ADITUM
Enrich your Research

Clinical Medical Case Reports and Case Series

Research Article

Antibacterial and Phytochemical Potentials of Ethanolic Leaf/Bulb Extracts of Chromolaena Odorata on Wound Pathogens Sourced From FMC, Owerri

Agu Emelda ogechi, Joy N Dike Ndudim, Dennis C Nwosu*, Nsonwu Cajetan Chibuike, Nnodim Johnkennedy, Ndubueze winners chizaram, Aguzie Charlotte chinwendu. and Agu Judith Chinyere
Department of Medical Laboratory Science Imo State University Owerri

Article Info

Received: August 17, 2024
Accepted: August 26, 2024
Published: September 04, 2024

***Corresponding author:** Nnodim Johnkennedy, Department of Medical Laboratory Science Imo State University Owerri.

Citation: Nnodim Johnkennedy, Agu Emelda ogechi, Joy N Dike Ndudim, Dennis C Nwosu, Nsonwu Cajetan Chibuike, Ndubueze winners chizaram, Aguzie Charlotte chinwendu. and Agu Judith chinyere. (2024) "Antibacterial and Phytochemical Potentials of Ethanolic Leaf/Bulb Extracts of Chromolaena Odorata on Wound Pathogens Sourced From FMC, Owerri.", Clinical Medical Case Reports and Case Series, Head, and Neck Surgery, 1(1); DOI: 10.61148/CMCRCS/005

Abstract:

Studies were conducted to assess the bactericidal abilities of Chromolaena odorata against wound infections obtained from the Federal Medical Center in Owerri. Chromolaena odorata leaves were air dried and powdered, for 48 hours, 50 grams (g) of the paste sample were soaked in 400 milliliters (ml) of ethanol and water. Utilizing Whatman No. 1 filter paper and the Soxhlet apparatus to prepare the homogenized plant extracts, the filtrate was then evaporated using a rotary evaporator. The disc diffusion method was used to assess the extract's antimicrobial activity. 20 discs were submerged in 0.2ml of 25mg/ml, 12.5mg/ml, 6.25g/ml, 3.12mg/ml, and 1.56mg/ml extracts, respectively, after being made by perforating Whatmann filter paper. They were placed on Petri dishes, left at room temperature for the night, and then dried in a 45 °C oven. Media was labeled and produced aseptically. Using sterile forceps, the test organisms (bacteria) were applied to the media and the disc was then placed on the agar. After 24 hours of incubation at 37°C, the media plates were checked, measured, and recorded for zones of inhibition. The control medication was 50 mg of ciprofloxacin. The plants' MIC, MBC, and phytochemical analyses were also carried out. The bacteria isolated from the wounds were Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. For Chromolaena odorata, the zones of inhibition ranged from 2.0 mm to 8.3 mm. For Chromolaena odorata, the MICs for S. aureus, E. coli, and P. aeruginosa were 3.125 mg/mL, 6.25 mg/mL, and 25 mg/mL, respectively. The plant extracts had bactericidal properties as well. For Chromolaena odorata, S. aureus, E. coli, and P. aeruginosa all had MBC of 12.5mg/mL, 12.5mg/mL, and 3.125mg/mL, respectively. The study's findings showed that Chromolaena odorata has antibacterial action against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The pharmaceutical firms ought to take this into consideration while developing new antibiotics.

Keywords: Antibacterial, Phytochemical, Extracts, Chromolaena Odorata, Wound Pathogens

Introduction:

Infections from wounds and subsequent death peaked in the 19th century [1]. The discovery of harmful microorganisms in the 20th century opened up a new area of study for the phenomenon of wound infection [2]. Exogenous or endogenous infections of the wound might occur [3]. Staphylococcus aureus is one of the germs that has been identified from wound infections in adults most frequently, and it is also the most common cause of wound infections in newborns, the majority of which are resistant to treatments [4]. Staphylococcus aureus,

Streptococcus pyogenes, Pneumococcus, and coliform bacteria such Escherichia coli, Proteus species, and Pseudomonas aeruginosa are the most prevalent pyogenic bacteria. It would be assumed that the etiologic agents of the infection would be present in the pus and exudates from an infected wound or open abscess [5].

