.

Plant Extracts Preparation

Different forms of extraction were carried out on the powder of the test plant by using water and ethanol.

a. Cold Ethanolic Extraction

30g of the powdered plant material was soaked in 150ml of 95% ethanol in a conical flask. This was placed on a shaker for about 5 days at about 28°C. The resulting extract was passed through muslin cloth and filtered through Whatman filter paper. The resulting extract was evaporated to dryness at 60°C in a hot air oven. The residue obtained was reconstituted back in 95% ethanol at stock concentration of 200mg/ml. The stock concentration was stored in an amber bottle at room temperature. The stock concentration was filtered with membrane filter before storage (Awe and Omojasola, 2003).

b. Hot Ethanolic Extraction

30g of the powdered plant material was soaked in 150ml of 95% ethanol in a conical flask. This was placed in water bath at 80°C for 2 hours. The resulting extract was passed through the muslin cloth and further filtered using Whatman filter paper. The resulting residue was reconstituted back in 95% ethanol at stock concentration of 200mg/ml. The stock concentration was filtered with membrane filter before being stored in an amber bottle at room temperature (Awe and Omojasola, 2003).

c. Hot Aqueous Extraction

The same quantity of the plant material and sterile distilled water was used as in hot ethanolic extractions, but the duration of extraction in the water bath at 80°C was 6 hours (Awe and Omojasola, 2003).

Assay of Plant Extracts

Each of the above extracts was tested for growth or contaminants by plating them on nutrient agar and incubated at 37°C for 24 hours. Where no visible growth was

observed, the extracts were then assessed for antibacterial activity (Omotayo, 1998).

Determination of pH of Extract of *Chromolaena odorata*

The pH of the extract of *Chromolaena odorata* was determined using pH meter Wag WT 3020 dipped into conical flasks containing the extracts. The constant reading on the pH meter was taken as the pH of the antimicrobial substance of extract of *Chromolaena odorata*.

Sensitivity Test of extract of *Chromolaena odorata* using Ditch Plate Method

Overnight broth culture of the test organisms were swabbed on sterile Muller Hinton agar in petri dishes using sterile cotton swabs. A sterile cork borer of size 6mm in diameter was used to make ditches on the plates. 0.3ml of the respective leaf extract were then put into each appropriately labeled ditches using sterile syringes. The incubated petri dishes were left for 1 hour for the extract to diffuse into the agar. The plates were incubated at 37°C for 24 hours and zone of inhibition were measured. The diameter of the zone of inhibition around each well was measured to the nearest millimeter along two axis 90° to each other and the mean of the reading was calculated (Omotayo, 1998). Stock concentration was 200mg/ml.

Determination of Effect of varying concentration of the Plant Extract on the Isolates

Overnight broth culture of the test organisms were swabbed on the sterile Mueller Hinton agar in the petri dishes using sterile cotton swabs. A sterile cork borer of size 6mm in diameter was used to make ditches on the plate. Stock concentration of the extract of *Chromolaena odorata* was diluted from 200mg/ml to 150mg/ml to 100mg/ml and finally to 50mg/ml with extraction solvent. Each of this concentration was introduced into ditches on the inoculated petri dishes. The plates were left for 1 hour for the extract to diffuse. The plates were subsequently incubated at 37°C for 24 hours and corresponding zone of inhibition measured.

Determination of Minimum Inhibitory Concentration (MIC)

Each test organism that is susceptible to the extract of *Chromolaena odorata* was inoculated unto one sterile nutrient broth and incubated overnight at 37°C. 1ml of the extract was serially diluted with nutrient broth from 50mg/ml to 25mg/ml to 15mg/ml and finally to 12.5mg/ml concentrations. Each of the diluted extract was then inoculated with 0.3ml of the overnight broth culture of the test organisms. A control was set up

which contains only nutrient broth and the extract. The inoculated and control tubes were incubated at 37°C for 24 hours after which they were observed for turbidity. The lowest concentration that shows no turbidity was taken as the MIC (Prescott *et al.*, 1999).

