

Full Length Research Paper

## Antibacterial and phytochemical screening of methanolic extract of *Celosia leptostachya* Benth leaves on some selected clinical isolates

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The antibacterial screening of *Celosia leptostachya* Benth leaves was carried out using the agar well diffusion technique and the methanolic extract of the leaves showed considerable activity on *Escherichia coli* and *Staphylococcus aureus* both at 60 and 80 mg/ml with zones of inhibition of 15.50, 18.00 and 16.50, 19.00 mm respectively. Whereas, it showed activity only at a concentration of 80 mg/ml with a zone of inhibition of 17.50 mm on *Salmonella typhi*. However, the *C. leptostachya* Benth extract showed no activity on *Shigella dysenteriae*. The phytochemical properties of the extract were also determined. The result reveals the presence of alkaloids, flavonoids, cardiac glycosides, and tannins, whereas phlobatannins, anthraquinones, steroids and saponins were not detected in the extract. The extract contains some major bioactive compound that inhibit the growth of microorganisms thereby proving very effective as alternative source of antibiotics.

**Key words:** Phytochemical, antibacterial, methanolic extract, *Celosia leptostachya* Benth.

### INTRODUCTION

Medicinal plants are used locally in the treatment of infections caused by bacteria, fungi, viruses and parasites. Over 60% of people in Nigeria rural areas depend on the traditional medicines for the treatment of their ailment (Ayandele and Adebisi, 2006). It has been reported that substantial population of people in the world are dependent on herbal medicines. The rural populations of a country are more disposed to traditional ways of treatment because of the ease of availability, affordability and cheapness of herbal drugs (Banquar, 1993). The medical value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents in plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga et al.,

2006). The use of orthodox medicine has resulted in the emergence of many resistant strains of microorganisms and has selectively enhanced their persistence (Cowan, 1999). As a result of this, a widespread dissemination of multiresistant strains of bacteria in developing countries has rendered many antibiotics ineffective (Aishatu and Deeni, 1994). Microorganisms developed resistance due to high rate of self medication, abuse or misuse of antibiotics, as well as therapeutic and prophylactic use of these antibiotics (Ozumba et al., 1993).

In recent years, the entire world population have shifted their interest to the development of medicinal plants and traditional medicine (Abdul, 1990). The shift of interest to tradomedicine could be explained with the fact that it is cheaper and more accessible to the common man than the orthodox medicine (Orjioko, 1995). It is also mostly compounded from natural products and thus more accessible than the orthodox medicine (Aishatu and Deeni, 1994). The plant *Celosia leptostachya* Benth belongs to the family Amaranthaceae of the order

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*Caryophyllales* (Soforowa, 1995). It grows in the raining season in Northern region of Nigeria. In Hausa language *C. leptostachya* is known as 'Nannafaa' while the Nupe and Yoruba called it 'ajefowo'. The leaf of the plant is used traditionally for the treatments of eclampsia, cardiac diseases, catarrh when boil with little potash, and for tape worms when taken with fresh milk (Personal communication with traditional healers). However, despite its promising potentials in tradomedicine little or no study has been conducted on the phytochemical and antibacterial qualities of this plant in this part of the country. Therefore, the objective of this study is to determine the antibacterial activity on some clinical isolates as well as the phytochemical properties of this plant.

## MATERIALS AND METHODS

### Sample collection and processing

Dried leaves of *C. leptostachya* were collected from Jega metropolis of Kebbi State, Nigeria. The leaves were placed into clean polythene bags and transported to the herbarium of Biological Sciences Department of Usmanu Danfodiyo University, Sokoto, Nigeria for identification. The leaves were air-dried and pulverized into powder form using pestle and mortar. Clinical isolates were obtained from the Microbiology Laboratory of Specialist Hospital, Sokoto, Nigeria. The isolates were transported to the Microbiology Department of Usmanu Danfodiyo University, Sokoto, Nigeria for identification and characterization. The isolates were characterized and identified as *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi* and *Escherichia coli*.

### Extraction procedures

This was carried out in accordance with the methods of Oyeleke and Manga (2008). Fifty grams of the leaves powder was weighed, placed into a sterile conical flask and 500 ml of methanol added. The flask was plugged with a cotton wool and left to stand overnight. Then the mixture was filtered using Whatmann No 1 filter paper to collect the extract. The extract collected was then concentrated at 60°C for 24 h using hot-air oven. The concentrated residue was weighed and varying concentrations of 20, 40, 60 and 80 mg/ml were prepared.

### Standardization of the inoculums

The standardization of the inoculum was carried out in accordance with the methods of Oyeleke et al. (2008). The isolates were subculture into fresh nutrient agar plates and incubated at 37°C for 24 h. After the incubation period, 5 ml of sterile distilled water was put into different universal bottles and were used to prepare the size of the inoculums using Mcfarland scale. The Mcfarland scale of 0.5 was used which is equivalent to  $1 \times 10^8$  cfu/ml.

### Antibacterial assay

The antibacterial assay was conducted in accordance with the methods described by Abalaka et al. (2010) using agar well diffusion technique. The standard inoculum was inoculated onto

already prepared plates of Mueller-Hinton agar using the streak plate method. Four holes were punched using sterilized cork borer from each plate. The wells (holes) were systematically labeled from the underneath the dishes with the name of the extract, test organism used and the concentration of the extracts used. The wells were filled with their corresponding labeled of the plant extract with 1% sterile plain agar into each designated well for each concentration. The plates were then allowed to set and finally inverted and incubated at 37°C for 24 h. After the incubation period, the plates were examined for zones of inhibition. The diameter of the zones of inhibition were measured and recorded in millimeter. Standard chloramphenicol antibiotic (10 mg) was obtained to serve as control and experiment carried out as described for the extract.

