



RESEARCH PAPER

Phytochemical Screening and Antibacterial activity of *Chromolaena odorata* leaves Extract against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

This study investigated the phytochemical properties and the in-vitro antibacterial activity of aqueous and ethanolic leaf extracts of *Chromolaena odorata*, also known as Siam weed. The extract challenged the *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method. Furthermore, the minimum inhibitory and minimum bactericidal concentrations of the extracts against the selected pathogens were also determined. The phytochemicals present in both the aqueous and ethanolic extracts were, terpenoids, tannins, saponin, phlobatannin, cardiac glycoside, alkaloid and phenol. Both *Staphylococcus aureus* and *Escherichia coli* were sensitive to different concentrations of the extracts. The highest zones of inhibition exhibited by *S. aureus* to both aqueous and ethanolic extracts were 16mm and 20mm respectively at a concentration of 200mg/ml. *E. coli* exhibited zones of inhibition measuring 22mm and 24mm to both aqueous and ethanolic extracts respectively at a concentration of 200mg/ml. The minimum inhibitory concentration (MIC) of the ethanolic extract against *Staphylococcus aureus* and *Escherichia coli* was at 25mg/ml and 56.25mg/ml respectively. The MIC of the aqueous extract recorded against *Staphylococcus aureus* and *Escherichia coli* were 25mg/ml and 12.5mg/ml respectively. The minimum bactericidal concentration (MBC) of the ethanolic extract on *Staphylococcus aureus* and *Escherichia coli* was 25mg/ml and 12.5mg/ml respectively, while that of the aqueous extract was 50mg/ml and 25mg/ml for *Staphylococcus aureus* and *Escherichia coli* respectively. The results indicated that *Chromolaena odorata* has great antibacterial potential. The ethanolic extract was more effective compared to the aqueous extract and *Escherichia coli* was more susceptible to both extracts than *Staphylococcus aureus*.

Keywords: Antibacterial Effects, Plant extracts, Pathogens, *Chromolaena odorata*, MIC, MBC

INTRODUCTION

Microorganisms have been known to cause diseases from time immemorial, and this has led to intensive research and development of effective chemotherapeutic agents in combatting diseases. Despite such achievements, the therapeutic potential of drugs have become limited as a result of multi-drug resistance (MDR). Microorganisms have developed numerous mechanisms to evade antimicrobial effects of drugs, which has led to the continued search for alternative, safer and effective natural drug sources such as plants. There are many plants with therapeutic potentials, the knowledge of which has birthed the advent of researches into discovery of new plant-derived drugs. The phytochemical screening of crude plant extracts using suitable solvents have revealed that some plants are naturally endowed with secondary metabolites which have antimicrobial properties that can be used in the treatment of infections. Among such useful plants is, *Chromolaena odorata*, a herbaceous

perennial that belongs to the family, Asteracea. It is a diffuse scrambling shrub that is mainly a weed of plantation crops and pastures of Southern Asia and Western Africa (Muriappan and Marcutani, 1998). It is commonly called Siam weed, Christmas bush, bitter biishjor and Jack in the bush. In Hausa language, it is called *Jiwene* and *Jinwinin*, *Awolowo* in Yoruba and *Obiarakara* in Igbo language (Okon and Amalu, 2003).

In tropical Africa, *Chromolaena odorata* is used as a medicinal herb for a variety of ailments including malaria, dysentery, toothache and fever (Taiwo *et al.*, 2000). Studies have also demonstrated that the leaf extract of *Chromolaena odorata* has antioxidant, anti-

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inflammatory, analgesic, antimicrobial cytoprotective and many other medicinally significant properties (Oyewole *et al.*, 2008 ; Alisi *et al.*, 2011). Phytochemical studies are very important in ethno medicinal researches because they reveal the likely active ingredients in crude extracts of plants under study. However, this in itself is not sufficient and needs to be complemented by in-vitro studies which indeed prove the likely roles played by these active secondary metabolites in inhibiting the growth of infectious disease agents, particularly bacteria and recently, parasites. Despite the increasing interest in the plant *Chromolaena odorata* due to its numerous medicinal attributes, there is limited information on the in-vitro activity of the plant against some of the pathogens implicated in the causation of some of the diseases highlighted earlier. Thus, this study was designed with the aim of determining the phytochemical constituents of *C. odorata* obtained from Zaria environs and establishing the in-vitro antibacterial potential of the leaves extract against *Escherichia coli* and *Staphylococcus aureus*. This may provide useful baseline information for plant-drug trials and understanding the dynamics of in-vivo actions or pathways of such plant-derived drugs and infusions.

METHODOLOGY

Collection of *Chromolaena odorata* plant

Fresh leaves of *Chromolaena odorata* were collected from the Ahmadu Bello University Botanical Garden, Zaria, Nigeria. Identification of the plant was done at the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The leaves were air-dried for two weeks at room temperature (30°C) after which they were ground into powder and ready for use.