The creation of numerous pharmaceuticals and chemotherapeutic agents from traditional plants that are abundant in the tropics is a result of the rising demand for medications derived from natural sources [6].

Originally from North and Central America, the flowering shrub Chromolaena odorata was later brought to portions of Asia, Africa, and Australia. Armstrong'sweed, baby tea, bitter bush, butterfly weed, Christmas bush, devil weed, eupatorium, Jack in the bush, king weed, paraffin bush, paraffin weed, Siam weed, turpentine weed, and triffid weed are some of the other names for C.odorata [7].

Traditional medical practitioners have treated human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds, leech bites, dyspepsia, and skin infections with the fresh leaves of C. odorata or the decoction [8]. Other pharmacological effects of this plant include anti-helminthic, analgesic, anti-inflammatory, antipyretic, antispasmodic [9], antimycobacterial, insecticidal, antioxidant [10], anti- gonorrheal, fungicidal, diuretic [11], blood coagulating, and antimicrobial effects. However, there is currently very little knowledge about the plant's capacity for healing wounds [12].

It is well recognized and established that some wound microorganisms are antibiotic resistant. Given that some of these species, such as the extremely refractory bacterium Pseudomonas aeruginosa, are resistant to most synthetic antibiotics, this study was carried out to determine whether a natural extract from a plant would be useful in treating illnesses brought on by these bacteria. The herb Chromolaena odorata was chosen because herbalists commonly utilized it in traditional medicine. They are inexpensive, simple to obtain, and may have significant therapeutic value.

Materials And Methods:

Study Area:

In Owerri, Nigeria's Federal Medical Center (FMC), this study was carried out. Along Orlu road in Owerri, there is a tertiary medical center called Federal Medical Centre. It is a sizable hospital with many fully functional arms. In addition to providing patient treatment, the hospital serves as a training ground for numerous health care professions. It has received accreditation from the Nigerian Medical and Dental Council for the training of house officers, the Nigerian Pharmacy Council for the training of pharmacy interns, the Nigerian Medical Laboratory Science Council for the training of laboratory scientists/interns, among other bodies.

Plant Material:

Chromolaena odorata leaves were bought in bulk from vendors at the Owerri relief market and authenticated at Imo State University's Department of Plant Science and Biotechnology. The bulbs and leaves were cleaned and air-dried for 21 days at room temperature.

Collection and Identification of Bacteria:

The Federal Medical Centre (F.M.C.) at Owerri's Medical Microbiology
Laboratory provided the cultures of the wound-isolate isolates used in this study.
Re-identified bacteria isolates were preserved on nutrient agar and kept at 4°C for a
short period of time.
Control Samples
Broad spectrum antibiotic ciprofloxacin was standardised and utilized as a positive
control, while ethanol and distilled sterile water were employed as negative controls for ethanolic extracts.

Preparation of Ciprofloxacin 15ug/ml:

One milliliter of distilled water was used to dissolve about 400 milligrams of ciprofloxacin. To make 1ml (15ul/dl), 985ul of distilled water was added after around 15ul had been pipetted out

Preparation of Plant Extract:

Chromolaena odorata leaves were air dried and powdered. A 100g sample of the paste was soaked for 48 hours in 1000ml of ethanol and water. Utilizing Whatman No. 1 filter paper and the Soxhlet apparatus to prepare the homogenized plant extracts, the filtrate was then evaporated using a rotary evaporator. According to Nagesh and Samreen, the extracts were collected in sterile screw-cap bottles and kept in the refrigerator at 4°C (2016).

