

Antimicrobial Activity of *Celosia argentea* L. Amaranthaceae

Eseoghene Okpako* and Kola' K. Ajibesin

Department of Pharmacognosy and Herbal Medicine,
Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

*E-mail: yonarever@yahoo.com

ABSTRACT

Aim: *Celosia argentea* L. Amaranthaceae, has been used traditionally in the treatment of sores, ulcers and skin eruptions and possesses laxative, antioxidant, and anti-inflammatory activities. This study aims to investigate its antimicrobial activity against a number of fungal and bacterial strains, especially those implicated in causing human microbial diseases such as skin diseases and wound putrefaction.

Methods: The leaves, stem and root of the plant were extracted cold in 50 % ethanol for 72 hours. The extracts were subjected to phytochemical screening to reveal classes of the chemical compounds. Using Agar Diffusion method, the extract was also subjected to antimicrobial tests such as sensitivity test by measuring the diameter of the zones of inhibition at a concentration of 20 mg/ml. Minimum Inhibitory and Minimum Bactericidal and Minimum Fungicidal Concentrations (MBC and MFC) were also determined for active extracts.

Results: Compounds such as alkaloids, flavonoids, tannins saponins and terpenes were detected in the plant. The leaves, stem and root extract elicited antibacterial activity against all the strains tested by zones of inhibition ranging between 7 mm to 26 mm. Only the leaf extract showed antifungal activity. Bactericidal and fungicidal effects of the leaves extract were same, with no cidal effect against *Aspergillus niger* and *Escherichia coli*. The stem extract had inhibitory effects at 25µg/ml and no bactericidal effect. The root extract was bacteriostatic and bactericidal at the same concentration for all bacterial strains with the exception of *Escherichia coli* that showed MIC of 50µg/ml.

Conclusion: Given the result of this study the leaf extract gave both antibacterial and antifungal activities while the stem and root extracts possessed only antibacterial effects. This however justifies the plant use ethno medicinally, in the treatment of sores, ulcers and skin eruptions.

Keywords: Antibacterial, Antifungal, leaf, stem, root, *Celosia argentea*

{**Citation:** Eseoghene Okpako, Kola' K. Ajibesin. Antimicrobial activity of *Celosia argentea* L. Amaranthaceae. American Journal of Research Communication, 2015, 3(5): 123-133} www.usa-journals.com, ISSN: 2325-4076.

1. INTRODUCTION

Majority of individuals in developing nations depend on plants for medicine ¹. In a survey by Nadembega et al. ², the most frequently sought parts for medicine in Burkina faso were leaves, accounting for 38.4%, followed by stem bark (30.0%), roots (29.5%), and whole plants (23.1%). With the increasing frequency in side effects and the resistance of pathogenic microorganisms build up against antibiotics, alternative antimicrobial strategies are persistently required, which may be to seek structurally novel antibiotics that have entirely different mechanisms of action from the currently used agents ^{3, 4} or to employ plants in crude form which show potent effect due to their complex chemical composition and synergistic action. This has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant based products ⁵. Antimicrobial activity so far considered empirical, have been scientifically confirmed in a number of plants ⁶. *Celosia argentea* L. known as Lagos spinach or cockscrew comb is a tropical, leaf vegetable crop belonging to the family Amaranthaceae, popularly known as “Shokoyokoto” among the Yorubas, meaning “make husbands fat” ⁷. The whole plant is known traditionally for its use in the treatment of wounds, ulcers, skin diseases, diarrhea, piles, bleeding nose, disinfectant, inflammation, haematological and gynaecologic disorders ^{8, 9}. Anti-inflammatory, immunostimulating, anticancer, hepatoprotective, antioxidant, wound healing, antidiabetic and antibacterial activities have been reported in the extracts and its constituents ¹⁰.

Consequently this study aims to investigate the antimicrobial activity of the different parts of *C. argentea* to confirm its ethno medicinal claims.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extract

The leaves, stem and root of *C. argentea* L. were collected from uncultivated farmlands located in Ondo State, Nigeria, in November 2012. The plant was identified and authenticated by Mrs H. A. Babalola of the Community of Agricultural Science Department, Akoko South West Local Government Isua - Akoko, still in Ondo State. The leaves, stems and roots were dried in open air for about 7 days. They were then pulverized and stored in air tight containers prior to further studies. 100g each of the powdered leaves were weighed out and extracted using cold maceration technique in 50% ethanol for 72 hours with occasional manual agitation. The mixture was filtered and the filtrate was concentrated to dryness under *in vacuo* at 35°C.