Determination of Minimum Bacteriocidal Concentration (MBC)

Samples from tubes used in the MIC assays which did not show signs of turbidity after period of incubation were streaked out on solidified sterile nutrient agar plates using sterile cotton swab and incubated at 37°C. The lowest concentration of the extract which shows the growth on plates after 24 hours of incubation indicate bacteriocidal effect and was taken as MBC (Alade and Irobi, 1993; Omotayo, 1998). The plates showing growth after 24 hours of incubation period was taken as having bacteriostatic effect.

Results and Discussion

Zone of inhibition given by the extract of *C. odorata* were measured and the results are shown in Table 1. No inhibition zone was formed by the plant extract on *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Klebsiella sp.* *Staphylococcus aureus*, *Proteus vulgaris* and *Escherichia coli* were sensitive to extract of *C. odorata* with varying degree. The stock concentration is 200mg/ml. The effect of different concentration of the extract of *Chromolaena odorata* on the susceptible organisms is shown on Table 2. Table 2a, 2 b and 2c show patterns of different concentration of HEE, CEE and HAE. Results of minimum inhibitory concentration of the extracts are shown in Table 3a, 3b and 3c.

The ethanolic extracts were observed to show more antibacterial activity than the hot aqueous extract. This may be due to the fact that accessibility of ethanol to the bioactive component of the leaf is greater than that of water. Brain and Turner (1975) indicated that the efficiency of the extraction procedure depends on the accessibility of the constituents to the solvent. The fact that ethanolic extraction of leaf of *Chromolaena odorata* is more active against test organisms than aqueous extraction may suggest that the active principle of the plant is more soluble in ethanol than in water. The herbalists claim that the plant is better extracted using local gin referred to as 'Ogogoro'.

All forms of the leaf extracts viz HEE, CEE and HAE showed antibacterial effect on *P. vulgaris*, *S. aureus* and *E. coli*. These bacteria fall in gram positive and gram negative groups which may suggest that the active principles in the extracts probably

possess a broad spectrum antibacterial activity (Jafri and Jalil-Subhani 1999; Samy and Ignacimuthu 2000). Both hot and cold ethanolic extracts were found to be 5.7 and that of hot aqueous extract was 6.5.

Pseudomonas aeruginosa, *Klebsiella sp* and *Streptococcus pyogenes* were found to be resistant to all forms of the extractions. Susceptible bacterial isolates (*P. vulgaris*, *S. aureus* and *E. coli*) were subjected to varying concentration of the different extractions as shown in Table 3. Table 3a showed the pattern of inhibition zone of susceptible isolates to different concentration of hot ethanolic extract. Result showed decrease in zone of inhibition with decrease in concentration of the extract except for *Proteus vulgaris* which showed no significant decrease in zone of inhibition with decrease in concentration. This suggests that the inhibition of growth of *Proteus vulgaris* by the extracts of *Chromolaena odorata* is not a function of its concentration. Tables 3b and table 3c also followed the same trend. The trend followed by *P. vulgaris* is contrary to the finding of Umeze and Abarikwu (1986) that stated that the potency of plant extract depends on the concentration and also method of extraction. The result obtained for *S. aureus* and *E. coli* however agreed with this fact.

It can also be deduced from Table 1 and Table 2 that activity of the plant extract also depends on the pH. Hot and cold ethanolic extracts which were found to both have pH of 5.7 is more potent than hot aqueous extract which has pH of 6.5. This agrees with Alade and Irobi (1993) which confirmed that the antibacterial activity of *Chromolaena odorata* significantly reduces with increase in pH. *Pseudomonas aeruginosa*, *Klebsiella sp*, *Streptococcus pyogenes* were not subjected to varying concentration because they are not susceptible to the plant extracts. This may suggest the ability of *P. aeruginosa* to metabolise many organic substrates, and viability and possession of extra cellular component (capsular material around the cell wall) by *Klebsiella sp*.