Preparation of Bacterial Inoculum:

On nutritional agar, test organism cultures were kept alive. Using an inoculating look, four to six colonies were selected quickly, suspended in 5 ml of broth, and incubated at 37 oC. The broth culture's turbidity was then adjusted to conform to 0.5 Macfarlands norms. This gives the pathogenic organism needed for the test, which ranges from 1 x 106 to 5 x 106 (colony forming unit/ml).

Preparation of the Extract's Serial Dilution:

The formula is 100g = 100,000mg = 100mg/ml (stock) 1000ml
1000ml
3.6 Preparation of Serial Dilution of the Extract
100g = 100,000mg = 100mg/ml (stock)
1000ml 1000ml
100mg/ml 50mg/ml 25mg/ml 12.5mg/ml
6.25mg/ml
3.125mg/ml.

The extract was serially diluted by two-fold from the stock (100 mg/ml), yielding 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml.

Antimicrobial Activity of the Crude Extract:

Using the agar well diffusion method, the extract's antibacterial activity was assessed. Satdive and other (2012). On the surface of a sterile Mueller-Hinton agar plate, a tenth of a milliliter of each standard test organism was streaked before being let to stand for 15 minutes. On the solidified Hinton agar plate, 0.1ml of the extracts (both stock and serially diluted) were cultivated into each well using a sterile 6mm cork-borer. Distilled water served as the negative control, and ciprofloxacin (15ug) as the positive control. The plate was incubated for 18 to 24 hours at 37°C. Using a transparent meter ruler, clear zones surrounding the wells were counted as signs of inhibition and measured in millimeters (ml).

Determination of Minimum Inhibitory Concentration (M.I.C):

Allium sativum and Chromolaena odorata ethanol extracts were tested for their minimum bacterial concentration (M.B.C.). The Minimum Inhibitory Concentration (M.I.C.) was established using the broth dilution method with ethanol as the M.I.C. and M.B.C. [13]. Test tubes were combined with an equal volume of extracts from Chromolaena odorata, and nutritional broth in various concentrations.

Test tubes were filled with 0.5 milliliters of the various ethanolic extract concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml). The equal volume (about 0.5ml) of various bacterial suspensions were injected into the appropriate test tubes for each pair. These test tubes, together with the positive and negative control test tubes, were infected at 37°C for 18 hours. The M.I.C was chosen as the lowest extract/antibiotic concentration at which the organism showed no signs of growth after 14 hours of incubation. The tubes that exhibited no signs of growth were plated out onto Mueller Hinton agar plates devoid of antibiotics to perform the M.B.C assay. After culturing on Mueller-Hinton agar plates without antibiotics, the M.B.C. was chosen as the lowest concentration of antibiotic/extract that completely stopped bacterial growth.

Phytochemical Analysis
According to Yadav and Agarwala, the ethanol extract underwent a phytochemical examination utilizing standardized methods to determine the phytochemical ingredients.

Qualitative Analysis:

Determination of saponins

A test tube containing 0.5g of the material was separately agitated; foaming that persisted after warming was considered proof of the presence of saponins.

Determination of tannins

Separately, 0.5g of the extract was mixed with 10ml of distilled water in a test tube before being filtered. 5% Iron III Chloride was added in two drips. The presence of tannins was indicated by the blue-black coloring.

Determination of alkaloids:

The material, which weighed 0.5g, was dissolved in 5ml of 1% hydrochloric acid (HCl). Dragendroff's reagent was used to process the filtrate. Alkaloids were present as evidenced by the red precipitate's formation.

Determination of glycosides:

One beaker received 1g of the material, 5ml of sulphuric acid was added, and the other beaker received 5ml of water. The contents of the two beakers were filtered into test tubes with labels after being heated for three minutes. After adding 0.5ml of sodium hydroxide to the filtrate to make it alkaline, the mixture was let to stand for three minutes. A good reaction for glycosides was shown by the reddish-brown precipitate that was present in the filtrate.

Determination of flavonoids:

1ml of the extract was combined with a piece of magnesium ribbon and 5ml of strong hydrochloric acid. Flavones were denoted by colors ranging from orange to red, flavonols from red to crimson, and flavonones from crimson to magenta.