2.2 Phytochemical Screening

Phytochemical tests were carried out to determine the presence the classes of chemical constituents were carried out using the methods as described by Sofowora ¹¹ and Trease and Evans ¹².

2.3 Antimicrobial Assay

2.3.1 Microorganisms

The pathogenic strains of two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*) and two fungi (*Candida albicans* and *Aspergillus niger*) species were obtained from the Delta State Teaching Hospital, Oghara, Delta state. They were maintained on Sabouraud Dextrose Agar and Mueller Hilton Agar slants for fungi and bacteria slants respectively at 4°C prior to use.

2.3.2 Determination of Antimicrobial activity using the Agar Well Diffusion Method

The extract was reconstituted in distilled water to obtain a stock solution of 20 mg/ml. 50 µl of this stock solution was introduced into each of the equidistant wells (8mm) bored on the agar plate surface previously inoculated with each of the test organisms. A control well containing Chloramphenicol (2 µg/ml) and Fluconazole (25 µg/ml) were placed in each of the plates seeded with bacteria and fungi respectively. The petri dishes were incubated at 37°C for 18-24 hours for bacteria and *C.albicans* and at 18°C for 5 days for fungi. Antimicrobial activity was expressed as diameter of the zones of inhibition calculated as the difference in diameter of the observed zones and those of the wells, comparing it with the corresponding standard antibacterial and antifungal drug^{13, 14}.

2.3.3 Determination of the Minimum Inhibitory, Bactericidal and Fungicidal Concentrations

The Minimum Inhibitory Concentration (MIC) of the leaf extract was determined by incorporating various amounts (3.125µg/ml to 50µg/ml) of the reconstituted extract by serial dilution into sets of test tubes containing the culture media. The culture of the test organisms were introduced into each of these test tubes. The set of tubes containing a mixture of the respective microorganism and the extracts only were then incubated at 37°C for 18-24 hours for bacteria and at 18°C for 5 days for fungi. The MIC for the standard procedural drugs Chloramphenicol and Fluconazole against bacteria and fungi was also carried out. The MIC was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with that of the control tubes. Samples from the tubes used in the MIC assay which did not show any visible growth after the period of incubation were sub cultured onto freshly prepared Mueller Hinton Agar and Sabouraud Dextrose Agar for bacteria and fungi respectively. The Minimum Bactericidal Concentration was regarded as the lowest concentration of the extract/drug that did not yield a single bacteria growth on Mueller Hinton Agar plates after 18-24 hours of incubation at 37°C, while the Minimum Fungicidal Concentration as the lowest concentration of the extract/drug that did not yield a single fungal growth on Sabouraud Dextrose Agar plates after incubation at 18°C for 5 days¹⁵.

RESULTS

The leaf, stem and root extracts were found to contain alkaloids, saponins, tannins and flavonoids in trace amounts with the exception of the leaf extract that had tannins in appreciable amounts. The stem extract in addition to the common constituents had traces of terpenes (Table 1). All the three extracts subjected to antimicrobial assay had antibacterial activity at various degrees, represented by the zones of inhibition that ranged between 7 to 26mm. However, only the leaf extract possessed antifungal activity (Table 2).

The leaf extract showed bactericidal and fungicidal activity against all the microbes except of *E. coli* and *A. niger*, even at a test concentration of 50µg/ml. The roots extract elicited bactericidal activity while the stem extract did not (Table 3).

Table 1: Phytochemical Screening of *C. argentea*

Components	Leaf extract	Stem extract	Root extract
Alkaloids`	+	+	+
Saponins	+	+	+
Tannins	++	+	+
Flavonoids	+	+	+
Cardiac glycosides	-	-	-
Anthraquinone	-	-	-
Terpenes	-	+	-

+: trace, ++: present, -: absent

Table 2: Antimicrobial effects of the leaf, stem and root extracts of *C. argentea*

	Leaf	Stem	Root	CHL	Fluconazole
Microorganisms	Zone of Inhibition in mm*				
<i>Escherichia coli</i>	13	10	8	25	NA
<i>P. aeruginosa</i>	26	8	7	41	NA
<i>Bacillus subtilis</i>	21	11	11	41	NA
<i>S. aureus</i>	21	11	7	13	NA
<i>Candida albicans</i>	10	0	0	NA	26
<i>Aspergillus niger</i>	9	0	0	NA	31

CHL: Chloramphenicol

NA: Not applicable

*: values are means of three replicates

Table 3 : Minimum Inhibitory, Bactericidal and Fungicidal concentrations of the extracts