Table 3 showed various result of minimum inhibitory concentration of the extraction. Table 3a showed that the MIC for hot ethanolic extract for each organism varies. The smallest concentration that shows no turbidity was taken as the MIC. Both *S. aureus* and *P. vulgaris* have the same MIC of 25mg/ml while *E. coli* has MIC of 15mg/ml. Table 3b showed the result of cold ethanolic extraction's MIC. It followed the same trend as Table 3a except that the MIC for *P. vulgaris*. 50mg/ml. hot aqueous

extraction's minimum inhibitory concentration is presented in Table 3c. *P. vulgaris* was found to have no MIC while *S. aureus* and *E. coli* have MIC of 50mg/ml. All the tubes with MIC were plated out on sterile nutrient agar to determine the minimum bacteriocidal concentration (MBC). All the tubes were found to grow on the plate except the tubes that contained hot ethanolic extract and *E. coli*. The MBC for *E. coli* was found to be 15mg/ml.

In conclusion, all the clinical bacterial isolates challenged with the extract of *Chromolaena odorata* are common pathogens associated with wound infections. Researches conducted in the past have shown that the rate at which pathogenic bacteria are developing resistance to common conventional antibiotics is alarming (Montefijore *et al.*, 1983; Olayemi and Oyabade, 1987). Broad spectrum activity of extract of *C. odorata* tested appears to be asset for the development of antibacterial drugs.

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Table 1: Susceptibility pattern of bacterial isolates to leaf extract of *Chromolaena odorata*.

S/n	Test Organism	HEE	CEE	HAE	C
1	<i>Proteus vulgaris</i>	14	11	7	-
2	<i>Pseudomonas aeruginosa</i>	-	-	-	-
3	<i>Staphylococcus aureus</i>	19	16	12	-
4	<i>Escherichia coli</i>	26	22	16	-
5	<i>Klebsiella sp</i>	-	-	-	-
6	<i>Streptococcus pyogenes</i>	-	-	-	-

Key

HEE—Hot ethanolic extraction
CEE—Cold ethanolic extraction
HAE—Hot aqueous extraction
- No zone of inhibition

Table 2a: Hot Ethanolic Extraction (HEE)

S/N	Susceptible isolate	Zone of inhibition of inhibition(mm) at varying concentration			
		200mg/ml	150mg/ml	100mg/ml	50mg/ml
1	<i>Staphylococcus</i>	19	15	11	7

	<i>aureus</i>				
2	<i>Escherichia coli</i>	26	24	20	13
3	<i>Proteus vulgaris</i>	14	14	14	13

Table 2b: Cold Ethanolic Extraction (CEE)

S/N	Susceptible isolate	Zone of inhibition(mm) at varying concentrations			
		200mg/ml	150mg/ml	100mg/ml	50mg/ml
1	<i>S. aureus</i>	16	13	10	6
2	<i>E. coli</i>	22	18	14	11
3	<i>P. vulgaris</i>	11	9	9	8

Table 2c: Hot Aqueous Extraction (HAE)

S/N	Susceptible isolate	Zone of inhibition(mm) at varying concentrations			
		200mg/ml	150mg/ml	100mg/ml	50mg/ml
1	<i>S. aureus</i>	12	10	8	3
2	<i>E. coli</i>	16	13	9	5
3	<i>P. vulgaris</i>	7	6	4	4

Table 3a: Hot ethanolic extraction (HEE)

S/N	Susceptible test organism	Concentration (mg/ml)			
		50	25	15	12.5
1	<i>S. aureus</i>	NT	NT	T	T
2	<i>E. coli</i>	NT	NT	NT	T
3	<i>P. vulgaris</i>	NT	NT	T	T

Key

NT-Not Turbid

T-Turbid

Table 3b: Cold Ethanolic Extraction (CEE)

S/N	Susceptible Test Organism	Concentrations (mg/ml)			
		50	25	15	12.5
1	<i>S. aureus</i>	NT	NT	T	T
2	<i>E. coli</i>	NT	NT	NT	T
3	<i>P. vulgaris</i>	NT	T	T	T

Key

NT-Not Turbid

T-Turbid

Table 3c: Hot Aqueous Extraction (HAE)

S/N	Susceptible Test Organism	Concentrations (mg/ml)			
		50	25	15	12.5
1	<i>S. aureus</i>	NT	T	T	T
2	<i>E. coli</i>	NT	T	T	T
3	<i>P. vulgaris</i>	T	T	T	T

Key

NT-Not Turbid

T-Turbid