Determination of phenols:

One milliliter of the extract was put to a test tube along with four drops of ferric chloride solution. Blueish black coloration formed suggested the presence of phenols.

Determination of carbohydrates:

5 ml of Benedict's reagent were applied to 1 ml of the filtrate. When the mixture was heated, a crimson precipitate formed, which indicates the presence of reducing sugar [14].

Chromatographic analysis:

For this analysis, 50 liters of Datura stramonium ethanolic extract were aspirated and injected into the sample inlet, which transports the sample to all other compartments. The spectra were analyzed, peak by peak and compound by compound, on the linked computer after a turnaround time of 27 minutes.

Statistical Analysis:

After data analysis, the results were displayed as means and standard deviations.

The zones of inhibition were compared using analysis of variance (ANOVA), which was used to identify the significant differences. When the P value is less than 0.05, statistical significance has been determined. Tables were used to display the results.

Results:

Test organisms		Concentrations						
	Raw	100mg/ml (Stock)	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Ciprofloxacin (15ug/ml)	Negative Control
S. aureus	16.05 ±0.04	11.10 ±0.05	8.15 ±0.26	6.10 ±0.10	0.00	0.00	25.67±0.15	0.00
E. coli	18.20 ±0.12	12.50 ±0.15	9.40 ±0.05	7.20 ±0.00	0.00	0.00	30.33±0.00	0.00
P. aeruginosa	22.00 ±0.20	14.00 ±0.00	11.10 ±0.11	8.50 ±0.10	0.00	0.00	30.33±0.50	0.00

Table 4.1 Mean ± standard deviation values of zones of inhibition (in mm) of ethanolic extracts of *Chromolaena odorata* leaves on the test organisms.

Key: ± Standard Deviation

Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	25	100
Escherichia coli	50	100
Pseudomonas aeruginosa	50	100

Table 4.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic extract of *Chromolaena odorata* on test organisms

Compound	Quantity (%)
Tannin	3.854
Phenol	5.329
Flavonoid	1.246
Alkaloid	9.602
Phytate	3.171
HCN	3.517

Table 4.3: Quantitative Phytochemical Analysis of *Chromolaena odorata* Results

Peak number	Retention time(minute)	Name	Percentage
1	1.511	Benzene-ethanamine	3.448
1	1.511	Bactolin	3.448
2	1.448	Methyl hydrogen disulfide	46.240
2	1.448	Hydrazine	46.240
3	2.010	Cyclohexane	1.587
3	2.010	Hexane	1.587
4	3.280	Thiazole	0.051
4	3.280	Butanoic acid	0.051
5	3.400	Cyclohexyl-ethylamine	0.085
5	3.400	Benzene-ethanamine	0.085
5	3.400	Dextroamphetamine	0.085
5	3.400	dl-phenylephrine	0.085
5	3.400	Phenylephrine	0.085
5	3.400	Thiophene-3-ol	0.085
6	7.890	Benzene methanol	0.041
6	7.890	2-iodohistidine	0.041
7	8.728	Ethanamine	0.037
7	8.728	Amphetamine	0.037