Microorganism	Leaf		Stem		Root		CHL		Fluconazole	
	MIC	MBC/ MFC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
	(Concentration in $\mu\text{g/ml}^{-1}$)									
<i>Escherichia coli</i>	12.5	-	25	-	25	50	12.5	12.5	NA	NA
<i>P. aeruginosa</i>	12.5	25	25	-	25	25	25	-	NA	NA
<i>Bacillus subtilis</i>	12.5	25	25	-	25	25	25	25	NA	NA
<i>S. aureus</i>	12.5	25	25	-	25	25	12.5	12.5	NA	NA
<i>Candida albicans</i>	25	25	NA	NA	NA	NA	NA	NA	12.5	25
<i>Aspergillus niger</i>	25	-	NA	NA	NA	NA	NA	NA	25	-

CHL: Chloramphenicol

NA: Not applicable,

-: No activity

DISCUSSION

The three parts of *C. argentea* showed antimicrobial activity against the microorganisms tested. All the three extracts had antibacterial activity against the four bacterial strains tested with the leaf having the best activity of the three followed by that of the stem. They were not as effective

as Chloramphenicol, the standard drug. However the effect of the leaf extract against *Staphylococcus aureus* was better than that of Chloramphenicol. This activity can be attributed to the phytochemical components of the plants which included saponins, tannins, alkaloids and flavonoids which have all been implicated in antimicrobial activity. These components are bioactive compounds provided by plants to cure human diseases, including microbial diseases¹⁶. The relative higher activity of the leaf extract may be due to its higher tannin content. Fiori et al.¹⁷ reported in a study that tannins elicit antimicrobial activity in the three following ways:

- (i) Inhibition of enzyme activity by complexation with substrates of bacteria and fungi;
- (ii) Direct action of tannins on the microorganism metabolism, through the inhibition of oxidative phosphorylation;
- (iii) A mechanism involving the complexation of tannins with metabolic ions, decreasing the availability of essential ions to the metabolism of the microorganisms.

Tannins have been shown to have antimicrobial effects that increased with increase in concentration by Min et al.¹⁸, in a study on the comparative antimicrobial activity of tannin extracts. This finding is also substantiated by Doss et al.¹⁹. This may also be responsible for its antifungal activity lacking in the other two extracts. This is corroborated by results of a study carried out by Sheh-Hong et al.²⁰, who observed that the tannins of *Rhizophora apiculata* barks exhibited various antibacterial and selective antifungal activity. The results of this study is contrary to the report by Kasim et al.²¹ which implied that the leaf extract of this plant has no antifungal activity against *C. albicans*. Conversely, it was reported by Mustapha²² to be used in the treatment of abnormal vaginal discharge, implicated as a fungal infection.

The inhibitory and killing effects of the leaf extracts were comparable with those of Chloramphenicol and Fluconazole for all the test organisms. This suggests that the leaf extract if taken through to some form of purification may have more potent antimicrobial compounds than some of the existing antimicrobial agents. The root extract showed appreciable bactericidal activity while the stem did not. This may due to the additional plant constituent, terpene which may antagonize the antimicrobial actions of the other constituents^{23, 24}. The leaf extract is bacteriostatic at lower concentrations and bactericidal at higher concentrations, while the root extract are bacteriostatic and bactericidal at the same concentration.

CONCLUSION

The results of this study justify the use of this herb in traditional medicine as an antimicrobial agent to treat sores, ulcers and skin eruptions, especially the leaf which showed the broadest spectrum of activity. Further purification of the leaf extract could give a lead to discovery of new compounds which may be more potent than the already existing antimicrobial agents in use.

COMPETING INTEREST

No competing interests exists

AUTHORS CONTRIBUTION

Author KKA designed the study and helped in the preparation of the manuscript.

Author EO wrote the manuscript and managed the literature search.

Both authors read the final manuscript and approved it.

ETHICAL APPROVAL

Not needed.

REFERENCES

1. Pirttila AM, Hirsikorpi M, Amarainen T, Jaakola I, Hohtola A. DNA isolation methods for medicinal and aromatic plants. *Plant Molecular Biology Reporter* 2011; 19 (3): 273a-f.