### Phytochemical screening

The phytochemical constituents of the plant were determined in accordance with the methods described by El-Mahmoud et al. (2008). A 100 g of the dried leaves were crushed using ceramic pestle and mortar into fine powder. A 40 g of the powdered material was then soaked with 300 ml of methanol and allowed to stay overnight. After a period of 24 h, the solution was filtered with a No 1 whatmann filter paper. The filtrate was used for the determination of the phytochemical constituents. Alkaloids, cardiac glycosides, tannins, flavonoids, anthraquinones, saponins and phlobatannins were determined in the extract.

## RESULTS AND DISCUSSION

The results of antibacterial activity of the methanolic extract of *C. leptostachya* were presented in Table 1. The result revealed that 20 and 40 mg/ml concentrations of the extract showed no activity on all the test isolates. Similarly, there was no activity against *Shigella dysenteriae* in all the different concentrations of the extract used. It is also evidently clear that highest activity of the extract of 19 mm was observed against *E. coli* when a concentration of 80 mg/ml was used. However, less activity (15.50 mm) was witnessed when a concentration of 60 mg/ml was used on *S. aureus*. This means that activity increases with increase in concentration. The inactivity of the extract at lower concentrations or on *S. dysenteriae* may be due to the ability of the isolates in degrading the active components of the extract (Croshaw, 1983). Furthermore, the age of inoculums and the number of variable cell it contains can affect the activity of the extract. The isolates may also have already developed resistance due to overlong exposure to the extract (Aishatu and Deeni, 1994).

However, the high activity of the extract recorded on *E. coli*, *S. typhi* and *S. aureus* is very encouraging. This could be due to the secondary metabolites contained within the plant since it is well documented that they possess antimicrobial activity (Cowan, 1999). These findings were in conformity with that of Yerima et al. (2007) who reported a high inhibitory activity of garlic extract on *S. aureus*. Similarly, Ekpo et al. (2007) reported high antimicrobial activity of the extracts and fractions of *Triumfetta cordifolia* A. Rich on *S. aureus*, *E.*

**Table 1.** Effect of *Celosia leptostachya* extract against bacterial isolates at different concentrations (mg/ml).

Clinical isolates	Zones of inhibition in (mm)				
	20	40	60	80	Control
<i>Staphylococcus aureus</i>	NA	NA	15.50	18.00	21.00
<i>Shigella dysenteriae</i>	NA	NA	NA	NA	20.00
<i>Salmonella typhi</i>	NA	NA	NA	17.50	23.00
<i>Escherichia coli</i>	NA	NA	NA	19.00	21.00

NA = No activity.

**Table 2.** Quantitative test of the phytochemical constituents of methanolic extract of *Celosia leptostachya* leaves.

Constituents	Methanolic extract
Alkaloids	
Mayer's reagent	-
Dragendorff's reagent	+
Wagner's reagent	+
Steroids	
Liebermann's test	+
Salkowski's test	-
Cardiac glycosides	+
Flavonoids	
Sodium hydroxide	+
Lead acetate	-
Anthraquinone	-
Saponins	-
Tannins	+
Phlobatannins	-

Key: += present -= absence

*coli* and *Klebsiella pneumoniae*. The positive control showed no activity against all the isolates. This could be explained by the fact that the control used was a standard antibiotic and well refined than the extract.

The phytochemical analysis on the methanolic extract of the leaf of *C. leptostachya* revealed the presence of alkaloids, steroids, cardiac glycosides, tannins and flavonoids. However, anthraquinone, saponins and phlobatannins are not detected in the extract (Table 2). Alkaloids are well known for their wide pharmacological activities ranging from antibacterial and antifungal activities (Trease and Evans, 1989). Pure isolated alkaloids and their synthetic derivatives are used as the basic medicinal agents because of their analgesic, antipyretic and antibacterial properties and show

marked physiological effects when administered to animals (Njoku and Akumefula, 2007).

The presence of tannins in the extract indicated the possible use of the plants in ethnobiological medicine (Trease and Evans, 1989). The tannins have the ability to react with protein forming stable water insoluble components and therefore, may have a profound effect on bacteria since bacterial cell wall are made up of proteins (Trease and Evans, 1989). They accelerate the healing of wounds and inflamed mucous membranes (Njoku and Akumefula, 2007). Cardiac glycosides comprise a group of glycosides that exert a cardiotonic action and therefore, are used to improve body circulation and heart function in congestive heart failure (El-Olemy et al., 1994). Furthermore, the leaves could be useful in the

treatment of heart diseases. Flavonoids are free radical scavengers, potent antioxidant and water soluble which prevent oxidative cell damage and have strong anticancer activity (Njoku and Akumefula, 2007). The flavonoids have been shown to be effective against allergies, platelets aggregation microbes, ulcer and tumors (Njoku and Akumefula, 2007).

Some substances that were determined in the extract possess antimicrobial activities. The observed antimicrobial properties of the extract could be attributable to these substances (Cowan, 1999). These findings are in conformity to that of Halilu et al. (2008) who reported the presence of flavonoids, tannins and cardiac glycosides from the stem bark extract of *Parinari curatellifolia*. Aliyu et al. (2009) also reported the presence of these secondary metabolites in the methenolic stem bark extract of *Bauhinia rufescens* lam. In a similar study, Omale et al. (2009) reported the presence of tannins and flavonoids in both the stem and leaf extracts *Ocimum gratissimum*. All these researchers reported the antimicrobial properties of these secondary metabolites.

## Conclusion

The result of the preliminary studies indicated that the plant contain some major bioactive compound that inhibit the growth of microorganism thereby proving very effective as alternative source of antibiotics. The result also showed high antibacterial activity of the plant extract which justifies the use of the plant in the treatment of bacterial and many infectious diseases.

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