8	10.650	6,9,12-octadecatrienoic acid	0.061
8	10.650	Allyl(dimethyl)benzyl oxysilone	0.061
9	11.114	1-hydroxy-4-dimethylhydrazonomethyl	0.033
9	11.114	3-(E)-octen-2-one	0.033
10	12.826	Diisopropyl(ethoxy)silane	0.051
11	13.658	2,5-methylene-1-thamnitol	0.041
12	14.275	1-ethoxy-1-methyl-1-silacyclohexane	0.051
12	14.275	Adipic acid	0.051
13	15.113	3-ethyl-4-hydroxy-4	0.034
14	15.359	2,3-O-Benzal-d-mannosan	0.051
14	15.359	2,3-O-Benzal-d-mannosan	0.051
15	16.553	Artemiseole	0.048
16	17.212	Amphetamine	0.051
17	17.258	9,10-secochola	0.051
18	17.649	Benzenamine	0.080
18	17.639	Benzene propanoic acid	0.069
19	20.700	4-hydroxy-4-(2-methylcychonex-3-enyl)	0.102
19	20.700	Hydrocinnamic acid	0.102
20	21.803	5,7-dodecadiyn-1,2-diol	0.488
20	21.803	3-cyclohexen-1-ol	0.488
21	22.590	3,7,11,15-tetramethyl-2-hexadeen 1-ol	0.035
21	22.590	Phytol, acetate	15.821
22	27.914	7-methyl-Z-tetradecen-1-ol acetate	15.821
23	28.596	Octadecanoic acid	1.400

Table 4.4: Chromatographic Analysis of the Ethanolic Extract of *Chromolaena* Using Gas Chromatography-Mass Spectrometry

Discussion:

According to the study's findings, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus are all susceptible to the antibacterial effects of Chromolaena odorata extracts. This is consistent with results from other scholars [14, 16], which also demonstrated the bactericidal efficacy of ethanolic extracts of Chromolaena odorata. It was foud that S. aureus was more vulnerable to the active compounds found in Chromolaena odorata in this investigation. S. aureus had an 8.30 mm for Chromolaena odorata extract zone of growth inhibition diameter. With diameters of 7.8 mm for Chromolaena odorata extract, P. aeruginosa is less sensitive. Of all the test microorganisms tested, E. coli was the least susceptible, with growth inhibition diameters for Chromolaena odorata extract of 7.5 mm, The production of enzymes by E. coli and P. aeruginosa may be the cause of these behaviors.These enzymes may degrade or inactivate some of the bioactive phytoconstituents in Chromolaena odorata. Additionally, it has been noted that the gram-negative bacteria's complex cell envelope slows or obstructs the passage of numerous antimicrobial drugs through the cell wall [17]. All test microorganisms had their development generally inhibited to varied degrees, which is consistent with the findings [18]. S. aureus was more susceptible than E. coli, according to the findings [19], and the same finding was made in our investigation. It is common practice to assess a variety of compounds, including antibiotics, antiseptics, disinfectants, and chemotherapeutic agents, using the MIC and MBC assay techniques [20, 21]. High MIC and MBC values are typically given by antimicrobial agents with low activity against an organism, while low MIC and MBC values are typically given by antimicrobial agents with high activity. Chromolaena odorata ethanol extract have different M.I.C. and M.B.C. values in this investigation, with M.I.C. values ranging from 25 to 50 mg/ml and M.B.C values of 100 mg/ml and higher. This indicates that the concentration or quantity of extract supplied to the medium has a significant impact on how well microorganisms are inhibited.

References:

1. Agu, G. and Thomas, B. (2011). Antibacterial activities of ethanol and aqueous extracts of five Nigerian medicinal plants on some wound pathogens. *Journal of the Healthcare Infection Society*, **78**(2): 12–19.
2. Sharifi-Rad, J. (2014). Antimicrobial synergic effect of Allicin