Ethanol extraction of *Chromolaena odorata* leaves

Five hundred (500ml) milliliters of ethanol was poured into a 1000ml capacity round bottom flask of a soxhlet apparatus and mixed together with some anti-bumping agent. Fifty (50g) gram of the leaf powder was measured and wrapped in a thimble and placed in the soxhlet extractor then connected to the round bottom flask. Cold water from the top was also connected to circulate through the condenser. The heating was adjusted to a constant temperature until the solvent was refluxed at a steady rate. After the

completion of the extraction, the unit was then allowed to cool and the solvent poured into a reagent bottle. The extract was recovered from the solvent by heating over a water bath set at 60°C until constant weight was obtained. The product was then aseptically transferred into a dry specimen bottle and stored at room temperature until required (Awe and Omojasola, 2009).

Aqueous Extraction of *Chromolaena odorata* leaves

Fifty (50g) gram of the leaf powder was weighed into a 500ml beaker, to which 300ml distilled water was added. It was shaken and boiled for 3hours at 60°C temperature. The mixture was allowed to cool at room temperature, and then it was decanted and filtered using Whatman filter paper with a diameter of 125mm and pore size of 0.7mm. The extract was then evaporated on a water bath set at 60°C to a constant weight. The final product was put into a specimen bottle, and stored at room temperature until required (Awe and Omojasola, 2009).

Phytochemical screening of aqueous and ethanolic extracts of *Chromolaena odorata* leaves

The active components of the extracted leaves of *Chromolaena odorata* were determined using standard methods. The phytochemical constituents screened were; terpenoids, anthraquinones, saponins (Harborne, (2003), tannins, alkaloids, flavonoids, phlobatannins, cardiac glycosides and phenols (Trease and Evans 2005).

Sensitivity test (Agar well dilution method).

Overnight broth cultures of the test organisms (standardized cultures of *E. coli* and *S. aureus*) were swabbed onto sterile Mueller-Hinton agar in Petri dishes using sterile cotton swabs. A sterile cork borer of size 5mm in diameter was used to make wells on the agar. Stock concentration of the extract of *Chromolaena odorata* was diluted from 200mg/ml to 100mg/ml to 50mg/ml and finally to 25mg/ml with sterile distilled water. Each of these concentrations was introduced into the wells on the inoculated Petri dishes. The plates were left for 1 hour for the extract to diffuse and subsequently incubated at 37°C for 24hours and the corresponding zones of inhibitions were measured (Omotayo, 1998).

Determination of minimum inhibitory concentration (MIC)

Each test organism that was susceptible to the extracts of *Chromolaena odorata* was inoculated into sterile Mueller-Hinton broth and incubated overnight at 37°C, then 1ml of the extract was serially diluted with sterile distilled water (4ml) from 50mg/ml to 25mg/ml to 12.5mg/ml, 6.25mg/ml and finally to 3.125mg/ml. From each of the diluted concentrations, 1ml was inoculated into sterile Mueller-Hinton broth (8ml) containing 0.2ml of the overnight broth culture of the test organisms. A control was set up which contained only Mueller-Hinton broth and the extract. The inoculated and control tubes were then incubated at 37°C for 24hours after which they were observed for turbidity. The lowest concentration which showed no turbidity was taken as the minimum inhibitory concentration (Prescott *et al.*, 2005).

Determination of minimum bactericidal concentration (MBC)

Samples from the tubes used in MIC assay which did not show signs of turbidity after the period of incubation were streaked out on solidified sterile Muller-Hinton agar in Petri dishes using sterile cotton swabs, and they were then incubated at 37°C for about 18-24hours. The lowest concentration of the extract which showed no growth was taken as the minimum bactericidal concentration. (Omotayo, 1998; Prescott *et al.*, 2005).

RESULTS

Table 1 presents the phytochemical analysis of the aqueous and ethanolic leaf extracts of *Chromolaena odorata*, indicating the active constituents. Out of the nine active ingredients screened for, six were present in the ethanolic extract, while five were present in the aqueous extract. Terpenoids, Tannins and Cardiac glycosides were abundantly present in the ethanolic extract compared to the aqueous

extract. Flavonoids was absent in both extracts, while terpenoids, cardiac glycosides and anthraquinones were absent in the aqueous extract.

Table 2 presents the result of the sensitivity of the microorganisms to both the aqueous and ethanolic extracts of the plant. The antibacterial effect of both extracts increased with increasing concentration. The ethanolic extract appeared to be more effective than the aqueous extract, while *S. aureus* was more susceptible to both extracts than *E. coli*.

Table 3 presents the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic and aqueous extracts of *Chromolaena odorata* on *Staphylococcus aureus*. The MIC and MBC of the aqueous extract were 25mg/ml and 50mg/ml respectively, while the MIC and MBC of the ethanolic extract were 25mg/ml and 25mg/ml respectively.