2. Nadembega P, Boussim JI, Nikiema JB, Poli F, Antognoni F. Medicinal plants in Baskoure; Kourittenga Province; Burkina Faso: an ethnobotanical study. *Journal of Ethnopharmacology* 2011; 133:378-95.
3. Sarac N, Ugu A. Antimicrobial activities and usage of folkloric medicine of some Lamiaceae species growing in Mugla Turkey. *EurAsian Journal of Biosciences* 2007; 1 (4): 28-34.
4. Abioye AO, Adesida SA, Bamiro SB. and Okpako E. In vitro kinetics of Staphylococcal death in the stem bark extracts of *Jatropha curcas* L. *Eurphobiaceae*. *Canadian Journal of Pure and Applied Sciences* 2011; 5 (2):1465-74.
5. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine* 2011; 1(2):154-60.
6. Silva NCC, Fernades A. Biological Properties of Medicinal plants a review of their antimicrobial activity. *The Journal of Venomous animals and toxins including tropical diseases* 2010; 16(3):402-13.
7. Tobih FO, Adegbite AA, Ononuju CC. Evaluation of some plant materials as organic mulch for the control of root-knot nematode *Meloidogyne sp* and its Impact on the yield of *Celosia argentea* L. *International Journal of Agricultural Science* 2011;1(14):195-202.
8. Nidavani RB, Mahalakshmi AM, Seema M, Krishna KL. Pharmacology of *Celosia argentea* L. *Journal of atoms and Molecules* 2014; 4(1):635-44.
9. Sharma PK, Vidyasagar G, Singh SS, Ghule SS, Kumar B. Antidiarrhoeal activity of leaf extract of *Celosia argentea* in experimentally induced diarrhea in rats. *Advanced Pharmaceutical Technology* 2010; 1: 41-8.
10. Nidavani RB, Mahalakshmi AM, Mallappa S. Towards a Better Understanding of an Updated Ethnopharmacology of *Celosia argentea* L. *International Journal of Pharmacy and Pharmaceutical Science* 2013; 5(3):54- 9.
11. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited, Ibadan, Nigeria 2008.
12. Trease and Evans WC, *Textbook of Pharmacognosy*. Elsevier Limited, 2009.
13. Valgas C, Machado SS, Smania EFA, Smania-Jr, A. Screening methods to determine the antibacterial activity of natural products. *Brazillian Journal of Microbiology* 2007; 38:369-80.

14. Ajibesin KK, Essien EE, Adesanya SA. Antibacterial constituents of the leaves of *Dacryodes edulis*. African Journal of Pharmacy and Pharmacology 2011; 5(15):1782-6.
15. Alade PI, ON Irobi, Antimicrobial activities of the crude leaf extracts of *Alcalypha wilkesiana*. Journal of Ethnopharmacology 1993; 39: 171-3.
16. Orhue PO, Momoh ARM, Igumbor EO, Esumeh FI. Antibacterial effect of *Azadirachta indica* (CN: Neem or Dongo Yaro) parts on some urinary tract bacterial isolates. Asian Journal of Plant Science and Research; 2014 4(2):64-7.
17. Fiori GML, Correa VSC, Bertoni BW, Giuliatti S, Amui SF, Franca SC, Pereira AMS. Antimicrobial activity and rates of antimicrobial tannins in *Stryphnodendron adstringens* Mart. Accessions collected in Brazillian Cerrado. American Journal of Plant Sciences 2013; 4:2193 – 8.
18. Min BR, Pinchak WE, Merkel K, Walker S, Tomta G, Anderson RC. Comparative antimicrobial activity of tannin extract from perennial plants on mastitis pathogens. Scientific Research and Essay 2008; 3(2):66-73.
19. Doss A, Mubarak HM, Dhanabalan R. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* L. Indian Journal of Science and Technology 2009; 2(2):41-3.
20. Sheh-Hong L, Darah I, Jain K. Antimicrobial activities of tannins extracted from *Rhizophora apiculata* barks. Journal of Tropical Forest Science 2006; 18(1):59-65.
21. Kasim LS, Ayodele O, Effedua HI, Adejumo OC, Ekor M, Fajemirokun TO. Antimicrobial activity of six selected plants against some strains of pathogenic organisms. Journal of Microbiology and Antimicrobials 2012; 4(3):54-9.
22. Mustapha AA. Ethnomedicinal studies of medicinal plants with antifungal activities in Keffi local government Nassarawa state Nigeria. Asian Journal of Plant Sciences and Research 2013; 3(4): 109-15.
23. Esimone CO, Adikwu MU, Okonta JM. Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usnea subfleridans* L. Journal of Pharmaceutical Research and Development 1998; 3(2): 99 – 102.
24. Politi FAS, de Mello JCP, Migliato KF, Nepomuceno ALA, Moriera RRD, Pietro RCIR, Antimicrobial, Cytotoxic and Antioxidant Activities and Determination of the Total Tannin Content of Bark Extracts *Endopleura uchi*. International Journal of Molecular Sciences 2011;12: 2757-68.