and silver nanoparticles on skin infection caused by methicillin resistant *Staphylococcus aureus* spp. *Journal of medical Scientists*, **14**(7): 66-72.
3. Mutawila, C. (2014). Improving pruning wound protection against grapevine truck disease pathogens. *Journal of Medical Microbiology*, **23**(2): 14-18.
4. Ranjan (2012). Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish. *Journal of Phytochemistry*, **6**(3):43-50.
5. Andualem, B. (2013). Combined antibacterial activity of stingless bee (*Apis mellipodae*) honey and garlic (*Allium sativum*) extracts against standard and clinical pathogenic bacteria. *Asian Pacific Journal of Tropical Biomedicine*, **4**(2): 45-56.
6. Falodun, A., Okenroba, L. O. and Uzoamaka, N. (2006). Phytochemical screening and anti -inflamentory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn (Euphorbiaceae). *African Journal of Biotechnology*, **5**(6): 529-531.
7. Nagesh, M. and Samreen, A. (2016). Antimicrobial Activity of *Carica papaya*, *Piper nigrum* and *Datura stramonium* Plants on Drug Resistant Pathogens Isolated from Clinical Specimens. *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 2(6): 56-61.
8. Panyaphu, K., Sirisa-Ard, P., Srisa-Nga, P., ChansaKaow, S. and Nathakarnkitkul, S. (2011). Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. *Journal of Ethnopharmacology*, **135**: 226–237.
9. Patel, J., Kumar, G. S., Qureshi, M. S. and Jena, P. K. (2010). Anthelminthic activity of ethanolic extract of whole plant of *Eupatorium odoratum*. *International Journal of Phytomedicine*, **2**: 127–132.
10. Phan, T. T., Wang, L., See, P., Grayer, R. J., Chan, S. Y. and Lee, S. T. (2011). Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. *Biological and Pharmaceutical Bulletin*, **24**: 1373–1379.
11. Gopinath, R., Sunilson, J.A., Radhamani, S., Das, A. and Nilugal, K. (2009). Diuretic activity of *Eupatorium odoratum* (L). *Journal of Pharmaceutical Research*, **2**: 844–846.
12. Biswal, P. R., Sardar K. K., Parija, S. C., Mishra, P. R. and Mishra, S. N. (1997). Wound healing effect of *Eupatorium odoratum* Linn, and Himax in rabbits. *Indian Journal of Indigenous Medicine*, **19**: 71–74.
13. Satdive, R. K. Chimata, R., Namdeo, A. and Fulzele, D. (2012). In Vitro Screening for Phytochemical and Antimicrobial Activity of Poisonous Plant *Fisus Tseila* Roxb. *International Journal of Pharmaceutical and Bioogical Sciences*, **3**(12): 213-221.17. Ahmadu, A. A., Akputu, I. N., Hassan, H. S., Sule, M. I. and Pateh, U. U. (2006). Preliminary phytochemical and antimicrobial screening of he leaves of *Byrsocarpas coccineus.* Schun and Thonn (Connaraceae). *Journal of Pharmacy and Bioresources*, **3**(2): 107-110.
14. Harborne, J. B. (1973). Phytochemical methods: A Guide to modern Techniques of Plant Analysis. Champman and Hall, London, p. 279.
15. Bharathi, T., Kolanjinathan, K. and Saranraj, P. (2014). Antimicrobial activity of solvent extracts of *Ocimum sanctum*, *Azadirachta indica* and *Phyllanthus amarus* Against Clinical Pathogens. *Global Journal of Pharmacology*, **22**(4): 33-45.
16. Mukhtar, S. and Ghori, I. (2012). Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against *Escherichia coli* ATCC 25922 and *Bacillus subtilis* DSm 3256. *International Journal of Applied Biopharmatechnology*, **10**(4)42-47.
17. Binggeli, P., Hall, J.B., Healey, J.R. (1998). Overview of Invasive woody plants in the Tropics, The Invasive Woody Species Database, *School of Agricultural and Forest Sciences, University of Wales, Bangor*, pp 103.
18. Ankri, S. and Mirelman, A. (1999). Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 2: 125-129
19. Jaber, M.A, Al-Mossawi A (2007). Susceptibility of some multiple resistant bacteria to garlic extract. *African Journal of Biotechnology*, **6** (6): 771-776.
20. Croshaw, B. (2003). Evaluation of non-antibiotic antimicrobial agents. In: Pharmaceutical Microbiology, 3rd edn.,(Hugo WB and Russell AD eds. Blackwell, Oxford, pp. 237-257.
21. Acheampong, Y. B., El-Mahmood, A. M. and Olurinola, P. F. (2008). Antibacterial properties of the liquid antiseptic TCP. *Indian Journal of Pharmaceutical Sciences*, **3:** 183-186.