Table 4 presents the MIC and MBC of the aqueous and ethanolic extracts of *Chromolaena odorata* on *Escherichia coli*. The MIC and MBC of the aqueous extract were 25mg/ml and 12.5mg/ml respectively, while the MIC and MBC of the ethanolic extract were 12.5mg/ml and 6.25mg/ml respectively.

Table 1: Phytochemical properties of aqueous and ethanolic extracts of *Chromolaena odorata*.

Phytochemicals	Aqueous	Ethanolic
Terpenoids	-	++
Tannins	+	++
Saponin	++	+
Phlobatannin	++	-
Cardiac glycosides	-	++
Flavonoids	-	-
Anthraquinones	-	+
Phenol	++	+
Alkaloid	++	-

Key: ++: abundant, +: trace, -: absent

Table 2: Zones of inhibition (mm) at different concentrations (mg/ml) of the aqueous extract.

Test organisms	Concentration (mg/ml)	Aqueous (mm)	Ethanollic (mm)	Positive control (mm)
<i>Staphylococcus aureus</i>	200	16	20	
	100	14	17	20
	50	10	13	
	25	7	10	
<i>Escherichia coli</i>	200	22	24	
	100	18	20	15
	50	14	18	
	25	10	16	

KEY: Positive control = ciprofloxacin

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (mg/ml) of aqueous and ethanolic extracts on *Staphylococcus aureus*.

Extract	MIC (mg/ml)	MBC (mg/ml)
Aqueous	25	
Ethanollic	25	

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in mg/ml of aqueous and ethanolic extracts on *Escherichia coli*

Extract	MIC (mg/ml)	MBC (mg/ml)
Aqueous	50	
Ethanollic	25	

DISCUSSION

Establishing the *in-vitro* antibacterial potential of *C. odorata* as well as other numerous plant extracts is dependent on quite a number of factors ranging from extraction processes to the type of laboratory media used (Parekh and Chanda, 2006). One major factor which may have played a role in this study was the type of solvent used for extraction of the active phytochemical constituents of the plant. The findings of this study revealed that both aqueous and ethanolic extracts of *C. odorata* had growth-inhibitory properties against both pathogens tested, which is attributed to the presence of phytochemical constituents. Phenolics, alkaloids, terpenoids and cardiac glycosides detected in *C. odorata* leaf extracts are compounds that have been documented to possess medicinal properties and health

promoting effects (Selah *et al.*, 1995; Del Rio *et al.*, 1997; Okwu, 2004; Liu, 2004). This suggests that the plant may possess haemolytic, anti-inflammatory, antioxidative, immunostimulant and antimicrobial properties (Lovkova *et al.*, 1999). Phlobatannins and alkaloids were present in the aqueous extract. The presence of phlobatannins indicated diuretic property, while the presence of alkaloids suggest potential of the aqueous extract to assist white blood cells in disposing harmful microorganisms and cell debris and also improve cardiac conditions by reducing blood pressure, increasing circulation and inhibiting accumulation of arteriosclerosis plaque and blood clots (Harborne, 2000). The presence of anthraquinones is very significant, because it is considered to be one of the most active anti-cancer agent in metastatic breast cancer (Ayoola *et al.*, 2013). The ethanolic leaf extract of *C. odorata* apparently performed better than the aqueous extract, which may be due to the solubility of the active components in this organic solvent (Parekh and Chanda, 2006). Another likely contributory factor to the difference in performance between both solvents is the polarity of the compounds being extracted by the solvents. These combined factors may have been responsible for certain phytochemical constituents being either present or absent in either of the extracts. The ethanolic extract exhibited more antibacterial effect probably because it contained more secondary metabolites combined, as revealed by the phytochemical analysis. This corroborates assertions of Geyid *et al.* (2005) that the more the number of secondary metabolites present in the extract, the stronger the antimicrobial

properties demonstrated by the plant. The result of the phytochemical analysis in this study is in agreement with the work done by Anyasor *et al.* (2011).

The leaf extracts of *C. odorata* exhibited potential antibacterial activity against both *S. aureus* and *E. coli*. *Staphylococcus aureus* being a Gram positive bacterium, appeared to be more susceptible to both aqueous and ethanolic crude extracts of the plant than *E. coli*. This difference in the level of susceptibility may be attributable to the difference in the cell wall structure of Gram negative and Gram positive organisms. It is therefore not surprising that this study corroborates other studies that have implicated that Gram negative organisms such as *E. coli* are less susceptible and more resistant to chemotherapeutic agents and even exhibit multi drug resistance (Gisene *et al.*, 2000; Du Sallah *et al.*, 2007).

In conclusion, *C. odorata* possesses in-vitro antibacterial activity against *S. aureus* and *E. coli*; and could be a potential to be harnessed and extended over other pathogens to establish its broad spectrum of activity. Both the ethanolic and aqueous extracts are suitable solvents that can be used to extract the active components of the plant, however, the ethanolic extract was more effective, as it extracted more secondary metabolites combined. There is however, need for further investigations into the phytochemical constituents of the plant leaves, using purified chemical components in contrast to the crude extracts.

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